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Monoclonal Antibodies: From Structure to Therapeutic Application

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INTRODUCTION

The exciting field of therapeutic monoclonal antibodies (MABs) had its origins as Milstein and Koehler presented their murine hybridoma technology in 1975 (Kohler and Milstein 1975). This technology provides a reproducible method for producing monoclonal antibodies with unique target selectivity in almost unlimited quantities. In 1984, both scientists received the Nobel Prize for their scientific breakthrough, and their work was viewed as a key milestone in the history of MABs as therapeutic modalities and their other applications. Although it took some time until the first therapeutic MAB got market authorization from the FDA in 1986 (Orthoclone OKT3, Chap. 19), monoclonal antibodies are now the standard of care in several disease areas. In particular, in the areas of oncology (Chap. 17), transplantation (Chap. 19), and inflammatory diseases (Chap. 20), patients now have novel life-changing treatment alternatives for diseases which had very limited or nonexistent medical treatment options before the emergence of MABs. To date more than 30 MABs and MAB derivatives including fusion proteins and MAB fragments are available for different therapies (Table 7.1). Eight MABs and three immunoconjugates in oncology; 11 MABs, one Fab conjugate, and four Fc fusion proteins in inflammation; and three MABs and one Fc fusion protein in transplantation comprise the majority of the approved therapies.

Technological evolutions have subsequently allowed much wider application of MABs via the ability to generate mouse/human chimeric, humanized, and fully humanized MABs from the pure murine origin. In particular, the reduction of the xenogenic portion of the MAB structure decreased the immunogenic potential of the murine MABs thus allowing their wider application. MABs are generally very safe drugs because of their target selectivity, thus avoiding unnecessary exposure to and consequently activity in nontarget organs. This is particularly apparent in the field of oncology, where MABs like rituximab, trastuzumab, and bevacizumab can offer a more favorable level of efficacy/safety ratios compared to common chemotherapeutic treatment regimens for some hematologic and solid tumors.

The dynamic utilization of these biotechnological methods resulted not only in new drugs, but it also triggered the development of an entirely new business model for drug research and development with hundreds of newly formed and rapidly growing biotech companies. Furthermore, the ability to selectively target disease-related molecules resulted in a new scientific area of molecular-targeted medicine, where the development of novel MABs probably contributed substantially to setting new standards for a successful drug research and development process. The term translational medicine was developed to cover the biochemical, biological, (patho) physiological understanding and using this knowledge to find intervening options to treat diseases. During this process, biomarkers (e.g., genetic expression levels of marker genes, protein expression of target proteins, molecular imaging) are used to get the best possible understanding of the biological activities of drugs in a qualitative and most importantly quantitative sense, which encompasses essentially also in the entire field of pharmacokinetics/pharmacodynamics (PK/PD). The application of those scientific methods together with the principle of molecular-targeted medicine

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Name	Therapeutic area	Type	Antibody isotype	Target		Behavior[a]	PK			Regimen
				Receptor	Type		Half-life	Clearance	Vss	
Abciximab	Cardiovascular	Fragment	Chimeric Fab: mVar-hIgG1	CD41		Linear	0.29 days (Kleiman et al. 1995)	0.068 L/h/kg (Mager et al. 2003)	0.12 L/kg	0.25 mg/kg IV bolus
Abatacept	Inflammation	Fusion protein	Extracellular domain of hCTLA-4 + hinge of hFc	CD80/CD86		Linear	13.1 days (Hervey and Keam 2006)	0.22 mL/h/kg (Hervey and Keam 2006)	0.07 L/kg	IV infusions 2 or 10 mg/kg Q2W for first three cycles and monthly thereafter
Adalimumab	Inflammation	MAB	hIgG1	TNFα	Soluble and cell bound	Linear	14.7–19.3 days (Weisman et al. 2003)	9–12 mL/h (Weisman et al. 2003)	5.1–5.75 L SC: $F=64\%$	40 mg SC every week
Alefacept	Inflammation	Fusion protein	LFA-3/hIgG1(Fc)	CD2		Nonlinear	11.3 days (Amevive 2003)	0.25 mL/h/kg (Amevive 2003)	IV:94 mL/kg IM: $F=63\%$	7.5 mg IV infusion Q1W/12 weeks or 10 mg and 15 mg IM injection
Alemtuzumab	Oncology	MAB	rCDR-hIgG1	CD52	Soluble	Nonlinear	11 h after single dose, 12 days following multiple doses (Morris et al. 2003)	NA	0.18 L/kg (Campath 2009)	IV infusion at 3 mg initially, escalated to 10 mg until tolerated, and maintained at 30 mg, 3 times a week for 12w
Basiliximab	Transplantation	MAB	Chimeric: mVar-hIgG1	CD25		NR	4.1 days (Kovarik et al. 2001)	75 mL/h (Kovarik et al. 2001)	5.5–13.9 L	IV bolus or infusion 20 mg on days 0 and 4 (40 mg total)

Drug	Indication	Format	Isotype	Antigen	Soluble/cell bound	Linearity	Half-life	Clearance	Volume	Dosing
Belatacept	Transplantation	Fusion protein	Extracellular domain of hCTLA-4+ hinge of hFc	CD80/CD86		Linear	9.8 days (Nulojix 2011)	0.49 mL/h/kg (Nulojix 2011)	0.11 L/kg	10 mg/kg IV infusion over 30 min
Belimumab	Inflammation	MAB	Fully humanized IgG1	BLyS (B lymphocyte stimulator)	Soluble and cell bound	Linear	19.4 days (Benlysta 2011)	215 mL/day (Benlysta 2011)	5.29 L (Benlysta 2011)	10 mg/kg IV infusion Q2W for first 3 doses, then Q4W
Bevacizumab	Oncology	MAB	hIgG1	VEGF	Soluble	Linear	20 days (Avastin 2004)	0.207–0.262 L/day (Avastin 2004)	2.66–3.25 L	IV infusion 5 or 10 mg/kg Q2W or 15 mg/kg Q3W depending on indication
Brentuximab vedotin	Oncology	MAB-ADC	Chimeric IgG1	CD30	Soluble	Linear	4.43 days	1.76 L/day (Younes et al. 2010)	8.21 L	IV infusion of 1.8 mg/kg over 30 min every 3 weeks
Canakinumab	Inflammation	MAB	hIgG1	IL-1beta	Soluble	Linear	26 days (Ilaris 2011)	0.174 L/day	6.01 L $F=70\%$	150 mg SC
Certolizumab pegol	Inflammation	Fragment	Fab conjugated with PEG2MAL40K	TNFα		Linear	14 days (Cimzia 2011)	9.21–14.38 mL/h (Cimzia 2011)	6–8 L $F=76\text{-}88\%$ (Cimzia 2011)	400 mg SC initially, and at Weeks 2 and 4; maintenance regimen 400 mg every 4 weeks
Cetuximab	Oncology	MAB	Chimeric: mVar-hIgG1	EGFR	Soluble	Nonlinear	4.8 days (Erbitux 2004) MRT: 12.6 days	0.02–0.08 L/h/m² (Erbitux 2004)	2–3 L/m²	IV infusion 400 mg/m² as first dose followed by weekly doses of 250 mg/m²
Daclizumab	Transplantation	MAB	Hyperchimeric: mCDRhIgG1	CD25		Linear	20 days (Zenapax 2005)	15 mL/h (Zenapax 2005)	5.9 L (Zenapax 2005)	IV infusion 1 mg/kg Q2W for 5 doses

Table 7.1 ■ The pharmacological properties of the approved therapeutic antibodies, antibody isotype, antigen target, clinical indication, and pharmacokinetic parameters.

Name	Therapeutic area	Type	Antibody isotype	Target		Behavior[a]	PK			Regimen
				Receptor	Type		Half-life	Clearance	Vss	
Denosumab	Oncology	MAB	Human IgG2	RANKL		Linear	28 days (Prolia 2010)	NA	$F=62\,\%$ (Prolia 2010)	120 mg SC injection Q4W
Eculizumab	Hemolysis	MAB	Humanized IgG2/4 k	Complement protein C5	Soluble	Linear	8–14.8 days	22 mL/h (Soliris 2007)	7.7 L (Soliris 2007)	600 mg Q1W for 4 weeks, 900 mg 7 days later, then 900 mg Q2W thereafter
Efalizumab	Inflammation	MAB	mCDR-hIgG1	CD11a	Cell-bound internalized	Nonlinear	NR[a]	6.6 mL/kg/day (Joshi et al. 2006)	58 mL/kg SC: $F=50\,\%$	single 0.7 mg/kg SC conditioning dose, then 1 mg/kg weekly
Etanercept	Inflammation	Fusion protein	TNF receptor/hIgG1(Fc)	TNFα	Soluble and cell bound	Linear	4 days (Lee et al. 2003)	120 mL/h (Lee et al. 2003)	F: 58 % Vd: 6–11 L	Biweekly 25 mg SC injection
Golimumab	Inflammation	MAB	Fully human IgG1	TNFα	Soluble and cell bound	Linear	2 weeks	4.9–6.7 mL/day/kg (Simponi 2009)	$F=53\,\%$ Vd= 58–126 mL/kg	50 or 100 mg SC injection Q2W or Q4W
Ibritumomab tiuxetan	Oncology	MAB	Murine IgG1	CD20	Cell-bound stable	NR	47 h (Wiseman et al. 2001)	NA	NA	14.8 MBq/kg
Infliximab	Inflammation	MAB	Chimeric: mVar-hIgG1	TNFα	Soluble and cell bound	Linear	7.7–9.5 days (Cornillie et al. 2001; Remicade 2006)	9.8 mL/h	NA	Single IV infusion 1, 5, 10, or 20 mg/ kg at 1, 2, 4, 8, and 12 weeks
Ipilimumab	Oncology	MAB	Fully humanized IgG1	CTLA-4		Linear	14.7 days (Yervoy 2011)	15.3 mL/h (Yervoy 2011)	7.21 L (Yervoy 2011)	3 mg/kg IV infusion Q3W
Muromonab-CD3	Transplantation	MAB	Murine IgG2α	CD3		NR	0.75 days (Hooks et al. 1991)	NA	NA	IV bolus of 5 mg every day for 10–14 days

Drug	Indication	Type	Structure	Target	Solubility	Linearity	Half-life	Clearance	Volume	Dosing
Natalizumab	Inflammation	MAB	Humanized IgG$_4$k	a$_4$b$_1$ and a$_4$b$_7$ integrins		NR	11 days (Tysabri 2006)	16 mL/h (Tysabri 2006)	NA	NA	300 mg IV infusion Q4W
Ofatumumab	Oncology	MAB	Human IgG1κ	CD-20	Cell bound	Nonlinear	14 days (Arzerra 2009)	0.01 L/h (Arzerra 2009)	1.7–5.1 L (Arzerra 2009)	NA	IV infusion 300 mg initial dose, followed 1 week later by 2000 mg Q1W for 7 doses, followed 4 weeks later by 2,000 mg Q4W for 4 doses
Omalizumab	Inflammation	MAB	mCDR-hIgG1	IgE	Soluble	Linear	26 days (Xolair 2003)	2.4 mL/day/kg	48 mL/kg SC: $F=62\%$		150–375 mg by subcutaneous (SC) injection every 2 or 4 weeks
Palivizumab	Antiviral	MAB	mCDR-hIgG1	RSV		NR	20 days (Synagis 2004)	NR	NA		IM 15 mg/kg Q4W
Panitumumab	Oncology	MAB	hIgG2	EGFR	Soluble	Nonlinear	7.5 days (Vectibix 2006)	4.9 mL/day/kg	82 mL/kg (Ma et al. 2009)		6 mg/kg given IV over 60 min, once every 2 weeks
Ranibizumab	Macular degeneration	Fragment	hIgG1κ	VEGF	Soluble	NA	9[b] days (Lucentis 2006)	NA	NA		0.5 mg intravitreal injection Q4W
Rilonacept	Inflammation	Fusion protein	hIgG1	IL-1beta	Soluble	NA	7.6 days (Radin et al. 2010)	CL/F: 0.866 L/day	Vz/F: 9.73 L		SC loading dose of 320 mg, then 160 mg once weekly
Rituximab	Inflammation	MAB	Chimeric: mVar-hIgG1	CD20	Cell-bound stable	Linear	19 days (Rituxan 2006)	10 mL/h (Rituxan 2006)	3.1 L		375 mg/m^2

Table 7.1 ■ (continued)

Name	Therapeutic area	Type	Antibody isotype	Target		PK				
				Receptor	Type	Behavior[a]	Half-life	Clearance	Vss	Regimen
Tocilizumab	Inflammation	MAB	Recombinant humanized IgG1	IL-6	Soluble and cell bound	Nonlinear	up to 13 days (Actemra® Tocilizumab 2010)	12.5 mL/h (Actemra® Tocilizumab 2010)	6.4 L (Actemra® Tocilizumab 2010)	8 mg/kg IV infusion either Q2W or Q4W depending on indication
Tositumomab	Oncology	MAB radiolabeled	Murine IgG2α	CD20	Cell-bound stable	Nonlinear	NA	68.2 mL/h (Bexxar 2003)	NA	485 mg IV infusion
Trastuzumab	Oncology	MAB	mCDR-hIgG1	Her2	Cell-bound shed	Nonlinear	1.7–12 days[c] (Herceptin 2006)	16–41 mL/h (Tokuda et al. 1999)	3.6–5.2 L	IV infusion 10–500 mg Q1W
Ustekinumab	Inflammation	MAB	Human IgG1	IL-12, IL-23		Linear	14.9–45.6 days (Stelara, 2009)	1.90–2.22 mL/ kg/day (Stelara, 2009)	V=0.1 L/ kg F ~57 % (Zhu et al. 2009)	45 mg SC initially followed by a further 45 mg dose after 4 weeks, then 45 mg q12w

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, *MRT* mean residence time, *mVar* murine variable, *RSV* respiratory syncytial virus, *TNF* tumor necrosis factor, *VEGF* vascular endothelial growth factor, *NA* information not found, *NR* not reported

[a]Where PK are nonlinear, parameters are reported at usual clinical dose

[b]Vitreous elimination half-life

[c]Dose-dependent pharmacokinetics

combined with the favorable pharmacokinetics and safety of MABs might at least partly explain why biotechnologically derived products have substantially higher success rates to become marketed therapy compared to chemically derived small molecule drugs.

This chapter tries to address the following questions: What are the structural elements of MABs? How do MABs turn functional differences into different functional activities? And how is a MAB protein turned from a potential clinical drug candidate into a therapeutic drug by using a translational medicine framework? In this sense, this chapter provides a general introduction to Chaps. 17, 19, and 20, where the currently marketed MABs and MAB derivatives are discussed in the context of their therapeutic applications. Efalizumab (anti-CD11a, Raptiva®), a MAB marketed as anti-psoriasis drug in the US and EU, was chosen to illustrate the application of pharmacokinetic/pharmacodynamic principles in the drug development process.

ANTIBODY STRUCTURE AND CLASSES

Antibodies (Abs) (immunoglobulin (Ig)) are roughly Y-shaped molecules or combinations of such molecules. There are five major classes of Ig: IgG, IgA, IgD, IgE, and IgM. Table 7.2 summarizes the characteristics of these molecules, particularly their structure (monomer, dimer, hexamer, or pentamer), molecular weight (ranging from ~150 to ~1,150 kDa), and functions (e.g., activate complement, FcγR binding). Among these classes, IgGs and their derivatives form the framework for the development of therapeutic antibodies. Figure 7.1 depicts the general structure of an IgG with its structural components as well as a conformational structure of efalizumab (anti-CD11a, Raptiva®). An IgG molecule has four peptide chains, including two identical heavy (H) chains (50~55 kDa) and two identical light (L) chains (25 kDa), which are linked via disulfide (S–S) bonds at the hinge region. The first ~110 amino acids of both chains form the variable regions (V_H and V_L) and are also the antigen-binding regions. Each V domain contains three short stretches of peptide with hypervariable sequences (HV1, HV2, and HV3), known as complementarity determining regions (CDRs), i.e., the region that binds antigen. The remaining sequences of each light chain consist of a single constant domain (C_L). The remainder of each heavy chain contains three constant regions (C_{H1}, C_{H2}, and C_{H3}). Constant regions are responsible for effector recognition and binding. IgG can be further divided into four subclasses (IgG1, IgG2, IgG3, and IgG4). The differences among these subclasses are also summarized in Table 7.2.

