PART I

ANTIBODY BASICS

CHAPTER 1

Therapeutic Monoclonal Antibodies: Past, Present, and Future

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ABSTRACT

In this chapter, an overview of the therapeutic antibody industry today, including the many commercial antibodies and Fc fusions and the rich clinical pipeline, is presented and analyzed. The long history of antibodies is given to bring context to the therapeutic antibody industry. This history includes serum therapy, the use of IVIG, and the evolution of those therapies into the development of the monoclonal

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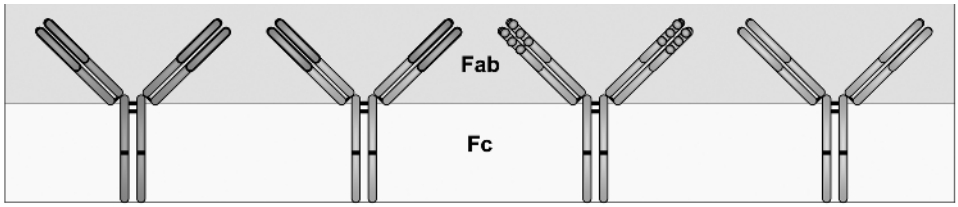
antibody business as we know it today. The history of technologies that fostered the revolution of therapeutic antibody development in the 1990s is also described. Finally, the future of the therapeutic monoclonal antibody and Fc fusion business is presented along with opportunities and challenges facing the business and those who work in it.

1.1 INTRODUCTION

Protein therapeutics in general, and more specifically, therapeutic monoclonal antibodies (Mabs; Fig. 1.1) and Fc fusion proteins, have become a significant addition to the pharmaceutical repertoire over the past 20 years, and promise to play an even more significant role in the future of pharmaceutical intervention in diseases (Carter 2006; Riley 2006; Dimitrov and Marks 2008; Leader, Baca, and Golan 2008). In total, protein therapeutics produced by the BioPharm industry had over $55 billion in sales in 2005 (Table 1.1), approximately 20 percent of the roughly $280 billion 2005 pharmaceutical market. Based on the increase in value of protein therapeutics already on the market, therapeutic proteins are projected to reach about $94 billion by 2010 (Table 1.1), which calculates to an approximately 12 percent compound annual growth rate over that period. The 27 currently marketed monoclonal antibodies and Fc fusion proteins (see Table 1.2 for a complete listing) combine to make up 35 percent of the market value of all therapeutic proteins (based on 2006 data; Table 1.1), but are projected to increase in proportion by 2010, especially now that the market for epoetins has weakened based on safety concerns raised in mid-2007. Sales in 2006 for therapeutic Mabs and Fc fusion proteins topped $23 billion (Table 1.1), led by Enbrel®, Rituxan®, Remicade®, and Herceptin®, all of which were approved in the 1997–1998 time frame (Fig. 1.2). Of the six Mabs and Fc fusions brought to market in 1997–1998,



Prefix	Target			Antibody Source		Suffix	Some Examples
Variable	Non-tumor Target	Viral	-vir-	-u-	Human	-mab	Pali-vi-zu-mab (humanized antiviral Mab)
		Bacterial	-bac-				
		Immune	-lim-	-o-	Murine		Ada-lim-u-mab (human Mab against immune disease target)
		Infectious lesions	-les-				
		Antifungal	-fung-	-a-	Rat		E-fung-u-mab (human antifungal Mab)
		Cardiovascular	-ci(r)-				
		Neurologic	-ne(r)-	-e-	Hamster		Bapi-neu-zu-mab (humanized Mab against neurobiology target)
		Interleukins	-kin-				
		Musculoskeletal	-mul-	-i-	Primate		Uste-kin-u-mab (human anticytokine Mab)
		Bone	-os-				
		Toxin as target	-toxa-	-xi-	Chimeric		Den-os-u-mab (human antibone target Mab
	Tumor target	Colon	-col-				
		Melanoma	-mel-	-zu-	Humanized		Ab-ci-xi-mab (chimeric Mab against CV target)
		Mammary	-mar-				
		Testis	-got-	-axo-	Rat/murine hybrid		Ore-gov-o-mab (murine Mab for ovarian cancer)
		Ovary	-gov-				
		Prostate	-pr(o)-	-xizu-	Chimeric + humanized		Adeca-tum-u-mab (human antibody against miscellaneous tumor target)
		Miscellaneous	-tu(m)-				

Figure 1.1 The different forms of therapeutic monoclonal antibodies that have been approved for marketing, including murine, chimeric, humanized, and fully human antibodies, as well as how the generic names are applied to each of them based on structure, source, and target. (See color insert.)

TABLE 1.1 Breakdown of Estimated Market for Protein Therapeutics Based on the Sales of the Top-Selling Biologics Drugs*

Category	2005 (in $B)	2006 (in $B)	2010 (Projected) (in $B)
Monoclonal antibodies and Fc fusions	17.3	23.1	41.2
Epoetins	11.2	12.0	12.8**
Insulin-related	7.6	9.0	13.0
Interferons	6.4	6.8	7.7
Antifibrinolytics	4.1	4.5	6.3
Immunostimulatory (xCSF)	3.9	4.3	5.5
Growth hormones	2.2	2.4	2.5
Other (mixed mechanisms)	2.9	3.2	4.7
Totals	55.6	65.3	93.7

*Based on published 2005 and 2006 sales, and 2010 projected sales of the top 70 biologics currently on the market, excluding vaccines (multiple sources).

**Projections were made prior to published safety concerns in mid-2007, which have depressed overall sales of epoetins.

four (Rituxan®, Remicade®, Enbrel®, and Herceptin®) are significant blockbusters, each with markets great than $3 billion (Fig. 1.2). Of the more recently approved Mabs, Humira®, Erbitux®, and Avastin®, all approved in the 2003–2004 time frame (Table 1.2, Fig. 1.2), had not yet hit their peak sales by 2006, the time at which these numbers were generated. Thus, it is expected that these successful new entries also will hit blockbuster status like some of their predecessors. The key inflection points for success in bringing these Mab-based biologics to market appear to be the late 1990s, 2003–2004, and the period 2007–2012 (Fig. 1.3), the latter being the period in which we are currently working. This near-term future inflection point is likely to be a direct result of the success of monoclonal antibodies marketed in the late 1990s, as well as a maturation of antibody engineering technologies and strategies to make more commercially successful biologics molecules.

At the time of this writing, Biologic License Application (BLAs) for four additional antibodies had been submitted for regulatory approval in the United States (Table 1.3), and an additional 30 Mabs and Fc fusion proteins are in advanced clinical trials (defined here as Phase III or entering into Phase III based on successful completion of Phase II clinical trials; Table 1.3). Between 2007 and 2012, the growth of marketed monoclonal antibodies promises to be extraordinary (Fig. 1.3). Although not probable, if all current Phase III candidates listed in Table 1.3 were to achieve registration, this would translate to over 60 monoclonal antibodies and Fc fusion proteins on the market by the 2012–2013 time frame (Fig. 1.3). Even if only 50 percent are successful in being marketed, that number still reaches 46, a 50 percent increase in numbers over the currently marketed antibodies and Fc fusion proteins (Fig. 1.3). With the current rate of success for monoclonal antibodies transitioning from Phase III to the market at 75 percent (KMR Group, Inc. 2007), this would translate into about 53 to 54 Mabs and Fc fusion proteins on the market by the 2012–2013 time frame. These additional marketed biologics should have a substantial impact on the pharmaceutical industry over the next five years. It has been projected that 60 percent of the total growth in the pharmaceutical industry between 2004 ($271 billion total) and 2010 ($317 billion) will be driven by biologics (Riley 2006), and the data presented herein (Table 1.1, Fig. 1.2) suggest that a significant fraction of that growth will be accounted for by Mab and Fc fusion proteins.

There are currently over 140 additional publicly stated, commercially funded monoclonal antibodies in early clinical trials (defined here as Phase I and Phase II candidates combined), many of which are listed in Table 1.4. With the probability of success (POS) for antibodies transitioning between Phase II and Phase III currently at about 62 percent (KMR Group, Inc. 2007), approximately 50 of the 84 Phase II candidates shown in Table 1.4 should result in Phase III candidates. Of the 58 known Phase I candidates listed in Table 1.4, 35 should transition to Phase II (based on 61 percent POS; KMR Group, Inc. 2007), and of those, 22 would be predicted to make it to Phase III based on the 62 percent POS for that transition. Thus, of the 142 early phase candidates listed in Table 1.4

TABLE 1.2 Marketed Monoclonal Antibodies and Fusion Proteins*

U.S. Trade Name (Generic Name)	Company	Approval Date (U.S.)	Molecular Target	Major Indication	Protein Format	Route & Form	Antibody Source	Production Cell Line
Orthoclone OKT3® (Muromonab-CD3)	Ortho Biotech (J&J)	06/19/1986	CD3 on T-cells	OTR	Murine IgG2a	IV, Liquid	Hybridoma	Hybridoma
ReoPro® (Abciximab)	Centocor (now J&J)/Lilly	12/22/1994	gPIIb/IIIa on platelets	CVD	Chimeric Fab	IV, Liquid	Hybridoma	*E. coli*
Rituxan® (Rituximab)	Biogen/Idec/ Genentech	11/26/1997	CD20 on B-cells	NHL, RA added 2/8/06	IgG1κ, Chimeric	IV, Liquid	Hybridoma	CHO
Zenapax® (Daclizumab)	PDL/Roche	12/10/1997	IL-2Rα (CD25; tac)	OTR	IgG1, Humanized	IV, Liquid	Hybridoma	NS0
Synagis® (Palivizumab)	MedImmune	06/19/1998	A-antigenic site of RSV F-protein	RSV (infant)	IgG1κ, Chimeric	IM, Lyo	Hybridoma	NS0
Remicade® (Infliximab)	Centocor (now J&J)	08/24/1998	TNF-α	RA	IgG1κ, Chimeric	IV, Lyo	Hybridoma	NS0
Herceptin® (Trastuzumab)	Genentech	09/25/1998	HER2/Neu	Breast cancer	IgG1κ, Humanized	IV, Lyo	Hybridoma	CHO
Enbrel® (Etanercept)	Immunex (now Amgen)	11/02/1998	TNF-α	RA	IgG1-Fc conjugated to p75exodomain of TNFR	SC, Lyo	Recombinant Fc fusion	CHO
Simulect® (Basiliximab)	Novartis	12/05/1998	IL-2Rα (CD25; tac)	OTR	IgG1κ, Chimeric	IV, Lyo	Hybridoma	SPII/0
Mylotarg® (Gemtuzumab ozogamicin)	Wyeth	05/17/2000	CD33	Leukemia	Humanized IgG4κ-Ozogamicin conjugate**	IV, Lyo	Hybridoma	NS0
Campath-1H® (Alemtuzumab)	ILEX/ Millenium	05/07/2001	CD52 on B- and T-cells	Leukemia	IgG1κ, Humanized	IV, Liquid	Hybridoma	CHO
Zevalin® (Ibritumomab tiuxetan)	Biogen/Idec	02/19/2002	CD20 on B-cells	NHL	Murine IgG1κ conjugate, Y-90 or In-111	IV, Liquid	Hybridoma	CHO

Humira® (Adalimumab)	CAT, Abbott	12/31/2002	TNF-α; Blocks interaction with p55 and p75	RA, Crohn disease	IgG1κ, Human	SC, Liquid	Phage display	HEK293
Amevive® (Alefacept)	Biogen	01/30/2003	CD2—inhibits CD2-LFA-3 interaction on activated T-cells	Psoriasis	CD2-binding domain of LFA-3::Fc fusion protein	IM/IV, Form not known	Recombinant Fc fusion	CHO
Xolair® (Omalizumab)	Genentech	06/20/2003	IgE	Asthma	IgG1κ, Humanized	SC, Lyo	Hybridoma	CHO
Raptiva® (Efalizumab)	Genentech	10/27/2003	CD11a, α-subunit of LFA-1; inhibits binding to ICAM-1	Psoriasis	IgG1κ, Humanized	SC, Lyo	Hybridoma	CHO
Bexxar® (Tositumomab-I131)	Corixa	06/27/2003	CD20 on B cells	NHL	Murine IgG2a/λ-I-131	IV, Liquid	Hybridoma	Mammalian
Erbitux® (Cetuximab)	ImClone/ BMS	02/12/2004	Binds EGF-R (HER1, c-ErbB-1)	Colorectal cancer	IgG1κ, Chimeric	IV, Liquid	Hybridoma	SPII/0
Avastin® (Bevacizumab)	Genentech	02/26/2004	VEGF (ligand)	Colorectal cancer	IgG1, Humanized	IV, Liquid	Hybridoma	CHO
Tysabri® (Natalizumab)	Biogen/Elan	11/23/2004***	α4 subunit of α4β1 or α4β7	Multiple sclerosis	IgG4 k, Humanized	IV, Liquid	Hybridoma	Murine myeloma
Orencia® (Abatacept)	BMS	12/23/2005	CD80/CD86 – T-cell ostimulatory	RA	CTLA4-Fc fusion protein	IV, Lyo	Recombinant Fc fusion	Mammalian
Lucentis® (Ranibizumab)	Genentech/ Novartis	06/30/2006	VEGF-A	Wet AMD	Humanized IgG1 k Fab fragment	Intravitreal injection	Hybridoma	*E. coli*
Vectibix® (Panitumumab)	Amgen	09/27/2006	EGFR	Colorectal cancer	Human IgG2 k	IV infusion, Lyo	Transgenic humanized mouse	CHO
Soliris® (Ecolizumab)	Alexion Pharma	03/16/2007	Complement C5	PNH (reduce hemolysis)	Humanized IgG2/4 hybrid	IV, Liquid	Hybridoma	Murine myeloma

(*Continued*)

TABLE 1.2 *Continued*

U.S. Trade Name (Generic Name)	Company	Approval Date (U.S.)	Molecular Target	Major Indication	Protein Format	Route & Form	Antibody Source	Production Cell Line
Arcalyst® (Rilonacept)	Regeneron	02/27/2008	IL-1	CAPS	Dimeric Fc fusion protein with IL-1R & IL-1 accessory protein in-line	Lyo	Recombinant Fc fusion	CHO
Cimzia® (Certolizumab pegol)	UCB/ Schwartz	04/22/2008	TNF-α	RA	PEGylated humanized Fab	SC	Hybridoma	*E. coli*
Nplate® (Romiplostim, AMG-531)	Amgen	08/22/08	TPO-R	Thrombocytopenia	Fc-peptide fusion (peptibody)	SC	Not applicable	*E. coli*

Abbreviations: OTR, organ transplantation rejection; CV, cardiovascular disease; RA, rheumatoid arthritis; RSV, respiratory syncytial virus; NHL, non-Hodgkin's lymphoma; TNF, tissue necrosis factor; PNH, paroxysmal nocturnal hemoglobinuria; AMD, age-related macular degeneration; CAPS, cropyrin-associated periodic syndrome; LYO, lyophilized; IV, intravenous; SC, subcutaneous; ND, not disclosed.

*Data obtained from prescribing information released by the manufacturers, company websites, Prous Science Integrity.

**Conjugate is ozogamicin, a calecheamycin (natural product cytotoxin).

***Suspended 2/28/05; reinstated under specified conditions.

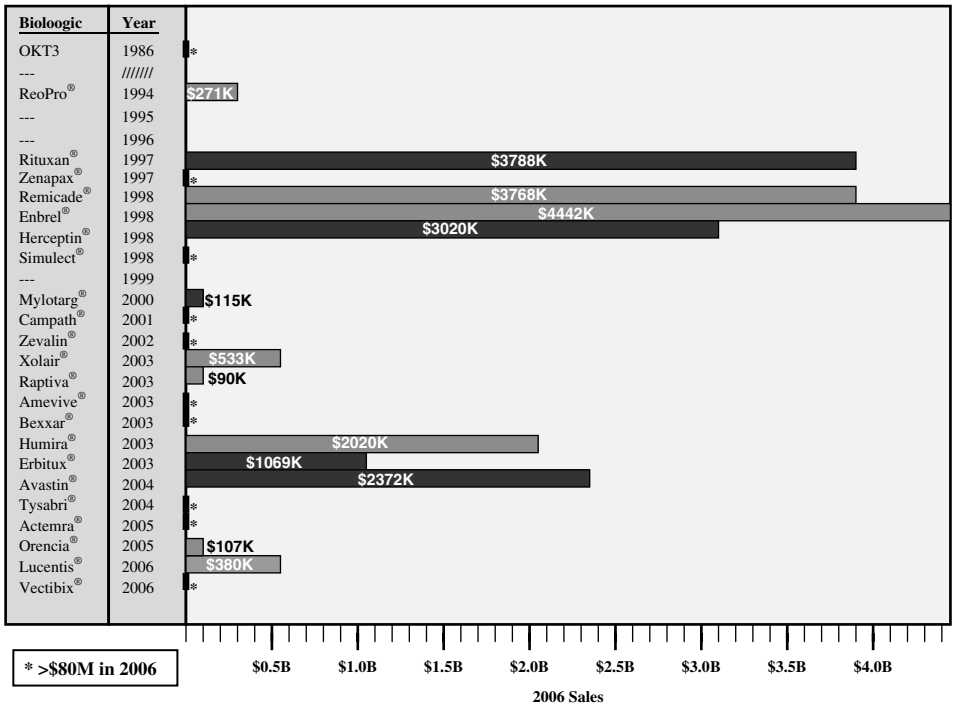


Figure 1.2 Sales of marketed antibodies in 2006 as a function of the year in which they reached the market. Two important features can be observed: (1) only 9 of 23 of the marketed antibodies and Fc fusion proteins have achieved substantial sales; (2) several of the Mabs and Fc fusion proteins on the market from the 1997–1998 period have been major blockbusters, which has driven broad interest by the pharmaceutical interest in biologics.

