Monoclonal antibody: the corner stone of modern biotherapeutics

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Abstract: Worldwide sales of biologic drugs exceeded 100 billion USD in 2011. About 32% is from therapeutic monoclonal antibody (mAb). With many blockbuster biopharmaceutical patents expiring over the next decade, there is a great opportunity for biosimilar to enter the worldwide especially emerging market. Both European Medicines Agency (EMA) and Food and Drug Administration (FDA) have introduced regulatory frameworks for the potential approval of biosimilar mAb therapeutics. Rather than providing a highly abbreviated path, as in the case for small molecule chemical drug, approval for biosimilar mAb will require clinical trial and the details will be very much on a case-by-case basis. Since mAb is the dominant category of biologic drugs, mAb will be the focus of this review. First, the United States (US) and European Union (EU) approved mAb and those in phase 3 trials will be reviewed, then strategies on how to win biosimilar competition will be reviewed.

Key words: monoclonal antibody; biotherapeutic; biosimilar; biobetter

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1 Introduction

Monoclonal antibody is the most dominant category of biological therapeutics on the market (total 28) and in the pipeline (total 350). MAb therapeutics are also the most mature and proven biologic treatment for human diseases. Among the 28 mAb products currently marketed in EU or US, 26 of them are approved in EU and 27 in US, with 25 approved in both US and EU. In terms of therapeutic indication, majority of these marketed mAbs are indicated for treating various cancer and immunological diseases. Catumaxomab is approved in EU but not US; tositumomab-I^{131} is marketed in US but not EU. Brentuximab vedotin was approved in US in 2011 and, in March 2012, a marketing application for this mAb is under review by the EMA.

2 Currently marketed mAbs in US and EU

2.1 Production system Of the 28 mAbs (Table 1) that are marketed in one or the other region, 43% (12/28) are produced in Chinese hamster ovary (CHO) cells, 25% (7/28) are produced in SP2/0 cells, 18% (5/28) are produced in NS0 cells, and 7% (2/28) are produced in hybridomas. The remaining two products (ranibizumab, certolizumab pegol) are antigen-binding fragments (Fab) that are produced in *E.coli*.

2.2 Type of mAbs Humanized and human mAbs comprise 36% (10/28) and 32% (9/28) of the total, respectively, while 21% (6/28) are chimeric and 11% (3/28) are murine. Most (75%; 21/28) are canonical full-length mAbs. Of the 7 non-canonical mAbs, three (abciximab, ranibizumab, certolizumab pegol) are Fab, with one of these (certolizumab pegol) pegylated; two (tositumomab-I^{131}, ibritumomab tiuxetan) are radio-labeled when administered to patients; one (brentuximab vedotin) is an antibody-drug conjugate (ADC); and one is bispecific (catumaxomab).

2.3 Clinically validated antigen targets for mAbs There are four approved mAbs targeting CD20 and TNF separately, two mAbs targeting EGFR and VEGF separately. Therefore these 4 antigens are the best clinically validated targets. Once approved, pertuzumab (which is under regulatory review in EU and US as a treatment for breast cancer) would be one of 2 mAbs that target human epidermal growth factor receptor 2 (HER2) on the market[1−3]. Other clinically validated targets are: Her2, EpCAM, IgE, CTLA-4, BLys, CD33, RANK-L, IL-1beta, IL-2R, CD52, C5, RSV, GbIIb/IIIa, IL12, IL23 and IL-16R. Humira, Avastin, Rituan and Herceptin are the best seller blockbuster mAbs targeting

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Table 1 Currently marketed mAb in US and EU. Note: Information is current as of May 1, 2012. Abbreviations: BLyS, B lymphocyte stimulator; C5, complement 5; CD, cluster of differentiation; CHO, Chinese hamster ovary; CTLA-4, cytotoxic T lymphocyte antigen 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; Fab, antigen-binding fragment; GP, glycoprotein; IL, interleukin; HER2, human epidermal growth factor receptor 2; PA, protective antigen; RANK-L, receptor activator of NFκB ligand; RSV, respiratory syncytial virus; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor

INN (Trade name)	Cell line	Target	Type	Indication	Approval year
Bevacizumab (Avastin®)	CHO	VEGF	Humanized IgG1κ	Colorectal, lung, breast (outside the USA), glioblastoma (USA only), kidney and ovarian	2004
Natalizumab (Tysabri®)	NS0	a4-integrin	Humanized IgG4κ	Multiple sclerosis and Crohn's disease	2004
Ranibizumab (Lucentis®)	*E. coli*	VEGF	Humanized IgG1κ	The "wet" type of age-related macular degeneration	2006
Panitumumab (Vectibix®)	CHO	EGFR	Human IgG2κ	Metastatic colorectal cancer	2006
Eculizumab (Soliris®)	NS0	C5	Humanized IgG2/4κ	Paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic-uremic syndrome (aHUS)	2007
Certolizumab pegol (Cimzia®)	*E. coli*	TNF	Humanized IgG1κ Fab	Crohn's disease and rheumatoid arthritis	2008
Abciximab (Reopro®)	Sp2/0	GPIIb/IIIa	Chimeric IgG1κ Fab	Percutaneous coronary intervention (angioplasty with or without stent placement).	1994
Rituximab (MabThera®, Rituxan®)	CHO	CD20	Chimeric IgG1κ	Lymphomas, leukemias, transplant rejection, and some autoimmune disorders	1997
Basiliximab (Simulect®)	Sp2/0	IL2R	Chimeric IgG1κ	Prevent rejection in organ transplantation, especially in kidney transplants	1998
Palivizumab (Synagis®)	NS0	RSV	Humanized IgG1κ	Prevention of respiratory syncytial virus (RSV) infections	1998
Infliximab (Remicade®)	Sp2/0	TNF	Chimeric IgG1κ	Psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, and ulcerative colitis	1998
Trastuzumab (Herceptin®)	CHO	HER2	Humanized IgG1κ	Her-2 positive breast cancer	1998
Alemtuzumab (Campath-1H®)	CHO	CD52	Humanized IgG1κ	Chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL) and T-cell lymphoma; bone marrow transplantation, kidney transplantation and islet cell transplantation	2001
Adalimumab (Humira®)	CHO	TNF	Human IgG1κ	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, moderate to severe chronic psoriasis, and juvenile idiopathic arthritis	2002
Tositumomab-I^{131} (Bexxar®)	Hybridoma	CD20	Murine IgG2α	Relapsed follicular lymphoma	2003
Cetuximab (Erbitux®)	Sp2/0	EGFR	Chimeric IgG1κ	Metastatic colorectal cancer and head and neck cancer	2004
Ibritumomab tiuxetan (Zevalin®)	CHO	CD20	Murine IgG1κ	B cell non-Hodgkin's lymphoma, a lymphoproliferative disorder	2004
Omalizumab (Xolair®)	CHO	IgE	Humanized IgG1κ	Severe, persistent allergic asthma	2002
Golimumab (Simponi®)	Sp2/0	TNF	Human IgG1κ	Moderately to severely active rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis	2003
Canakinumab (Ilaris®)	Sp2/0	IL1b	Human IgG1κ	Cryopyrin-associated periodic syndromes (CAPS)	2009
Catumaxomab (Removab®)	Hybridoma	EpCAM/CD3	Rat IgG2β/mouse IgG2α	EpCAM-positive cancer	2009
Ustekinumab (Stelara®)	Sp2/0	IL12/23	Human IgG1κ	Psoriatic arthritis	2009
Tocilizumab (Actemra®)	CHO	IL16R	Humanized IgG1κ	Autoimmune diseases, multiple myeloma and prostate cancer	2010
Ofatumumab (Arzerra®)	NS0	CD20	Human IgG1κ	Follicular non-Hodgkin's lymphoma, diffuse large B cell lymphoma, rheumatoid arthritis and relapsing remitting multiple sclerosis	2009
Denosumab (Prolia®)	CHO	RANK-L	Human IgG2κ	Osteoporosis, treatment-induced bone loss, bone metastases, rheumatoid arthritis, multiple myeloma, and giant cell tumor of bone	2010
Belimumab (Benlysta®)	NS0	BLyS	Human IgG1κ	Systemic lupus erythematosus (SLE)	2011
Raxibacumab (Pending)	NS0	B.anthrasis PA	Human IgG1κ	Prophylaxis and inhaled anthrax	In review
Ipilimumab (Yervoy®)	CHO	CTLA-4	Human IgG1κ	Non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC) and metastatic hormone-refractory prostate cancer	2011

TNF, VEGF, CD20 and HER2 respectively. They are the most aimed biologics by biosimilar developers around the globe.

