Regulatory Framework and Challenges in Developing Biosimilar Monoclonal Antibodies and Related Biological Products

Bobby George
Reliance Life Sciences Pvt. Ltd, Dhirubhai Ambani Life Sciences Centre,R-282, Thane Belapur Road, Rabale, Navi Mumbai, India.

Received June 9, 2012; accepted July 25, 2012

ABSTRACT
Monoclonal antibodies (mAbs) were initially used as laboratory reagents, later they were adopted as clinical diagnostic reagents, and eventually as therapeutic agents. The development of therapeutic mAbs commenced in the early 1980s and by 1986 the first mAb for human use "Orthoclone OKT3®" was approved by the US Food and Drug Administration (FDA). The next wave of antibody products were mostly anticancer agents which were approved in the US and Europe in the 1990s. The technological evolution from murine-based therapeutic mAbs to chimeric (part murine part human protein), humanized, and fully human antibodies has led to a reduction in immune-mediated clearance and hypersensitivity, improved the safety and feasibility of repeated administration making them therapeutically viable. Since the commercialization of the first therapeutic mAb, these products have become a dominant component of the biopharmaceutical market generating revenues of several billion dollars. The area of biosimilar antibody development is positioned for substantial growth with regulatory agencies like the European Medicines Agency (EMA) coming up with new guidelines on similar biological medicinal products followed by Health Canada, the US FDA and others, addressing biosimilar product development. With few of the blockbuster mAbs going off-patent in the next decade, companies with expertise in manufacturing biosimilar mAbs with the right kind of business and regulatory strategy are likely to have good value proposition.

KEYWORDS: Antibodies; biosimilars; European Medicines Agency; guidelines; monoclonal antibodies; regulations.

Introduction
Antibodies (Abs) are a key component of the adaptive immune response, playing a central role both in the recognition of foreign antigens and the stimulation of an immune response to them. Over a century ago Paul Ehrlich’s postulated that, if a compound could be made that selectively targeted against a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity (Ehrlich, 1900). The mouse hybridoma technology in 1975 as described by Köhler and Milstein was the triggering event that led to the development of antibody technology and emergence of therapeutic mAbs (Kohler and Milstein, 1975). This technology turned the magic bullet concept into a realistic option. Abs are grouped into five classes based on the sequence of their heavy chain constant regions: IgM, IgD, IgG, IgE and IgA (Louis et al., 2010). The mAbs are monospecific Abs that are the same because they are made by identical immune cells that are all clones of a unique parent cell. The Abs have monovalent affinity, in that they bind to the same epitope. They are structurally complex, and may have several functional domains within a single molecule, depending on the isotype (antigen-binding region, complement-binding region, constant part interacting

ABBREVIATIONS:
Antibodies: Abs; Antibody-dependent cell-mediated cytotoxicity: ADCC; Antibody drug conjugates: ADC; Biologics Price Competition and Innovation: BPCI; Biologics and Genetic Therapies Directorate: BGTD; Central Drugs Standard Control Organization: CDSCO; Central nervous system: CNS; Complement-dependent cytotoxicity: CDC; Constant fragment: Fc; Committee for Medicinal Products for Human Use: CHMP; Clinical trial: CT; Department of Biotechnology: DBT; Drug Controller General of India: DCGI; European Medicines Agency: EMA; European Pharmacopoeia: EP; European Union: EU; Federal Food, Drug and Cosmetic: FFDC; Food and Drug Administration: FDA; Fragment of antigen binding: Fab; Human anti-mouse antibodies: HAMA; International non-proprietary name: INN; Monoclonal antibodies: mAbs; National Health and Medical Research Council: NHMRC; Notice of allegation: NOA; Notice of compliance: NOC; Overall survival: OS; Overall Response Rate: ORR; Pharmacodynamic: PD; Pharmacokinetic: PK; Public Health Service: PHS; Progression free survival: PFS; Quality by design: QbD; Reference biological product: RBP; Reference Medicinal Product: RMP; Review Committee on Genetic Manipulation: RCGM; Similar Biologics products: SBPs; Subsequent Entry Biologics: SEBs; Tumor necrosis factor: TNF; United States: US; World Health Organization: WHO.
with Fc receptors). They are typically/traditionally made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen (Hybridoma technology). The desired Abs are later extracted and purified by separating the impurities/contaminants using various methods. Murine mAbs when injected into humans evoke an immune response which results in their rapid removal from the bloodstream/systemic inflammatory effects, and production of human anti-mouse antibodies (HAMA). Orthoclone OKT3® was the first such commercially approved therapeutic mAb. Murine analogues, contributed to the early lack of success of mAbs as they had a short half-life in vivo (due to immune complex formation), limited penetration into tumor sites, and inadequate host effector functions.