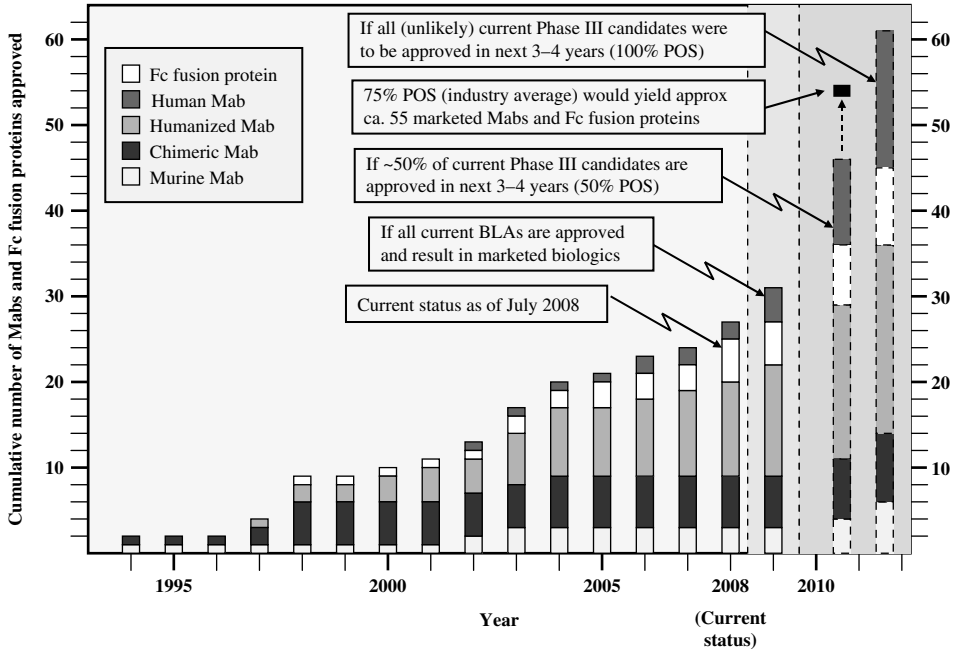


Figure 1.3 Accumulated Mabs and Fc fusion proteins on the market in the United States up to 2008, with projections for near-term (2012–2013 timeframe) future numbers based on current Phase III clinical candidates.

TABLE 1.3 Examples of Important Monoclonal Antibodies and Fc Fusion Proteins in Advanced Clinical Trials*

U.S. Trade Name (Generic Name)	Company	Current Status (U.S.)	Molecular Target	Major Indication	Protein Format	Antibody Source
Golimumab (CNTO-148)	Centocor	BLA filed June 2008	TNF-α	Psoriasis	Human IgG1	Transgenic humanized mouse
Ustikinumab (CNTO-1275)	Centocor	BLA filed November 2007	P40 subunit of IL-12 & IL-23	Psoriasis	Human IgG1	Transgenic humanized mouse
ABT-874	Abbott	Phase III initiated	P40 subunit of IL-12 & IL-23	Psoriasis, Crohn disease	Human IgG	Phage displayed human antibody library
Belatacept (LEA-29Y)	Bristol-Myers Squibb	Phase II/III	CD80/CD86	Renal transplantation	CTLA-4 Fc fusion LEA (higher affinity than abatacept)	Not applicable; Fc fusion
Lymphostat B® (Belimumab)	Glaxo Smith-Kline	Phase III	BLyS	Lupus	Human IgG	Phage displayed human antibody library
Atacicept (TACI-Ig)	Merck-Serono/ Zymogenetics	Phase III	BlyS, April antagonist	SLE (PII for MS, RA)	Fc fusion protein	Not applicable; Fc fusion
Ocrelizumab (2nd gen. anti-CD20)	Genentech	Phase III	CD20	RA, lupus, relapsing MS	Humanized IgG1	Hybridoma
ACZ-885	Novartis	Phase III	IL-1β	Muckle Wells syndrome	Human IgG1 k	Transgenic humanized mouse
Actemra® (Tocilizumab; Atlizumab)	Roche/Chugai	BLA filed Nov 2007	IL-6R	Castlemans disease	IgG1, humanized	Hybridoma
Bosatria® (Mepolizumab)	Glaxo Smith-Kline	Phase III	IL-5	Hyper eosinophilic syndrome	Humanized IgG	Hybridoma
Omnitarg® (Pertuzumab)	Genentech	Phase III	Her2	Oncology	Humanized IgG1	Hybridoma
Ofatumumab (Humax CD20)	GenMab/Glaxo Smith-Kline	Phase III	N-terminal epitope of CD20	B-cell chronic leukemia; NHL, RA	Humanized IgG1	Hybridoma
Tremelimumab (CP-675,206; Ticilimumab)	Pfizer	Phase III	CTLA4	Oncology	IgG2 human antibody	Transgenic humanized mice

Ipilimumab (MDX-010)	Medarex/Bristol-Myers Squibb	Phase III	CTLA4	Melanoma, other cancers	Human IgG1	Transgenic humanized mouse
Galiximab (IDEC-114)	Biogen/IDEC	Phase III	CD80 (B7-1)	NHL	Primatized IgG1λ	Hybridoma
Inotuzumab ozogamycin	Wyeth	Phase III	CD22	NHL	Humanized IgG4, Mab-conjugate	Hybridoma
Zalutumumab (HuMax EGFR)	GenMab/Medarex	Phase III	EGFR	Head and neck cancer	Human IgG1	Transgenic humanized mice
Aflibercept	Sanofi-Aventis/ Regeneron	Phase III	VEGF	NSCLC	Fc fusion	Exodomain 1 of human VEGFR1 and 2 of VEGFR2 – Fc fusion
VEGF Trap-Eye	Bayer-Schering Pharma/ Regeneron	Phase III	VEGF	Wet age-related macular degeneration	Fc fusion	Exodomain 1 of human VEGFR1 and 2 of VEGFR2 – Fc fusion
Zanolimumab (HuMax-CD4)	GenMab/Medarex	Phase III	CD4	Cutaneous T-cell lymphoma	Human IgG1	Transgenic humanized mice
Teplizumab; HOKT3γ1(Ala-Ala)	Macrogenics/Eli Lilly	Phase III	CD3	Diabetes	Humanized modified Fc	Hybridoma
Otelixizumab (ChAglyCD3; TRX4)	GSK/Tolerx	Phase III	CD3	Type 1 diabetes	Aglycosylated, humanized IgG	Hybridoma
Removab® (Catumaxomab)	Fresenius/Trion	Phase II/III	EpCAM and CD3	Malignant ascites; cancer	Rat-murine hybrid	Hybridomas; modified Fc; trifunctional bispecific
IGN101	Aphton	Phase II/III	EpCAM (CD326)	Oncology, specifically non-small-cell lung cancer	Murine Mab 17A-1 absorbed on aluminum hydroxide to provoke immune response on cells containing EpCAM	Hybridoma
Adecatumumab (MT-201)	Micromet/Merck-Serono	Phase II completed	EpCAM (CD326)	Oncology	Human IgG1	Phage displayed human antibody library

(*Continued*)

TABLE 1.3 *Continued*

U.S. Trade Name (Generic Name)	Company	Current Status (U.S.)	Molecular Target	Major Indication	Protein Format	Antibody Source
OvaRex® (Oregovomab)	ViRess/United Therapeutics	Phase III	CA125 tumor antigen	Ovarian cancer	Murine IgG	Hybridoma
CH-14.18	NCI	Phase III	GD2 ganglioside	Neuroblastoma	Chimeric IgG1 k	Hybridoma
Rencarex® (WX-G250)	Wilex AG	Phase III	Carbonic anhydrase IX	Nonmetastatic renal cell cancer	Chimeric IgG1	Hybridoma
Denosumab (AMG-162)	Amgen	Phase III	RANK-ligand	Osteoporosis	Human IgG2	Transgenic humanized mouse
Bapineuzumab (AAB-001)	Wyeth	Phase II completed	Amyloid beta	Alzheimer disease	Humanized	Hybridoma
Numax® (Motavizumab; MEDI-524)	Astra-Zeneca	BLA filed January 2008	Respiratory syncytial virus	Respiratory infection	Humanized IgG1; affinity optimized	Hybridoma
Mycograb® (efungumab)	Novartis	Phase III	Fungal HSP90	Fungal diseases	Human scFv	Phage displayed human antibody library
Aurograb®	Novartis/Neutec Pharma (now part of Novartis)	Phase III	Staph ABC transporter GrfA	MRSA, to be used with vancomycin	Human scFv	Phage displayed human antibody library
Abthrax® (Raxibacumab)	Human Genome Sciences	Phase III	B. anthracis PA toxin	Anthrax biodefense	Human IgG	Phage displayed human antibody library

Abbreviations: MS, multiple sclerosis; NHL, non-Hodgkin's lymphoma; MRSA, methicillin-resistant *Staphylococcus aureus*; NSCLC, non-small cell lung cancer; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

*Data as of August 2008; data obtained from company websites, Prous Science Integrity, and www.clinicaltrials.gov.

TABLE 1.4 Key Phase I and Phase II Clinical Candidates by Indication and Target*

U.S. Trade Name (Generic Name)	Company	Current Status (U.S.)	Molecular Target	Major Indication	Protein Format
			Mostly Inflammatory Diseases		
Anrukinzumab (IMA-638)	Wyeth	Phase II	IL-13	Asthma	Humanized IgG
CAT-354	Astra-Zeneca	Phase II	IL-13	Asthma	Human IgG4
QAX-576	Novartis	Phase II	IL-13	Asthma	IgG
Anti-IL-13 Ab	Genentech	Phase I	IL-13	Asthma	IgG
AMG-317	Amgen	Phase II	IL-4 R	Asthma	Human IgG
MEDI-528	Astra-Zeneca	Phase II	IL-9	Asthma	Humanized IgG
GSK-679586A	GSK	Phase II	Not disclosed	Asthma	IgG
AMG-714 (HuMax-IL-15)	Genmab/Amgen	Phase II	IL-15	RA	Human IgG
CNTO-328	Johnson & Johnson	Phase II	IL-6	Multiple myeloma	IgG
CNTO-136	Johnson & Johnson	Phase II	IL-6	RA	IgG
REGN-88	Sanofi-Aventis	Phase I	IL-6	RA	IL-6R Fc-fusion trap
AMG-220	Amgen	Phase I	IL-6	Crohn disease	IgG
Baminercept alpha (LTβR-Ig)	Biogen/IDEC	Phase II	LTβR-Ig	RA	Lymphotoxin-βR – Fc fusion protein
IL-1βAb (Hu-007)	Eli Lilly	Phase II	IL-1β	RA	Humanized IgG
AMG-108	Amgen	Phase II	IL-1	Osteoarthritis	Human IgG
Xoma 052	Xoma	Phase I	IL-1β	Type 2 diabetes	Humanized IgG2
AMG-827	Amgen	Phase I	IL-17	RA	Human IgG
AIN-457	Norvartis	Phase I	IL-17A	Psoriasis	IgG
HuMax IL-8 (MDX-018)	Genmab/Medarex	Phase I/II	IL-8	Palmoplantar pustulosis	Human IgG
MEDI-563 (formerly BIW-8405)	Astra-Zeneca	Phase II	IL-5R	SLE	Afucosylated IgG based on BioWa's Potelligent technol.
MEDI-545	Astra-Zeneca/ Medarex	Phase II	IFNα	SLE	IgG
Fontolizumab (HuZAF)	Biogen IDEC/PDL	Phase II	IFNγ	IBD, inflammatory disorders	Humanized IgG1
AMG-811	Amgen	Phase I	IFN-γ	SLE	Human IgG
Anti-IFNα	Genentech	Phase I	IFN-R	SLE	IgG
MEDI-502	Astra-Zeneca	Phase II	CD2	Psoriasis	Humanized IgG

(*Continued*)

TABLE 1.4 *Continued*

U.S. Trade Name (Generic Name)	Company	Current Status (U.S.)	Molecular Target	Major Indication	Protein Format
Nuvion® (Visilizumab)	PDL	Phase II	CD3	GVHD; ulcerative colitis	Humanized IgG with modified Fc for decreased FcγR binding
NI-0401	NovImmune	Phase I/II	CD3	Crohn disease; renal transplantation	Human IgG
MT-110	Micromet	Phase I	CD3, EpCAM (CD326)	Gastrointestinal cancer	CD3, EpCAM bispecific scFv-based BiTE
TRX1	Genentech	Phase I	CD4	RA	IgG
MLN-0002	Millenium	Phase II	α4β7 on T-cells	Crohn disease	Humanized IgG
Anti-beta7	Genentech	Phase I	Beta7	Ulcerative colitis	IgG
MDX-1100	Medarex	Phase II	CXCL10 (IP-10)	Ulcerative colitis, RA	Human IgG
MLN-1202	Millenium	Phase II	CCR2	Scleroderma	Humanized IgG
Anti-OX40L	Genentech	Phase I	OX40-L	Asthma	IgG
PD-360324	Pfizer	Phase I	GM-CSF	RA	IgG
CAM-3001	Zenith	Phase I	GM-CSF R	RA	Human IgG (CAT Library)
Anti-VAP1 MAb	BioTie	Phase I	VAP1	Inflammatory disease	Human IgG
AMG-557	Amgen	Phase I	B7RP-1	SLE	Human IgG
FG-3019	Fibrogen	Phase I	CTGF	IPF, diabetic nephropathy	Human IgG
GC-1008	Genzyme	Phase I/II	TGF-β	IPF	Human IgG (CAT)
			Mostly Oncology		
Milatuzumab	Immunomedics	Phase I	CD74	MM, other tumors	IgG
MDX-1411	Medarex	Phase I	CD70 (ligand for CD27)	RCC	Human IgG
SGN-70	Seattle Genetics	Phase I	CD70 (ligand for CD27)	Autoimmune diseases	Human IgG
IMGN901 (formerly huN901-DM1)	Immunogen	Phase II	CD56	SCLC, MM	Humanized IgG-DM1 cytotoxin conjugate
CP-870893	Pfizer	Phase II	CD40 agonist	Oncology	IgG
Dacetuzumab (SGN-40)	Genentech	Phase II	CD40	Oncology	Humanized IgG
HCD122 (formerly CHIR-12.12)	Xoma/Novartis	Phase I	CD40	B-cell malignancies	IgG
HuMax CD-38	GenMab	Phase II	CD38	Multiple myeloma	IgG
Lintuzumab (SGN-33)	Seattle Genetics	Phase II	CD33	AML	IgG
AVE-9633 (huMy9-6)	Sanofi-Aventis	Phase I	CD33	AML	Humanized IgG-DM4 cytotoxin conjugate