■ Murine, Chimeric, Humanized, and Fully Humanized MABs

With the advancement of technology, early murine MABs have been engineered further to chimeric (mouse CDR human Fc), humanized, and fully humanized MABs (Fig. 7.2). Murine MABs, chimeric MABs, humanized MABs, and fully humanized MABs have 0 %, ~60–70 %, ~90–95 %, and ~100 % sequences that are similar to human MABs, respectively. Decreasing the xenogenic portion of the MAB potentially reduces the immunogenic risks of generating anti-therapeutic antibodies (ATAs). The first therapeutic MABs were murine MABs produced via hybridomas; however, these murine antibodies easily elicited formation of neutralizing human anti-mouse antibodies (HAMA) (Kuus-Reichel et al. 1994). Muromonab-CD3 (Orthoclone OKT3), a first-generation MAB of murine origin, has shown efficacy in the treatment of acute transplant rejection and was the first MAB licensed for use in humans. It is reported that 50 % of the patients who received OKT3 produced HAMA after the first dose. HAMA interfered with OKT3's binding to T cells, thus decreasing the therapeutic efficacy of the MAB (Norman et al. 1993). Later, molecular cloning and the expression of the variable region genes of IgGs have facilitated the generation of engineered antibodies. A second generation of MABs, chimeric MABs, consists of human constant regions and mouse variable regions. The antigen specificity of chimeric MAB is the same as the parental mouse antibodies; however, the human Fc region renders a longer in vivo half-life than the parent murine MAB, and similar effector functions as the human Ab. Currently, there are 5 chimeric antibodies and fragments on the market (abciximab, basiliximab, cetuximab, infliximab, and rituximab). These antibodies can still induce human anti-chimeric antibodies (HACA). For example, about 61 % of patients who received infliximab had HACA response associated with shorter duration of therapeutic efficacy and increased risk of infusion reactions (Baert et al. 2003). The development of ATA is currently not predictable, as 6 of 17 patients with systemic lupus erythematosus receiving rituximab developed hightiter HACA (Looney et al. 2004), whereas only 1 of 166 lymphoma patients developed HACA (McLaughlin et al. 1998). Humanized MABs contain significant portions of human sequence except the CDR which is still of murine origin. There are eleven humanized antibodies on the market (alemtuzumab, bevacizumab, daclizumab, eculizumab, efalizumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, and trastuzumab). The incidence rate of antidrug antibody (i.e., human antihuman antibody (HAHA)) was greatly decreased for these humanized MABs. Trastuzumab has a reported HAHA incidence rate of

Property		IgA		IgG				IgM	IgD	IgE
		IgA1	IgA2	IgG1	IgG2	IgG3	IgG4			
Serum concentration in adult (mg/mL)		1.4–4.2	0.2–0.5	5–12	2–6	0.5–1	0.2–1	0.25–3.1	0.03–0.4	0.0001–0.0002
Molecular form		Monomer, dimer		Monomer				Pentamer, hexamer	Monomer	Monomer
Functional valency		2 or 4		2				5 or 10	2	2
Molecular weight (kDa)		160 (m), 300 (d)	160 (m), 350 (d)	150	150	160	150	950(p)	175	190
Serum half-life (days)		5–7	4–6	21–24	21–24	7–8	21–24	5–10	2–8	1–5
% Total IgG in adult serum		11–14	1–4	45–53	11–15	3–6	1–4	10	0.2	50
Function	Activate classical complement pathway	–		+	+/–	++	–	+++	–	–
	Activate alternative complement pathway	+	–	–	–	–	–	–	–	–
	Cross placenta	–		+	+/–	+	+	–	–	–
	Present on membrane of mature B cell	–		–	–	–	–	+	–	+
	Bind to Fc Receptors of phagocytes	–		++	+/–	++	+	?	–	–
	Mucosal transport	++		–	–	–	–	+	–	–
	Induces mast cell degranulation	–		–	–	–	–	–	+	–
Biological properties		Secretory Ig, binds to polymeric Ig receptor		Placental transfer, secondary antibody for most response to pathogen , binds macrophage and other phagocytic cells by Fcγ receptor				Primary antibody response, some binding to polymeric Ig receptor, some binding to phagocytes	Mature B cell marker	Allergy and parasite reactivity, binds FcεR on mast cells and basophiles

Table 7.2 ■ Important properties of endogenous immunoglobulin subclass (Goldsby et al. 1999; Kolar and Capra 2003).

only 0.1 % (1 of 903 cases) (Herceptin 2006), but daclizumab had a HAHA rate as high as 34 % (Zenapax 2005). Another way to achieve full biocompatibility of MABs is to develop fully humanized antibodies, which can be produced by two approaches: through phage-display library and by using transgenic XenoMouse® with human heavy and light chain gene fragments (Weiner 2006). Adalimumab is the first licensed fully humanized MAB generated by the phage-display library. Adalimumab was approved in 2002 and 2007 for the treatment of rheumatoid arthritis and Crohn's

diseases, respectively (Humira 2007). However, despite its fully humanized Ab structure, the incidence of HAHA was about 5 % (58 of 1,062 patients) in three randomized clinical trials with adalimumab (Cohenuram and Saif 2007; Humira 2007). Panitumumab is the first approved fully humanized monoclonal antibody generated by using transgenic mouse technology. No HAHA responses have been reported yet in clinical trial after chronic dosing with panitumumab to date (Vectibix 2006; Cohenuram and Saif 2007). Of note, typically ATAs are measured using ELISA, and the
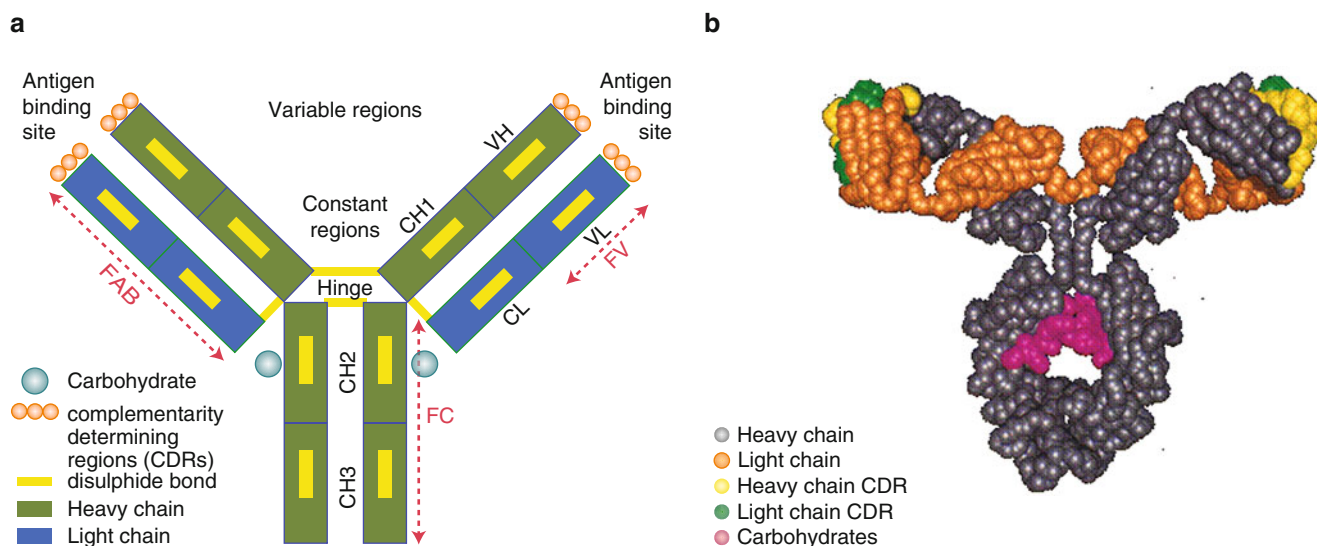
Figure 7.1 ■ (**a**) IgG1 antibody structure. Antigen is bound via the variable range of the antibody, whereas the Fc part of the IgG determines the mode of action (also called effector function). (**b**) Example efalizumab (anti-CD11a), Raptiva®. *H chain* heavy chain consisting of VH, CH1, CH2, CH3, *L chain* light chain consisting of VL, CL, *VH, VL* variable light and heavy chain, *CHn, CL* constant light and heavy chain, *Fv* variable fraction, *Fc* crystallizable fraction, *Fab* antigen-binding fraction (http://people.cryst.bbk.ac.uk/~ubcg07s/gifs/IgG.gif).
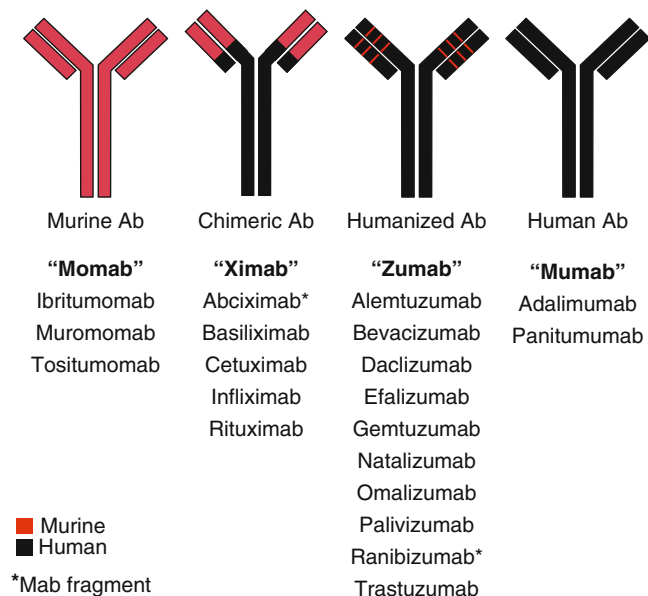
Figure 7.2 ■ Different generations of therapeutic antibodies.

reported incidence rates of ATAs for a given MAB can be influenced by the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be also influenced by several other factors including sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of a specific MAB with the incidence of antibodies to other products may be misleading.

■ Key Structural Components of MABs

Proteolytic digestion of antibodies releases different fragments termed Fv (fragment variable), Fab (fragment antigen binding), and Fc (fragment crystallization). These different forms have been reviewed by others (Wang et al. 2007). These fragments can also be generated by recombinant engineering. Treatment with papain generates two identical Fab's and one Fc. Pepsin treatment generates a F(ab')2 and several smaller fragments. Reduction of F(ab')2 will produce two Fab's. The Fv consists of the heavy chain variable domain (V_H) and the light chain variable domain (V_L) held together by strong noncovalent interaction. Stabilization of the Fv by a peptide linker generates a single chain Fv (scFv).

■ Modifying Fc Structures

The Fc regions of MABs play a critical role not only in their function but also in their disposition in the body. Monoclonal antibodies elicit effector functions (antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)) following interaction between their Fc regions and different Fcγ receptors and complement fixation (C1q, C3b). The CH2 domain or the hinge region joining CH1 and CH2 has been identified as the crucial regions for binding to FcγR (Presta et al. 2002). Engineered MABs with enhanced or decreased ADCC and CDC activity have been produced by manipulation of the critical Fc regions. Umana et al. (1999) engineered an anti-neuroblastomal IgG1 with enhanced ADCC activity

compared with wild type (WT). Shields et al. (2001) demonstrated that selected IgG1 variants with improved binding to FcγRIIIA showed an enhancement in ADCC for peripheral blood monocyte cells or natural killer cells. These findings indicate that Fc-engineered antibodies may have important applications for improving therapeutic efficacy. It was found that the FCGR3A gene dimorphism generates two allotypes, FcγRIIIa-158V and FcγRIIIa-158F, and the polymorphism in FcγRIIIA is associated with favorable clinical response following rituximab administration in non-Hodgkin's lymphoma patients (Cartron et al. 2004; Dall'Ozzo et al. 2004). Currently, several anti-CD20 MABs with increased binding affinity to FcγRIIIA are in clinical trials. The efficacy of antibody-interleukin 2 fusion protein (Ab-IL-2) was improved by reducing its interaction with Fc receptors (Gillies et al. 1999). In addition, the Fc portion of MABs also binds to the Fc receptor (FcRn named based on discovery in neonatal rats as neonatal FcRn), an Fc receptor belonging to the major histocompatibility complex structure, which is involved in IgG transport and clearance (Junghans 1997). Engineered MABs with a decreased or increased FcRn binding affinity have been investigated for the potential of modifying the pharmacokinetic behavior of MAB (see the section on Antibody Clearance for detail).

■ Antibody Derivatives (F(ab')2, Fab, Antibody Drug Conjugates) and Fusion Proteins

The fragments of antibodies (Fab, F(ab')2, and scFv) have a shorter half-life compared with the full-sized corresponding antibodies. scFv can be further engineered into a bivalent dimer (diabody) (~60 kDa, or trimer: triabody ~90 kDa). Two diabodies can be further

linked together to generate bispecific tandem diabody (tandab). Figure 7.3 illustrates the structure of different antibody fragments. Of note, abciximab and ranibizumab are two Fab approved by FDA. Abciximab is a chimeric Fab used for keeping blood from clotting with 20–30 min half-life in serum and 4 h half-life in platelets (Schror and Weber 2003). Ranibizumab, which is administrated via an intravitreal (IVT) injection, was approved for the treatment of macular degeneration in 2006 and exhibits a vitreous elimination half-life of 9 days (Albrecht and DeNardo 2006).

The half-life of Fc is more similar to that of full-sized IgGs (Lobo et al. 2004). Therefore, Fc portions of IgGs have been used to form fusions with molecules such as cytokines, growth factor enzymes, or the ligand-binding region of receptor or adhesion molecules to improve their half-life and stability. Alefacept, abatacept, and etanercept are three Fc fusion proteins on the market. Etanercept, a dimeric fusion molecule consisting of the TNF-α receptor fused to the Fc region of human IgG1, has a half-life of approximately 70–100 h (Zhou 2005), which is much longer than the TNF-α receptor itself (30 min ~2 h) (Watanabe et al. 1988).

Antibodies and antibody fragments can also be linked covalently with cytotoxic radionuclides or drugs to form radioimmunotherapeutic (RIT) agents or antibody drug conjugates (ADCs), respectively. In each case, the Ab is used as a delivery mechanism to selectively target the cytotoxic moiety to tumors (Prabhu et al. 2011). For both ADCs and RIT agents, the therapeutic strategy involves selective delivery of a cytotoxin (drug or radionuclide) to tumors via the antibody. As targeted approaches, both technologies exploit the overexpression of target on the surface of the cancer
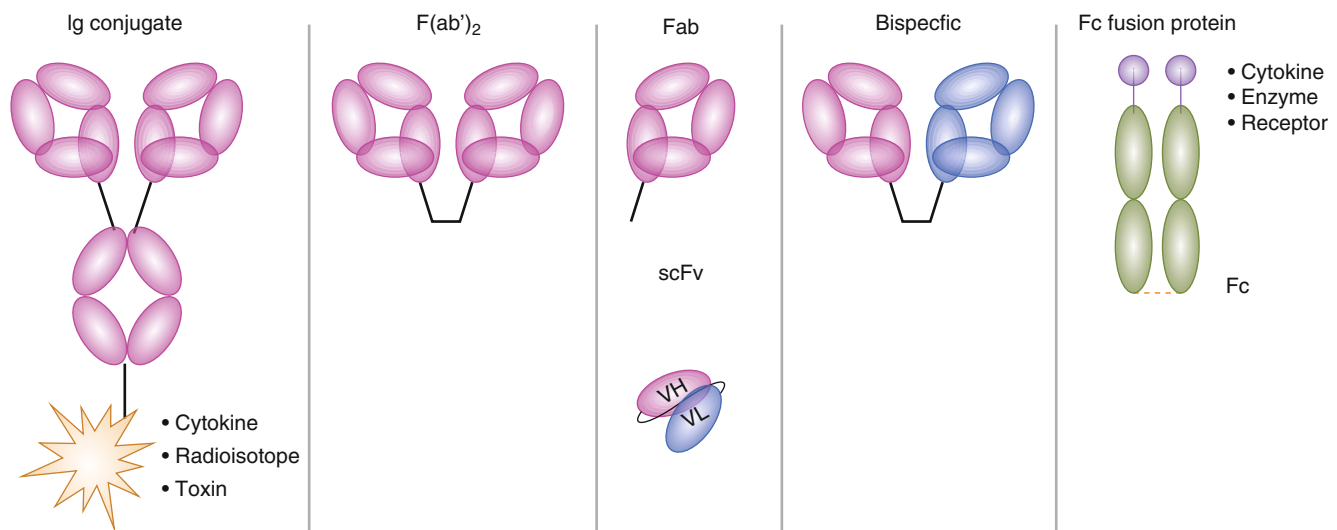

Figure 7.3 ■ Schematic representation of antibody derivatives (F(ab')2, Fab, scFv, and antibody conjugates) and Fc fusion proteins.

cells and thereby minimize damage to normal tissues. Such approaches are anticipated to minimize the significant side effects encountered when cytotoxic small molecule drugs or radionuclides are administered as single agents, thus leading to enhanced therapeutic windows. However, important distinctions exist between these two therapeutic modalities. For example, ADCs often require internalization into the endosomes and/or lysosomes for efficacy, while RIT agents are able to emit radiation, even from the cell surface, to achieve cell killing following direct binding to membrane antigens. Furthermore, RIT can deliver high levels of radiation even with very low doses of radioimmunoconjugate compared to ADCs. Importantly, most clinically successful ADC and RIT agents to date have been against hematologic tumors (Boswell and Brechbiel 2007). Various impediments to the delivery of antibodies and other macromolecules to solid tumors have been widely discussed and studied, especially in the context of microspatial distribution (Thurber et al. 2008).