To overcome the HAMA responses occurring from the usage of murine mAbs, efforts were made to make mAbs more human-like and less immunogenic. In the early 1990s, using molecular biology techniques “chimeric” Abs were created by linking the murine genes encoding the antigen binding portion of the Ab (the variable region) to the genes encoding the constant region of human immunoglobulin light and heavy chains. Many of the mAbs approved for commercialization in the 1990’s and early 2000’s were chimeric Abs, including the anticancer Abs Rituxan® and Erbitux®, as well as the anti-inflammatory product Remicade®. Chimeric Ab products are superior to murine Ab products but they still pose a moderate risk of immunogenicity to patients from their residual murine components (Susan et al., 2007).

In 1991, Protein Design Labs developed and patented the first technology for successfully humanizing mAbs (Co and Queen, 1991). The resulting humanized Ab has the same antigen binding properties as the original murine Ab but contains minimal murine sequences and, therefore, elicits a lower HAMA response in patients. Two other potential advantages of humanized Abs are improved effector function and longer circulating half-life in patients. Some of the humanized antibody products which have been approved include Synagis®, Herceptin®, Mylotarg®, Xolair®, and Avastin®.

Further advancement in creating less immunogenic therapeutic Ab products was the ability to generate fully human mAbs using transgenic mice or phage display libraries (Lonberg, 2008). Human mAbs are produced by transferring human immunoglobulin genes into the murine genome, after which the transgenic mouse is vaccinated against the desired antigen, leading to the production of mAbs. Humira® was the first fully human mAb to be approved in 2002. Many mAb products approved or currently in clinical development are fully human. Raxibacumab (for inhalational anthrax) was under review by the US FDA/EMA (Aaron et al., 2010; Alejandro and Anthony, 2007; Andrew and Paul, 2010; Louis et al., 2010; Janice, 2012a; Xu-Rong et al., 2011). The first patient treated in the United States (US) with mAb therapy (a murine Ab, designated AB 69) was a patient with non-Hodgkin’s lymphoma—NHL (Nadler et al., 1980). Although treatment was not successful in inducing a significant clinical response, it did represent the first proof of principle in humans that a mAb could induce transient decreases in the number of circulating tumor cells, induce circulating dead cells, and form complexes with circulating antigen, all with minimal toxicity to the patient. During the 12 years period of 1985-1996, 16 human mAbs that could fit the selection criteria entered clinical development. By contrast, 131 human mAbs were first studied in the clinic during the following 12 years period (1997-2008) (Aaron et al., 2010). Presently around 29 mAbs are marketed in the European Union (EU) or the US.

Aside from targeting antigens that are involved in cancer cell proliferation and survival, mAbs can also function to either activate or antagonize immunological pathways that are important in cancer immune surveillance. The mAbs due to their high affinity and specificity, with a differential expression of target antigen in tumor cells versus normal cells makes an ideal agent for cancer immunotherapy. They have been used either as single agents; in combination therapy or as antibody drug conjugates (ADC) (Alejandro and Anthony, 2007).

Six mAbs were subsequently withdrawn or discontinued from marketing by their sponsors for various reasons (Table 2) (Janice, 2012a).