MDX-060	Medarex	Phase II	CD30	Hodgkin lymphoma	Human IgG
MDX-1401	Medarex	Phase I	CD30 (backup to MDX-060)	Oncology	Human IgG
SGN-35	Seattle Genetics	Phase I	CD30 auristatin conjugate	Hodgkin lymphoma	IgG conjugated NP-toxin
Lumiliximab (IDEC-152)	Biogen/IDEC	Phase II	CD23	CLL	Primatized IgG1 k
Epratuzumab	Immunomedics/UCB	Phase II	CD22	NHL, SLE	Humanized IgG
Epratuzumab tetraxetan	Immunomedics	Phase I/II	CD22	NHL, SLE	Humanized IgG-Y90 radio-conjugate
Anti-CD22-PE (CAT-8015)	Astra-Zeneca	Phase I	CD22	Oncology; CLL	anti-CD22-pseudomonas exotoxin fusion protein
Veltuzumab (IMMU-106)	Immunomedics	Phase I/II	CD20	NHL, autoimmune diseases	Humanized IgG
PRO-131921	Genentech	Phase I/II	CD20 (third generation)	Oncology	IgG with modified Fc for increased ADCC
AME-133v (LY2469298)	Eli Lilly	Phase I/II	CD20	NHL	IgG with modified Fc for increased ADCC
TRU-015	Wyeth	Phase II	CD20	RA	Small modular immunopharmaceutical product (SMIP)
R-7159	Roche	Phase II	CD20	NHL	IgG
Blinatumomab (MT103/MEDI-538)	Astra Zeneca/Micromet	Phase II	CD19, CD3	ALL, NHL, CLL	CD19, CD3 bispecific scFv-based BiTE
MDX-1342	Medarex	Phase I	CD19	CLL, RA	Human IgG
SAR-3419	Sanofi-Aventis/Immunogen	Phase I	CD19	NHL	IgG
AMG-102	Amgen	Phase II	HGF/SF (cmet-L)	RCC	Human IgG
MetMAb	Roche/Genentech	Phase I/II	c-met	Oncology	IgG
AMG-386	Amgen	Phase II	Angiopoietin-related target	RCC	Fc-peptide “peptibody”
Mapatumumab (HGS-ETR1)	HGS	Phase II	TRAIL-R1	Oncology	Agonist MAb
AMG-951; RhApo2L/TRAIL	Amgen; Genentech	Phase II	rhApo2L/TRAIL	Oncology	Therapeutic protein
AMG-655	Amgen	Phase I/II	DR5 (TRAIL-2)	Pancreatic cancer	Human IgG
Apomab	Genentech	Phase II	DR5 (TRAIL-R2) agonist	Oncology	Human IgG
Lexatumumab (HGS-ETR2)	GSK	Phase I	TRAIL-2	Solid tumors	Human IgG (agonist)

(*Continued*)

TABLE 1.4 *Continued*

U.S. Trade Name (Generic Name)	Company	Current Status (U.S.)	Molecular Target	Major Indication	Protein Format
LBY-135	Norvartis	Phase I	DR5	Solid tumors	Chimeric IgG
CT011	CureTech	Phase II	PD-1	Oncology	Humanized IgG
MDX-1106 (ONO-4538)	Ono Pharma/ Medarex	Phase I	PD-1	Oncology; infectious diseases	Human IgG
MDX-1105	Medarex	Phase I	PD-L1	Oncology	Human IgG
Farletuzumab (MORAb-003)	Morphotek	Phase II	Folate Receptor Alpha	Oncology	Humanized IgG
Trastuzumab-DM1	Genentech	Phase II	HER2	Oncology	Humanized IgG- DM1 cytotoxin conjugate
Ertumaxomab (Rexomun)	Fresenius/Trion	Phase II/III	Her2/neu and CD3	Malignant ascites; Cancer	Rat-murine hybrid; modified Fc; trifunctional bispecific
CP-751871	Pfizer	Phase II	IGF-1R	NSCLC	Human IgG2
MK-0646 (H7C10)	Merck/Pierre-Fabre	Phase II	IGF-1R	Oncology	Humanized IgG
AMG-479	Amgen	Phase II	IGF-1R	Ewings sarcoma	Human IgG
IMC-A12	ImClone	Phase II	IGF-1R	Oncology (multiple)	Human IgG
R-1507 (formerly Roche 1)	GenMab	Phase II	IGF-1R	Solid tumors	Human IgG
AVE-1642	Sanofi-Aventis/ Immunogen	Phase I	IGF-1R	Solid tumors	Human IgG
Anti-IGF-1R Mab	Biogen/IDEC	Phase I	IGF-1R	Solid tumors	IgG
Nimotuzumab (DE-766)	Oncoscience/YM Biosciences	Phase II (launched in India)	EGFR	Oncology, several indications	Humanized IgG1
IMC-11F8	ImClone, Dyax	Phase II	EGFR	Solid tumors	Human IgG (CAT Library)
IMC-1121B	Imclone, Dyax	Phase II	VEGF-B	Solid tumors	Human IgG (CAT Library)
Alacizumab pegol (CDP-791)	UCB-Celltech/ ImClone	Phase II	VEGF-R2	Lung cancer	Di-Fab-PEG conjugate
Angiocept (CT-322)	BMS	Phase II	VEGF-R2 (FLK-1/ KDR)	Glioblastoma	PEGylated adnectin
IMC-18F1	ImClone	Phase I	VEGF-R1	Oncology	Human IgG
IMC-3G3	ImClone	Phase I	PDGFRα	Oncology	Human IgG
CVX-045	Pfizer	Phase I	Angiogenesis inhibitor	Oncology	Thrombospondin-1 mimetic-IgG conjugate
Volociximab (M200)	Biogen Idec/PDL	Phase II	α5β1 integrin	Solid tumors	Chimeric MAb
CNTO-95	Johnson & Johnson	Phase II	αV integrins	Melanoma	IgG

MEDI-522	Astra-Zeneca	Phase II	αVβ3 integrin	Systemic psoriasis	Humanized IgG
IMGN388	Immunogen	Phase I	αV integrins	Oncology	Humanized IgG-DM4 cytotoxin conjugate
CNTO-888	J&J	Phase I	MCP-1	Oncology	Human IgG1
CME-548	Wyeth	Phase II	5T4	Solid tumors	IgG-calicheamicin conjugate
MK-4721	Merck/Astellas	Phase II	PSCA	Oncology	Human IgG
Apolizumab (Hu1D10)	NCI/PDL	Phase II	HLA-DR beta-chain epitope	NHL, other oncology	Humanized IgG1
3F8	NCI	Phase II	GD2 ganglioside	Oncology	Murine IgG
Tucotuzumab celmo-leukin (EMD 273066)	Merck-Serono	Phase II	GD-2 tumor antigen	SCLC	Humanized IgG linked to cytokine IL-2
IMGN242	Immunogen	Phase II	CanAg	Oncology	Humanized IgG- DM4 cytotoxin conjugate
BrevaRex(R) AR20.5	AltaRex	Phase I/II	MUC1	Multiple myeloma	IgG
Y90-hPAM4	Immunomedics	Phase I	MUC1	Oncology	IgG-Y90 radioconjugate
MORAb-009	Morphotek	Phase II	Mesothelin on ovarian cancer	Ovarian cancer	Humanized IgG
CDX-1307	Celldex/Medarex	Phase I	Mannose receptor; hCGβ	Oncology	Human IgG
Elotuzumab (HuLuc 63)	PDL/BMS	Phase I	CS1 surface antigen	Myeloma	IgG
BMS-663513	BMS	Phase I/II	CD137 agonist	Oncology	Human IgG
anti-ALK1 Ab	Pfizer	Phase I	ALK1	Solid tumors	IgG
anti-P-cadherin Ab	Pfizer	Phase I	P-cadherin	Solid tumors	IgG
MT-293	Micromet/Tracon Pharma	Phase I	Cleaved collagen	Oncology	IgG
BIIB-015	Biogen/IDEC	Phase I	Cripto	Solid tumors	IgG
Mostly Nonimmunology/Nononcology Mabs					
Stamulumab (MYO-029)	Wyeth	Phase II	Myostatin (GDF8)	Muscular dystrophy; sarcopenia	IgG
AMG-745	Amgen	Phase I	Myostatin	Muscle loss	IgG
CAL	Chugai/Roche	Phase II	PTHrP	Hypercalcemia, bone metastases	IgG
NPVBHQ-880	Norvartis	Phase I	DKK-1	Osteoporosis	IgG
AMG-785	Amgen/UCB	Phase I	Sclerostin	Osteoporosis	IgG
LY-2062430	Eli Lilly	Phase II	Amyloid-β	Alzheimer disease	IgG

(*Continued*)

TABLE 1.4 *Continued*

U.S. Trade Name (Generic Name)	Company	Current Status (U.S.)	Molecular Target	Major Indication	Protein Format
PF-4260365	Pfizer	Phase II	Amyloid-β	Alzheimer disease	IgG
Gantenerumab (R-1450)	Roche/MorphoSys	Phase I	Amyloid-β	Alzheimer disease	Human IgG
TTP-4000	Pfizer	Phase I	Amyloid-β	Alzheimer disease	RAGE Fc fusion protein
anti-Nogo Ab	Norvartis	Phase I	NOGO	Spinal cord injury	IgG
Tanezumab (RI-624)	Pfizer	Phase II	NGF	Pain	IgG
AMG-403	Johnson & Johnson/ Amgen	Phase I	NGF	Pain	IgG
Bertilimumab (iCo-008, CAT-213)	iCo Therpaeutics	Phase II	Eotaxin (CCL11)	Vernal keratoconjunctivitis	Human IgG4 (CAT Library)
CVX-096	Pfizer	Phase I	GLP-1R	Diabetes	Peptide mimetic-IgG conjugate
AMG-477	Amgen	Phase I	Glucagon receptor	Type 2 diabetes	IgG
TGFβ Ab	Eli Lilly	Phase I	TGFβ Ab	Diabetic nephropathy	IgG
R-7025	Roche	Phase I	HCV	Antiviral therapy	Therapeutic protein
Ibalizumab (TNX-355; Hu5A)	Genentech (Tanox)	Phase II	CD4	HIV	Humanized IgG1
Pagibaximab (BSYX-A110)	Biosynexis/ Medimmune	Phase II	Staphylococcus lipoteichoic acid	Staphylococcus infections	Chimeric IgG1
Aurexis (Tefibazumab)	Inhibitex	Phase II	Staphylococcus clumping factor A	Staphylococcus infections	Humanized IgG1
MDX-066 (CDA-1)+ MDX-1388 (CDA-2)	Medarex/MBL	Phase II	*Clostridium difficile* toxins A and B	*C. difficile*-associated diarrhea (CDAD)	Human IgGs
MEDI-557	Astra-Zeneca	Phase I	F-protein on RSV	RSV	YTE mutant—longer half-life Mab
Valortim™ (MDX-1303)	Pharm Athene/ Medarex	Phase I	*Bacillus anthracis* PA toxin	Anthrax—biodefense	Human IgG
CytoFab™	Astra-Zeneca	Phase II	TNF-α	Severe sepsis	IgG

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; GVHD, graft-versus-host disease; IBD, irritable bowel disease; IPF, idiopathic pulmonary fibrosis; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; RA, rheumatoid arthritis; RCC, renal cell carcinoma; RSV, respiratory syncytial virus; SCLC, small cell lung cancer; SLE, systemic lupus erythematosus.

*Data as of August 2008; data obtained from company websites, Prous Science Integrity, and www.clinicaltrials.gov.

(these represent the majority, but likely not all, early phase commercially funded clinical candidates), 72 (50 from current Phase II candidates and another 22 from the current Phase I candidates) should eventually reach Phase III. Based on the 75 percent POS for transition from Phase III to marketing approval (KMR Group, Inc. 2007), this could result approximately in an additional 54 Mabs and Fc fusion proteins on the market between 2013 and 2018 (Fig. 1.3).

Taking into account the currently marketed Mabs and Fc fusion proteins (Table 1.2, Fig. 1.3), as well as the POS-adjusted clinical candidates listed in Table 1.3 (late clinical phase) and Table 1.4 (early clinical phase), there could potentially be a total of 135 Mabs and Fc fusion proteins on the U.S. market by the 2018 time frame, a decade from now. This number is approximately five times the current 27 Mabs and Fc fusion products on the market today, most of which have been marketed in the 10 year period of 1997–2007. These calculations, while necessarily forward projecting, suggest that there will be significant expansion of Mab and Fc fusion products reaching the market over the next decade, which could have a profound and lasting impact on the pharmaceutical industry in general. This could be especially noticeable if the overall POS for Mabs remains in the 18 to 20 percent range, as compared with the historical POS for small molecules, at about 7 to 8 percent. Thus, by 2018, a decade from now, therapeutic proteins in general and, more specifically, Mabs and Fc fusion proteins, should comprise a significant fraction of worldwide pharmaceutical revenues, considerably higher than the 20 percent fraction that biologics make up today.

In a sampling of the more than 1500 clinical trials testing Mabs today (www.clinicaltrials.gov), approximately 45 percent of these represent oncology studies using "naked" antibodies (i.e., no toxin- or radioconjugate attached). Another 31 percent, many funded by the National Cancer Institue (NCI) and/or academic groups, are being conducted using radioconjugated Mabs to kill tumors, and 2 percent represent toxin-conjugates for oncology. Thus, 78 percent of all current clinical trials on Mabs are focused on the therapeutic area of oncology. Fourteen percent are focused on immunology-related indications, and the final eight percent on non-oncology, non-immunology-related indications. These clinical trials data are somewhat skewed, however, by the large number of academic- and government-funded Phase I clinical trials focused on testing radioconjugated monoclonal antibodies for oncology indications. In another view of the therapeutic area breakdown, of the more than 200 combined clinical candidate Mabs and Fc fusion proteins listed in Tables 1.3 and 1.4, approximately 50 percent are either used for, or are being tested primarily for, oncology indications, 32 percent for immunology-related indications, and about 18 percent for non-oncology, non-immunology indications. This final category includes a wide range of indications, including, for examples, atherosclerosis, diabetes, infectious diseases, bone loss, muscle wasting and dystrophy, and other assorted indications.

1.2 HISTORICAL ASPECTS

It now has been approximately a third of a century since Köhler and Milstein described methods for producing murine hybridomas, in what is accepted by most as the dawn of the era of therapeutic monoclonal antibodies (Köhler and Milstein 1975). After Mab hybridoma technology was first described in 1975, it took 11 years, until 1986, before the first commercial therapeutic antibody, Orthoclone OKT3® (muronomab-CD3), was licensed by Ortho Biotech, a subsidiary of Johnson & Johnson, for inhibition of transplanted organ rejection. It was yet another eight years before the second antibody, the chimeric Fab antibody, ReoPro® (abciximab), was developed by Centocor (now a subsidiary of Johnson & Johnson), and marketed by Eli Lilly to inhibit platelet aggregation post-cardiovascular surgery. Thus, even two decades after the seminal paper was published on monoclonal antibodies, only two monoclonal antibody products had been brought to the market. This changed dramatically in 1997–1998, when a total of five monoclonal antibody drugs were introduced to the market (Table 1.2, Fig. 1.3), generating considerable interest in the field of therapeutic monoclonal antibodies. How did we get from discovery to market, and why did it take so long? In the next section, the history leading up to the current status of the monoclonal antibody field will be addressed.

1.2.1 Historical Aspects: Origins of Serum Therapy, Forerunner to the Monoclonal Antibody Business

The first concept of using antibodies as therapeutics came long before the generation of hybridomas, as shown in Figure 1.4. It started when Robert Koch, discoverer of the tubercle bacillus and 1905 Nobel Laureate, was named director of the Institute of Hygiene in Berlin in 1885. There he assembled a team of the brightest minds in the newly forming field of immunotherapeutics, including Paul Ehrlich (known for the "magic bullet" hypothesis; 1908 Nobel Laureate), Emil von Behring (father of immunotherapy; 1901 Nobel Laureate), Erich Wernicke, and Shibasaburo Kitasato (eventual founder of Japan's famed Kitasato Institute), all of whom would have a significant impact on the beginnings of antibody-based therapy (Winau, Westphal, and Winau 2004). Working initially on iodoform chemotherapeutics, Behring made several key observations that led to the concept of *Blutserumtherapie*, or serum therapy. He noticed that the blood of those rats resistant to anthrax was able to kill the anthrax bacterium (Chung n.d.), and together with his friend Wernicke, he developed the first working serum therapy for diphtheria. Behring and Kitasato, a student of Koch's who had isolated the tetanus-forming bacillus and had determined that its pathogenesis lay in the activity of its toxin, together demonstrated that the transfer of serum from a guinea pig immunized with diphtheria toxin to another guinea pig offered protection from the toxin (Behring and Kitasato 1890). Behring and Kitasato also obtained anti-sera against tetanus toxin, demonstrating the breadth of the principle.