Gemtuzumab ozogamicin (Mylotarg®, Dowell et al 2001), an anti-CD33 MAB linked to the cytotoxic antitumor antibiotic drug calicheamicin, became the first approved ADC in 2000 when it was granted accelerated approval for the treatment of acute myelogenous leukemia (AML). Calicheamicin binds to the minor groove of DNA, causing double-strand DNA breaks and resulting in inhibition of DNA synthesis. However, gemtuzumab ozogamicin was removed from the US market in June 2010, based on negative results in a follow-up confirmatory trial. In August 2011, the FDA approved a second ADC, brentuximab vedotin (Adcetris® 2011), for treatment of Hodgkin's lymphoma and systemic anaplastic large-cell lymphoma. Like gemtuzumab ozogamicin, brentuximab vedotin is directed against a soluble target (CD30). Most recently, in February 2013, FDA approved ado-trastuzumab emtansine Kadcyla®, a human epidermal growth factor receptor (HER2)-targeted ADC for treatment of HER2-positive breast cancer (LoRusso et al. 2011).

The only current radioimmunotherapeutic agents licensed by the FDA are ibritumomab tiuxetan (Zevalin® 2002) and tositumomab plus ^{131}I tositumomab (Bexxar® 2003), both for non-Hodgkin's lymphoma. Both of the above intact murine MABs bind CD20 and carry a potent beta particle-emitting radioisotope (^{90}Y for ibritumomab/tiuxetan and ^{131}I for tositumomab). In the case of ibritumomab, the bifunctional chelating agent, tiuxetan, is used to covalently link the radionuclide to the antibody, ibritumomab. However, another approved anti-CD20 antibody, rituximab, is included in the dosing regimen as a nonradioactive predose to improve the biodistribution of the radiolabeled antibody. Despite impressive clinical results, radioimmunotherapeutic antibodies have not generated considerable commercial success; various financial, regulatory, and commercial barriers have been cited as contributing factors to this trend (Boswell and Brechbiel 2007).

HOW DO ANTIBODIES FUNCTION AS THERAPEUTICS?

The pharmacological effects of antibodies are first initiated by the specific interaction between antibody and antigen. Monoclonal antibodies generally exhibit exquisite specificity for the target antigen. The binding site on the antigen called the epitope can be linear or conformational and may comprise continuous or discontinuous amino acid sequences. The epitope is the primary determinant of the antibody's modulatory functions, and depending on the epitope, the antibody may exert antagonist or agonist effects, or it may be nonmodulatory. The epitope may also influence the antibody's ability to induce ADCC and CDC. Monoclonal antibodies exert their pharmacological effects via multiple mechanisms that include direct modulation of the target antigen, CDC and ADCC, and delivery of a radionuclide or immunotoxin to target cells.

■ Direct Modulation of Target Antigen
Examples of direct modulation of the target antigen include anti-TNFα, anti-IgE, and anti-CD11a therapies that are involved in blocking and removal of the target antigen. Most monoclonal antibodies act through multiple mechanisms and may exhibit cooperativity with concurrent therapies.

■ Complement-Dependent Cytotoxicity (CDC)
The complement system is an important part of the innate (i.e., nonadaptive) immune system. It consists of many enzymes that form a cascade with each enzyme acting as a catalyst for the next. CDC results from interaction of cell-bound monoclonal antibodies with proteins of the complement system. CDC is initiated by binding of the complement protein, C1q, to the Fc domain. The IgG1 and IgG3 isotypes have the highest CDC activity, while the IgG4 isotype lacks C1q binding and complement activation (Presta 2002). Upon binding to immune complexes, C1q undergoes a conformational change, and the resulting activated complex initiates an enzymatic cascade involving complement proteins C2 to C9 and several other factors. This cascade spreads rapidly and ends in the formation of the membrane attack complex (MAC), which inserts into the membrane of the target and causes osmotic disruption and lysis of the target. Figure 7.4 illustrates the mechanism for CDC with rituximab (a chimeric antibody, which targets the CD20 antigen) as an example.
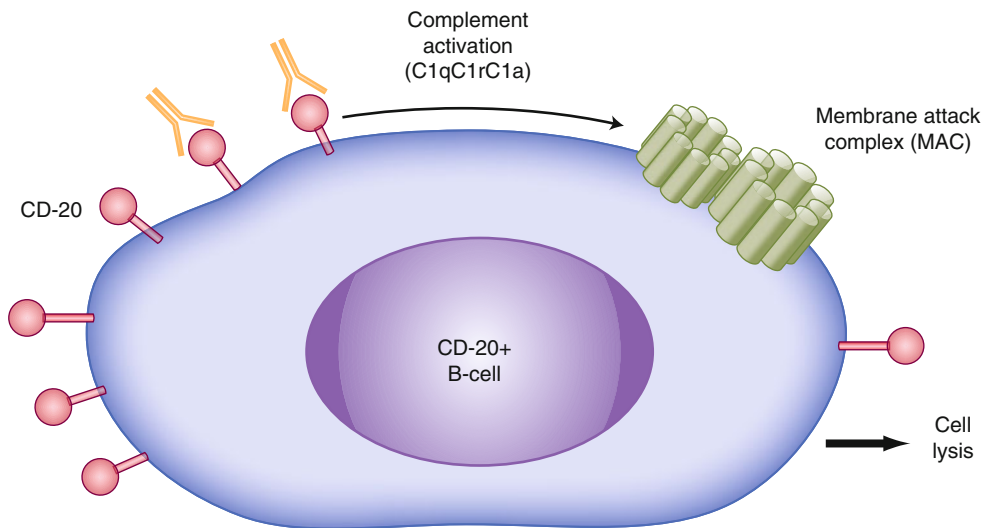
Figure 7.4 ■ An example of CDC, using a B cell lymphoma model, where the monoclonal antibody rituximab binds to the receptor and initiates the complement system, also known as the "complement cascade." The end result is a membrane attack complex (*MAC*), which leads to cell lysis and death.
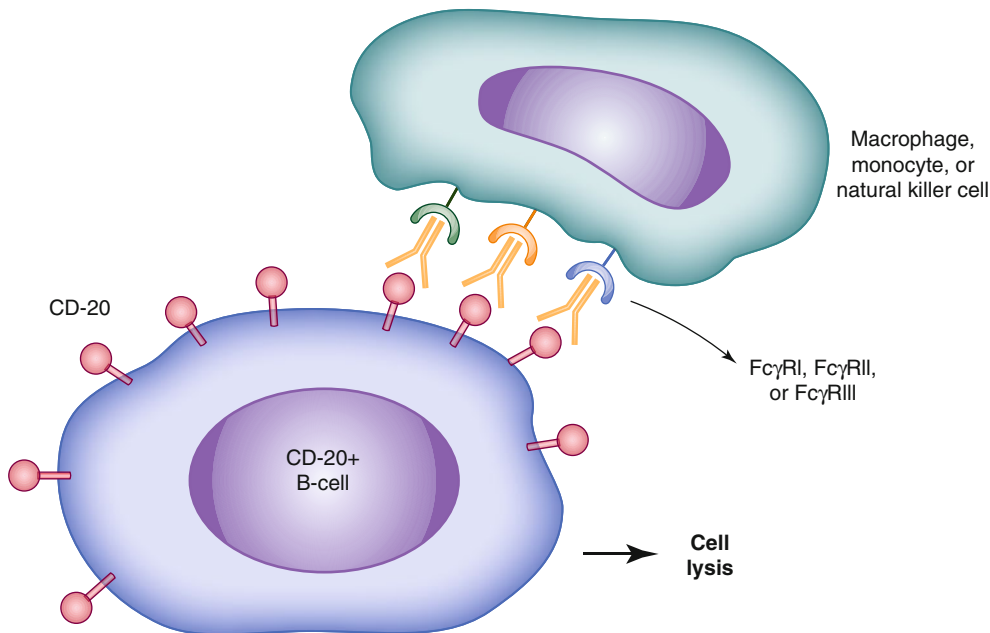

Figure 7.5 ■ An example of ADCC. In this situation rituximab targets the CD20 antigen. This antigen is expressed on a significant number of B cell malignancies. The Fc fragment of the monoclonal antibody binds the Fc receptors found on monocytes, macrophages, and NK cells. These cells in turn engulf the bound tumor cell and destroy it. NK cells secrete cytokines that lead to cell death, and they also recruit B cells.

■ Antibody-Dependent Cellular Cytotoxicity (ADCC)

ADCC is a mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies. It is one of the mechanisms through which antibodies, as part of the humoral immune response, can act to limit and contain infection. Classical ADCC is mediated by natural killer (NK) cells, monocytes, or macrophages, but an alternate ADCC is used by eosinophils to kill certain parasitic worms known as helminths. ADCC is part of the adaptive immune response due to its dependence on a prior antibody response. The typical ADCC involves activation of NK cells, monocytes, or macrophages and is dependent on the recognition of antibody-coated infected cells by Fc receptors on the surface of these cells. The Fc receptors recognize the Fc portion of antibodies such as IgG, which bind to the surface of a pathogen-infected target cell. The Fc receptor that exists on the surface of NK cell is called CD16 or FcγRIII. Once bound to the Fc receptor of IgG, the NK cell releases cytokines such as IFN-γ and cytotoxic granules like perforin and granzyme that enter the target cell and promote cell death by triggering apoptosis. This is similar to, but independent of, responses by cytotoxic T cells. Figure 7.5 illustrates the mechanism for ADCC with rituximab as an example.

■ Apoptosis

Monoclonal antibodies achieve their therapeutic effect through various mechanisms. In addition to the above-mentioned effector functions, they can have direct

effects in producing apoptosis or programmed cell death. It is characterized by nuclear DNA degradation, nuclear degeneration and condensation, and the phagocytosis of cell remains.

TRANSLATIONAL MEDICINE/DEVELOPMENT PROCESS

The tight connection of basic to clinical sciences is an essential part of translational medicine trying to *translate* the knowledge of basic science into practical therapeutic applications for patients. This knowledge transfer is also often entitled as *from-bench-to-bedside* process emphasizing the transition of scientific advancements into clinical applications. This framework of translational medicine is applied during the discovery and drug development process of a specific antibody against a certain disease. It includes major steps such as identifying an important and viable pathophysiological target antigen to modify the disease in a beneficial way, producing MABs with structural elements providing optimal pharmacokinetics, and safety and efficacy by testing the MAB in nonclinical safety and efficacy models and finally in patients. An overview of the development phases of the molecules comprising the nonclinical activities is outlined in Fig. 7.6. Furthermore, the critical components of the entire development process of MABs from a PK/PD perspective is explained in detail within the following sections.

■ Preclinical Safety Assessment of MABs

Preclinical safety assessment of MABs offers unique challenges, as many of the classical evaluations employed for small molecules are not appropriate for protein therapeutics in general and MABs in particular. For example, in vitro genotoxicology tests such as the Ames and chromosome aberration assays are generally not conducted for MABs given their limited interaction with nuclear material and the lack of appropriate receptor/target expression in these systems. As MAB binding tends to be highly species specific, suitable animal models are often limited to nonhuman primates, and for this reason, many common in vivo models such as rodent carcinogenesis bioassays and some safety pharmacology bioassays are not viable for MAB therapeutic candidates. For general toxicology studies, cynomolgus and rhesus monkeys are most commonly employed and offer many advantages given their close phylogenetic relationship with humans; however, due to logistics, animal availability, and costs, group sizes tend to be much smaller than typically used for lower species thus limiting statistical power. In some cases, alternative models are employed to enable studies in rodents. Rather than directly testing the therapeutic candidate, analogous monoclonal antibodies that can bind to target epitopes in lower species (e.g., mice) can be engineered and used as a surrogate MAB for safety evaluation (Clarke et al. 2004). Often the antibody framework amino acid sequence is modified to reduce antigenicity thus enabling longer-term studies (Albrecht and DeNardo 2006; Weiner 2006; Cohenuram and Saif 2007). Another approach is to use transgenic models that express the human receptor/target of interest (Bugelski et al. 2000); although, results must be interpreted with caution as transgenic models often have altered physiology and typically lack historical background data for the model.
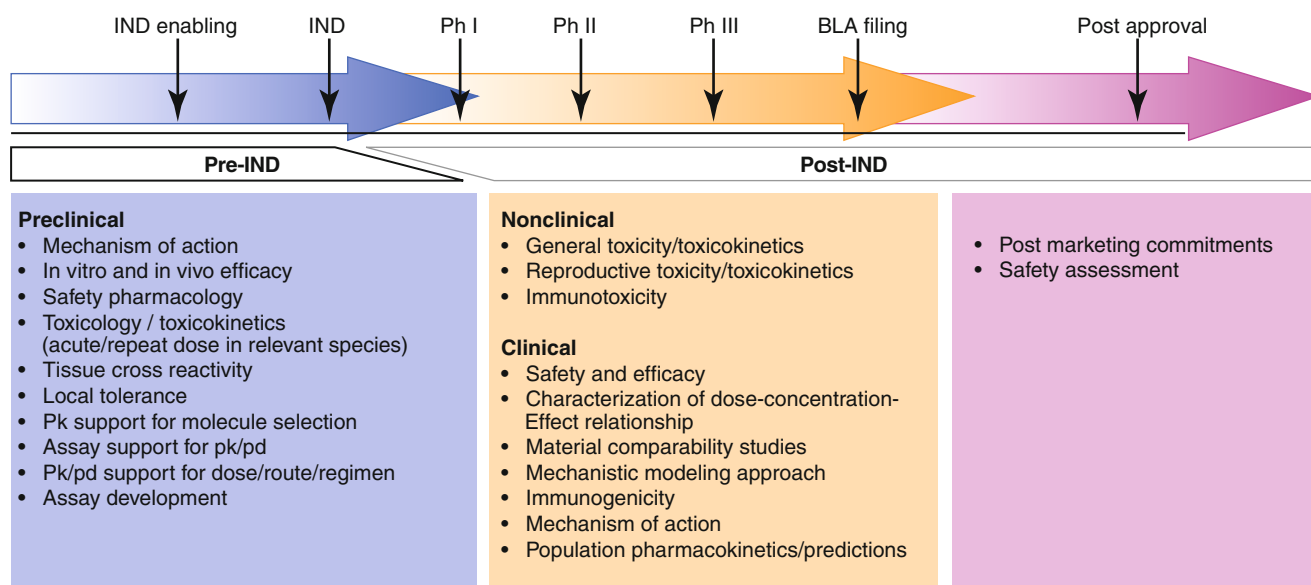

Figure 7.6 ■ Flowchart depicting PK/PD/toxicology study requirements during preclinical and clinical drug product development.

To address development issues that are specific to monoclonal antibodies and other protein therapeutics, the International Conference of Harmonization (ICH) has developed guidelines specific to the preclinical evaluation of biotechnology-derived pharmaceuticals (ICH 1997a, b).