- Orthoclone OKT3®: Was discontinued by Janssen-Cilag due to availability of other treatments with similar efficacy and fewer side effects and declining sales (Janssen-Cilag, 2010).
- Panorex®: Was withdrawn by GlaxoSmithKline due to the product’s lack of efficacy (Punt et al., 2002).
- Zenpax®: Hoffman La Roche decided to stop making this drug in 2008. This decision was apparently taken in view of available alternative treatments and the diminishing market demand for Zenpax® and was not due to any safety issue.
- Raptiva®: The drug was found to be associated with risk of progressive multifocal leukoencephalopathy (PML), a fatal brain infection. It was withdrawn from the market by Genentech in 2009 (EMA, 2009; Major 2010).
- Mylotarg®: Pﬁzer voluntarily withdrew its product from the market in 2010 as the post-approval study required by the FDA that combined chemotherapy and Mylotarg®, did not demonstrate improved survival. Furthermore, the rate of fatal toxicity was higher in the combination arm compared with patients treated with chemotherapy alone (Bethan, 2010).
- Centoxin®: Was withdrawn by Centocor due to safety, efficacy and commercial reasons (McCloskey et al., 1994).

**Clinically Approved mAbs**

**mAbs as Therapeutics**

mAbs have been approved for use as therapeutics in a broad range of medical indications (Table 1) (Aaron et al., 2010; Alejandro and Anthony, 2007; Andrew and Paul, 2010; Louis et al., 2010; Janice, 2012a; Xu-Rong et al., 2011). The first patient treated in the United States (US) with mAb therapy (a murine Ab, designated AB 69) was a patient with non-Hodgkin’s lymphoma—NHL (Nadler et al., 1980). Although treatment was not successful in inducing a significant clinical response, it did represent the first proof of principle in humans that a mAb could induce transient decreases in the number of circulating tumor cells, induce circulating dead cells, and form complexes with circulating antigen, all with minimal toxicity to the patient. During the 12 years period of 1985-1996, 16 human mAbs that could fit the selection criteria entered clinical development. By contrast, 131 human mAbs were first studied in the clinic during the following 12 years period (1997-2008) (Aaron et al., 2010). Presently around 29 mAbs are marketed in the European Union (EU) or the US.

Aside from targeting antigens that are involved in cancer cell proliferation and survival, mAbs can also function to either activate or antagonize immunological pathways that are important in cancer immune surveillance. The mAbs due to their high affinity and specificity, with a differential expression of target antigen in tumor cells versus normal cells makes an ideal agent for cancer immunotherapy. They have been used either as single agents; in combination therapy or as antibody drug conjugates (ADC) (Alejandro and Anthony, 2007).

Six mAbs were subsequently withdrawn or discontinued from marketing by their sponsors for various reasons (Table 2) (Janice, 2012a).

- Orthoclone OKT3®: Was discontinued by Janssen-Cilag due to availability of other treatments with similar efficacy and fewer side effects and declining sales (Janssen-Cilag, 2010).
- Panorex®: Was withdrawn by GlaxoSmithKline due to the product’s lack of efficacy (Punt et al., 2002).
- Zenpax®: Hoffman La Roche decided to stop making this drug in 2008. This decision was apparently taken in view of available alternative treatments and the diminishing market demand for Zenpax® and was not due to any safety issue.
- Raptiva®: The drug was found to be associated with risk of progressive multifocal leukoencephalopathy (PML), a fatal brain infection. It was withdrawn from the market by Genentech in 2009 (EMA, 2009; Major 2010).
- Mylotarg®: Pﬁzer voluntarily withdrew its product from the market in 2010 as the post-approval study required by the FDA that combined chemotherapy and Mylotarg®, did not demonstrate improved survival. Furthermore, the rate of fatal toxicity was higher in the combination arm compared with patients treated with chemotherapy alone (Bethan, 2010).
- Centoxin®: Was withdrawn by Centocor due to safety, efficacy and commercial reasons (McCloskey et al., 1994).
### TABLE 1

Few of the approved therapeutic mAbs and fusion proteins.