Behring's diphtheria serum therapy was first tested clinically in 1891 at Charite' Hospital in Berlin. A year later, Behring began working with the pharmaceutical manufacturer Faberwerke Hoechst to develop the diphtheria serum treatment. In 1894, Hoechst launched the first immunobiological therapeutic, dispatching the first 25,000 doses of anti-diphtheria serum to fight the diphtheria epidemic that was claiming the lives of 50,000 children annually in Germany alone (Fig. 1.4). The serum therapy was

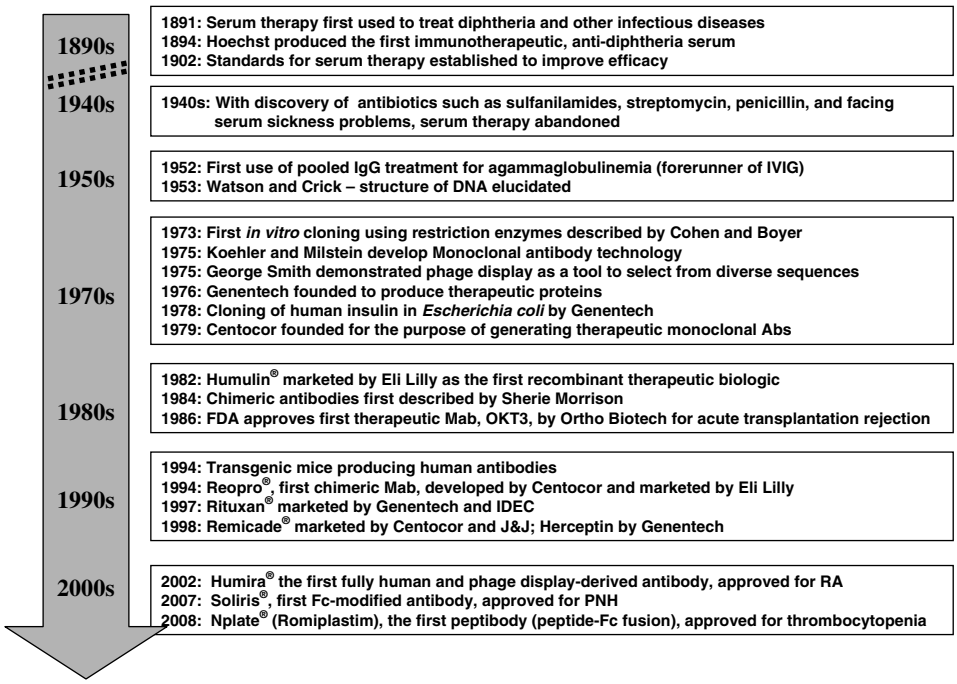


Figure 1.4 Timeline for important discoveries leading to therapeutic Mabs and Fc fusion proteins.

not without detractors—several scientists and politicians across Europe criticized, and some even poked fun at, the serum-based therapeutic approach (Winau and Winau 2002). However, with introduction of the serum therapy, mortality in Paris dropped from 52 percent to 25 percent (Llewelyn, Hawkins, and Russell 1992), silencing most doubters. The discovery of serum therapy led to the awarding of the first Nobel Prize in Medicine and Physiology to Behring in 1901. In what was likely the first example of venture capital funding in biotechnology, Behring used the funds from his Nobel Prize to seed a new company in 1904, which still exists today as Novartis (Chiron) Behring, a vaccine manufacturer, located on Emil Von Behring Strasse in Marburg (Winau and Winau 2002).

The serum used for serum therapy was crude and from immunized, nonhuman (heterologous) sources (e.g., rabbits, horses), containing many foreign proteins as well as the antibodies, and gave rise to a phenomenon that has been generally called "serum sickness" (Gronski, Seiler, and Schwick 1991; Lawley et al. 1984). The natural onset of serum sickness in virtually 100 percent of patients treated with serum therapy led to a variety of efforts to improve on the therapeutic approach. Behring even came to realize that the toxic side effects of serum therapy were interrelated with the efficacy of the preparation (Gronski, Seiler, and Schwick 1991). He tried many methods of purifying the serum, without substantial success. At about the same time, Paul Ehrlich recognized the need for standardization of serum therapies, which led to the development of methods for quantitation of the serum therapeutic effect, including the concept of LD50 (dilution of serum preventing death of 50 percent of animals treated), still used today. In 1908, Ehrlich was awarded the Nobel Prize (shared with Ilya Metchnikoff, who discovered the basis of phagocytosis), for his characterization and standardization of the anti-serum therapies. Others worked on the concept of protease-treated sera, with the notion of deriving a formulation that would remain efficacious but would lose the side effects caused by the serum itself. The use of proteases to purify heterologous (nonhuman origin) immune sera (dubbed *fermo-sera*) was not perfected until the late 1930s (Weil, Pafentjiev, and Bowman 1938), but these preparations still resulted in serum sickness and also were prone to sensitizing and anaphylactic reactions.

Besides the obvious issues with serum sickness, other significant problems besetting serum therapy included lack of batch-to-batch consistency, difficult administration, and variable pharmacokinetics (Casadevall 1996). Nevertheless, heterologous serum therapy was used widely until approximately the onset of World War II for a variety of diseases, including the bacterial diseases: diphtheria, streptococcal pneumonia, meningitis, tularemia, shigella dysentery, brucellosis, gas gangrene, tetanus, botulism, anthrax, whooping cough; and the viral diseases: measles, poliomyelitis, mumps, and chickenpox (Casadevall and Scharff 1995). Some of these treatments, for example, for diphtheria, meningitis, and pneumonia, proved to be fairly successful, whereas others, for example, anthrax, whooping cough, and shigella dysentery, were apparently less so (Casadevall and Scharff 1995). An example for how widespread serum therapy was used is that 86 percent of patients diagnosed with type I streptococcal pneumonia in the late 1930s at Boston City Hospital were treated with a type-specific serum therapy (Casadevall and Scharff 1995).

With the discovery of sulfonamides in the mid-1930s, and later penicillin, streptomycin and other natural product antibiotics (many of which were broad spectrum), the practice of passive immunization using heterologous serum declined precipitously. The combination of serum sickness, lack of consistency, narrow spectrum of use, unknown pharmacokinetics, and intravenous administration made heterologous serum therapy largely noncompetitive with the, then, newly found chemotherapeutics (Casadevall 1996). The exception to this paradigm is the third world, in which health care is substantially different from that in Western nations or nations with large, robust economies. In many countries in which antimicrobial chemotherapeutics are not readily available, serum-based therapy still plays an important role in overall health care (Wilde et al. 1996).

1.2.2 IVIG Therapeutics and Prophylactics

Later, the concept of serum therapy was modified by isolating natural antibodies in either vaccinated (or convalescing, called "specific immunoglobulins") or "naïve" humans, followed by isolation of

the IgG fraction from the pool sera for therapeutic use. The first reported use of fractionated IgG as a therapeutic agent was in 1952, in which a patient with primary immunodeficiency was treated with intramuscular (IM) injections of purified human IgG (Bruton 1952; Fig. 1.4). IM delivery resulted in limited dosing regimens, marginal IgG replenishment, and thus marginal clinical benefit. Early attempts at intravenous delivery of human IgG fractions (IVIG or, in the United States, IGIV), however, resulted in immunological reactions thought at the time to be due to activation of complement (Weiler 2004). Later methods for producing IVIG were able to remove the cause for this immune reaction, allowing for transfusions to take place. Thus, widespread use of IVIG to treat primary antibody deficiencies did not occur until the early 1980s (Mouthon and Lortholary 2003).

There are two types of IVIG, specific immunoglobulins and normal immunoglobulins. Specific immunoglobulins are obtained from convalescent donors or from healthy volunteers specifically vaccinated to provide the antibodies (as in the case of anti-rhesus D antigen) (Llewelyn, Hawkins, and Russell 1992). Another example of a specific immunoglobulin is the passive administration of human anti-rabies IgG to patients not previously vaccinated against rabies. The huIgG, generated by hyperimmunized human donors, provides virus-neutralizing antibodies immediately to bridge the gap until the patient produces his or her own antibodies in response to concomitant vaccine administration. Two anti-rabies IgG formulations are licensed for use in the United States: Imogam® Rabies-HT (Sanofi-Pasteur) and HyperRab™ S/D (Talecris Biotherapeutics).

The term *normal immunoglobulin* has been used for IgG pools obtained from a large number of random donors. These antibodies generally provide four to six weeks of protection against pathogens that are relatively widespread in populations, including hepatitis A, measles, mumps, and other viral diseases (Llewelyn, Hawkins, and Russell 1992). This approach, generally known as IVIG or gamma globulin treatment, was initially used as replacement therapy for patients unable to generate their own immunoglobulins (Orange et al. 2006). As of 2006, the U.S. Food and Drug Administration (FDA) had approved 11 products for primary immunodeficiency or humoral immunodeficiency, another 5 for idiopathic thrombocytopenic purpurea, 3 for Kawasaki syndrome, 2 for B-cell chronic lymphocytic leukemia, and one each for HIV infection and bone marrow transplantation (Weiler 2004; Orange et al. 2006). Doses typically are in the range of 300 to 600 mg/kg on a monthly or biweekly basis (Orange et al. 2006). It is noteworthy as well as ironic that the approved use for IVIG in the United States for various infectious diseases is severely limited, even though the conceptual origins of IVIG use sprang directly from Berhing's work on serum therapy for infectious diseases. Several new uses for IVIG have been proposed recently, including the expansion of use for infectious diseases (Wallington 2004; Casadevall and Scharff 1995) and protection from potential biological warfare agents (Casadevall 2002).

Significantly, the IVIG approach led directly to the development of one of the early significant licensed monoclonal antibodies, Synagis®. Medimmune first developed an IVIG prophylactic, Respigam®, which was licensed in 1996 to protect infants from respiratory syncytial virus (RSV). While pushing forward with their development of Respigam®, MedImmune already had begun clinical trial development of Synagis® as early as 1994, understanding that a monoclonal antibody would be both preferable over IVIG and ultimately more profitable. Synagis®, a human-mouse chimeric IgG1 targeting a key epitope of the A-antigenic site of RSV F-protein, was then licensed in 1998, essentially replacing Respigam® as the primary anti-RSV prophylactic for premature infants.

The concept of IVIG also has led to an approach similar to IVIG, but yet significantly more refined and sophisticated, as developed by the biotech company, Symphogen. The scientists at Symphogen isolate multiple antibodies directed against a single target or target entity (a virus in the case of antiviral, or a cell for antibacterial or antitumor) and then produce the multiple Mabs in a single pot cell culture based on a mixed inoculum from individual master cell banks (Rasmussen et al., 2007). This concept apparently is meant to simulate the natural mechanism the body uses to defeat a foreign antigen or invader, while lacking the huge volume of nonspecific antibodies that would be present in an IVIG type of preparation. Symphogen is currently in Phase I with an anti-RhD product candidate containing 25 different Mabs (Wilberg et al. 2006).

1.3 TECHNOLOGIES LEADING TO THE CURRENT MONOCLONAL ANTIBODY ENGINEERING ENVIRONMENT

1.3.1 Fundamental Breakthroughs Allowing for Recombinant Monoclonal Antibodies

The 1970s and 1980s proved to be an incubator period that spawned the dawn of the biologics revolution (Fig. 1.4). A series of technologies were developed in this time period that ultimately converged to provide all of the technological and fundamental bases for development of the therapeutic monoclonal antibody industry. These technologies include the use of restriction enzymes to clone a gene into a plasmid (Cohen et al. 1973), development of hybridoma technology by Köhler and Milstein (1975), site-directed mutagenesis as a tool for protein engineering (Hutchinson et al. 1978; Zoller and Smith 1982; Dalbadie-McFarland et al. 1982), and development of an understanding of the genetics of antibody expression [Hozumi and Tonegawa 1976; Early et al. 1980; Gough and Bernard 1981; Tonegawa 1983; also, the debate concerning germline versus somatic mutation as the basis for diversity was laid out nicely by Silverstein (2003)]. Additional technologies and scientific knowledge leading to the development of recombinant antibodies were added in the 1980s, including phage display technology (Smith 1985), polymerase chain reaction (PCR; Mullis et al. 1986; Saiki et al. 1988), sequencing and characterization of human germline antibody genes (Kabat et al. 1987), and expression of antibody genes in cell cultures (Neuberger 1983; Neuberger and Williams 1986) and in *Escherichia coli* (Better et al. 1988; Skerra and Plückthun 1988). In the next few sections, the fundamental breakthroughs in antibody engineering, built on the shoulders of the technologies mentioned above, are described.

The commercial path for therapeutic monoclonal antibodies was paved by Genentech and Eli Lilly, who teamed up to produce the first recombinant human protein, the human insulin product Humulin®, approved on October 30, 1982 for marketing in the United States. Leading up to this achievement, Genentech had produced somatostatin, the first recombinant human protein from a chemically synthesized gene, in *E. coli* (Itakura et al. 1977). Shortly thereafter, scientists at Genentech used the same approach to clone out the human insulin gene for expression in *E. coli* (Goeddel et al. 1979b). Genentech then licensed the recombinant human insulin to Eli Lilly, who developed it clinically and obtained marketing approval for the first recombinant human protein, Humulin®, in 1982 (Fig. 1.4). Prior to this seminal achievement, diabetic patients had been limited to taking Iletin®, a heterologous insulin product purified from the pancreas of animals (mostly pigs and cows), since 1923 when Eli Lilly had developed the first commercial process for its production (Shook 2007). Genentech scientists then went on to clone and express human growth hormone in *E. coli* (Goeddel et al. 1979a), which in 1985 became their first internally marketed product, Protropin®.

1.3.2 Hybridoma Technology

In the early 1970s, Georges Köhler was having difficulty finding a way to obtain antibodies from mortal B-cells in culture. Caesar Milstein and his colleagues, on the other hand, had worked out how to transform myeloma cell lines and generate myeloma-myeloma fusions to secrete antibodies (Milstein 1985). These myeloma fusions, however, produced antibodies lacking specificity (Alkan 2004). Another key piece to the puzzle was a critically important hemolytic plaque assay developed by Jerne, which allows direct visualization of antibody-producing B-cells (Jerne and Nordin 1963). Köhler joined Milstein's lab as a postdoctoral fellow in 1973, where the two joined forces to generate B-cell-myeloma fusions that secreted single (i.e., monoclonal) antibodies that recognized a specific antigen (Köhler and Milstein 1975), as visualized using Jerne's plaque assay (Alkan 2004). This discovery led to the awarding of the Nobel Prize in Physiology or Medicine in 1984 to Milstein, Köhler, and Jerne. As has been discussed on many occasions, Köhler and Milstein did not patent their discovery, which opened up the use of their hybridoma technology to academics and industry alike for generation of future potential therapeutic monoclonal antibodies.

1.3.3 Transfectomas and Chimeric Antibodies

The leap from the use of purely murine antibodies from hybridomas, as originally described by Köhler and Milstein (1975) and developed into an early industrial process by Ortho Biotech for licensure of Orthoclone OKT3® in 1984 (Table 1.2), to recombinant antibodies with human Fc domains that could be developed more fully and used more widely came with three significant developments, as described below.

The first development was the ability to clone out, using PCR methodology, the murine VH and VL genes for recombinant expression (Orlandi et al. 1989). The second requirement was to express both heavy and light chain antibody genes in stable human cell lines after transfection (originally called *transfectomas*; Neuberger 1983; Neuberger and Williams 1986; Beidler et al. 1988). Coupled with that was the third development, which was the method of making chimeric antibodies possessing murine VH and VL chains fused with human constant regions (Morrison et al. 1984; Boulianne, Hozumi, and Shulman 1984). Chimeric antibodies possess about one-third murine sequences (2VH and 2VL subunits) and two-thirds human sequences, including a human Fc. The first descriptions for the construction of chimeric antibodies occurred in 1984 (Fig. 1.4).

Vectors such as pSV2 and murine myeloma cell lines such as SP2/0 were popular early on (Shin and Morrison 1989). CHO-dg44-DHFR was the expression system of choice for several years, but in more recent years, Chinese hamster ovary (CHO) cell lines, and in particular the glutamine synthetase (GS) expression system coupled with the cell line CHO-K1SV (de la Cruz Edmonds et al. 2006), as widely licensed by Lonza, has seen more widespread use. Using the GS-CHO expression system, current levels of production routinely hit 1 g/L, with ranges of 3 to 5 g/L antibody production often achieved after cell cloning and process optimization (Kalwy, Rance, and Young 2006; personal communications with several bioprocess colleagues in the industry).

The first chimeric antibody to be marketed was ReoPro®, which was made chimeric and then cleaved to a Fab and purified to make the drug. The first chimeric IgG was Rituxan®, which is still a strong product with worldwide sales of more than $3 billion annually (Fig. 1.2). There are a total of six chimeric antibodies on the market, with an additional two in Phase III clinical trials (Fig. 1.5). With the development of humanization technologies and the concomitant reduction in immunogenicity that humanization brings, it is likely that there will be very few additional chimeric antibodies brought through clinical trials these days, as indicated by the trends shown in Figure 1.5.