For general safety studies, species selection is an important consideration given the exquisite species specificity often encountered with MABs. Model selection needs to be justified based on appropriate expression of the target epitope, appropriate binding affinity with the therapeutic candidate, and appropriate biologic activity in the test system. To aid in the interpretation of results, tissue cross-reactivity studies offer the ability to compare drug localization in both animal and human tissues. For MAB therapeutic candidates, a range of three or more dose levels are typically selected to attain pharmacologically relevant serum concentrations, to approximate levels anticipated in the clinic, and to provide information at doses higher than anticipated in the clinic. For most indications, it is important to include dose levels that allow identification of a no observable adverse effect level (NOAEL). If feasible, the highest dose should fall within the range where toxicity is anticipated; although, in practice, many monoclonal antibodies do not exhibit toxicity, and other factors limit the maximum dose. To best reflect human exposures, doses are often normalized and selected to match and exceed anticipated human therapeutic exposure in plasma, serum, or blood based upon the exposure parameters, area under the concentration-time curve (AUC), maximum concentrations (C_{max}), or concentration prior to next treatment (C_{trough}). The route of administration, dosing regimen, and dosing duration should be selected to best model the anticipated use in clinical trials (ICH 1997a, b).

To adequately interpret nonclinical study results, it is important to characterize anti-therapeutic antibody (ATA) responses. For human MABs, ATA responses are particularly prominent in lower species but also evident in nonhuman primates albeit to a lesser degree, making these species more viable for chronic toxicity studies. ATAs can impact drug activity in a variety of ways. Neutralizing ATAs are those that bind to the therapeutic in a manner that prevents activity, often by inhibiting direct binding to the target epitope. Non-neutralizing antibodies may also indirectly impact drug activity, for example, rapid clearance of drug–ATA complexes can effectively reduce serum drug concentrations. In situations where prominent ATA responses are expected, administration of high-dose multiples of the anticipated clinical dose may overcome these issues by maintaining sufficient circulating concentrations of active drug. To properly interpret study results, it is important to characterize ATA incidence and magnitude as the occurrence of ATA responses could mask toxicities. Alternatively, robust ATA responses may induce significant signs of toxicity such as infusion-related anaphylaxis that may not be predictive of human outcome where ATA formation is likely to be less of an issue. If ATA formation is clearly impacting circulating drug levels, ATA-positive individuals are often removed from consideration when evaluating pharmacokinetic parameters to better reflect the anticipated pharmacokinetics in human populations.

■ Pharmacokinetics

A thorough and rigorous PK program in the early learning phase of preclinical drug development can provide a linkage between drug discovery and preclinical development. PK information can be linked to PD by mathematical modeling, which allows characterizing the time course of the effect intensity resulting from a certain dosing regimen. Antibodies often exhibit pharmacokinetic properties that are much more complex than those typically associated with small molecule drugs (Meibohm and Derendorf 2002). In the following sections, the basic characteristics of antibody pharmacokinetics are summarized.

The pharmacokinetics of antibodies is very different from small molecules. Table 7.3 summarizes the PK differences between small molecule drugs and therapeutic antibodies regarding pharmacokinetics. Precise, sensitive, and accurate bioanalytical methods are essential for PK interpretation. However, for MABs, the immunoassays and bioassay methodologies are often less specific as compared to assays used for small molecule drugs (e.g., LC/MS/MS). Monoclonal antibodies are handled by the body very differently than small molecules. In contrast to small molecule drugs, the typical metabolic enzymes and transporter proteins such as cytochrome P450, multidrug resistance (MDR) efflux pumps are not involved in the disposition of MABs. Consequently, drug–drug interactions at the level of these drug-metabolizing enzymes and transporters are not complicating factors in the drug development process of MABs and do not need to be addressed by in vitro and in vivo studies. Intact MABs are not cleared by normal kidneys because of their large molecular weight; however, renal clearance processes can play an important role in the elimination of molecules of smaller molecular weight such as Fab's and chemically derived small molecule drugs. The different *ADME* (*A*bsorption, *D*istribution, *M*etabolism, and *E*limination) processes comprising the pharmacokinetics of MABs will be discussed separately to address their individual specifics.

Small molecule drugs	Monoclonal antibodies			
	Target is soluble antigen	Target is cell-bound antigen	Target is cell-bound antigen that is internalized and downregulated	Target is cell-/tissue-bound antigen that can be shed
PK usually independent of PD	PK often independent of PD	PK often dependent of PD		
Binding generally nonspecific (can affect multiple enzymes)	Binding very specific for target protein or antigen			
Usually linear PK Nonlinear PK problematic	Linear PK	Nonlinear PK		
Relatively short $t_{1/2}$	Long $t_{1/2}$	Low dose: short $t_{1/2}$ High dose: long $t_{1/2}$		
Not always orally available	Need parenteral dosing. SC or IM is possible			
Metabolism by P450s or other enzymes	Metabolism by nonspecific clearance mechanisms. No P450s involved	Metabolism by specific and nonspecific clearance mechanisms. No P450s involved		
Renal clearance often important	MABs: No renal clearance of intact antibody. May be cleared by damaged kidneys Antibody fragment might be eliminated by renal clearance			
Binding to tissues, high Vd	Distribution usually limited to blood and extracellular space			

Table 7.3 ■ Comparing the pharmacokinetics of small molecule drugs and monoclonal antibodies (Lobo et al. 2004; Roskos et al. 2004; Mould and Sweeney 2007).

ABSORPTION

Most of the MABs are not administrated orally because of their limited gastrointestinal stability, lipophilicity, and size all of which result in insufficient resistance against the hostile proteolytic gastrointestinal milieu and very limited permeation through the lipophilic intestinal wall. Therefore, intravenous administration is still the most frequently used route, which allows for immediate systemic delivery of large volume of drug product and provides complete systemic availability. Of note, 13 of over 30 FDA-approved antibody therapies listed in Table 7.1 are administered by an extravascular route (adalimumab (SC), alefacept (IM), canakinumab (SC), certolizumab pegol (SC), denosumab (SC), efalizumab (SC), etanercept (SC), golimumab (SC), omalizumab (SC), palivizumab (IM), ranibizumab (intravitreal), rilonacept (SC), and ustekinumab (SC)). The absorption mechanisms of SC or IM administration are poorly understood. However, it is believed that the absorption of MABs after IM or SC is likely via lymphatic drainage due to its large molecular weight, leading to a slow absorption rate (see Chap. 5). The bioavailability for antibodies after SC or IM administration has been reported to be around 50–100 % with maximal plasma concentrations observed 1–8 days following administration (Lobo et al. 2004). For example, following an IM injection, the bioavailability of alefacept was ~60 % in healthy male volunteers; its C_{max} was threefold (0.96 versus 3.1 μg/mL) lower, and its T_{max} was 30 times longer (86 versus 2.8 h) than a 30-min IV infusion (Vaishnaw and TenHoor 2002). Interestingly differences in PK have also been observed between different sites of IM dosing. The pharmacokinetics of MAB after IM injection is also dependent on the injection site. PAMAB, a fully humanized MAB against *Bacillus anthracis* protective antigen, has a significantly different pharmacokinetics between IM-GM (gluteus maximus site) and IM-VL (vastus lateralis site) injection in healthy volunteers (Subramanian et al. 2005). The bioavailability of PAMAB is 50–54 % for IM-GM injection and 71–85 % for IM-VL injection (Subramanian et al. 2005). Of note, MABs appear to have greater bioavailability after SC administration in monkeys than in humans (Oitate et al. 2011). The mean bioavailability of adalimumab is 52–82 % after a single 40 mg SC administration in healthy adult subjects, whereas it was observed to be 94–100 % in monkeys. Similarly the mean bioavailability of omalizumab is 66–71 % after a single SC dose in patients with asthma versus 88–104 % in monkeys.

DISTRIBUTION

After reaching the bloodstream, MABs undergo biphasic elimination from serum, beginning with a rapid distribution phase. The distribution volume of the rapid distribution compartment is relatively small, approximating plasma volume. It is reported that the volume of the central compartment (Vc) is about 2–3 L, and the

steady-state volume of distribution (Vss) is around 3.5–7 L for MABs in humans (Lobo et al. 2004; Roskos et al. 2004). The small Vc and Vss for MABs indicate that the distribution of MABs is restricted to the blood and extracellular spaces, which is in agreement with their hydrophilic nature and their large molecular weight, limiting access to the lipophilic tissue compartments. Small volumes of distributions are consistent with relatively small tissue: blood ratios for most antibodies typically ranging from 0.1 to 0.5 (Baxter et al. 1994; Baxter et al. 1995, Berger et al. 2005). For example, the tissue to blood concentration ratios for a murine IgG1 MAB against the human ovarian cancer antigen CA125 in mice at 24 h after injection are 0.44, 0.39, 0.48, 0.34, 0.10, and 0.13 for the spleen, liver, lung, kidney, stomach, and muscle, respectively (Berger et al. 2005). Brain and cerebrospinal fluid are anatomically protected by blood–tissue barriers. Therefore, both compartments are very limited distribution compartments for Abs hindering the access for therapeutic MABs. For example, endogenous IgG levels in CSF were shown to be in the range of only 0.1–1 % of their respective serum levels (Wurster and Haas 1994). However, it has been repeatedly noted that the reported Vss obtained by traditional non-compartmental or compartmental analysis may be not correct for some MABs with high extent of catabolism within tissue (Tang et al. 2004; Lobo et al. 2004; Straughn 2006). The rate and extent of antibody distribution will be dependent on the kinetics of antibody extravasation within tissue, distribution within tissue, and elimination from tissue. Convection, diffusion, transcytosis, binding, and catabolism are important determining factors for antibody distribution (Lobo et al. 2004). Therefore, Vss might be substantially greater than the plasma volume in particular for those MABs demonstrating high binding affinity in the tissue. Effects of the presence of specific receptors (i.e., antigen sink) on the distribution for MAB have been reported by different research groups (Danilov et al. 2001; Kairemo et al. 2001). Danilov et al. (2001) found that anti-PECAM-1 (CD31) MABs show tissue to blood concentration ratios of 13.1, 10.9, and 5.96 for the lung, liver, and spleen, respectively, in rats at 2 h after injection. Therefore, the true Vss of the anti-PECAM-1 is likely to be 15-fold greater than plasma volume.

Another complexity which needs to be considered is that tissue distribution via interaction with target proteins (e.g., cell surface proteins) and subsequent internalization of the antigen-MAB complex might be dose dependent. For the murine analog MAB of efalizumab (M17), a pronounced dose-dependent distribution was demonstrated by comparing tissue to blood concentration ratios for liver, spleen, bone marrow, and lymph node after a tracer dose of radiolabeled M17 and a high-dose treatment (Coffey et al. 2005).

The tracer dose of M17 resulted into substantially higher tissue to blood concentration ratios of 6.4, 2.8, 1.6, and 1.3 for the lung, spleen, bone marrow, and lymph node, respectively, in mice at 72 h after injection. Whereas, the saturation of the target antigen at the high-dose level reduced the tissue distribution to the target independent distribution and resulted consequently into substantially lower tissue to blood concentration ratios (less than 1).

FcRn may play an important role in the transport of IgGs from plasma to the interstitial fluid of tissue. However, the effects of FcRn on the MABs' tissue distribution have not been fully understood. Ferl et al. (2005) reported that a physiologically based pharmacokinetic (PBPK) model, including the kinetic interaction between the MAB and the FcRn receptor within intracellular compartments, could describe the biodistribution of an anti-CEA MAB in a variety of tissue compartments such as plasma, lung, spleen, tumor, skin, muscle, kidney, heart, bone, and liver. FcRn was also reported to mediate the IgG across the placental barriers (Junghans 1997) and the vectorial transport of IgG into the lumen of intestine (Dickinson et al. 1999) and lung (Spiekermann et al. 2002).

ANTIBODY CLEARANCE

Antibodies are mainly cleared by catabolism and broken down into peptide fragments and amino acids, which can be recycled – to be used as energy supply or for new protein synthesis. Due to the small molecular weight of antibodies fragments (e.g., Fab and Fv), elimination of these fragments is faster than intact IgGs, and they can be filtered through the glomerus and reabsorbed and/or metabolized by proximal tubular cells of the nephron (Lobo et al. 2004). Murine monoclonal anti-digoxin Fab, F(ab')2, and IgG1 have half-lives of 0.41, 0.70, and 8.10 h in rats, respectively (Bazin-Redureau et al. 1997). Several studies reported that the kidney is the major route for the catabolism of Fab and elimination of unchanged Fab (Druet et al. 1978; McClurkan et al. 1993).

Typically, IgGs have serum half-life of approximately 21 days, resulting from clearance values of about 3–5 mL/day/kg, and Vss of 50–100 mL/kg. The exception is IgG3, which has only a half-life of 7 days. The half-life of IgG is much longer than other Igs (IgA 6 days, IgE 2.5 days, IgM 5 days, IgD 3 days). The FcRn receptor has been demonstrated to be a prime determinant of the disposition of IgG antibodies (Ghetie et al. 1996; Junghans and Anderson 1996; Junghans 1997). FcRn, which protects IgG from catabolism and contributes to the long plasma half-life of IgG, was first postulated by Brambell in 1964 (Brambell et al. 1964) and cloned in the late 1980s (Simister and Mostov 1989a, b).
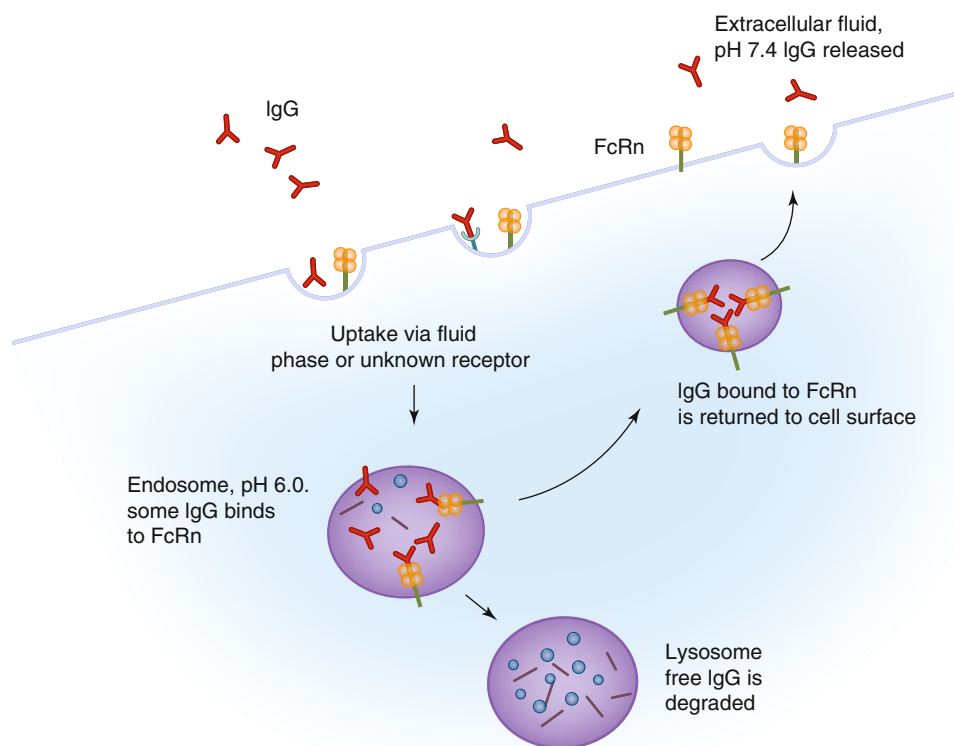
Figure 7.7 ■ Schematic disposition pathway of IgG antibodies via interaction with FcRn in endosomes. (1) IgGs enter cells by receptor-mediated endocytosis by binding of the Fc part to FcRn. (2) The intracellular vesicles (endosomes) can fuse with lysosome containing proteases. (3) Proteases can degrade non-bound IgG molecules, whereas IgG bound to FcRn is protected. (4) The intact IgG bound to FcRn is transported back to the cell surface and released back to the extracellular fluid.

FcRn is a heterodimer comprising of a β_2m light chain and a MHC class I-like heavy chain. The receptor is ubiquitously expressed in cells and tissues. Several studies have shown that IgG clearance in β_2m knockout mice (Ghetie et al. 1996; Junghans and Anderson 1996) and FcRn heavy chain knockout mice (Roopenian et al. 2003) is increased 10–15-fold, with no changes in the elimination of other Igs. Figure 7.7 illustrates how the FcRn receptor protects IgG from catabolism and contributes to its long half-life. The FcRn receptor binds to IgG in a pH-dependent manner: binding to IgG at acidic pH (6.0) at endosome and releasing IgG at physiological pH (7.4). The unbound IgG proceeds to the lysosome and undergoes proteolysis.