<table>
<thead>
<tr>
<th>No.</th>
<th>Generic Name</th>
<th>Trade Name</th>
<th>Type/Format</th>
<th>First EU/US approval year</th>
<th>Clinical Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90Y-1biritumomab/Tuxetan</td>
<td>Zevalin®</td>
<td>Murine</td>
<td>2002</td>
<td>NHL</td>
</tr>
<tr>
<td>2</td>
<td>131I-Tositumomab</td>
<td>Bexxar®</td>
<td>Murine</td>
<td>2003</td>
<td>NHL</td>
</tr>
<tr>
<td>3</td>
<td>Abciximab</td>
<td>ReoPro®</td>
<td>Chimeric</td>
<td>1994</td>
<td>Adjunct to PCI for the prevention of cardiac ischemic complications</td>
</tr>
<tr>
<td>4</td>
<td>Basiliximab</td>
<td>Simulect®</td>
<td>Chimeric</td>
<td>1998</td>
<td>Prophylaxis of acute organ rejection in renal transplant</td>
</tr>
<tr>
<td>5</td>
<td>Brentuximab vedotin</td>
<td>Ad cetris®</td>
<td>Chimeric</td>
<td>2011</td>
<td>Hodgkin’s Lymphoma; Systemic anaplastic large cell lymphoma</td>
</tr>
<tr>
<td>6</td>
<td>Cetuximab</td>
<td>Erbitux®</td>
<td>Chimeric</td>
<td>2004</td>
<td>Colorectal Ca</td>
</tr>
<tr>
<td>7</td>
<td>Rituximab</td>
<td>MabThera®</td>
<td>Humanized</td>
<td>1997</td>
<td>NHL, RA</td>
</tr>
<tr>
<td>8</td>
<td>Inflimab</td>
<td>Remicade®</td>
<td>Chimeric</td>
<td>1998</td>
<td>CD, RA, PA, PP, AS, UC</td>
</tr>
<tr>
<td>9</td>
<td>Alemtuzumab</td>
<td>Campath-IH®</td>
<td>Humanized</td>
<td>2001</td>
<td>CLL</td>
</tr>
<tr>
<td>10</td>
<td>Bevacizumab</td>
<td>Avastin®</td>
<td>Humanized</td>
<td>2004</td>
<td>Colorectal, Ovarian &amp; Lung Ca</td>
</tr>
<tr>
<td>11</td>
<td>Certolizumab pegol</td>
<td>Cimzia®</td>
<td>Humanized</td>
<td>2008</td>
<td>RA, CD</td>
</tr>
<tr>
<td>12</td>
<td>Eculizumab</td>
<td>Soliris®</td>
<td>Humanized</td>
<td>2007</td>
<td>Paroxysmal nocturnal haemoglobinuria</td>
</tr>
<tr>
<td>13</td>
<td>Natalizumab</td>
<td>Ty sabri®</td>
<td>Humanized</td>
<td>2004</td>
<td>MS, CD</td>
</tr>
<tr>
<td>14</td>
<td>Ofatumumab</td>
<td>Campath-IH®</td>
<td>Humanized</td>
<td>2001</td>
<td>CLL, RA, AS, PA, PP, CD</td>
</tr>
<tr>
<td>15</td>
<td>Palivizumab</td>
<td>Orthoclone OKT3®</td>
<td>Murine</td>
<td>1986</td>
<td>Acute allograft rejection in heart, liver and renal transplantation</td>
</tr>
<tr>
<td>16</td>
<td>Edrecolomab</td>
<td>Panorex®</td>
<td>Murine</td>
<td>1995*</td>
<td>Colon Ca</td>
</tr>
<tr>
<td>17</td>
<td>Daclizumab</td>
<td>Zenpax®</td>
<td>Humanized</td>
<td>1997</td>
<td>For the prevention of rejection in renal transplantation</td>
</tr>
<tr>
<td>18</td>
<td>Efalizumab</td>
<td>Raptiva®</td>
<td>Humanized</td>
<td>2003</td>
<td>PP</td>
</tr>
<tr>
<td>19</td>
<td>Gemtuzumab ozogamicin</td>
<td>Mylotarg®</td>
<td>Humanized</td>
<td>2000</td>
<td>AML</td>
</tr>
<tr>
<td>20</td>
<td>Nabumetone</td>
<td>Celgosivir®</td>
<td>Humanized</td>
<td>1999*</td>
<td>H&amp;N Ca, Gioma, Lymphoma</td>
</tr>
<tr>
<td>21</td>
<td>Natalizumab</td>
<td>Tysabri®</td>
<td>Humanized</td>
<td>2001</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>22</td>
<td>Ofatumumab</td>
<td>Arzerra®</td>
<td>Humanized</td>
<td>2009</td>
<td>Colorectal Ca</td>
</tr>
<tr>
<td>23</td>
<td>Palivizumab</td>
<td>Orthoclone OKT3®</td>
<td>Humanized</td>
<td>1986</td>
<td>Acute allograft rejection in heart, liver and renal transplantation</td>
</tr>
<tr>
<td>24</td>
<td>Infliximab</td>
<td>Remicade®</td>
<td>Chimeric</td>
<td>1998</td>
<td>CD, RA, PA, PP, AS, UC</td>
</tr>
<tr>
<td>25</td>
<td>Alefacept</td>
<td>Amgen®</td>
<td>Humanized</td>
<td>2009</td>
<td>CAPS</td>
</tr>
<tr>
<td>26</td>
<td>Efalizumab</td>
<td>Raptiva®</td>
<td>Humanized</td>
<td>2003</td>
<td>PP</td>
</tr>
<tr>
<td>27</td>
<td>Alefacept</td>
<td>Amgen®</td>
<td>Humanized</td>
<td>2009</td>
<td>PP</td>
</tr>
<tr>
<td>28</td>
<td>Daclizumab</td>
<td>Zenpax®</td>
<td>Humanized</td>
<td>1997</td>
<td>For the prevention of rejection in renal transplantation</td>
</tr>
<tr>
<td>29</td>
<td>Natalizumab</td>
<td>Tysabri®</td>
<td>Humanized</td>
<td>2004</td>
<td>Multiple sclerosis</td>
</tr>
</tbody>
</table>