1.3.4 Humanization Technology

Chimeric antibodies, as described earlier in this chapter, still retain 30 to 35 percent murine sequence, which may lead to enhanced immunogenicity (Pendley, Schantz, and Wagner 2003; Hwang and Foote 2005; Almagro and Strohl, Chapter 13 in this volume). Humanization, the idea of making the V-chains from a murine or other mammalian antibody "more human," was first described in 1986 by Winter and colleagues (Jones et al. 1986). They grafted the complementary determining regions (CDRs) from a murine antibody into the most closely related human framework, followed by making amino acid changes required to stabilize the engineered constructs. Queen and colleagues (Queen et al. 1989; Co and Queen 1991; Ostberg and Queen 1995) at Protein Design Labs (now PDL BioPharma) developed a detailed process for humanizing antibodies via CDR grafting, which has been the basis for humanization of many of the antibodies currently on the market or in advanced clinical trials. Most of the humanized antibodies on the market, or currently in development, have been humanized by some form of CDR grafting. Other forms of humanization that do not include CDR grafting also have been developed, however, most notably resurfacing (also called veneering) of antibodies to remove B-cell epitopes (Roguska et al. 1994, 1996; Staelens et al. 2006).

The first humanized antibody to reach the marketplace was Zenapax®, an anti-CD25 (IL-2 alpha subunit) Mab which was humanized and developed at Protein Design Labs and licensed by Roche, to combat transplant rejection. As can be seen in Figure 1.5 and in Tables 1.3 and 1.4, humanization

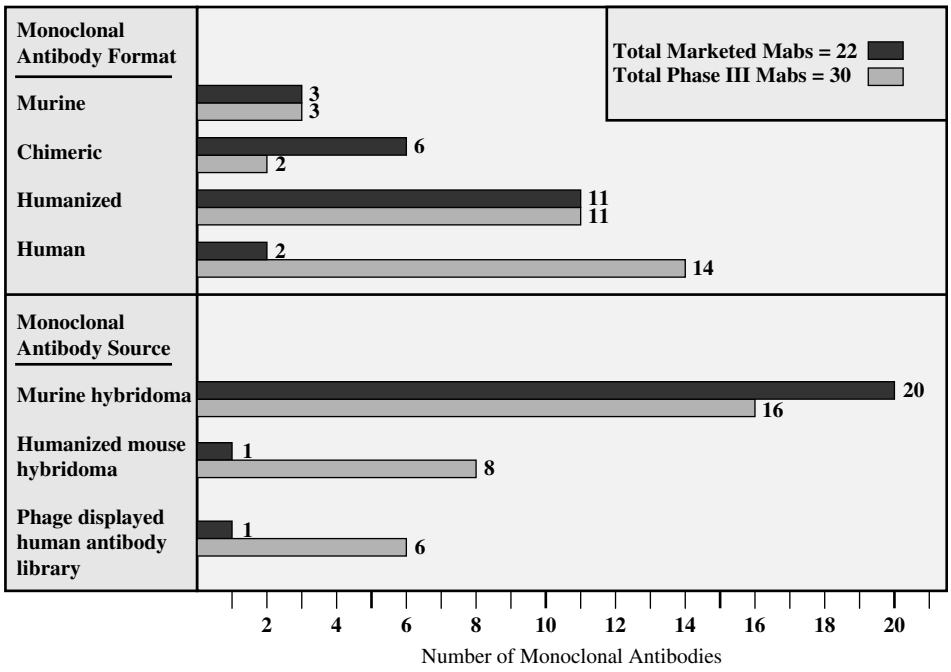


Figure 1.5 Format and primary source of current commercial Mabs and Phase III clinical candidates.

of murine hybridoma-derived monoclonal antibodies remains a major source of therapeutic candidates in the biopharm industry pipeline.

1.3.5 Humanized Mice

It became apparent after Orthoclone OKT3® was marketed that murine antibodies were not going to be acceptable as mainstream therapeutics, especially for chronic indications. As mentioned previously, *in vitro* manipulations were being used to generate chimeric and humanized monoclonal antibodies, and even libraries of phage-displayed human antibodies. Alt, Blackwell, and Yancopoulos (1985) suggested a different possibility altogether, that is, the generation of transgenic mice producing human antibodies ("humanized mice"). Subsequently, two groups separately and independently were successful in developing approaches to generate functional human antibodies directly from transgenic mice. Scientists at Cell Genesys (which later spun off Abgenix, which was acquired by Amgen in 2007; Table 1.5) and GenPharm (acquired in 1997 by Medarex) each engineered mice by disabling the ability of the mice to produce their own murine antibodies, and replacing that function with human antibody genes (Lonberg et al. 1994; Green et al. 1994). Thus, with both systems, immunization of the resultant transgenic "humanized" mice would result in the generation of fully human antibodies by those mice against the antigen (reviewed by Green 1999; Lonberg 2005). The first fully human antibody to be developed and marketed from one of these humanized mouse systems was Vectibix®, a human IgG2 antibody discovered using the Abgenix XenoMouse™ technology, in 2006, 12 years after the publication of the key paper demonstrating the construction of the mice (Fig. 1.6). Fully human antibodies derived from humanized mice make up 27 percent of the current Phase III candidates (Table 1.3, Fig. 1.5), and are expected to continue to feed the pipeline. Amgen, for example, has more than a dozen fully human antibodies in clinical trials (Tables 1.2 and 1.3) derived from the Abgenix mouse platform, which likely led to their acquisition of Abgenix in 2005 (Table 1.5).

TABLE 1.5 Significant Acquisitions in the Therapeutic Monoclonal Antibody Space*

Acquirer	Acquired	Date	Apparent Driver(s) for Acquisition
Amgen	Immunex	2002	Additional rights to Enbrel
	Abgenix	2005	Vectibix® (anti-EGFR); transgenic humanized mouse technology
	Avidia	2006	Anti-IL6 avimer; avimer technology
Astra-Zeneca	Cambridge Antibody Technology	2006	CAT354 (anti-IL-13) and CAT-3888 (anti-CD22-Ps. immunotoxin fusion); phage displayed human antibody libraries
	Medimmune	2007	Synagis®, Numax®, extended pipeline
Bristol-Meyers Squibb	Adnexus	2007	Adnectin (alternative scaffold) technology; discovery engine
Eli Lilly	Applied Molecular Evolution (AME)	2004	AME-133 (second generation anti-CD20), AME-527 (second generation anti-TNF-α), and discovery engine
Genentech	Tanox	2006	Additional rights to Xolair
Glaxo Smith-Kline	Domantis	2006	Domain antibodies; discovery engine
Johnson & Johnson	Centocor	1999	Remicade® antibody discovery and development capabilities
	Egea	2004	Advanced protein optimization technology
Merck	GlycoFi	2006	*Pichia*-based expression and glycosylation technology
	Abmaxis	2006	Antibody structure-based optimization technology
Novartis	Chiron	2006	Vaccines, but also antibody discovery and early development experience
	Neutec	2006	Aurograb® (antistaphylococcal Mab) and Mycograb® (antifungal Mab)
Pfizer	Bioren	2005	Antibody optimization technology
	Rinat	2006	RN624 (anti-NGF Mab), RN219 (anti-Aβ mab), pipeline, discovery engine
	Biorexis	2007	GLP-1 lead; transferring-fusion protein technology
	Coley	2007	Vaccines; TLR technology
	CovX	2007	CVX-045 (thrombospondin 1 mimetic); CVX-60 (angiopoietin-2 binder); CVX-096 (GLP-1 mimetic); peptide-Mab conjugation technology
Roche	Genentech	1990	Biologics capabilities
	Chugai	2001	Expansion of biologics capabilities and market in Japan
	Therapeutic Human Polyclonals	2007	Polyclonal antibody technology
	Glycart	2007	Afucosyl glycosylation and cell culture technology
Schering-Plough	DNAX	1982	Fundamental biology expertise, especially in cytokines
	Canji	1996	Gene therapy
	Organon	2007	Biologics manufacturing
Wyeth	Haptogen	2007	Shark antibodies; discovery engine

*Data as of August 2008; data obtained from company websites and news releases.

1.3.6 Phage Display Technology

George Smith (1985) demonstrated that peptides could be displayed as fusions of P3 on the tail fibers of the *Escherichia coli* filamentous phage M13. Shortly afterwards, it was realized that this method of display could be used more broadly, including the display of proteins (Markland et al. 1991). It became apparent that phage display would make a great tool for selection of mutants for protein engineering (Bass, Greene, and Wells 1990; Lowman et al. 1991; Markland et al. 1991). Additionally, a phagemid system was described which allowed for monovalent display, which helped considerably in selections,

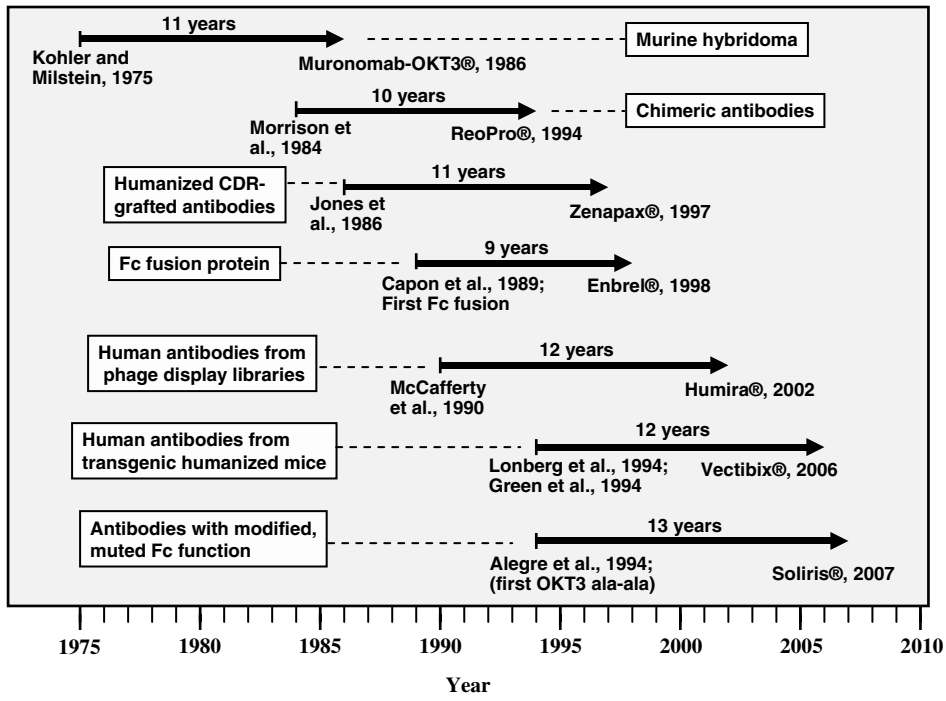


Figure 1.6 Timelines for maturation of technologies to reach the market in the form of a therapeutic Mab or Fc fusion protein. Given is the year of discovery or first publication and the year the technology resulted in the first therapeutic using that technology. Adapted, updated, and modified from Lonberg (2005).

especially for high affinity binders rather than high avidity binders (Barbas et al. 1991). Since antibodies function via binding ligands, M13 P3-based phage display technology became an optimal methodology for selecting modified antibody fragments capable of increased binding capabilities (Gram et al. 1992; Hawkins, Russell, and Winter 1992; Marks et al. 1992a,b).

1.3.7 Human Antibody Libraries

McCafferty et al. (1990) first demonstrated the potential for bypassing immunization altogether by building a library of antibody genes, displayed on the P3 protein of M13 phage, using PCR methodology to recover the human genes from either B-cells or hybridomas. This was followed by the construction of large, human libraries from either synthetic repertoires (Barbas et al. 1992; Griffiths et al. 1994) or from multiple "naïve" human donors (Marks et al. 1991; Vaughan et al. 1996). The latter library from Cambridge Antibody Technology (CaT, now part of MedImmune, a wholly owned subsidiary of Astra-Zeneca; Table 1.5), constructed using single chain Fv constructs fused with His and myc tags, produced sub-nM binders and became the prototype for many later libraries. Since then, there have been many fully human antibody libraries displayed on M13 P3 using either Fab or scFv formats, including (among others), the following examples: a synthetic library used by Crucell (de Kruif et al., 1995), synthetic libraries by MorphoSys (Knappik et al. 2000; Rothe et al. 2008), a large Fab-based library built by scientists at Dyax (Hoet et al. 2005), and a minimalist library generated at Genentech based on a single, well-characterized framework (Fellouse et al. 2007). A wide variety of strategies for building either large libraries or more focused libraries have been recently published and have been reviewed by Hoogenboom (2005), Sidhu and Fellouse (2006), and Mondon et al. (2008). The first antibody from a phage-displayed human antibody library to be approved for therapeutic

use is Humira®, which was approved in 2002 (Table 1.2). Interestingly enough, Humira® was not isolated *de novo* from the CaT human antibody library, but instead was isolated via a "guided selection" strategy, using a murine antibody as the primary binder, as has been described by Osbourn, Groves, and Vaughan (2005), and reviewed in Chapter 13 in this book by Almagro and Strohl.

1.3.8 Summary of Core Therapeutic Mab Technologies Leading to Therapeutics

In his 2005 review on human antibodies from transgenic animals, Nils Lonberg (2005) showed timelines from first reports on technologies, such as chimerization, to approval of the first antibody to utilize that technology. Figure 1.6 shows a similar set of timelines, adapted and updated from Lonberg's paper. The first monoclonal antibody, Orthoclone OKT3® was approved in 1986, 11 years after Köhler and Milstein first described the generation of monoclonal antibodies. The first chimeric antibody, ReoPro®, was approved in 1994, 10 years after the first papers on chimeric antibodies (Morrison et al. 1984; Boulianne, Hozumi, and Shulman 1984). The first humanized antibody, Zenapax®, was approved in 1997, 11 years after the first report on CDR grafting by Jones et al. (1986), and the first antibody from a phage-displayed human antibody library, Humira®, was approved in 2002, 12 years after the initial paper by McCafferty et al. (1990) describing the construction of phage-displayed human antibody libraries. Similarly, the first antibody from a transgenic humanized mouse, Vectibix®, was approved in 2006, 12 years after the first descriptions of the generation of functional transgenic humanized mice producing human antibodies (Lonberg et al. 1994; Green et al. 1994). In an antibody-related technology development, Capon et al. (1989) described the first Fc fusion proteins (then termed *immunoadhesin*) using the Fc portion of IgG attached to the exodomain of CD4. Enbrel®, the first Fc fusion protein to be marketed, was approved in 1998 (Table 1.2), nine years later (Fig. 1.6). Finally, in 2007, Soliris® became the first antibody with a modified, or nonnatural IgG (the Fc of Soliris® is an IgG2-4 chimera), to be approved for marketing in the United States (Rother et al. 2007). This antibody, which has significantly decreased ability to bind Fcγ receptors, and thus substantially diminished immunological activity, was approved 13 years after Alegre et al. (1994) described the generation and characterization of the ala-ala mutations of a humanized OKT3, the first detailed description of an Fc-muted antibody.

In summary, it has taken approximately 9 to 13 years for each of these key technologies to result in an FDA-approved therapeutic monoclonal antibody or Fc fusion protein. Considering that discovery, preclinical development, preclinical toxicology studies, clinical trials, and registration of a new molecular entity often take from 8 to 10 years, it is apparent that these technologies were taken up rapidly by the industry and incorporated into the pipeline without significant delay.

1.4 FROM BIOTECHNOLOGY TO BIOPHARMA

1.4.1 From OKT3 to Remicade: Early Successes and Disappointments

The first therapeutic antibody, Orthoclone OKT3® (muronomab CD3), from Ortho Biotech, a subsidiary of Johnson & Johnson, was approved by the U.S. FDA for use in transplantation in June 1986. The original OKT3 monoclonal antibody was isolated in 1979 as a T-cell recognizing antibody (Kung et al. 1979). It took a total of nine years from the discovery of hybridoma technology to put Orthoclone OKT3® on the market, which is remarkably short, considering the time required for development of the technology at Ortho Biotech, use of the technology to target T-cell binding antibodies, understanding of the biology enough to determine a therapeutic indication, and then adding the time required for preclinical toxicology, clinical development, and registration (Fig. 1.6).

While the marketing of Orthoclone OKT3® was a huge breakthrough for the biotechnology industry as a whole, it also came with a serious reality check for two significant issues that have laid the foundation for modern therapeutic monoclonal antibodies. Muronomab-CD3®, a fully murine antibody of the IgG2a murine isotype, was found to be highly immunogenic in people (Kimball et al. 1995), which,

given its murine nature, is hardly surprising. Muronomab-CD3® also generated a cytokine storm known as systemic cytokine release syndrome (Chatenoud et al. 1990), shown to be via the interaction of its murine IgG2a Fc with FcγRs on human immune effector cells (Tax et al. 1984; Lobo and Patel 1997). This led to several important studies concerning the nature of Fc-based functionality in therapeutic monoclonal antibodies (as exemplified by Brüggeman et al. 1987; Xu et al. 2000). Ultimately, this has led to the generation of three different humanized versions of anti-CD3 (Teplizumab [hOKT3γ1ala-ala], Visilizumab, and Otelixizumab [ChAglyCD3; TRX4]), all of which lack significant Fc functionality; these antibodies are all currently in clinical trials.