It has been demonstrated that IgG half-life is dependent on its affinity to FcRn receptors. The shorter half-life of IgG3 was attributed to its low binding affinity to the FcRn receptor (Junghans 1997; Medesan et al. 1997). Murine MABs have serum half-lives of 1–2 days in human. The shorter half-life of murine antibodies in human is due to their low binding affinity to the human FcRn receptor. It is reported that human FcRn binds to human, rabbit, and guinea pig IgG, but not to rat, mouse, sheep, and bovine IgG; however, mouse FcRn binds to IgG from all of these species (Ober et al. 2001). Interestingly, human IgG1 has greater affinity to murine FcRn (Petkova et al. 2006), which indicates potential limitations of using mice as preclinical models for human IgG1 pharmacokinetic evaluations.

Ward's group confirmed that an engineered human IgG1 had disparate properties in murine and human systems (Vaccaro et al. 2006). Engineered IgGs with higher affinity to FcRn receptor have a two to three-fold higher half-life compared with wild type in mice and monkeys (Hinton et al. 2006; Petkova et al. 2006). Two engineered human IgG1 mutants with enhanced binding affinity to human FcRn show a considerably extended half-life compared with wild type in hFcRn transgenic mice (4.35 ± 0.53, 3.85 ± 0.55 days versus 1.72 ± 0.08 days) (Petkova et al. 2006). Hinton et al. (2006) found that the half-life of IgG1 FcRn mutants with increasing binding affinity to human FcRn at pH 6.0 is about 2.5-fold longer that the wild-type Ab in monkey (838 ± 187 h versus 336 ± 34 h).

Dose-proportional, linear clearance has been observed for MAB against soluble antigens with low endogenous levels (such as TNF-α, IFN-α, VEGF, and IL-5). For example, linear PK has been observed for a humanized MAB directed to human interleukin-5 following intravenous administration over a 6,000-fold dose range (0.05–300 mg/kg) in monkeys (Zia-Amirhosseini et al. 1999). The clearance of rhuMAB against vascular endothelial growth factor after IV dosing (2–50 mg/kg) ranged from 4.81 to 5.59 mL/day/kg and did not depend on dose (Lin et al. 1999). The mean total serum clearance and the estimated mean terminal half-life of adalimumab were reported to range from 0.012 to 0.017 L/h and 10.0 to 13.6 days,
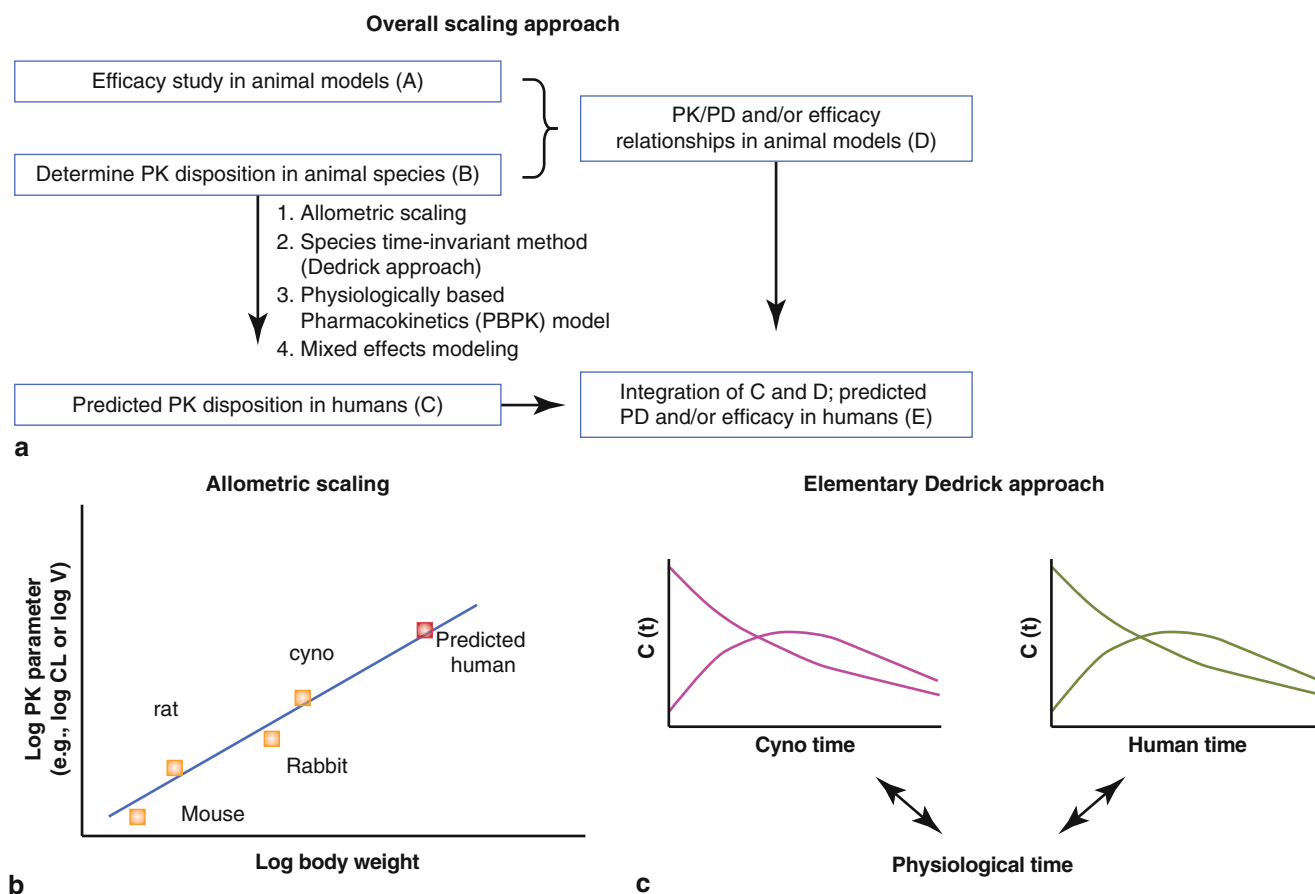
Overall scaling approach

a

b

c

Figure 7.8 ■ PK/PD scaling approach from preclinical studies to humans. (**a**) Overall scaling approach. (**b**) Allometric scaling. (**c**) Elementary dedrick approach.

respectively, for a 5-cohort clinical trial (0.5–10 mg/kg), with an overall mean half-life of 12 days (den Broeder et al. 2002). However, MABs against soluble antigens with high endogenous levels (such as IgE) exhibit nonlinear pharmacokinetics. The pharmacokinetics of omalizumab, an antibody against IgE, is linear only at doses greater than 0.5 mg/kg (Petkova et al. 2006; Xolair 2003).

Elimination of MABs may also be impacted by interaction with the targeted cell-bound antigen, and this phenomenon was demonstrated by dose-dependent clearance and half-life. At low dose, MABs show a shorter half-life and a faster clearance due to receptor-mediated elimination. With increasing doses, receptors become saturated; the half-life gradually increases to a constant; and the clearance gradually decreases to a constant. The binding affinity (K_d), antigen density, and antigen turnover rate may influence the receptor-mediated elimination. Koon et al. (2006) found a strong inverse correlation between CD25+ cell expression and apparent daclizumab (a MAB specifically binding to CD25) half-life. It has been shown that the pharmacokinetics of murine antihuman CD3 antibodies may be

determined by the disappearance of target antigen (Meijer et al. 2002). In monkeys and mice, clearance of SGN-40, a humanized monoclonal anti-CD40 antibody, was much faster at low dose, suggesting nonlinear pharmacokinetics (Kelley et al. 2006). In addition, Ng et al. (2006) demonstrated that an anti-CD4 monoclonal antibody (TRX-1) had ~5-fold faster CL at 1 mg/kg dose compared with 10 mg/kg dose (37.4±2.4 versus 7.8±0.6 mL/day/kg) in healthy volunteers. They also found that receptor-mediated CL via endocytosis became saturated at higher doses; nonspecific clearance of TRX-1 contributed 8.6, 27.1, and 41.7 % of total CL when dose was 1, 5, and 10 mg/kg, respectively.

In addition to FcRn and antigen–antibody interaction, other factors may also contribute to MAB elimination (Lobo et al. 2004; Roskos et al. 2004; Tabrizi et al. 2006):

1. *Immunogenicity of antibody*: The elimination of MABs in humans often increases with increasing level of immunogenicity (Ternant and Paintaud 2005; Tabrizi et al. 2006).
2. *The degree and the nature of antibody glycosylation*: The study conducted by Newkirk et al. (1996) shows that

the state of glycosylation of IgG affects the half-life in mice and that by removing the terminal sugars (sialic acid and galactose), the antibody (IgG2a) will remain in circulation significantly longer. However, Huang et al. (2006) demonstrated that a humanized anti-Aβ MAB with different glycans in the Fc region had the same clearance in mice.

3. *Susceptibility of antibody to proteolysis*: Gillies and his coworkers (2002) improved the circulating half-life of antibody-interleukin 2 immunocytokine twofold compared with wild type (1.0 h versus 0.54 h) by increasing the resistance to intracellular degradation.

4. *Effector function*, such as interactions with FcγR, could also regulate elimination and PK of MABs (Mahmood and Green 2005). Mutation of the binding site of FcγR has dramatic effects on the clearance of the Ab-IL-2 fusion protein (Gillies et al. 1999).

5. *Concomitant medications*: Methotrexate reduced adalimumab apparent clearance after single dose and multiple dosing by 29 and 44 %, respectively, in patients with rheumatoid arthritis (Humira 2007). In addition, azathioprine and mycophenolate mofetil were reported to reduce clearance of basiliximab by approximately 22 and 51 %, respectively (Simulect 2005). These interactions could be explained by the effects of small molecule drugs on the expression of Fcγ receptors. It has been found that methotrexate has the impact on the expression profiles of FcγRI on monocytes significantly in rheumatoid arthritis patients (Bunescu et al. 2004).

6. *Body weight, age, disease state, and other demography factors* can also change MAB pharmacokinetics (Mould and Sweeney 2007) (see discussion on Population Pharmacokinetics).

THERAPEUTIC MAB–DRUG INTERACTIONS

MABs and other therapeutic proteins are increasingly combined with small molecule drugs to treat various diseases. Assessment of the potential for PK- and/or PD-based MAB–drug interactions is more frequently incorporated into the drug development process (Girish et al. 2011). The exposure and/or response of concomitantly administered drugs can be altered by MABs (MAB as perpetrator). Alternately, PK and/or PD of therapeutic MABs can be affected by other drugs (MAB as victim).

Several different mechanisms have been proposed for MAB–drug interactions. Various cytokines and cytokine modulators can influence the expression and activity of cytochrome P450 (CYP) enzymes and drug transporters (Lee et al. 2010). Therefore, if a therapeutic MAB is a cytokine or cytokine modulator, it can potentially alter the systemic exposure and/or clinical response of concomitantly administered drugs that are substrates of CYPs or transporters (Huang et al. 2010) particularly those with narrow therapeutic windows. For example, an increase in cyclosporin A (CsA) trough level was observed when given in combination with muromomab (Vasquez and Pollak 1997). Similarly, basiliximab has been shown to increase CsA and tacrolimus level when used in combination (Sifontis et al. 2002). In diseases states, such as infection or inflammation, cytokines or cytokine modulators can also normalize previously changed activity of CYPs or transporters, thereby alter the exposure of coadministered drugs. Examples include tocilizumab coadministered with omeprazole and tocilizumab coadministered with simvastatin (Actemra® 2010).

MAB–drug interactions can also occur through changing the formation of anti-therapeutic antibody (ATA), which may enhance MAB clearance from the body. For example, methotrexate (MTX) reduced the apparent clearance of adalimumab by 29 and 44 % after single and repeated dosing (Humira® 2010). MTX also had similar effect on infliximab (Marni et al. 1998). PD-based interactions can result from alteration of target biology, such as information on the site of expression, relative abundance of expression, and the pharmacology of the target (Girish et al. 2011). Examples include efalizumab in combination with triple immune-suppressant therapy (Vincenti et al. 2007) and anakinra in combination with etanercept (Genovese et al. 2004).

To date, evidence of MAB–drug interactions via nonspecific clearance appears to be limited, although downregulation of Fcγ receptors by MTX is observed in patients with rheumatoid arthritis. It is possible that changes in Fcγ receptors can affect MAB clearance in the presence of MTX (Girish et al. 2011).

ADCs can also interact with drugs or MABs vis mechanisms described above. However, evidence of ADC–drug or ADC–MAB interaction appears to be absent. Lu et al. reported lack of interaction between ado-trastuzumab emtansine (T-DM1) and pertuzumab in patients with HER2-positive metastatic breast cancer (Lu et al. 2011). Similarly no interaction was observed between T-DM1 and paclitaxel or T-DM1 and docetaxel (Lu et al. 2012). With the theoretical potential for and current experiences with MAB–drug interactions, a question and risk-based integrated approach depending on the mechanism of the MABs and patient population have been progressively adopted during drug development to address important questions regarding the safety and efficacy of MAB and drug combinations (Girish et al. 2011). Various in vitro test systems have been used to provide some insight into the MAB–drug interactions, such as isolated hepatocytes and liver microsomes. However, the interpretation of these in vitro data is difficult. More importantly,

prospective predictions of drug interactions based on in vitro findings have not been feasible for MABs. Therefore, clinical methods are primarily used to assess MAB–drug interactions. Three common methods are dedicated drug interaction studies, although rare, population pharmacokinetics, and clinical cocktail studies. Details of various strategies used in pharmaceutical industry were reviewed in a 2011 AAPS white paper (Girish et al. 2011).

Prediction of Human PK/PD Based on Preclinical Information

Prior to the first-in-human (FIH) clinical study, a number of preclinical in vivo and in vitro experiments are conducted to evaluate the PK/PD, safety, and efficacy of a new drug candidate. However, the ultimate goal is at all times to predict how these preclinical results on pharmacokinetics, safety, and efficacy translate into a given patient population. Therefore, the objective of translational research is to predict PK/PD/safety outcomes in a target patient population, acknowledging the similarities and differences between preclinical and clinical settings.

Over the years, many theories and approaches have been proposed and used for scaling preclinical PK data to humans. Allometric scaling, based on a power–law relationship between size of the body and physiological and anatomical parameters, is the simplest and most widely used approach (Dedrick 1973; Mahmood 2005, 2009). Physiologically based PK modeling (Shah and Betts 2012), species-invariant time method (Dedrick approach) (Oitate et al. 2012), and nonlinear mixed effect modeling based on allometry (Jolling et al. 2005; Martin-Jimenez and Riviere 2002) have also been used for interspecies scaling of PK. While no single scaling method has been shown to definitively predict human PK in all cases, especially for small molecule drugs (Tang and Mayersohn 2005), the PK for MABs can be predicted reasonably well, especially for MAB at doses where the dominant clearance route is likely to be independent of concentration. Most therapeutic MABs bind to nonhuman primate antigens more often than to rodent antigens, due to the greater sequence homology observed between nonhuman primates and humans. The binding epitope, in vitro binding affinity to antigen, binding affinity to FcRn, tissue cross-reactivity profiles, and disposition and elimination pathways of MABs are often comparable in nonhuman primates and humans. It has recently been demonstrated that clearance and distribution volume of MABs with linear PK in humans can be reasonably projected based on data from nonhuman primates alone, with a fixed scaling exponent ranging from 0.75 to 0.9 for clearance and a fixed scaling exponent 1 for volume (Ling et al. 2009; Wang and Prueksaritanont 2010; Deng et al. 2011;

Dong et al. 2011; Oitate et al. 2011). For MABs that exhibited nonlinear pharmacokinetics, the best predictive performance was obtained above doses that saturated the target of the MAB (Dong et al. 2011). Pharmacokinetic prediction for low doses of a MAB with nonlinear elimination remains challenging and will likely require further exploration of species difference in target expression level, target antibody binding and target kinetics, as well as strategic animal in vivo PK studies, designed with relevant dose ranges. Immunogenicity is an additional challenge for prediction of MAB PK. Alterations in the PK profile due to immune-mediated clearance mechanisms in preclinical species cannot be scaled up to humans, since animal models are not predictive of human immune response to human MABs. Thus, either excluding antidrug antibody (ADA)-positive animals from PK scaling analysis or using only the early time points prior to their observation in ADA positive animals has been a standard practice in the industry.