*Approval outside of EU/US

AML: Acute myeloid leukemia; AS: Ankylosing spondylitis; Ca: Cancer; CAPS: Cryopyrin-associated periodic syndromes; CD: Crohn’s disease; CLL: Chronic lymphocytic leukemia; H&N: Head and neck; JIA: Juvenile idiopathic arthritis; MM: Metastatic melanoma; MS: Multiple sclerosis; NHL: Non-Hodgkin’s lymphoma; PA: Psoriatic arthritis; PP: Plaque psoriasis; PCI: Percutaneous coronary intervention; RA: Rheumatoid arthritis; RSV: Respiratory syncytial virus; SLE: Systemic lupus erythematosus; UC: Ulcerative colitis

### TABLE 2

Monoclonal antibodies withdrawn/discontinued post approval.

<table>
<thead>
<tr>
<th>No.</th>
<th>Generic Name</th>
<th>Trade Name</th>
<th>Type/Format</th>
<th>First EU/US approval year</th>
<th>Clinical Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Murinomab</td>
<td>Orthoclone OKT3®</td>
<td>Murine</td>
<td>1986</td>
<td>Acute allograft rejection in heart, liver and renal transplantation</td>
</tr>
<tr>
<td>2</td>
<td>Edrecolomab</td>
<td>Panorex®</td>
<td>Murine</td>
<td>1995*</td>
<td>Colon Ca</td>
</tr>
<tr>
<td>3</td>
<td>Daclizumab</td>
<td>Zenpax®</td>
<td>Humanized</td>
<td>1997</td>
<td>For the prevention of rejection in renal transplantation</td>
</tr>
<tr>
<td>4</td>
<td>Efalizumab</td>
<td>Raptiva®</td>
<td>Humanized</td>
<td>2003</td>
<td>PP</td>
</tr>
<tr>
<td>5</td>
<td>Gentuzumab ozogamicin</td>
<td>Mylotarg®</td>
<td>Humanized</td>
<td>2000</td>
<td>AML</td>
</tr>
<tr>
<td>6</td>
<td>Nebucumab</td>
<td>Centoxin®</td>
<td>Human</td>
<td>1991</td>
<td>Sepsis</td>
</tr>
</tbody>
</table>

*Approval outside of EU/US
mAbs as Diagnostics

mAbs can also be used as disease-specific contrast agents for diagnostic imaging. Once mAbs for a given substance have been produced, they can be used to detect the presence of this substance. They are useful in immunohistochemistry, which detect antigen in fixed tissue sections and immunofluorescence test, which detect the substance in a frozen tissue section or in live cells. For diagnostic purposes, mAbs have been labeled with γ-emitting radionuclides and imaged with a single photon emission computerized tomography camera (Guaset al., 2007). Some of the mAbs clinically used for diagnostic purpose (mainly for staging disease in patients suspected of recurrent or metastatic cancer) are enlisted below (Table 3).