In part because Orthoclone OKT3® caused these significant adverse events, in part because the biotechnology field was rapidly changing with new technologies being proposed and developed, in part because the discovery and development timelines for drugs are so long (averaging approximately 8 to 10 years from discovery to market), and in part because of a devastating clinical failure, the next therapeutic Mab to be approved in the United States was not until 1994, eight years later. This next market entry was ReoPro®, an anti-gpIIb/IIIa murine-human chimeric Fab developed by Centocor and marketed by Eli Lilly. The situation surrounding the development of ReoPro® by Centocor and its licensing to Eli Lilly have been described in detail by Shook (2007). ReoPro® was not intended, initially, to be the first therapeutic monoclonal antibody from Centocor. Instead, in the early 1990s, most of the resources were focused on the development of a sepsis monoclonal antibody, targeting bacterial lipopolysaccharide, named Centoxin. With Centoxin having looked positive in late stage clinical trials, and even achieving licensure in several European countries, Centocor invested heavily in development and manufacturing capabilities as it ran Phase III clinical trials to demonstrate unequivocal efficacy. Unfortunately, those trials were not unequivocal and the FDA ordered additional trials to be run. At that time, Centocor was operating with substantial and rising losses, and no drug on the market to buffer the costs with incoming revenue, so they made a licensing deal with Eli Lilly to share the risk, as well as give rights to Lilly for another Centocor drug in development, ReoPro®. The final clinical trials on Centoxin ultimately demonstrated not only lack of efficacy, but also higher death rates in one group of patients, resulting in a complete cessation in the development of that candidate. At this point, Centocor licensed the rights to Panorex® (murine monoclonal IgG2a antibody known originally as 171A, which recognizes a 37 to 40 kDa cell surface glycoprotein expressed on malignant and normal epithelial cells; approved in 1995 in Europe for colorectal cancer therapy) to Wellcome, reduced its workforce and overhead costs, and focused all remaining resources on development of ReoPro®, which eventually was approved in 1994, effectively keeping Centocor afloat as an independent company (Shook 2007). By 1997, Centocor became Pennsylvania's first biotech company to make a profit (Shook 2007).

Shortly thereafter, Centocor initiated collaborative studies with Jan Vilcek, New York University School of Medicine, on a murine anti-TNF-α antibody named mA2. The antibody was converted to a murine-human chimeric antibody (cA2), and developed both for Crohn disease (FDA approval granted in 1998) and rheumatoid arthritis (FDA approval granted in 1999), marketed by Centocor in the United States and Schering-Plough overseas. The successes of ReoPro and Remicade ultimately led to the acquisition of Centocor by Johnson & Johnson in 1999 (Table 1.6). Notably, through its ownership of Ortho Biotech and its acquisition of Centocor in 1999, J&J had a stake in four (Orthoclone OKT3®, ReoPro®, and Remicade® worldwide; Panorex® in Europe) of the first seven monoclonal antibodies to be approved.

1.4.2 Examples of Other Early Mabs

In the meantime, Rituxan®, a mouse-human chimeric anti-CD20 monoclonal antibody (originally known as C2B8), was being developed by IDEC for non-Hodgkin's lymphoma. IDEC, which was founded in 1985, had begun work on C2B8 in the early 1990s (Reff et al. 1994), and entered Phase I clinical trials with it in 1993. Significantly, it was recognized from the very beginning that C2B8 possessed the ability to kill target B-cells via antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CDC), which laid the groundwork for literally thousands of

TABLE 1.6 Comparison of the Top Biopharmaceutical Companies with Respect to Biopharma Pipelines and Future Potential*

Company	Estimated Sales of Top Biologics (2006) in millions	Public Late Pipeline (Phase II or III)*	Key Biologics Pipeline Candidates	Key Technologies	Potential Future Ranking
Amgen	12,140	$N = 10$	Romiplostim; Denosumab	Peptibody; Abgenix mice; Avidia avimers	2
Roche (not including Genentech)	6,895	$N = 4$	Omnitarg; Ocrelizumab	Glycart afucosyl glycosylation; THP polyclonals; alternative scaffolds (MP)	1**
Genentech	6,765	$N = 7$	Omnitarg; Ocrelizumab	Fc engineering; Afucosyl Mabs; antibody libraries, humanization; protein engineering; toxin conjugates	See Roche**
Novo Nordisk	5,930	$N = 11$	NovoSeven; Liraglutide	Focus on diabetes and hormone replacement therapies; no Mabs	3
Johnson & Johnson	5,590	$N = 5$	Ustikinumab; Golimumab	Library; Medarex mice; humanization	4
Eli Lilly	3,635	$N = 5$	Teplizumab	Focus on diabetes and neurobiology	8
Merck-Serono	2,535	$N = 1$	Cetuximab	—	—
Sanofi-Aventis	2,485	$N = 3$	Aflibercept (VEGF trap)	Regeneron license—humanized mice	9
Schering Plough	2,305	$N = 2$	Golimumab; Acadesine	—	10
Wyeth	2,265	$N = 7$	Bapineuumab (anti-Aβ)	Trubion, alternative scaffolds, shark domain Abs (small binders)	5
Abbott	2,190	$N = 1$	ABT874 (anti-IL12/23)	—	—
Bayer AG	2,145	$N = ?$	—	—	—
Biogen Idec	1,775	$N = 4$	Galixumab (anti-CD80); Lumilixumab (anti-CD23)	Bispecifics; protein engineering	—
Baxter	1,700	$N = ?$	—	—	—
Astra-Zeneca (including Medimmune and CaT)	910	$N = 5$	Motavizumab; anti-IL-5R, IL-9, IL-13 MAbs	CaT Library; half-life extension; BiTE technology (Micromet)	7
Pfizer	880	$N = 10$	Ticilizumab (anti-CTLA4)	Medarex license; Biorexus transferrin fusions; CovX Ab-peptide fusions	6
Chugai	855	$N = 1$	Tocilizumab (Actemra)	—	—
Bristol-Myers Squibb	771	$N = 3$	Belatacept; Ipilizumab	Adnectin alternative scaffold	11
Kirin	515	$N = 1$	TPO mimetic	Humanized mice	—
Glaxo-Smith Kline	—	$N = 6$	Belimumab; Mepolizumab; Ofatumumab	Domantis domain antibodies; Afucosyl Mabs (via BioWa license)	12

*Based on publicly stated, novel biologics (not market extension or expansion of existing biologics) in Phase II or III; data as of August 2008; data obtained from company websites, Prous Science Integrity, and www.clinicaltrials.gov.

**Roche and Genentech combined, based on the 7/21/08 offer by Roche to acquire remaining shares of Genentech they currently do not own.

studies since then on these important mechanisms of action for oncology indications. In 1995, after strong Phase II clinical results were reported, IDEC signed an agreement with Genentech to collaborate on the Phase III clinical development and marketing of C2B8. Simultaneously, Genentech also was conducting Phase III clinical trials on Herceptin® for breast cancer and Actimmune® (interferon-γ1b; eventually discontinued in 1996 by Genentech) for renal cell carcinoma, as well as preclinical studies on an anti-VEGF monoclonal antibody (which eventually was to be developed into bevacizimab, approved as Avastin® in 2004). In 1997, Rituxan® (C2B8) was approved as the third monoclonal antibody to reach the marketplace in the United States, and in 1998, Herceptin® was approved as the sixth Mab to be marketed in the United States.

1.4.3 Evolution of the Biotechnology Industry to the New BioPharma Industry

The period of technology development in the 1970s and 1980s quickly gave way to an era of biotechnology start-up companies, many of which were in the San Francisco Bay area, that effectively drove the biotechnology boom. Cetus was the first major biotechnology company, formed in 1971 in the Berkeley area. Genentech was founded in South San Francisco in 1976 as a shortened name for "genetic engineering technology," Amgen was formed in 1980 as AMGen (for Applied Molecular Genetics), Xoma was started in 1980, Chiron was founded in 1981 to find a vaccine for hepatitis B, and SCIOS was formed in 1981 as Cal Bio (for California Biotechnology). In all, 112 biotechnology companies were started in the Bay Area by 1987. In Europe, Biogen was formed in 1979, and Celltech, the first biotechnology company founded in the United Kingdom, was started in 1980. Toward the end of this period, Medimmune was started in Maryland in 1987 as Molecular Vaccines, Centocor was founded in Philadelphia in 1989 to take advantage of the new antibody revolution, and Cambridge Antibody Technology was opened in Cambridge, UK, in 1989.

Since then, there have been many changes in the biopharma industry, with a significant number of acquisitions, mergers, and licensing deals, driven by the need of large pharmaceutical companies to build their pipelines. As mentioned earlier in this chapter, Mabs and Fc fusions proteins have been playing an increasing role in pharma pipeline portfolios, so the intense competition for the most important technologies, drug candidates, and intellectual property has driven and will continue to drive such mergers and acquisitions (Table 1.5). Notably, of the companies mentioned above, Roche now owns a majority stake in Genentech and has recently (July 21, 2008) tendered an offer for all remaining shares it does not own. This would make Roche/Genentech the largest biopharma player with the strongest pipeline in the business (Table 1.6), taking over from Amgen, who had the strongest combined sales and portfolio in 2006 when the marketing data used in this analysis were gathered. Additionally, of those biotechnology companies listed above, Biogen has since merged with IDEC, Celltech has become a part of UCB, MedImmune and Cambridge Antibody Technology were acquired by AstraZeneca in 2006 and 2007, respectively, and, as mentioned previously in this chapter, Centocor was acquired in 1999 by Johnson & Johnson (Table 1.5). Other major acquisitions relevant to the monoclonal antibody field are also listed in Table 1.5.

Table 1.6 shows the top 20 major biopharma, their public pipelines, important advanced clinical candidates, key technologies, a current ranking (based on 2006 market information), and a projected future ranking, taking the factors mentioned into consideration. Other than Novo Nordisk, whose pipeline is focused almost entirely on its strong diabetes franchise, and Bayer-Schering and Baxter, which have diversified biologics pipelines, the top biopharma companies have placed significant efforts and resources into the discovery, development, and commercialization of monoclonal antibodies and Fc fusion proteins (Table 1.6).

Significantly, virtually all of the major large pharma companies have joined in the search for monoclonal antibody and Fc fusion protein drugs. Even companies not invested, or not significantly so, in the late 1990s, such as Merck, Sanofi-Aventis, and Astra-Zeneca, are now players, along with other large pharma that have been in the field longer, for example, Johnson & Johnson (the first company to bring any monoclonal antibody to the market), Roche, Novartis, and Wyeth. With the greater number of players, and the increased significance placed on biologics approaches by all of these

companies, the competition for new biologics, well validated biologics-friendly targets, and biologics markets has become incredibly intense.

1.5 CHALLENGES AND OPPORTUNITIES FOR MONOCLONAL ANTIBODIES AND Fc FUSION PROTEINS

1.5.1 SWOT Analysis

Figure 1.7 shows a SWOT (strengths, weaknesses, opportunities, threats) diagram for the Mab and Fc fusion market. The key threats include safety concerns in the post-Tegenero TGN-1412 debacle (Haller, Cosenza, and Sullivan 2008), the high cost-of-goods (including production, purification, formulation, packaging, and delivery) of Mabs, which lead to significant market pressures from third-part payors, and small molecules that could enter markets currently dominated by biologics (Ziegelbauer and Light 2008). Additionally, the impending follow-on biologics revolution is eventually expected to impact several Mabs coming off patent, although the regulatory environment supporting this still remains unclear today (see Williams and Strohl, Chapter 32 in this volume). The most significant of these threats to innovator biologics is probably cost and impending follow-on biologics. A final threat, or perhaps weakness, in the field is that there is the perception that there is a limited number of targets, and thus, many companies compete for market share on the same "hot" targets.

1.5.2 Competition on "Hot" Targets

As pointed out earlier in this chapter, there are 27 marketed therapeutic antibodies and Fc fusion proteins, and another 170+ in various stages of clinical trials. While many of these clinical candidate biologics target novel mechanisms, there are also several that target the same molecule. Some of these already face significant competition on the market. For example, Remicade®, Enbrel®, Humira®, and Cimzia® are now all marketed as anti-TNF-α therapeutics for the treatment of rheumatoid arthritis (Table 1.2). A BLA was recently submitted for golimumab in the same field and CytoFab is still in

Major Strengths:	**Major Weaknesses:**
• Targets that cannot be addressed with small molecules (e.g., protein–protein interactions) • Half-life leads to less frequent dosing • Efficacy • ADCC/CDC (i.e., immune system functionality)	• Parenteral delivery (IV, SC) • Limitation to extracellular and cell-surface targets • Cost, and cost of goods driving the pricing • Immunogenicity and injection site reactions
Major Opportunities:	**Major Threats:**
• Delivery improvements (e.g., transdermal, oral, intranasal) • Modified Fc; fine-tuning immune system functionality • Extended and/or tunable T1/2 • Multispecificity (e.g., ability to engage multiple targets while retaining long T1/2 • Novel scaffolds, approaches • Tissue targeting, e.g., ability to cross BBB	• Safety concerns in a post-Tegenero TGN-1412 era • Small molecules functioning in same pathways as biologic • Third party payer restrictions on reimbursement • Follow-on-biologics • Perception of a limited number of high quality targets leading to intense competition on certain "hot" targets (e.g., TNF-α, CD20)

Figure 1.7 SWOT diagram for therapeutic Mabs and Fc fusion proteins.

clinical trials for sepsis (Tables 1.2 and 1.3), bringing the total to six competitive molecules directed against TNF-α. Those anti-TNF-α antibodies able to out-compete on the basis of potency, dosing frequency, route of administration, and safety (particularly low infection rates and injection site reactions, and lack of immunogenicity) should ultimately garner the most significant shares of the steady-state market. It should be noted that there are public reports on efforts to make follow-on anti-TNF-α biologics as well, although how these will be developed and commercialized is still unknown due to a lack of clarity concerning U.S. policy in this area (see Chapter 32).

The most competitive target, based on the number of known molecules under development, is CD20 (Table 1.7). To date, there are at least 11 known anti-CD20 Mabs in development for oncology and/or rheumatoid arthritis indications (Table 1.7). These known candidates roughly fall into two categories known as Type I and Type II anti-CD20 Mabs. The type I anti-CD20 antibodies, such as Rituxan®, Ofatumumab (Humax CD20; Hagenbook et al. 2005), Ocrelizumab (Genentech's second generation anti-CD20), and Veltuzumab, are thought to function primarily by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and promote translocation into lipid rafts, but probably do not induce apoptosis to a substantial degree (data summarized from Teeling et al. 2004; Bello and Sotomayor 2007; Maloney 2007; Glennie et al. 2007; Leonard et al. 2008; Beers et al. 2008). Type II anti-CD20 antibodies, such as Tositumumab, AME-133v (Weiner et al. 2005), and GA-101 (Cragg and Glennie 2004; Umana et al. 2006), are generally thought to function through ADCC and apoptosis, but do not significantly function via CDC nor allow translocation into lipid rafts (Table 1.7).

The competition and efforts to sort out the biology of CD20 and the Mabs attacking it will likely lead to clear winners and losers in the marketplace based on efficacy measured by patient survival, as well as use in patients who are heterozygous FcγRIIIa 158V/F or homozygous FcγRIIIa 158F/F, polymorphs of FcγRIIIa for which IgGs have lower affinity (Cartron et al. 2002; Weng and Levy 2003; Ghielmini et al. 2005). These patients have been shown clinically to respond more poorly to Rituxan® than patients who are homozygous FcγRIIIa 158V/V, the higher affinity form of the receptor. It is believed that anti-CD20 antibodies with modified Fc functionality to impart tighter binding to FcγRIIIa will help to overcome the problems observed with the V/F and F/F polymorphisms (Bello and Sotomayer, 2007). The intense competition also should lead to a significantly greater understanding of tumor biology and disease mechanisms, as well as tumor killing mechanisms, and ultimately should help to improve the efficacy of future antibodies targeting CD20, as well as other oncology targets.