2.4 mAbs withdrawn or discontinued In addition to the 28 mAbs currently marketed, six mAbs were approved in at least one country of EU or in US[4], but were subsequently withdrawn or discontinued from marketing for various reasons (Table 2). First approved in US in 1986, muromonab-CD3 (Orthoclone OKT3®) was a murine IgG2α used to treat acute kidney allograft rejection; however, manufacturing was discontinued in 2010 due to the availability of other treatments with similar efficacy and fewer side effects, and declining sales. Nebacumab (Centoxin®), a human IgM, was approved in Netherlands, Britain, Germany and France during 1991 as a treatment for gram-negative sepsis, but it was subsequently withdrawn for safety, efficacy and commercial reasons. The murine anti-epithelial cell adhesion molecule (EpCAM) edrecolomab (Panorex®) was approved in Germany in 1995 as an adjuvant treatment for colon cancer, but subsequently withdrawn because of the product's lack of efficacy. Daclizumab was first approved in 1997 for prophylactic treatment of acute organ rejection in kidney transplant patients, but the product was voluntarily withdrawn from the market in EU effective on January 1st, 2009 and discontinued in US market because of the availability of alternative therapy and the diminished market share and demand. Gemtuzumab ozogamicin is the first antibody drug conjugate (ADC) approved. It was marketed in the US for a decade before being voluntarily withdrawn in 2010. The product was approved under the accelerated approval mechanism as a treatment for acute myeloid leukemia (AML), but was withdrawn when a confirmatory clinical trial and post-approval use did not show evidence of clinical benefit in AML patients. Efalizumab (Raptiva®) was approved in US and EU in 2003 and 2004, respectively, as a treatment for adults with moderate to severe plaque psoriasis, but the product was voluntarily withdrawn from both markets in 2009 because of the risk of side effects, including progressive multifocal leukoencephalopathy.

3 mAbs currently in phase 3 trials

Also there are many novel 2nd and 3rd generation and biobetter mAbs targeting these same antigens are currently in preclinical development or various stages of clinical trials (Table 3). Human mAb is increasingly favored over humanized and chimeric mAb. More and more recent approved mAbs are derived directly from human B cells isolated from patient or immunized individual[5]. Antibody drug conjugate [6] and bispecific antibody[7] are also become popular due to the recent technology advancement in chemical labeling and bispecific antibody production platform. CHO is the most dominant, validated and proven host cell for mAb manufacturing. Many non-CHO host cells, especially human cell line such as HEK293 and PerC6, are gradually gaining popularity. But its viral safety and robustiness as a manufacturing system remain to be proved. Therefore, CHO system is still the safest production system for biosimilar developers. Novel cell line is only good for innovative biobetter developers.

The commercial pipeline includes roughly 350 mAbs now being evaluated in clinical studies around the world as treatments for many diseases, including cancer, immunological disorders and infectious diseases. Total of 165 anti-cancer antibodies are currently at various phases of clinical trials. Among them, 84 (51%) are unmodified naked IgG, 25 (15%) antibody drug conjugates, 16 (10%) are antibody fragments, 17 (10%) engineered, and 10 (6%) bispecific. Listed in Table 3 are mAbs currently in phase3 trials. Vast

Table 2 Therapeutic mAbs withdrawn or discontinued. Note: Information current as of May 1, 2012. Abbreviations: CD, cluster of differentiation; CHO, Chinese hamster ovary; EpCAM, epithelial cell adhesion molecule; IL, interleukin

INN (Trade name)	Cell line	Target	Type	Indication	Approval year
Muromonab-CD3 (Orthoclone OKT3®)	Hybridoma	CD3	Murine IgG2α	Acute, glucocorticoid resistant rejection of allogeneic renal, heart and liver transplants; T-cell acute lymphoblastic leukemia	1986
Nebacumab (Centoxin®)	Hybridoma	Endotoxin	Human IgM	Gram negative bacteraemia (gnb) sepsis	1991
Edrecolomab (Panorex®)	Hybridoma	EpCAM	Murine IgG2α	Colorectal carcinoma	1995
Daclizumab (Zenapax®)	NS0	IL21R	Humanized IgG1κ	Prevent rejection in organ transplantation, especially in kidney transplants	1997
Gemtuzumab ozogamicin (Mylotarg®)	NS0	CD33	Humanized IgG4κ	Acute myelogenous leukemia	2000
Efalizumab (Raptiva®)	CHO	CD11a	Humanized IgG1κ	Psoriasis	2003

majority of them are for oncology and immune disease indications[8]. The list of marketed therapeutic antibodies will be substantially larger in the near future.