TABLE 3
Monoclonal antibodies approved as imaging agents.

<table>
<thead>
<tr>
<th>No.</th>
<th>Generic Name</th>
<th>Trade Name</th>
<th>Used for</th>
<th>First EU/US approval year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acritumomab</td>
<td>CEAScan™</td>
<td>Colorectal Ca</td>
<td>2003</td>
</tr>
<tr>
<td>2</td>
<td>Satumom-abpentetide</td>
<td>OncosciTM</td>
<td>Ovarian and Colorectal Ca</td>
<td>1995</td>
</tr>
<tr>
<td>3</td>
<td>Nofetumom-ahmerpentan</td>
<td>Verluma™ **</td>
<td>Staging Small cell lung Ca</td>
<td>1996</td>
</tr>
<tr>
<td>4</td>
<td>Imcirom-abpentatate</td>
<td>MyosciTM **</td>
<td>Identifying presence and location of Myocardial necrosis</td>
<td>1996</td>
</tr>
</tbody>
</table>

**No longer marketed by sponsor owning to economic consideration

mAbsBeing Evaluated in Phase III Studies

Modified Abs such as ADCs, biospecific Abs, Fc or glyco-engineered Abs and Ab fragments/ domains comprise 40% of the those mAbs in Phase II or Phase III clinical trials (CTs). Majority of these are undergoing evaluation as treatments for cancer or for immunological diseases; with very few being tried in other indications (Table 4). Among the other mAbs, Solanezumab and Bipinezumab are the ones being studied in Phase III setting (Janice MR, 2012b).

TABLE 4
Antibody-based therapeutics in Phase III clinical trials.

<table>
<thead>
<tr>
<th>No.</th>
<th>For Cancer indications</th>
<th>Immunological Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INN</td>
<td>Indication in Phase III</td>
</tr>
<tr>
<td>1</td>
<td>Girentuximab</td>
<td>Renal cell Ca</td>
</tr>
<tr>
<td>2</td>
<td>Elotuzumab</td>
<td>MM</td>
</tr>
<tr>
<td>3</td>
<td>Ohninituzumab</td>
<td>CLL, NHL</td>
</tr>
<tr>
<td>4</td>
<td>Inotuzumabgozabicin</td>
<td>NHL</td>
</tr>
<tr>
<td>5</td>
<td>Necitumumab</td>
<td>Non Small Cell Lung Ca</td>
</tr>
<tr>
<td>6</td>
<td>Pertuzumab</td>
<td>Breast Ca</td>
</tr>
</tbody>
</table>

Development of Biosimilar mAbs

Given the prohibitive development costs of novel mAbs, the high degree of risk from attrition in developing a molecule against a novel target and the imminent expiration of patents for some currently marketed mAb products, the decision of developing mAbs that recognize known, validated targets is becoming more lucrative. Couple of biosimilar mAbs has already been approved in few of the emerging markets. Dr. Reddy’s Labs launched Redix® in India as a biosimilar version of Genentech’s cancer drug Rituxan® (rituximab) way back in 2007. South Korea approved Clotinab®, from ISU Abxis, as biosimilar form of abciximab (ReoPro®) also in 2007. However, developing a biosimilar mAb has its inherent set of concerns/challenges, some of which are highlighted below:

A biosimilar developer of a mAb will need to establish an expression system that will produce the biosimilar product, and develop a commercial scale manufacturing process that will involve a closely monitored process of purification, formulation and testing of the product. A biosimilar mAb needs to have the identical amino-acid sequence and a similar glycosylation profile compared to a reference product. Defining the comparability of two mAbs (test and reference product) will require consideration of a wide range of aspects, including analytical and physicochemical characterization by several orthogonal methods, comparative biological assays and comparative immunogenicity assessment.