Another hot target for oncology is IGF-1R, for which there are at least seven anti-IGF-1R clinical candidates in Phase I or II, as noted in Table 1.4. Similarly, Feng and Dimitrov (2008) described eight different antibodies in clinical programs targeting that receptor, making it perhaps the single most competitive Mab target for which there is not yet a marketed drug. Other targets on which there is considerable competition include: IL-6/IL-6R (five known competitors in the clinic), VEGF/VEGFR (6 known competitors in the clinic, although two receptor types covered), IL-13 (four known competitors in the clinic), and amyloid-beta (four known competitors in the clinic). Targets for which there are at least three known competitor Mabs/Fc fusion proteins in clinical trial include: CD19, CD22, CD30, CD40, IFN-γ/IFN-R, IL-1, EpCAM, and alpha-V integrin (Tables 1.2 to 1.4).

1.5.3 Targets

Perhaps the two most important opportunities in the field of Mabs and Fc fusion proteins are (1) the increase in basic scientific knowledge around many novel targets that may provide new opportunities for therapeutic interventions using biologics; and (2) the burgeoning understanding of antibody biology and how to engineer antibodies to function optimally for a desired target.

Figure 1.8 shows the general classes of targets for all of the marketed therapeutic Mabs and Fc fusion proteins, as well as those in Phase III clinical trials. Interestingly, nearly two-thirds of these targets are receptor or cell-surface targets, whereas only approximately 30 percent fall into the cytokine or soluble protein group. Five belong to infectious disease entities. Of the receptor-based targets, there are no marketed Mabs or Phase III candidates for proteases, G-protein coupled receptors (GPCRs), or ion

TABLE 1.7 Intense Competition: Well-Known Antibodies Targeting CD20

Name of Molecule	Company	Date Approved or Current Phase	Type*	Primary Indication	Comments
Rituxan® (Rituximab)	Biogen/Idec/ Genentech	11/26/1997	I	NHL, RA added 2/8/06	IgG1κ, chimeric
Zevalin® (Ibritumomab tiuxetan)	Biogen/Idec	02/19/2002	UNK	NHL	Murine IgG1κ conjugate, Y-90 or In-111
Bexxar® (Tositumomab-I131; B-1 Mab)	Corixa	06/27/2003	II	NHL	Murine IgG2a/ λ-I-131
Ofatumumab (Humax CD20)	GenMab/Glaxo Smith-Kline	Phase III	I	B-cell chronic leukemia; NHL, RA	Humanized IgG1; Targets N-terminus of CD20; strong CDC activity
Ocrelizumab (Second gen. anti-CD20; 2H7; PRO-70769; R-1594)	Genentech	Phase III	I	RA, Lupus, relapsing MS	Humanized IgG1
Veltuzumab (Ha-20; IMMU-106)	Immunomedics	Phase I/II	I	NHL, autoimmune diseases	Humanized IgG
PRO-131921 (Third generation ant-CD20)	Genentech	Phase I/II	I	Oncology	IgG with modified Fc for increased ADCC
AME-133v (LY2469298)	Eli Lilly	Phase I/II	II	NHL	IgG with modified Fc for increased ADCC
TRU-015	Wyeth	Phase II	I	RA	Small modular immunopharmaceutical product (SMIP)
R-7159	Roche	Phase II	II	NHL	IgG; glycoengineered, afucosylated Mab; strong apoptotic efect
GA-101	Roche	Preclinical	II	Oncology	Afucosylated form generated using Glycart technology

Abbreviations: MS, multiple sclerosis; NHL, non-Hodgkin's lymphoma; RA, rheumatoid arthritis; UNK, unknown.

*Type I anti-CD20 antibodies are thought to possess the following general characteristics: function by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and allow translocation by lipid rafts, but probably do not induce apoptosis to a substantial degree. Type II anti-CD20 antibodies function through ADCC and apoptosis but do not function via CDC, nor promote translocation into lipid rafts. Data accumulated and summarized from Cragg and Glennie (2004); Teeling et al. (2004); Hagenbook et al. (2005); Weiner et al. (2005); Umana et al. (2006); Bello and Sotomayor (2007); Maloney (2007); Glennie et al. (2007); Leonard et al. (2008); Beers et al. (2008).

Therapeutic Target Classes	%	Accessible?	TA**
Soluble proteins	11%	yes	8, 11 = 19
Receptors (including GPCRs)	45%	yes	17, 20 = 37
Ion channels	5%	yes	0, 0
Enzymes*	28%	some*	0, 0
DNA	2%	no	na
Nuclear receptors	2%	no	na
Other	7%	no	na

* Those present in serum, on cell surface, or extracellular spaces
Source: CSFB; *Science* 17 Mar 2000

** Therapeutic Antibodies and Fc Fusion proteins
(Marketed, black; Phase III, green)

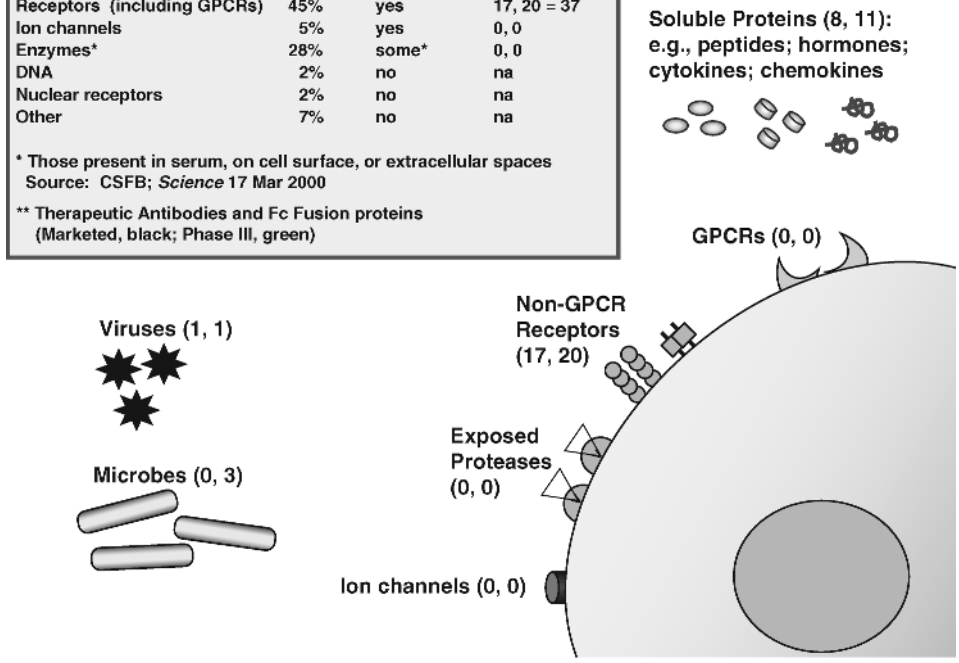


Figure 1.8 Cartoon exemplifying the types of targets for currently marketed and Phase III candidate Mabs and Fc fusion proteins. The first number in each pair indicates the current marketed number, while the second in each pair indicates the number in Phase III clinical trials (based on data from Tables 1.1 and 1.2).

channels. The overall trend also was observed with the earlier clinical candidates, in which 78 percent of the candidates targeted receptors, 37 percent targeted soluble ligands, and 5 percent targeted infectious agents. Figure 1.9 shows a comparison of a profile on what might be considered a perfect target for a therapeutic Mab or Fc fusion protein versus a profile that might be considered more challenging. This comparison, which reflects the author's bias, is interesting in that the majority of clinically validated targets are more closely aligned with what may be considered the more challenging type of targets. This type of comparison, however, can be skewed significantly by the needs of a therapeutic area. The significant number of Mabs against oncology cell-surface targets is a prime example of this kind of skewing.

Obtaining intellectual property rights on important targets appropriate for biologics approaches will be a critical issue that cannot ignored. There are only a limited number of new targets coming available each year for which the biology is compelling; that is, the target is appropriate for a biologic, and the human or rodent genetics data are strongly supportive. A few recent new targets (i.e., not yet validated with a marketed drug, but possessing strong preclinical proof-of-pharmacology) that fall into this class are IGF-1R, IL-13, TRAIL-R1, IL-1β, IL-6, and myostatin, all of which have attracted significant competition (Tables 1.3 and 1.4). It is clear that as more companies focus on biologics approaches, the competition on each new target will become only greater, and will require companies to figure out strategies to differentiate their biologics molecules from the entries of their competitors.

1.5.4 Differentiation and Fit-for-Purpose Biologics

The first antibodies to be marketed were largely based on a standard IgG1 platform. The concept of making antibodies that go beyond what nature gives us, that is, to design antibodies that fit the

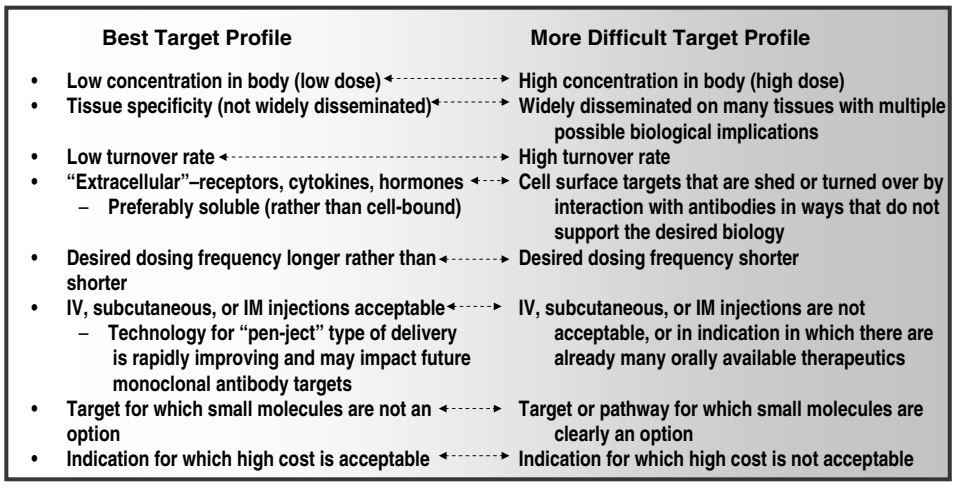


Figure 1.9 Characteristics of an optimal target for a therapeutic Mab as compared with the characteristics of a significantly more challenging target.

needs rather than just accept the biology dictated by natural IgG isotypes (e.g., IgG1, 2, or 4), has been around since the early 1980s (see Neuberger, Williams, and Fox 1984), so it is nothing new to speak of "fit-for-purpose" biologics. In another striking example of this, a search of "monoclonal + ADCC" in PubMed reveals over 1700 references; another for "monoclonal + engineered" yielded more than 1100 references. Moreover, dozens of recent reviews have, in fact, highlighted the need for making antibodies that are more tuned to the biology required of them; a few are listed here for reference (Stockwin and Holmes 2003; Chowdhury and Wu 2005; Laffly and Sodoyer 2005; Carter 2006; Presta 2006, 2008; Jefferis 2007; Dimitrov and Marks 2008).

Several companies already have made significant efforts in modifying Mabs or Fc fusion proteins to make them more fit-for-purpose, represented by the presence of marketed products or advanced clinical candidates. As shown in Table 1.8, several antibodies and Fc fusion proteins already on the market have incorporated significant modifications or alternative formats to a standard human IgG1 to be considered engineered for fit-for-purpose. Certainly, the efforts to generate humanized, and then fully human, Mabs to reduce immunogenicity were the first step in this direction (review by Almagro and Strohl, Chapter 13). Antibodies and Fc fusion proteins concentrated enough for dosing subcutaneously, such as Enbrel®, Xolair®, and Humira®, also fall into this category, as they provide the patient and health care workers with a preferred form of dosing (Table 1.8). Important firsts also include the first alternative isotypes (IgG4 [Bexxar®], IgG2 [Vectibix®]), conjugation with either cytotoxic chemicals or radionuclides for targeting cancer tissue, the peptibody (NPlate®) from Amgen, recently approved for marketing by the FDA, and alternative formats such as a site-specifically PEGylated Fab (Cimzia®) (Table 1.8).

Table 1.9 shows an impressive list of fit-for-purpose engineered molecules currently in clinical trials. These molecules generally fall into three categories: (1) modification of Fc functionality relating to either FcγR, complement, or FcRn interactions; (2) half-life extension of peptides, scFvs, or Fabs using either PEGylation or fusion to Ig domains; and (3) multispecificity. It is clear that several companies are working hard to identify strategies to develop molecules that can be differentiated from the competition (Carter 2006; Dimitrov and Marks 2008).

From a therapeutic standpoint, the most critical feature of a product is that it must have demonstrated clinical efficacy, which relies on a combination of many factors, including target biology, potency, safety, proper dosing, selection of patient population, and so forth. These are all fundamental issues that must be considered when starting a therapeutic biologics project. Nevertheless, there are several

TABLE 1.8 Table of Significant "Marketplace Firsts" in Therapeutic Antibody and Fc-Fusion Protein Drug Discovery

Technology or Process "First"	Product	Company or Inventor	Effective Date	Notes/Comments
IgG marketed	Orthoclone OKT3® (Muromonab-CD3)	Ortho Biotech (Johnson & Johnson)	06/19/1986	Murine IgG
Fab marketed	ReoPro® (Abciximab)	Centocor (now Johnson & Johnson)/Lilly	12/22/1994	Also first chimeric antibody of any kind and first recombinant antibody produced in *E. coli*
Recombinant antibody produced by CHO or NS0	Rituxan® (Rituximab)	Biogen/Idec/Genentech	11/26/1997	Also first chimeric Mab (IgG) marketed
Humanized Mab marketed	Zenapax® (Daclizumab)	PDL/Roche	12/10/1997	Now, 11 humanized antibodies are on the market
Subcutaneous formulation and administration	Enbrel® (Etanercept)	Immunex (now Amgen)	11/02/1998	Now also available with a "SureClick®" Autoinjector device
Mab-conjugate marketed	Mylotarg® (Gemtuzumab ozogamicin)	Wyeth	05/17/2000	Conjugated to the cytotoxic drug, ozogamicin, a calecheamycin (natural product cytotoxin)
Phage displayed human antibody marketed	Humira® (Adalimumab)	Cambridge Antibody Technology/Abbott	12/31/2002	Known before marketing as "D2E7," the indicators for ELISA plate and wells from which it was isolated
Mab radionuclide conj	Bexxar® (Tositumomab-I131)	Corixa	06/27/2003	Reconstitution with radiolabel takes place at clinical site
First IgG4 isotype (or any other than IgG1) antibody to be approved	Mylotarg® (Gemtuzumab ozogamicin)	Wyeth	05/17/2000	Tysabri (Natalizumab) is second example of IgG4 to be marketed; Vectibix is first IgG2 to be marketed
Intravitreal injection	Lucentis® (Ranibizumab)	Genentech/Novartis	06/30/2006	Different V chains than used in Avastin
Mab from transgenic humanized mouse source	Vectibix® (Panitumumab)	Amgen	09/27/2006	Also first IgG2 isotype antibody to be approved
Modified Fc functionality	Soliris® (Ecolizumab)	Alexion Pharma	03/16/2007	Humanized IgG2/4 hybrid to significantly reduce Fc functionality while retaining FcRn-binding mediated half-life
PEGylated antibody marketed	Cimzia® (Certolizumab pegol)	UCB/Schwartz	04/22/2008	Site-specific PEGylation
Peptide-Fc construct	Nplate® (AMG531; Romiplostim)	Amgen	08/22/2008	"Peptibody" construct recently approved for marketing

TABLE 1.9 Examples of Mab and Fc Fusion Protein Engineering to Generate Greater Molecule Fitness for Therapeutic Purpose

Property	Effect	Example(s)
Fc mutations resulting in increased binding to FcγRIIIa	Increased antibody-dependent cellular cytotoxicity (ADCC) as measured preclinically in antibody-dependent cell-killing assays. While widely hypothesized, clinical efficacy improvements attributed to ADCC are still to be determined.	PRO-131921; AME-133v (LY2469298)
Afucosylated antibody	Increased ADCC as measured preclinically in antibody-dependent cell-killing assays. Multiple approaches to achieve afucosylated Mabs have been published, as described in the text.	Anti-CD20 GA-101
Fc mutations resulting in increased binding to FcγRIIa	Increased opsonophagocytosis or ADCP (antibody-dependent phagocytic cytotoxicity), as measured preclinically, in antibody-dependent bacterial or tumor cell-killing assays. Stills need clinical proof-of-concept.	Richards et al., 2008
Fc mutations resulting in decreased binding to FcγRIIa and FcγRIIIa for safety, or to provide a greater efficacy to toxicity window	Murine Orthoclone OKT3® elicited both strong anti-antibody responses because it was a murine antibody, as well as systemic cytokine release syndrome (SCRS), as described in the text. Teplizumab, humanized OKT3γ1ala-ala, yields lowered immunogenicity as well as significantly decreased incidence of SCRS.	Teplizumab, humanized OKT3γ1(ala-ala)
Aglycosylated Mab	Descreased binding to FcγRs resulting in no ADCC; complement activation relatively unaffected; Otelixizumab (ChAglyCD3) to reduce FcγR interaction.	Aglycosylated anti-CD3
Modified pH-dependent binding to FcRn	Longer or shorter half-life as compared with wild-type Fc.	MEDI-557 (Numax-YTE; MEDI-524-YTE)
Tissue distribution	> In making the Numax® FcRn mutant of MED-524 (yielding MEDI-557), it was observed that the change in FcRn binding significantly altered tissue distribution into the lung (Dall'Acqua, Kiener, and Wu 2006). > Also, it has long been known that the size of an antibody construct can have an impact on biodistribution and tissue penetration (Colcher et al. 1998).	MEDI-557 (Numax-YTE)
Affinity—K_{on} vs K_{off}	> In the affinity maturation of Numax®, differences in K_{on} vs K_{off} resulted in significant differences in potency, indicating the importance of both parameters contributing to K_D (Wu et al. 2005). > Additionally, affinity has been shown to be an important factor in both tumor penetration and strength of ADCC response, as described in the text (Adams et al. 2001; Tang et al. 2007).	MEDI-557 (Numax-YTE)