Due to its complexity, any extrapolation of PD to humans requires more thorough consideration than for PK. Little is known about allometric relationships in PD parameters. It is expected that the physiological turnover rate constants of most general structures and functions among species should obey allometric principles, whereas capacity and sensitivity tend to be similar across species (Mager et al. 2009). Through integration of PK/PD modeling and interspecies scaling, PD effects in humans may be predicted if the PK/PD relationship is assumed to be similar between animal models and humans (Duconge et al. 2004; Kagan et al. 2010). For example, a PK/PD model was first developed to optimize the dosing regimen of a MAB against EGF/r3 using tumor-bearing nude mice as an animal model of human disease (Duconge et al. 2004). This PK/PD model was subsequently integrated with allometric scaling to calculate the dosing schedule required in a potential clinical trial to achieve a specific effect (Duconge et al. 2004).

In summary, species differences in antigen expression level, antigen–antibody binding and antigen kinetics, differences in FcRn binding between species, the immunogenicity, and other factors must be considered during PK/PD scaling of a MAB from animals to humans.

PK/PD in Clinical Development of Antibody Therapeutics

Several new developments have taken place in the antibody therapeutics in the last years. The emphasis in the field has grown and is obvious by the fact that many of the companies are now involved in building antibody product-based collaborations. Drug development has traditionally been performed in sequen-

tial phases, divided into preclinical as well as clinical phases I–IV. During the development phases of the molecules, the safety and PK/PD characteristics are established in order to narrow down on the compound selected for development and its dosing regimen. This information-gathering process has been characterized as two successive learning–confirming cycles (Sheiner 1997; Sheiner and Wakefield 1999).

The first cycle (phases I and IIa) comprises learning about the dose that is tolerated in healthy subjects and confirming that this dose has some measurable benefits in the targeted patients. An affirmative answer at this first cycle provides the justification for a larger and more costly second learn–confirm cycle (phases IIb and III), where the learning step is focused on how to use the drug benefit/risk ratio, whereas the confirm step is aimed at demonstrating acceptable benefit/risk in a large patient population (Meibohm and Derendorf 2002). In the following sections, the approved therapeutic antibody efalizumab is provided as a case study to understand the various steps during the development of antibodies for various indications.

A summary of the overall PK/PD data from multiple studies within the efalizumab (Raptiva®) clinical development program and an integrated overview of how these data were used for development and the selection of the approved dosage of efalizumab for psoriasis will be discussed in detail. Psoriasis is a chronic skin disease characterized by abnormal keratinocyte differentiation and hyperproliferation and by an aberrant inflammatory process in the dermis and epidermis. T cell infiltration and activation in the skin and subsequent T cell-mediated processes have been implicated in the pathogenesis of psoriasis (Krueger 2002).

Efalizumab is a subcutaneously (SC) administered recombinant humanized monoclonal IgG1 antibody that has received approval for the treatment of patients with psoriasis in more than 30 countries, including the United States and the European Union (Raptiva 2004). Efalizumab is a targeted inhibitor of T cell interactions (Werther et al. 1996). An extensive preclinical research program was conducted to study the safety and mechanism of action (MOA) of efalizumab. Multiple clinical studies have also been conducted to investigate the efficacy, safety, pharmacokinetics (PK), pharmacodynamics (PD), and MOA of efalizumab in patients with psoriasis.

PRE-PHASE I STUDIES

In the process of developing therapeutic antibodies, integrated understanding of the pharmacokinetic/pharmacodynamic (PK/PD) concepts provides a highly promising tool. A thorough and rigorous preclinical program in the early learning phase of preclinical drug development can provide a linkage between drug discovery and preclinical development. As it sets the stage for any further development activities, the obtained information at this point is key to subsequent steps (Meibohm and Derendorf 2002). At the preclinical stage, potential applications might comprise the evaluation of in vivo potency and intrinsic activity, the identification of bio-/surrogate markers, understanding the MOA, as well as dosage form/regimen selection and optimization. A few of these specific aims are described below with information on efalizumab as an example.

IDENTIFICATION OF MOA AND PD BIOMARKERS

The identification of appropriate PD endpoints is crucial to the process of drug development. Thus, biomarkers are usually tested early during exploratory preclinical development for their potential use as pharmacodynamic or surrogate endpoints.

Through an extensive preclinical research program, the MOA and PD biomarkers for efalizumab have been established. Efalizumab binds to CD11a, the α-subunit of leukocyte function antigen-1 (LFA-1), which is expressed on all leukocytes, and decreases cell surface expression of CD11a. Efalizumab inhibits the binding of LFA-1 to intercellular adhesion molecule-1 (ICAM-1), thereby inhibiting the adhesion of leukocytes to other cell types. Interaction between LFA-1 and ICAM-1 contributes to the initiation and maintenance of multiple processes, including activation of T lymphocytes, adhesion of T lymphocytes to endothelial cells, and migration of T lymphocytes to sites of inflammation, including skin. Consistent with the proposed MOA for efalizumab, in vitro experiments have demonstrated that efalizumab binds strongly to human lymphocytes with a K_d of approximately 110 ng/mL (Werther et al. 1996; Dedrick et al. 2002) and blocks the interaction of human T lymphocytes with tissue-specific cells such as keratinocytes in a concentration-dependent manner.

Upon understanding the MOA, PD effects relevant to the MOA of efalizumab are usually measured in order to identify the efficacious dosage of antibody therapeutics. As saturation of CD11a binding sites by efalizumab has been shown to increase while T cell activation is increasingly inhibited, maximum saturation of CD11a binding sites occurs at efalizumab concentrations >10 μg/mL, resulting in maximum T cell inhibition (Werther et al. 1996; Dedrick et al. 2002). Therefore, CD11a expression and saturation have been chosen as relevant PD markers for this molecule.
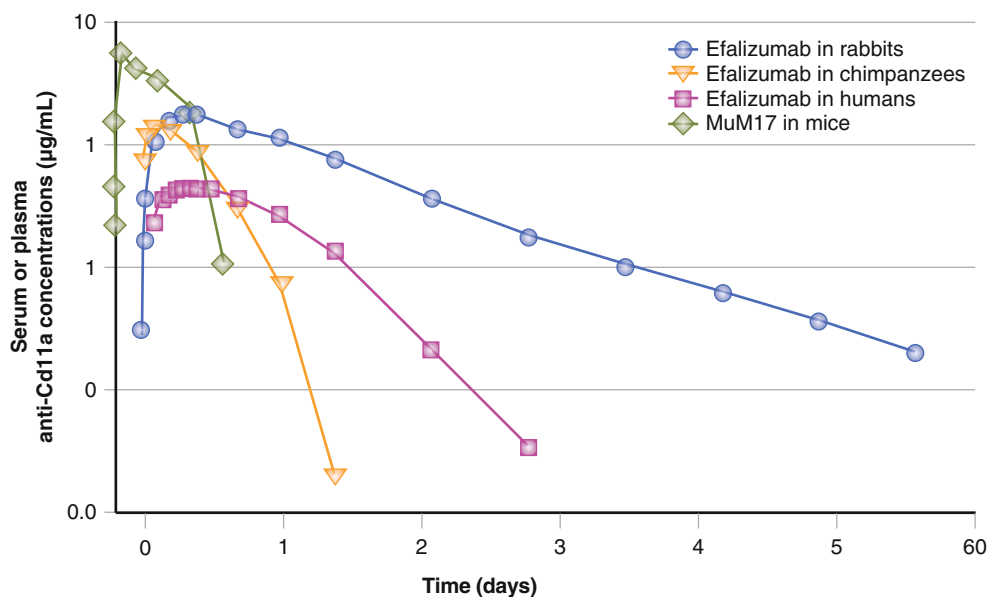
Figure 7.9 ■ Anti-CD11a molecules comparative PK profiles in humans, chimpanzees, rabbits, and mice following SC dose. Due to the species differences in binding, the pharmacokinetics of efalizumab are nonlinear (i.e., dose dependent) in humans and chimpanzees, while being linear in rabbits (nonbinding species). muM17, on the other hand binds to the mouse anti-CD11a and exhibits dose-dependent pharmacokinetics in mice.

ROLE OF SURROGATE MOLECULES

The role of surrogate molecules in assessing ADME of therapeutic antibodies is important as the antigen specificity limits ADME studies of humanized monoclonal antibodies in rodents. In the development of therapeutic antibodies, various molecules may be used to provide a comprehensive view of their PK/PD properties. Studies with surrogates might lead to important information regarding safety, mechanism of action, disposition of the drug, tissue distribution, and receptor pharmacology, which might be too cumbersome and expensive to conduct in nonhuman primates. Surrogates (mouse/rat) provide a means to gaining knowledge of PK and PD in a preclinical rodent model thus allowing rational dose optimization in the clinic. Therefore, in the case of efalizumab to complete a more comprehensive safety assessment, a chimeric rat anti-mouse CD11a antibody, muM17, was developed and evaluated as a species-specific surrogate molecule for efalizumab. muM17 binds mouse CD11a with specificity and affinity similar to those of efalizumab to human. In addition, muM17 in mice was demonstrated to have similar pharmacological activities as that of efalizumab in human (Nakakura et al. 1993; Clarke et al. 2004). Representative PK profiles of efalizumab and muM17 in various species are depicted in Fig. 7.9 to help understand the species differences in the PK behavior of molecules.

PHARMACOKINETICS OF EFALIZUMAB

A brief overview of efalizumab nonclinical PK/PD results is provided in the following sections to summarize the key observations that led to decisions in designing the subsequent clinical programs. The ADME program consisted of pharmacokinetic, pharmacodynamic (CD11a down-modulation and saturation), and toxicokinetic data from pharmacokinetic, pharmacodynamic, and toxicology studies with efalizumab in chimpanzees and with muM17 in mice. The use of efalizumab in the chimpanzee and muM17 in mice for pharmacokinetic and pharmacodynamic and safety studies was supported by in vitro activity assessments. The nonclinical data were used for pharmacokinetic and pharmacodynamic characterization, pharmacodynamic-based dose selection, and toxicokinetic support for confirming exposure in toxicology studies. Together, these data have supported both the design of the nonclinical program and its relevance to the clinical program.

The observed pharmacodynamics as well as the mechanism of action of efalizumab and muM17 is attributed to bind CD11a present on cells and tissues. The binding affinities of efalizumab to human and chimpanzee CD11a on CD3 lymphocytes are comparable confirming the use of chimpanzees as a valid nonclinical model for humans. CD11a expression has been observed to be greatly reduced on T lymphocytes in chimpanzees and mice treated with efalizumab and muM17, respectively. Expression of CD11a is restored as efalizumab and muM17 are eliminated from the plasma. The bioavailability of efalizumab in chimpanzees and muM17 in mice after an SC dose was dose-dependent and ranged from 35 to 48 % and 63 to 89 % in chimpanzees and mice, respectively. Binding to CD11a serves as a major pathway for clearance of these molecules, which leads to nonlinear pharmacokinetics depending on the relative amounts of CD11a and efalizumab or muM17 (Coffey et al. 2005).
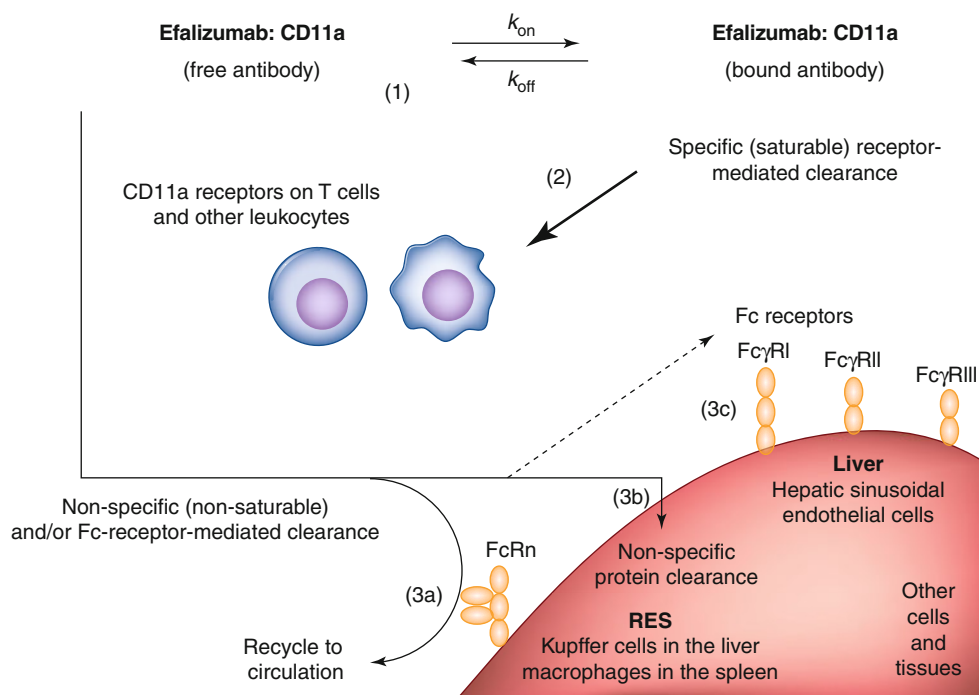
Figure 7.10 ■ Clearance pathways for efalizumab.

The disposition of efalizumab and the mouse surrogate muM17 is mainly determined by the combination of both specific interactions with the ligand CD11a and by their IgG1 framework and is discussed in detail as follows. The factors controlling the disposition of these antibodies are shown in Fig. 7.10 and include the following:

1. The binding of the free antibody with its ligand CD11a present on both circulating lymphocytes and tissues leads to its removal from circulation. Data suggests that anti-CD11a antibodies are internalized by purified T cells, and upon internalization, the antibodies appeared to be targeted to lysosomes and cleared from within the cells in a time-dependent manner. CD11a-mediated internalization and lysosomal targeting of efalizumab may constitute one pathway by which this antibody is cleared in vivo (Coffey et al. 2005).

2. Binding to CD11a is both specific and saturable as demonstrated by the dose-dependent clearance of efalizumab in chimpanzees and humans or muM17 in mice.

3. Because of its IgG1 framework, free or unbound efalizumab or muM17 levels are also likely to be influenced by:

   (a) Recycling and circulation following binding to and internalization by the neonatal Fc receptor (FcRn)

   (b) Nonspecific uptake and clearance by tissues

   (c) Binding via its Fc framework to Fcγ receptors present on hepatic sinusoidal endothelial cells

The disposition of efalizumab is governed by the species specificity and affinity of the antibody for its ligand CD11a, the amount of CD11a in the system, and the administered dose.

Based on the safety studies, efalizumab was considered to be generally well tolerated in chimpanzees at doses up to 40 mg/kg/week IV for 6 months, providing an exposure ratio of 339-fold based on cumulative dose and 174-fold based on the cumulative AUC, compared with a clinical dose of 1 mg/kg/week. The surrogate antibody muM17 was also well tolerated in mice at doses up to 30 mg/kg/week SC. In summary efalizumab was considered to have an excellent nonclinical safety profile thereby supporting the use in adult patients.

CLINICAL PROGRAM OF EFALIZUMAB: PK/PD STUDIES, ASSESSMENT OF DOSE, ROUTE, AND REGIMEN

The drug development process at the clinical stage provides several opportunities for integration of PK/PD concepts. Clinical phase I dose escalation studies provide, from a PK/PD standpoint, the unique chance to evaluate the dose–concentration–effect relationship for therapeutic and toxic effects over a wide range of doses up to or even beyond the maximum tolerated dose under controlled conditions (Meredith et al. 1991). PK/PD evaluations at this stage of drug development can provide crucial information regarding the potency and tolerability of the drug in vivo and the verification and

suitability of the PK/PD concept established during preclinical studies.

Efalizumab PK and PD data are available from ten studies in which more than 1,700 patients with psoriasis received IV or SC efalizumab. In the phase I studies, PK and PD parameters were characterized by extensive sampling during treatment; in the phase III trials, steady-state trough levels were measured once or twice during the first 12-week treatment period for all the studies and during extended treatment periods for some studies. Several early phase I and II trials have examined IV injection of efalizumab, and dose-ranging findings from these trials have served as the basis for SC dosing levels used in several subsequent phase I and all phase III trials.