4 Biosimilar or biobetter?

Biosimilars are structural mimics of the innovator biologics whereas biobetters are improvements to innovator biological molecular entity[8]. Biobetter mAbs target the same protein as the originator already on the market, but possess some molecular or chemical modification that constitutes an improvement over the originator drug, such as enhanced bioavailability or reduced immunogenicity.

The major advantage to manufacturers of biobetters is the fact that they have significantly lower early-stage R & D costs compared to originator drugs. In addition, biobetters have an advantage over biosimilars as they constitute improvements over the originator and any biosimilar competitors, and some of them should be patentable. However, it can also be the case that, because the active ingredient is so similar to an already marketed product, you may not be able to patent some of the biobetters you developed. Patents are granted only to mAb products that are shown to be a significant advance over technology already known to the public. Many biobetters may not meet that standard. But how will the pharmaceutical industry choose to penetrate this lucrative market and benefit from patent expirations on therapeutic biologics? Will biobetters or biosimilars be the winners?

Recent advance in this field include cell line with targeted genetic modifications (including Selexis' UCOE, ubiquitous chromatin opening element, Cellectis' meganuclease mediated targeted integration and Sigma's ZFN, zinc finger nuclease, platforms), alternative production hosts such as EB66, PerC6, engineered CHO and yeast, engineered expression vectors with weak promoter on selection marker gene. Host cell engineering, single-use technologies, and rapid transient expression may play a major role in future mAb biosimilar development[9]. However it remains to be

Table 3 mAbs currently in phase 3 trials. Note: Information current as of May 1, 2012. Abbreviations: CD, cluster of differentiation; CHO, Chinese hamster ovary; IL, interleukin; IGF, insulin growth factor. Sources: European Medicines Agency public assessment reports, United States Food and Drug Administration (drugs@fda), the international ImMunoGeneTics information system® (www.imgt.org/mAb-DB/index)

Sponsor	INN name	Target/Type	Indication
Abbott/BMS	Elotuzumab	CD2/IgG1	Cancer
Amgen	AMG-386	Angiopoietin/peptide-Fc fusion	Cancer
Amgen	AMG-479	IGF-1R	Cancer
Wilex AG	Girentuximab	Carbonic anhydrase IX/IgG1	Cancer
Active Biotech Research	Naptumomab	5T4/Fab-enterotoix A	Cancer
TenX Biopharma/Genmab	Zanolimumab	CD4/IgG1	Cancer
Glycart/Genetech/Biogen	Obinutuzumab	CD20/IgG1	Cancer
Imclone	Necitumumab	EGFR/IgG1	Cancer
Morphotek	Farletuzumab	Folate R alpha/IgG1	Cancer
Genetech	Trastuzumab emtansine	HER2/IgG1 conjugate to DM1	Cancer
Genetech	Pertuzumab	HER2/IgG1	Cancer
Merck/Pierre Fabre	Dalotuzumab	IGF-1R/IgG1	Cancer
Imclone/Lilly	Ramucirumab	VEGFR2/IgG1	Cancer
GSK	Mepolizumab	CD22/IgG1	Systemic lupus
Lilly	Tabalumab	B cell activating factor/IgG4	Systemic lupus
Takeda/Millennium	Vedolizumab	a4b7Integrin/IgG1	Crohn's disease/ulcerative colitis
Biocon/CIMAB SA	T1h	CD6/IgG1	Psoriasis
UCB/Immunomedics	Epratuzumab	CD22/IgG1	Systemic lupus
Cephalon	Reslizumab	IL-5/IgG4	Eosinophilic esophagitis
Regeneron	REGN88	IL-6R/human IgG1	RA/Ankylosing spondylitis
Novartis	AIN-457	IL-17A/human IgG1	Uveitis
Lilly	Solanezumab	Beta Amyloid/IgG1	Alzheimer's disease
Pfizer/Janssen	Bapineuzumab	Beta Amyloid/IgG1	Alzheimer's disease
Pfizer	Figitumumab	IGF-1R/IgG2	Cancer
Pfizer	Inotuzumab	CD22/IgG1	Cancer

seen how novel cell line generation strategies and the above new technologies will affect product equivalence and regulatory approval in the emerging biosimilar age.