(*Continued*)

TABLE 1.9 *Continued*

Property	Effect	Example(s)
Peptibody or mimetibody approach	Peptide agonists fused with Fc to impart substantially longer half-life; replacement of PEGylation as a method for improving serum half-life of peptides.	Romiplostim
Antibody-peptide fusions or conjugates	An agonist or tissue-targeting peptide is conjugated with a biologically active antibody possessing antagonist or agonist activity. CovX (now Pfizer) utilizes a semisynthetic linker-based method to achieve peptide stabilization.	Pfizer/CovX CVX-045 (thrombospondin 1 mimetic); CVX-60 (angiopoietin-2 binder); CVX-096 (GLP-1 mimetic)
Antibody conjugates	Toxin-conjugates, radioconjugates, siRNA conjugates, and perhaps small molecule conjugates, all fundamentally tissue targeting modalities, are likely to become more sophisticated approaches to delivery of small molecule entities to specific tissues, targets, and compartments.	Toxin conjugates (Mylotarg®) and radio-conjugate (Bexxar®, Zevalin®)
Antibody fragments such as Fabs, scFvs, domain antibodies, etc.	Small, short half-life molecules that may possess increased ability to penetrate tissues; or retain other desired properties inherent with their smaller size, lack of Fc effector activity, and/or short serum half-life.	ReoPro and Lucentis are Fab constructs; Cimzia is a site-specifically PEGylated Fab construct
Bispecific antibodies lacking Fc domains	Bispecificity coupled with short serum half-life may be perfect fit for some targets and indications.	BiTEs (Micromet MT103/MEDI-538)
Bispecific approaches using IgG scaffolds	Ability to engage two targets simultaneously while retaining long half-life of typically IgGs and effector functionality (Ridgeway, Presta, and Carter 1996; Wu et al. 2007b). One clinical example is the Trion Triomab® technology, using a bispecific CD3 and either CD20 or Her2/neu built into IgG with modified Fc for improved FcγR binding (Shen et al. 2006).	Catumaxomab; Ertumaxomab

additional factors that can help, in some cases, to differentiate molecules against the same target from one another. So what are the technologies of the future that will help to differentiate a clinical candidate from the competition? A few of the high-level considerations are listed below, followed by a more in-depth treatise on a few of them:

- Molecules that provide a greater margin of safety, or larger efficacy to toxicity window
- Delivery—route and/or ease of administration (subcutaneous route being more preferred in most cases over IV)
- Tissue distribution and penetration
- Tuning Fc functionality to desired biology—interaction of the Fc with FcγRs and complement and the biology they confer
- Affinity, including differentiation of K_{on} and K_{off} where appropriate
- Epitope—it is clear that for many targets, especially cell surface targets, epitope differences can lead to significant differences in biology, and therefore efficacy
- Multispecificity—in a molecular format that can be stabilized and manufactured

- Size, shape, and flexibility—affects biodistribution and tissue penetration
- Behavior of the molecule—stability and efficient folding (Honegger 2008), solubility (especially at high concentrations), aggregation characteristics, degradation and amino acid reactions, spurious glycosylation sites, and so forth
- Mixed modality—using antibodies as carriers for peptides, siRNA, toxins, small molecules either for half-life extension and/or for tissue targeting

Included below are several examples highlighting how fit-for-purpose Mabs and Fc fusion proteins are being pursued. Soliris® is the first example of a marketed Mab in which the Fc domain has been mutated away from that of a natural Fc (Rother et al. 2007). Examples of other Mabs in late clinical trials having modified Fc domains include Teplizumab (humanized OKT3-γ1-ala-ala, an anti-CD3 Mab with substantially reduced FcγR binding to down-modulate mitogenic response; H. Li et al. 2006), Visilizumab (anti-CD3 Mab with substantially reduced FcγR binding), AME-133v (anti-CD20 with increased affinity to FcγRIIIa; Weiner et al. 2005), and rhuMab V114 (anti-CD20 with increased affinity to FcγRIIIa). As mentioned previously, the field of Fc engineering is incredibly competitive, with significant activity in both the research and development phases; a few reviews and key papers are cited for reference (Shields et al. 2001; Lazar et al. 2006; Presta 2006, 2008; Richards et al. 2008).

There are two important findings with respect to how glycosylation can affect the functionality of IgG Fc. In the first example, now quite well known, it was determined that lack of a fucose residue in the glycoside that binds to residue ser297 in the CH2 domain of IgG results in a tighter binding of the antibody to FcγRIIIa, and with that, higher ADCC (Shields et al. 2002; Niwa et al. 2004; Masuda et al. 2007). Interestingly, the second example for glycosylation effects on biological function is the opposite in nature. Ravetch and colleagues (Kaneko, Nimmerjahn, and Ravetch 2006; Nimmerjahn and Ravetch 2007; Anthony et al. 2008) have shown that sialylated antibodies or Fc domains can have an immunosuppressive effect, and have proposed that this might explain at least part of the mechanism for why IVIG treatment has immunosuppressive properties. It is likely that as we understand more about the relationship between Mab glycoform and immune-related functionality, additional unique glycoform-specific activities may be found. The greatest challenge to these experiments is that normal CHO or other mammalian cell systems produce heterogeneous *N*-glycans. It seems likely that GlycoFi, now a wholly owned subsidiary of Merck (Table 1.5), is the company that should have the greatest opportunity in the near future to address these kinds of questions, as they have shown that they can produce Mabs that possess a single major species of glycoside in recombinant humanized *Pichia pastoris* (see H. Li et al. 2006).

Another example of Fc engineering to improve biologic molecule fitness would be the modification of Fc sequence to potentially improve half-life, and therefore reduce frequency of administration (Petkova et al. 2006). Perhaps the best example of this modification is the YTE mutant Fc from Medimmune (now part of Astra-Zeneca), in which residues M252Y, S254T, and T256E were modified to increase the binding of the IgG1 Fc to human FcRn specifically at pH 6.0, while at neutral pH there was little binding (Dall'Acqua, Kiener, and Wu 2006). This pH-dependent increase in binding to FcRn has been hypothesized to be a way to increase the half-life of antibodies through an improvement in the recycling mechanism (Dall'Acqua, Kiener, and Wu 2006). Indeed, the YTE Fc mutant of an IgG1 possessed approximately four times the half-life in nonhuman primates as compared to the wild-type version of the same antibody (Dall'Acqua, Kiener, and Wu 2006). Another benefit of engineering FcRn was that the biodistribution of the resultant antibody into the lungs was significantly increased (Dall'Acqua, Kiener, and Wu 2006), which is perhaps not too surprising since it had been determined previously that antibodies are transcytosed across lung epithelium via FcRn (Bitonti et al. 2004). The lead molecule, MEDI-557 (also known as MEDI-524-YTE and Numax-YTE), entered Phase I clinical trials in December 2007, so clinical validation (or refutation) of this mechanism should be publicly available soon. If these results hold true in human trials, with little or no mutant-associated immunogenicity, this type of approach could result in many enhanced half-life antibodies in the future, as well

as antibodies that distribute better into the lungs. Such a biodistribution pattern could be quite attractive with many of the antibody candidates targeting asthma (e.g., anti-IL-13, anti-IL-9, anti-IL-4R, and anti-IL-13R).

Another area of Mab engineering that has been of significant interest for both research and development concerns antibody fragments such as Fabs, scFvs, and domain antibody fragments. Thus far, there are three marketed Fab-based products, ReoPro®, Lucentis®, and Cimzia® (Table 1.2). Additionally, both Aurograb® and Mycograb® are in Phase III clinical trials (Table 1.3), and Alacizumuab pegol, a di-Fab-PEGylated construct, is in Phase II trials (Table 1.4). BiTEs, as described below, are constructs consisting of two scFvs linked together (Baeuerle, Reinhardt, and Kufer 2008). The constructs that have recently made the most visible splash are domain antibodies (Dumoulin et al. 2002; Harmsen and De Haard 2007), as also evidenced by the recent acquisition of Domantis by Glaxo-Smith Kline (Table 1.5).

The idea of making a bispecific antibody has been around for over 20 years (Paulus 1985; Brennan, Davison, and Paulus 1985). The concepts of using a bispecific antibody to either bind and neutralize two targets simultaneously, to carry a molecule, for example, toxin, to a specific targeted site, or, alternatively, to bring two targets together, were already being discussed by the mid-1980s (Paulus 1985). The first bispecific construct to bring together an effector cell (e.g., T-cell) and its target cell was published in 1986 (Staerz and Bevan 1986), which was essentially the forerunner to what is now known as the BiTE technology from Micromet (Baeuerle, Reinhardt, and Kufer 2008). There are currently four bispecific constructs in clinical trials, two from Micromet and two using the Trion Triomab® technology. MT-103 and MT-110 are bispecific T-cell engagers (BiTE) scFv constructs from Micromet targeting CD3 on T-cells and CD19 and EpCAM, respectively. Blinatumomab (MT-103) is in Phase II clinical trials for non-Hodgkin's lymphoma and is being tested for several other B-cell malignancies. Catumaxomab is a trifunctional, bispecific hybrid mouse-rat monoclonal antibody (Triomab® technology) against human EpCAM and human CD3. The tri-specificity comes from the fact that the hybrid murine IgG2a/rat IgG2a Fc also binds to FcγRs I and III to trigger ADCC (Zeidler et al. 1999; Shen and Zhu 2008). Catumaxomab is currently in the preregistration phase, so it is expected to reach the marketplace very shortly. A second Triomab® is Ertumaxomab, a trifunctional, bispecific hybrid Mab consisting of a dimer comprised of the subunits anti-HER2/neu mouse IgG2a and anti-CD3 rat IgG2a. This antibody also is functional on FcγRs I and III, giving it its reported trifunctionality. There are many reports in the literature highlighting other strategies to make bispecific antibodies containing functional Fc domains (e.g., Ridgeway, Presta, and Carter 1996; Coloma and Morrison 1997; Shen et al. 2006; Wu et al. 2007b), but none of these approaches has yet been incorporated into Mabs that made it into the clinic. A new approach just recently published is the construction of Surrobodies, which are antibody-pre-BCR subunit chimeras that lend themselves to multispecificity due to an extra fragment hanging off the surrogate light chain components (Xu et al. 2008).

Affinity has always been an issue of discussion. There are now several examples of antibodies being affinity matured to K_D values of 1 to 10 pM or even sub-pM (reviewed in Chapter 13). The question of how tight is tight enough will be debated for some time to come, and is likely to have target-specific answers. The most highly successful antibodies on the market, for the most part, are not particularly high affinity antibodies, many of them having K_D values in the 0.1 to 3 nM range (Carter 2006). There are a few examples, however, of cases in which affinity, and the type of affinity, matter. In a classical study, Wu et al. (2005) showed that affinity matured mutants of an anti-RSV antibody possessed very different characteristics based on whether the maturation improved K_{on} or K_{off}, the two components that make up K_D. This study exemplifies the importance of understanding the details of the biology of a system and how the antibody will interact with that system. In another interesting study of affinity versus functionality, Adams et al. (2001) demonstrated that affinity of an scFv (monovalent) antibody had a significant impact on the ability of that antibody to penetrate tumors; the higher the affinity, the poorer the tissue penetration. The same group, however, later showed that the higher the affinity of an antitumor IgG, the stronger the ADCC (Tang et al. 2007). Taken together, these two studies suggest that there might be a delicate balance in affinity when targeting solid tumors with antibodies.

A novel approach to the stabilization of peptides is the fusion of those peptides to Ig domains, followed by engineering to stabilize the fusion construct (Kuter 2007). The most advanced molecule in this class is Romiplostim, a "peptibody" from Amgen comprised of an Fc derived from IgG fused to a TPO peptide mimetic for specific binding to TPO receptor (Kuter 2007). Romiplostim (Nplate®) was approved by the FDA for marketing in August 2008, making it the first of this type of construct to reach the market. Scientists at Centocor also have reported the construction of EPO- (Bugelski et al. 2008) and GLP-1 (Picha et al. 2008) "mimetibody constructs" which should extend the half-life of the biologically active peptide mimetics. An alternative approach for using the IgG scaffold to stabilize and extend the half-life of peptides or small molecules is the CovX body™, as recently described by Doppalapudi et al. (2007). They used an aldolase antibody engineered so that it possessed a highly reactive lysine in the V-chain, which allowed for highly specific placement of a chemical linker. This linker can then be used to attach a pharmacophore of interest, such as a biologically active peptide, a small molecule, or any other molecule for which a longer half-life may be desired (Doppalapudi et al. 2007).

1.6 SUMMARY, AND "WHERE DO WE GO FROM HERE"?

The examples mentioned above are a few of the many ways in which scientists are looking to engineer Mabs and Fc fusion proteins to impact biology conferred by them. It is probably fair to say that most of the conceptual modifications that could be made to alter the activity of an antibody have been proposed, and most of them are either being made now or have already been made and tested (see Tables 1.8 and 1.9). Variable chain maturation and humanization are well worked out, and now there are several approaches to apply information from *in silico* and *in vitro* deimmunization to improve, at least in theory, the humanness of the molecules made (Abhinanden and Martin 2007; see also Chapter 13). In the near future (e.g., the next decade), the major advancements that will come to the fore will be from the clinical validation (or lack thereof) of constructs that have already been conceptualized. Additionally, recent advances have been made in generation of domain antibodies, nonantibody binding scaffolds, bispecific and multispecific antibodies, and generation of Fc mutants that result in differential pharmacology, that is, increased or decreased ADCC, CMC, and half-life. Many of these types of molecules will make their way into clinical validation. In some cases, this has already begun. Phase I trials are being conducted on MedImmune's YTE half-life extension mutant, MEDI-557. The BiTE technology (CD3/CD19) is already in advanced clinical trials and holds significant promise as a cancer therapeutic (Baeuerle, Reinhardt, and Kufer 2008). As stated previously, Fc modified versions of IgGs already are in the clinic that have improved binding to FcγRs, with the concept of improving ADCC functionality (a hypothesis that still requires clinical validation). By the 2010–2015 time frame, it is expected that many antibodies entering the clinic will be modified from the natural IgG isotypes, IgG1, IgG2, or IgG4, either to increase effector function or, like with Soliris®, to down-modulate it.

In summary, Dimitrov and Marks (2008) recently proposed that there are two major eras in antibody discovery, the original serum therapy period in the early 1900s and, today, in which significant changes are impacting the way we design and make therapeutic proteins. I propose that the "antibody era" be considered in four phases: For the first phase, I concur with Dimitrov and Marks that the seminal work done by von Behring and his colleagues in the early 1900s set the stage for treatment of infectious diseases and the field of immunology. For the second phase, I propose that the era of IVIG therapy (starting in 1952), which led to both the concept and practice of monoclonal therapy, was a crucial step to get to where we are today. The third phase is the 1990s, which is the decade of the "first generation antibody therapeutics," exemplified by therapeutic chimeric and humanized Mabs based largely on standard IgG1 scaffolds.

Finally, I propose that in the 2006–2008 period, we entered the fourth phase, the expansion decade, an era in which many engineered antibodies and antibody-like constructs will be developed and commercialized. Additionally, over the next 10 years, nonantibody scaffolds (not covered here) will likely

be validated and made commercially successful for certain applications, and a wide variety of new IgG isoytpes, modified Fc constructs, peptibodies, and similar second-generation biologics will be built and tested clinically for validation. Collectively, these "fit-for-purpose" molecules will revolutionize how we view biologics. This fourth era also will likely usher in follow-on biologics to those marketed in the first era, which should add pressure on the innovators to continue to innovate.

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