Being high molecular weight proteins (~150 kilo Dalton), they have highly complex secondary and tertiary structures, subject to post-translational modifications. Product heterogeneity is common to mAb production and is typically introduced either upstream during expression or downstream during manufacturing. These variants are typically aggregates, deamidation products, glycosylation variants, oxidized amino acid side chains, as well as amino and carboxyl terminal amino acid additions. These seemingly minute changes in an mAb structure can have a profound effect on preclinical stability and process optimization as well as therapeutic product potency, bioavailability and immunogenicity. Even small changes such as those relating to pH in cell cultures, temperature and culture media ingredients - may not seem significant, but can have an adverse impact on the final product making each step of manufacture, highly relevant (Janice and Alain, 2009).

As the clinical applications and needs for recombinant mAbs and Ab fragments have increased, the need for improvements in expression technologies to support these demands has correspondingly increased. Another reason for this extra demand for bio-manufacturing capacity is the dose requirement for the novel therapeutic humanized mAbs that are now being commercialized. The extra demand for production has to be met by construction of increased bioreactor capacity. Apart from capacity crunch, there is also a shortage of skilled qualified manpower to manage this. The manufacturers are also striving to develop final product...
formulations containing high concentrations of Ab with sufficient stability to address the increasing doses of Ab products without adversely impacting the quality of these products.

Vigorous competition is bound to be strengthened by expiring patents. However, one needs to be extra cautious as the mAbs are usually protected by ‘patent thickets’, i.e., multiple patents which cover not only the product itself, but also the formulation and the associated manufacturing processes (McCabe, 2009; Holliday, 2009). Patents also cover the technologies used to generate the mAbs (e.g., humanization, phage display technology, transgenic mice with a human immune repertoire), as well as the vectors and cell lines used to produce the mAbs (e.g., CHO, CHO-K1SV, GS-NS0)/(Chartrain and Chu, 2008). Estimated patent expiration dates for blockbuster mAbs and related products in the near offering are 2012 for etanercept; 2014 for infliximab; 2015 for rituximab, trastuzumab and palivizumab; 2016 for adalimumab; and 2017 for bevacizumab. Again the opinions of experts differ on when certain protein products will lose their patent protection, and unpredictable legal and legislative events could influence how biosimilar developers will navigate through the existing patent landscape.

As mAbs have diverse functional activities and could be possibly approved in diverse indications, extrapolation of efficacy across indications for a biosimilar mAb should be adequately justified. Reason being different indications can require different activities and receptors (or combinations of these) in different sites over different time courses, and in different pharmacologic milieu. As a consequence, mAbs with similar effects in one disease may have different effects in a second indication if the second indication involves a different mechanism of action. There are cases where two products share the same mechanism of action but still have different clinical activity. This is exemplified in the case of two marketed tumor necrosis factor alpha (TNF-alpha) antagonists, Enbrel® (etanercept; a fusion protein consisting of the p75 binding unit of the TNF-alpha receptor and the Fc portion of human IgG1) and Remicade® (infliximab; a chimeric anti-TNF-alpha IgG1 antibody). While Remicade® is efficacious in Crohn’s disease; Enbrel® is not, though both share the same mechanism of action which is TNF-alpha antagonism (Sandborn et al., 2001). Ideally, extrapolating data between indications should only be done when mechanisms of action in both indications are understood and highly similar, bearing in mind that implications of immunogenicity for mAbs are always potentially substantial.

Immunogenicity is among the primary concerns for the biotechnological medicinal products being developed as biosimilars and mAbs are no exception to that. The clinical consequences described following Ab development against mAbs include loss or reduction of efficacy, local reactions, serum sickness/immune complex-mediated disease, and major allergic reactions (e.g. urticaria, bronchospasm, bronchoconstriction). The severity of the consequences of these different reactions can be affected by the underlying health status of the patient. It is important during the clinical development to measure Ab levels, pharmacokinetic (PK), pharmacodynamic (PD) markers, efficacy and safety simultaneously and over a period of repeated treatments. This would allow assessment of the clinical significance of Ab development, and also whether the Ab effect changes over time. Every therapeutic mAb needs to be evaluated for immunogenicity individually and all immunogenicity strategies should be adapted for each mAb development program (EMA/CHMP/BWP/86289/2010).