■ IV Administration of Efalizumab

The PK of monoclonal antibodies varies greatly, depending primarily on their affinity for and the distribution of their target antigen (Lobo et al. 2004). Efalizumab exhibits concentration-dependent nonlinear PK after administration of single IV doses of 0.03, 0.1, 0.3, 0.6, 1.0, 2.0, 3.0, and 10.0 mg/kg in a phase I study. This nonlinearity is directly related to specific and saturable binding of efalizumab to its cell surface receptor, CD11a, and has been described by a PK/PD model developed by Bauer et al. (Bauer et al. 1999) which is discussed in the following sections. The PK profiles of efalizumab following single IV doses with observed data and model predicted fit are presented in Fig. 7.11. Mean clearance (CL) decreased from 380 to 6.6 mL/kg/day for doses of 0.03 mg/kg to 10 mg/kg,
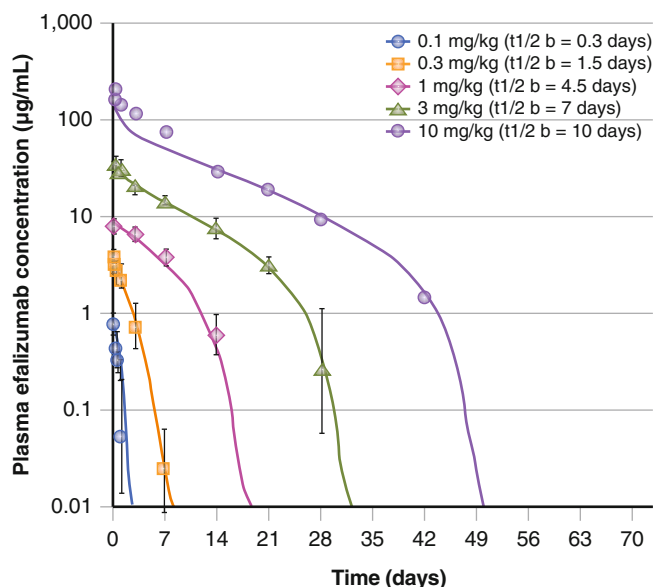
respectively. The volume of distribution of the central compartment (Vc) of efalizumab was 110 mL/kg at 0.03 mg/kg (approximately twice the plasma volume) and decreased to 58 mL/kg at 10 mg/kg (approximately equal to plasma volume), consistent with saturable binding of efalizumab to CD11a in the vascular compartment. Because of efalizumab's nonlinear PK, its half-life ($t_{1/2}$) is dose dependent.

In a phase II study of efalizumab, it was shown that at a weekly dosage of 0.1 mg/kg IV, patients did not maintain maximal down-modulation of CD11a expression and did not maintain maximal saturation. Also at the end of 8 weeks of efalizumab treatment, 0.1 mg/kg/week IV, patients did not have statistically significant histological improvement and did not achieve a full clinical response. The minimum weekly IV dosage of efalizumab tested that produced histological improvements in skin biopsies was 0.3 mg/kg/week, and this dosage resulted in submaximal saturation of CD11a binding sites but maximal down-modulation of CD11a expression. Improvements in patients' psoriasis were also observed, as determined by histology and by the Psoriasis Area and Severity Index (PASI) (Papp et al. 2001).

■ Determination of SC Doses

Although efficacy was observed in phase I and II studies with 0.3 mg/kg/week IV efalizumab, dosages of 0.6 mg/kg/week and greater (given for 7–12 weeks) provided more consistent T lymphocyte CD11a saturation and maximal PD effect. At dosages ≤0.3 mg/kg/week, large between-subject variability was observed, whereas at dosages of 0.6 or 1.0 mg/kg/week, patients experienced better improvement in PASI scores, with lower between-patient variability in CD11a saturation and down-modulation. Therefore, this dosage was used to estimate an appropriate minimum SC dose of 1 mg/kg/week (based on a 50 % bioavailability) that would induce similar changes in PASI, PD measures, and histology. The safety, PK, and PD of a range of SC efalizumab doses (0.5–4.0 mg/kg/week administered for 8–12 weeks) were evaluated initially in 2 phase I studies (Gottlieb et al. 2003). To establish whether a higher SC dosage might produce better results, several phase III clinical trials assessed a 2.0 mg/kg/week SC dosage in addition to the 1.0 mg/kg/week dosage. A dose of 1.0 mg/kg/week SC efalizumab was selected as it produced sufficient trough levels in patients to maintain the maximal down-modulation of CD11a expression and binding-site saturation between weekly doses (Joshi et al. 2006). Figure 7.12 depicts the serum efalizumab levels, CD11a expression, and available CD11a binding sites on T lymphocytes (mean ± SD) after subcutaneous administration of 1 mg/kg efalizumab.
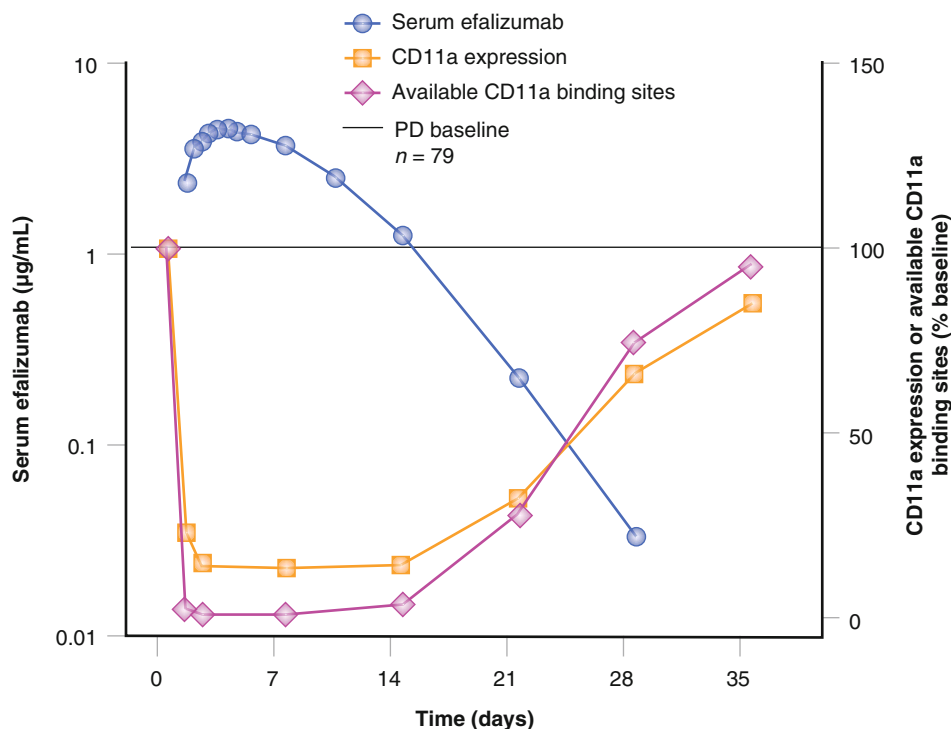

Figure 7.11 ■ Plasma concentration versus time profile for efalizumab following single IV doses in psoriasis patients.

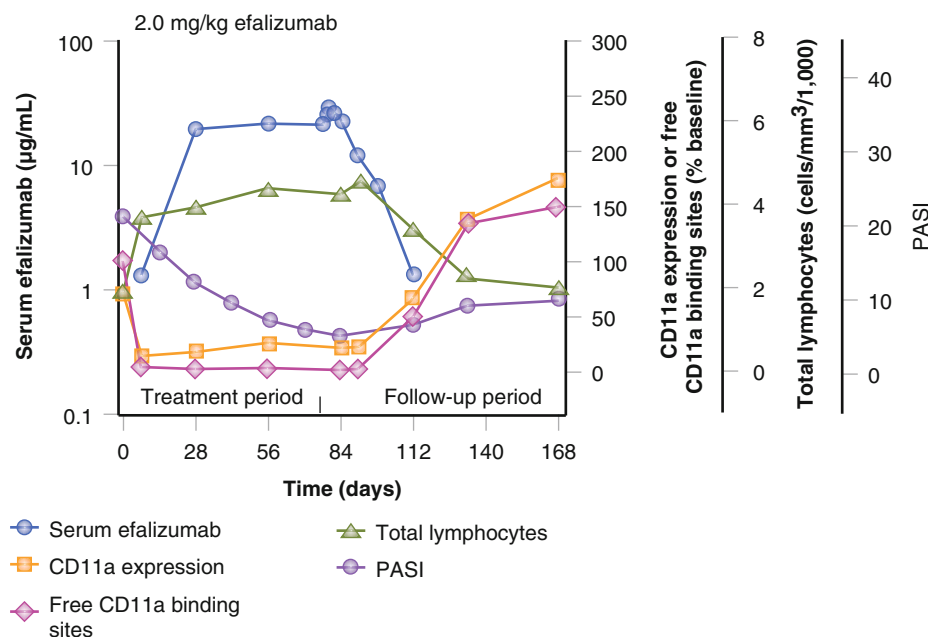**Figure 7.12** ■ PK/PD profile following efalizumab in humans (1 mg/kg SC).

Figure 7.13 ■ Serum efalizumab, CD11a expression, and free CD11a binding sites on T lymphocytes, absolute lymphocyte counts, and Psoriasis Area and Severity Index (PASI) score (mean) following 1.0 mg/kg/week SC efalizumab for 12 weeks and 12 weeks posttreatment.

■ SC Administration of Efalizumab

The PK of SC efalizumab has been well characterized following multiple SC doses of 1.0 and 2.0 mg/kg/week (Mortensen et al. 2005; Joshi et al. 2006). A phase I study that collected steady-state PK and PD data for 12 weekly SC doses of 1.0 and 2.0 mg/kg in psoriasis patients provided most of the pharmacologic data relevant to the marketed product. Although peak serum concentration after the last dose (C_{max}) was observed to be higher for the 2.0 mg/kg/week (30.9 µg/mL) than for the 1.0 mg/kg/week dosage (12.4 µg/mL), no additional changes in PD effects were observed at the higher dosages (Mortensen et al. 2005). Following a dose of 1.0 mg/kg/week, serum efalizumab concentrations were adequate to induce maximal down-modulation of CD11a expression and a reduction in free CD11a binding sites on T lymphocytes (Fig. 7.13). Steady-state serum efalizumab levels

were reached more quickly with the 1.0 mg/kg/week dosage at 4 weeks compared with the 2.0 mg/kg/week dosage at 8 weeks (Mortensen et al. 2005), which is in agreement with the average effective $t_{1/2}$ for SC efalizumab 1.0 mg/kg/week of 5.5 days (Boxenbaum and Battle 1995). The bioavailability was estimated at approximately 50 %. Population PK analyses indicated that body weight was the most significant covariate affecting efalizumab SC clearance, thus supporting body weight-based dosing for efalizumab (Sun et al. 2005).

Mechanistic Modeling Approaches

In clinical drug development, PK/PD modeling approaches can be applied as analytical tools for identifying and characterizing the dose–response relationships of drugs and the mechanisms and modulating factors involved. Additionally, they may be used as predictive tools for exploring various dosage regimens as well as for optimizing further clinical trial designs, which might allow one to perform fewer, more focused studies with improved efficiency and cost-effectiveness. The PK/PD database established during the preclinical and clinical learning phases in the development process and supplemented by population data analysis provides the backbone for these assessments.

PK/PD modeling has been used to characterize efalizumab plasma concentrations and CD11a expression on CD3-positive lymphocytes in chimpanzees and in subjects with psoriasis (Bauer et al. 1999). As the PK data revealed that CL of efalizumab was not constant across dose levels, one of the models described by Bauer et al. (1999) incorporated a Michaelis–Menten clearance term into the pharmacokinetic equations and utilized an indirect response relationship to describe CD11a turnover. However, in the above model, the exposure–response relationship of efalizumab was not addressed, and another report expanded on the developed receptor-mediated pharmacokinetic and pharmacodynamic model by incorporating data from five phase I and II studies to develop a pharmacokinetic (PK) pharmacodynamic (PD) efficacy (E) model to further increase the understanding of efalizumab interaction with CD11a on T cells and consequent reduction in severity of psoriasis (Ng et al. 2005). A general outline of the mechanistic modeling approach for various molecules is presented alongside the model for efalizumab in Fig. 7.14a. The description of the pharmacokinetic–pharmacodynamic–efficacy model of efalizumab in psoriasis patients is described below and is schematically represented in Fig. 7.14b. Details on parameters utilized in the model can be found in the paper by Ng et al. (2005).

PHARMACOKINETIC ANALYSIS

A first-order absorption, two-compartment model with both linear and Michaelis–Menten elimination was used to describe the plasma efalizumab concentration data. This model is schematically represented in Fig. 7.14b (iv).

PHARMACODYNAMIC ANALYSIS

A receptor-mediated pharmacodynamic model previously developed was used to describe the dynamic interaction of efalizumab to CD11a, resulting in the removal of efalizumab from the circulation and reduction of cell surface CD11a (Bauer et al. 1999). This model is schematically represented in Fig. 7.14b (v).

EFFICACY ANALYSIS

The severity of the disease has been assessed by the PASI score that is assumed to be directly related to the psoriasis skin production. The rate of psoriasis skin production was then modeled to be directly proportional to the amount of free surface CD11a on T cells, which is offset by the rate of skin healing (Fig. 7.14b (vi)).

MODEL RESULTS

Upon evaluation and development, the model was used to fit the PK/PD/efficacy data simultaneously. The plasma concentration-time profile of efalizumab was reasonably described by use of the first-order absorption, two-compartment model with Michaelis–Menten elimination from the central compartment. In addition, the pharmacodynamic model described the observed CD11a-time data from all the studies reasonably well. In the efficacy model, an additional CD11a-independent component to psoriasis skin production accounted for incomplete response to efalizumab therapy and the model described the observed data well. Figure 7.15 depicts the fit of the model to the PK/PD/efficacy data.

The pharmacokinetic-pharmacodynamic-efficacy model developed for efalizumab has a broad application to antibodies that target cell-bound receptors, subjected to receptor-mediated clearance, and for which coating and modulation of the receptors are expected to be related to clinical response (Mould et al. 1999). Despite the nonlinear pharmacokinetics of these agents, the model can be used to describe the time course of the pharmacodynamic effect and efficacy after different dosing regimens.
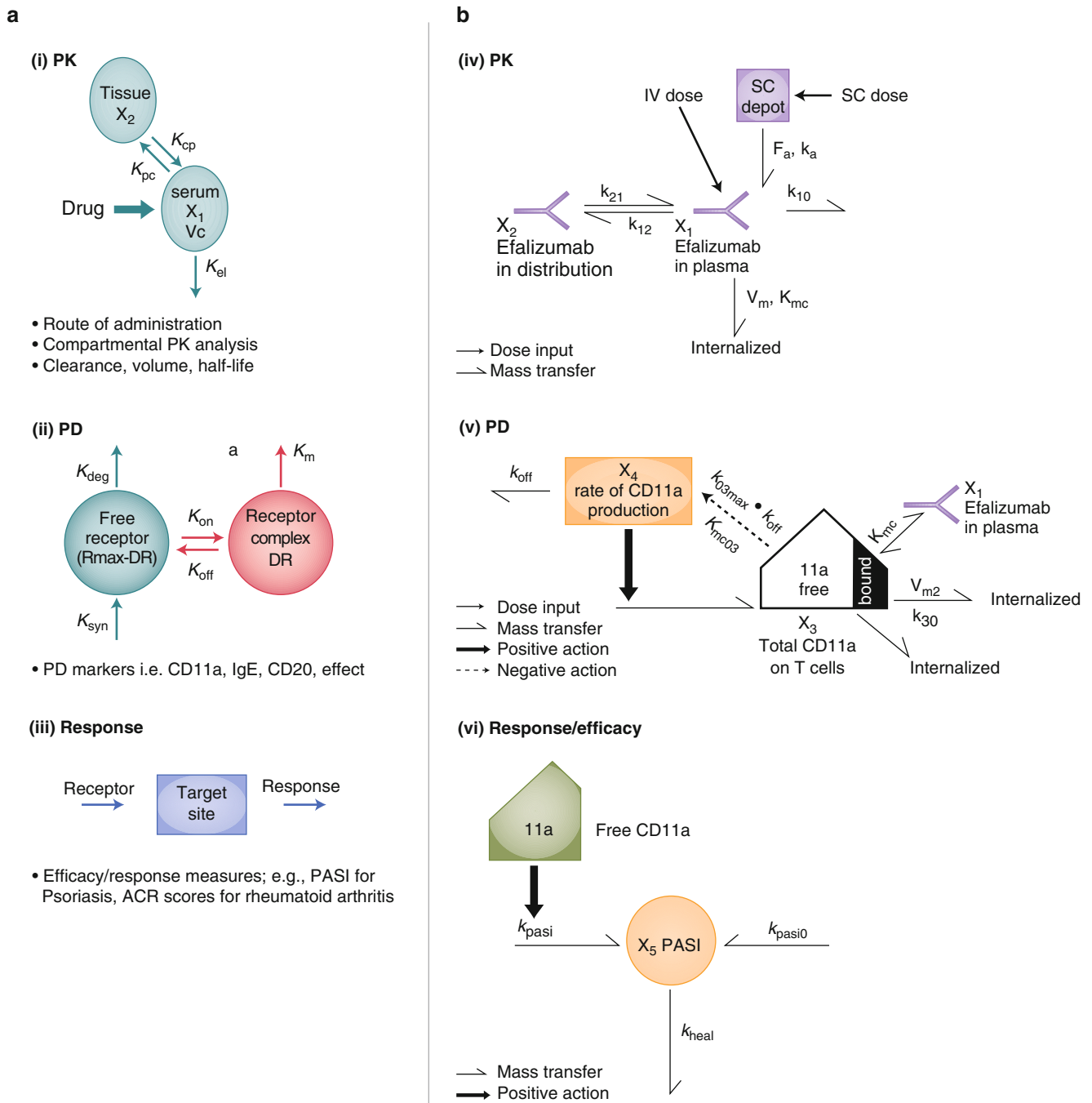
Figure 7.14 ■ (**a**) A schematic for pharmacokinetic–pharmacodynamic–response model for antibody therapeutics (*i*) PK, (*ii*) PD, and (*iii*) response. (**b**) Schematic representation of pharmacokinetic–pharmacodynamic–efficacy model of efalizumab in psoriasis patients. (*iv*) First-order absorption, two-compartment pharmacokinetic model with linear and nonlinear elimination from the central compartment. (*v*) Pharmacodynamic model with negative feedback mechanism. (*vi*) Efficacy model with CD11a-dependent and -independent pathway.