Biosimilar manufacturers have to decide whether traditional methods of cell line development will be sufficient or if emerging technologies can provide greater reproducibility and speed. Expression level of 1−2 $g \cdot L^{-1}$ is adequate for most production processes. Product quality (protein homogeneity and glycan profile) and reproducibility (cell line stability) are more important than maximum possible mAb titer in cell line/process development.

From the Table 4, it is predicted, by year 2016, top selling drug will be mostly antibody related biologics. Among them, Humira, Avastin and Rituxan will take over the top spots of the list. Since many of the blockbuster mAbs, such as Humira, Rituxan, Avastin, Herceptin, named just a few of the top sellers, who's patents will expire in a few years, generic drug developers as well as R&D driven big pharmas are all rushed into this biosimilar gold mine[10]. This is because biosimilars (some people call it follow-on biologics) significantly reduce development risk due to the fact that targets and molecular entity for these mAb therapies are clinically (both safety and efficacy) validated, the risk of development failure is much lower comparing to novel targets[11].

Table 4 Top selling antibody related biologics *vs* Lipitor

Drug	2010 (billion)	Drug	2016 (billion, projected)
Lipitor	12.1	Humira	10.1
Enbrel	6.8	Avastin	9.8
Humira	6.6	Rituxan	9.3
Remicade	6.5	Enbrel	8.5
Avastin	6.2	Herceptin	6.2
Rituxan	6.1	Remicade	5.4
Herceptin	5.2	Lipitor	<1.0

Drug developer's risk tolerance is drastically reduced in the current economic environment, which is not in favor of high price medicine and high cost of drug development program. Therefore there is a great opportunity for biosimilar mAb in next 5−10 years, not only in developing countries but also in developed ones.

There is no doubt that biosimilars are part of the future of the pharmaceutical industry. The global biosimilars market is expected to grow from 243 million USD in 2010 to 3.7 billion USD in 2015 according to 2011 data from Datamonitor. Major generics players (Table 5) such as Dr Reddy's, Teva, Sandoz and Hospira have already signalled that biosimilars are a priority for growth, as has newcomer to the biosimilars market Merck[12].

Table 5 Key players in the biosimilars field. CRO: Contract Research Organization; CMO: Contract Manufacture Organization

Innovator company	Generic company	CRO/CMO	Other player
Amgen	Actavis	Biocon	Fujifilm
Biogen Idec	Cipla	Bioton	GE
Boehringer Ingelheim	Dr Reddy's	Celltrion	Samsung
Merck	Gedeon Richter	Harvest Moon	Mitsubishi
Pfizer	Hospira	Intas	
Lilly	Mylan	Lonza	
Astrazeneca	Sandoz	Parexel	
Sanofi	Stada Arzneimittel	Quintiles	
Roche	Teva	Wockhardt	
BMS	Watson	Zydus	

China and India are seen as being areas of major opportunity for biosimilar manufacturers, both in terms of exclusivity and in terms of costs. Both countries have relatively short (China) or no (India) market and data exclusivity provisions to protect originator drugs. Cost savings of 30%−50% are reported for manufacturing biosimilars in China compared to in Europe or US, while in India cost reductions of around 40% have been suggested. The Indian government has also been actively encouraging biosimilar development by investment in government-led initiatives and by constructing biotechnology parks.

Despite the fact that patent protection may not be available, provided that the market is sufficiently large to warrant full-scale clinical trials, regulatory pathways in EU and US could still encourage the development of biobetters rather than biosimilars. These biobetters would use the standard biological approval route, rather than the abbreviated pathway used by biosimilars. This would mean that biobetters, as 'new drugs' would benefit from market exclusivity, even if they are not different enough to gain patent protection. Therefore, it's a good strategy to develop biosimilar in Asia and other developing countries and biobetter for US and EU.

Biosimilars are very attractive not only to the traditional pharmaceutical sector, non-pharmaceutical companies are also very interested in getting into the game. For example, Korean electronics giant Samsung and US domestic electronic manufacturer GE Healthcare joined force to develop biosimilars, and

Japanese film and digital camera maker Fujifilm, making a deal in November 2011 with biotech firm Kyowa Hakko Kirin (Kyowa), creating a dedicated biosimilars division called Fujifilm Kyowa Kirin Biologics. Last year Samsung and Biogen formed a 300 M joint venture to develop biosimilars. The best strategy is to develop mAb biosimilars for Asia and other developing country markets and biobetter for US and EU.

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