■ Population Pharmacokinetics of Monoclonal Antibodies

Compared to many small molecule drugs, monoclonal antibodies typically exhibit less inter- and intra-subject variability of the standard pharmacokinetic parameters such as volume of distribution and clearance. However, it is possible that certain pathophysiological conditions may result into substantially increased intra- and inter-patient variability. In addition, patients are usually not very homogeneous; patients vary in sex, age, body weight; they may have concomitant disease and may be receiving multiple drug treatments.

Even the diet, lifestyle, ethnicity, and geographic location can differ from a selected group of "normal" subjects. These covariates can have substantial influence on pharmacokinetic parameters. Therefore, good therapeutic practice should always be based on an understanding of both the influence of covariates on pharmacokinetic parameters as well as the pharmacokinetic variability in a given patient population. With this knowledge, dosage adjustments can be made to accommodate differences in pharmacokinetics due to genetic, environmental, physiological, or pathological factors, for instance, in case of compounds with a relatively small therapeutic index. The framework of application of population pharmacokinetics during drug development is summarized in the FDA guidance document entitled "*Guidance for Industry – Population Pharmacokinetics*" (www.fda.gov).

For population pharmacokinetic data analysis, there are generally two reliable and practical approaches. One approach is the standard two-stage (STS) method, which estimates parameters from the plasma drug concentration data for an individual subject during the first stage. The estimates from all subjects are then combined to obtain a population mean and variability estimates for the parameters of interest. The method works well when sufficient drug concentration-time data are available for each individual patient; typically these data are gathered in phase 1 clinical trials. A second approach, nonlinear mixed effect modeling (NonMEM), attempts to fit the data and partition the differences between theoretical and observed values into random error terms. The influence of fixed effect (i.e., age, sex, body weight) can be identified through a regression model building process.

The original scope for the NonMEM approach was its applicability even when the amount of time-concentration data obtained from each individual is sparse and conventional compartmental PK analyses are not feasible. This is usually the case during the routine visits in phase III or IV clinical studies. Nowadays the NonMEM approach is applied far beyond its original scope due to its flexibility and robustness. It has been used to describe data-rich phase I and phase IIa studies or even preclinical data to guide and expedite drug development from early preclinical to clinical studies (Aarons et al. 2001; Chien et al. 2005).

There has been increasing interest in the use of population PK and pharmacodynamic (PD) analyses for different antibody products (i.e., antibodies, antibody fragments, or antibody fusion proteins) over the past 10 years (Lee et al. 2003; Nestorov et al. 2004; Zhou et al. 2004; Yim et al. 2005; Hayashi et al. 2007; Agoram et al. 2007; Gibiansky and Gibiansky 2009; Dirks and Meibohm 2010; Zheng et al. 2011; Gibiansky and Frey

2012). One example involving analysis of population plasma concentration data involved a dimeric fusion protein, etanercept (Enbrel®). A one-compartment first-order absorption and elimination population PK model with interindividual and inter-occasion variability on clearance, volume of distribution, and absorption rate constant, with covariates of sex and race on apparent clearance and body weight on clearance and volume of distribution, was developed for etanercept in rheumatoid arthritis adult patients (Lee et al. 2003). The population PK model for etanercept was further applied to pediatric patients with juvenile rheumatoid arthritis and established the basis of the 0.8 mg/kg once weekly regimen in pediatric patients with juvenile rheumatoid arthritis (Yim et al. 2005). Unaltered etanercept PK with concurrent methotrexate in patients with rheumatoid arthritis has been demonstrated in a phase IIIb study using population PK modeling approach (Zhou et al. 2004). Thus, no etanercept dose adjustment is needed for patients taking concurrent methotrexate. A simulation exercise of using the final population PK model of subcutaneously administered etanercept in patients with psoriasis indicated that the two different dosing regimens (50 mg QWk versus 25 mg BIWk) provide a similar steady-state exposure (Nestorov et al. 2004). Therefore, their respective efficacy and safety profiles are likely to be similar as well.

An added feature is the development of a population model involving both pharmacokinetics and pharmacodynamics. Population PK/PD modeling has been used to characterize drug PK and PD with models ranging from simple empirical PK/PD models to advanced mechanistic models by using drug–receptor binding principles or other physiologically based principles. A mechanism-based population PK and PD binding model was developed for a recombinant DNA-derived humanized IgG1 monoclonal antibody, omalizumab (Xolair®) (Hayashi et al. 2007). Clearance and volume of distribution for omalizumab varied with body weight, whereas clearance and rate of production of IgE were predicted accurately by baseline IgE, and overall, these covariates explained much of the interindividual variability. Furthermore, this mechanism-based population PK/PD model enabled the estimation of not only omalizumab disposition but also the binding with its target, IgE, and the rate of production, distribution, and elimination of IgE.

Population PK/PD analysis can capture uncertainty and the expected variability in PK/PD data generated in preclinical studies or early phases of clinical development. Understanding the associated PK or PD variability and performing clinical trial simulation by incorporating the uncertainty from the existing PK/PD data allows projecting a plausible range of doses for future clinical studies and final practical uses.
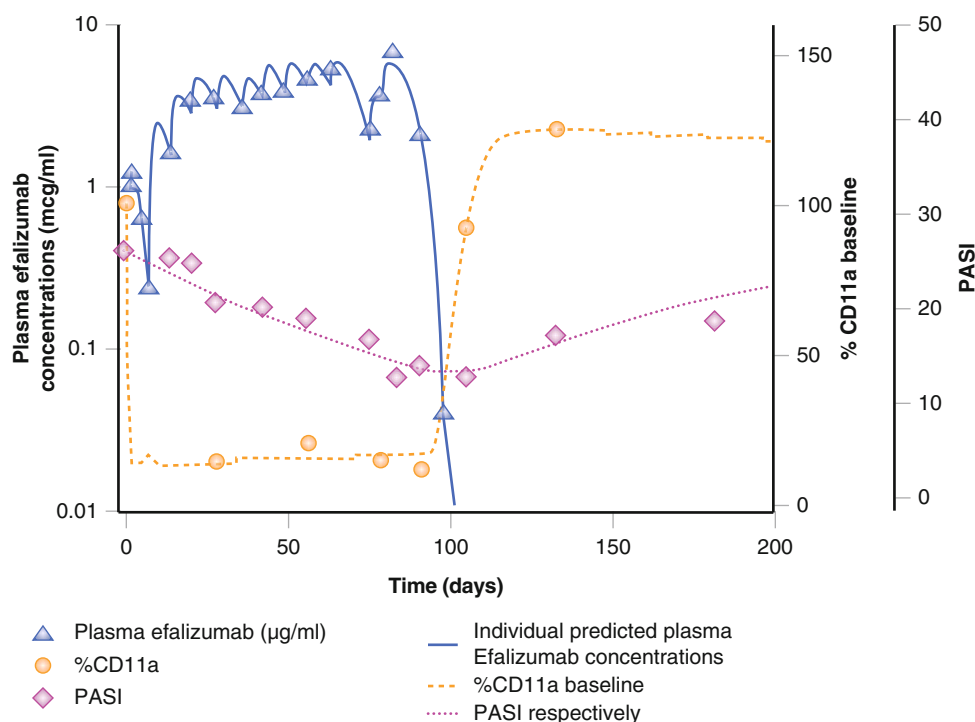
Figure 7.15 ■ Representative pharmacokinetic–pharmacodynamic–efficacy profiles from a patient receiving a 1 mg/kg weekly dose of efalizumab subcutaneously for 12 weeks. *Solid triangle*, plasma efalizumab (µg/mL); *open circles*, %CD11a; and *solid circles*, PASI. *Solid*, *dashed*, and *dotted lines* represent individual predicted plasma efalizumab concentrations, %CD11a baseline, and PASI, respectively.

FUTURE PERSPECTIVE

The success of monoclonal antibodies as new therapeutic agents in several disease areas such as oncology, inflammatory diseases, autoimmune diseases, and transplantation has triggered growing scientific, therapeutic, and business interest in the MAB technology. The market for therapeutic MABs is one of the most dynamic sectors within the pharmaceutical industry. Further growth is expected by developing MABs towards other surface protein targets, which are not covered yet by marketed MABs. Particularly, the technological advancement in the area of immunoconjugates and MAB fragments may overcome some of the limitations of MABs by providing highly potent drugs selectively to effect compartments and to extend the distribution of the active moiety, which are typically not reached by MABs. Immunoconjugates hold great promise for selective drug delivery of potent drugs with unfavorable own selectivity to target cells (e.g., highly potent cytotoxic drugs). Several of such immunoconjugates are under development to target different tumor types and are expected to reach the market in the next years. Modification of the MAB structure allows adjusting the properties according to therapeutic needs (e.g., adjusting half-life, increasing volume of distribution, changing clearance pathways). By using modified MAB derivatives, optimized therapeutic agents might become available. So far this technology has been successfully used for two antibody fragments marketed in inflammatory disease and antiangiogenesis (abciximab, ranibizumab).

Bispecific antibodies represent another promising new methodological approach to antibody therapy. Technological refinements in antibody engineering have allowed the production of bispecific antibodies that are simultaneously directed towards two distinct target antigens (Holmes 2011). For instance, the CDR consisting of the variable domains (V_L and V_H) at the tip of one arm of an IgG may be asymmetrically designed to bind to a different target than that of the other arm (Fig. 7.1). Symmetrical formats in which each arm can bind two targets are also possible.

MABs have become a key part of the pharmaceutical armamentarium, especially in the oncology and immunology settings and will continue to be a focus area for drug discovery and development.

SELF-ASSESSMENT QUESTIONS

■ Questions

1. What are the structural differences between the five immunoglobulin classes?
2. (a) What are key differences in PK/PD between MABs and small molecule drugs?
   (b) Why do IgGs typically show nonlinear PK in the lower plasma (serum) concentration range?
3. What is a surrogate MAB and how can it potentially be used in the drug development process of MABs?
4. Which other modes of actions apart from ADCC – antibody dependent cellular cytotoxicity are known for MABs? What are the key steps of ADCC?
5. Why do IgGs have a longer in vivo half-life compared with other Igs?

6. What are the development phases for antibody therapeutics? What major activities are involved in the each phase?

■ Answers

1. The following structural properties distinguish MABs:
   • The molecular form can be different for the 5 immunoglobulin classes: IgG, IgD, and IgE are monomer; IgM appears as pentamer or hexamer, and IgA are either monomer or dimer.
   • Consequently, the molecular weight of the different Igs is different (IgG 150–169 kD, IgA 160–300 kD, IgD 175 kD, IgE 190, IgM 950 kD).

2. (a) I. Metabolism of MABs appears to be simpler than for small molecules. In contrast to small molecule drugs, the typical metabolic enzymes and transporter proteins such as cytochrome P450, multidrug resistance (MDR) efflux pumps are not involved in the disposition of MABs. Therefore, drug–drug interaction studies for those disposition processes are only part of the standard safety assessment for small molecules and not for MABs. Monoclonal antibodies, which have a protein structure, are metabolized by proteases. These enzymes are ubiquitously available in mammalian organisms. In contrast, small molecule drugs are primarily metabolized in the liver.

   II. Because of the large molecular weight, intact MABs are typically not cleared by the renal elimination route in the kidneys. However, renal clearance processes can play a major role in the elimination of small molecule drugs.

   III. Pharmacokinetics of MABs usually is dependent on the binding to the pharmacological target protein and shows nonlinear behavior as consequence of its saturation kinetics.

   IV. In general, MABs have a longer half-life (in the order of days and weeks) than small molecule drugs (typically in the order of hours).

   V. The distribution of MABs is very restricted (volume of distribution in the range of 0.1 L/kg). As a consequence, MABs do have limited access to tissue compartments as potential target sites via passive, energy-independent distribution processes only (e.g., brain).

   (b) At lower concentrations, MABs generally show nonlinear pharmacokinetics due to receptor-mediated clearance processes, which are characterized by small capacity of the clearance pathway and high affinity to the target protein. Consequently at these low concentrations, MABs exhibit typically shorter half-life. With increasing doses, these receptors become saturated, and the clearance as well as elimination half-life decreases until it becomes constant. The clearance in the higher concentration range, which is dominated by linear, nontarget-related clearance processes, is therefore also called nonspecific clearance in contrast to the target-related, specific clearance.

3. A surrogate MAB has similar antigen specificity and affinity in experimental animals (e.g., mice and rats) compared to those of the corresponding human antibody in humans. It is quite common that the antigen specificity limits ADME studies of humanized monoclonal antibodies in rodents. Studies using surrogate antibodies might lead to important information regarding safety, mechanism of action, disposition of the drug, tissue distribution, and receptor pharmacology in the respective animal species, which might be too cumbersome and expensive to be conducted in nonhuman primates. Surrogate MABs (from mouse or rat) provide a means to gain knowledge of ADME and PD in preclinical rodent models and might facilitate the dose selection for clinical studies.

4. Apart from ADCC, monoclonal antibodies can exert pharmacological effects by multiple mechanisms that include direct modulation of the target antigen, complement-dependent cytotoxicity (CDC) and apoptosis.

   The key steps of ADCC are (1) opsonization of the targeted cells, (2) recognition of antibody-coated targeted cells by Fc receptors on the surface of monocytes, macrophages, natural killer cells, and other cells, and (3) destruction of the opsonized targets by phagocytosis of the opsonized targets and/or by toxic substances released after activation of monocytes, macrophages, natural killer cells, and other cells.

5. IgG can bind to neonatal Fc receptor (FcRn) in the endosome, which protects IgG from catabolism via proteolytic degradation. This protection results into a slower clearance and thus longer plasma half-life of IgGs. Consequently, changing the FcRn affinity allows to adjust the clearance of MABs (higher affinity – lower clearance), which can be employed to tailor the pharmacokinetics of these molecules.

6. Pre-IND, phase I, II, III, and IV are the major development phases for antibody therapies. Safety pharmacology, toxicokinetics, toxicology, tissue cross reactivity, local tolerance, PK support for molecules selection, assay support for PK/PD, and PK/PD support for dose/route/regimen are major activities in the pre-IND phase. General toxicity, reproductive toxicity, carcinogenicity, immunogenicity, characterization of dose–concentration–effect relationship, material comparability studies, mechanistic modeling approach, and population pharmacokinetics/

predictions are major activities from phase I to phase III. Further studies might be performed as needed after the MAB got market authorization. These studies are called phase IV studies.

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