Another point to consider is the design of the clinical comparative trial. An equivalence study to demonstrate biosimilarity against a reference mAb might be expected to require a large sample size of patients, and might thus be far more extensive than the pivotal trial for a stand-alone development. Alternatives like a non-inferiority trial against the reference mAb might in few cases be possible, but this also would depend on the approach/consideration adopted by the regulators toward the specific biosimilar product (Christian and Ulrich, 2008). Populations used for PK/PD measurement should be selected carefully because PK or PK/PD can be different due to mechanism of action, patient age, other medication or disease state. For example, PK and immunogenicity of mAbs are different in pediatric and adult patient populations.

Differences in starting materials, manufacturing processes and other characteristics mean that biosimilar mAbs, are likely to have attributes that cannot be detected through pre-market testing, e.g., rare adverse events (especially immunologically mediated events), or medically significant increases in such events. It is therefore important to consider what special post-market requirements should be imposed to facilitate detection of such events. The above factors need to be kept in mind while designing/developing a biosimilar mAb or related products/substances (like fusion proteins), their derivatives and products of which they are components e.g., conjugates.

### Applicable Regulations for Biosimilar mAbs and Related Products

#### Europe

The European regulatory system is based on Article 10(4) of Directive 2001/83/EC and Section 4, Part II, Annex I of this Directive. The European Union (EU) utilizes a centralized regulatory system for evaluation of all biotechnology-derived medicinal products since 2004. The Committee for Medicinal Products for Human Use (CHMP) has issued several guidelines to further delineate this framework. Guideline CHMP/437/04, the so-called overarching guideline puts forward the general concept of biosimilars. The scope of the guideline includes “any biological product,” and it explicitly also mentions complex biotech-derived medicinal products, such as immunologicals (vaccines), blood products and mAbs. EMA has been at the forefront of regulatory
agency activities concerning biosimilars including mAbs. As regards general regulatory requirements, European Pharmacopoeia (EP) documentation is available for mAbs, like the monograph for mAbs (EP Monograph 2031) or the monograph for products of recombinant DNA technology (EP Monograph 0784). Further guidance from CHMP for production and quality control of mAbs and on immunogenicity assessment for in vivo clinical use (EMA/CHMP/BMWP/86289/2010) is also available.

The principles mentioned in this guideline can be applied to related products/substances like, for example, fusion proteins. The requirements laid therein are detailed below:

**Non-clinical Requirements**

**Step 1**

*In vitro* studies should be conducted first and a decision then made as to the extent of what, if any, *in vivo* work will be required.

**In vitro studies**

Data from a number of comparative *in vitro* studies, some of which may already be available from quality-related assays, should be provided. *In vitro* non-clinical studies should include relevant studies on:

- Binding to the target antigens and binding to representative isoforms of the relevant three Fc gamma receptors, Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation or blockade).
- Fc-associated functions (e.g. antibody-dependent cell-mediated cytotoxicity (ADCC); complement-dependent cytotoxicity (CDC); complement activation).

These concentration/activity studies should be comparative in nature and should be designed to be sensitive enough to detect differences of importance in the concentration. Together these assays should cover all functional aspects of the mAb even though some may not be considered necessary for the therapeutic mode of action. As these assays may be more specific and sensitive than studies in animals, these assays can be considered paramount in the non-clinical comparability exercise.

**Step 2**

Identification of factors to consider need for additional *in-vivo* non-clinical studies. These factors include but are not restricted to:

- Presence of relevant quality attributes that have not been detected in the reference product (e.g. new post-translational modification structure).
- Presence of quality attributes in significantly different amounts than those measured in the reference product.
- Relevant differences in formulation, e.g. use of excipients not widely used for mAbs.

Although each of the factors mentioned above do not necessarily warrant *in vivo* testing, these issues should be considered together to assess the level of concern and need for *in vivo* testing.

**Step 3**

*In vivo studies*

If the comparability exercise in the *in vitro* PD studies in step 1 is considered satisfactory and no factors of concern are identified in step 2, an *in vivo* animal study is not considered necessary. However, if an *in vivo* study is deemed necessary, the focus of the study depends on the need for additional information, and the availability of a relevant animal model.

The possibility of performing *in vivo* comparative PK and PD studies depends on the characteristics of the product, and on the availability of a relevant animal species, or other relevant models and their sensitivity. A relevant species, as defined in EMA's Note for Guidance on preclinical safety evaluation of biotechnology derived pharmaceuticals (CPMP/ICH/302/95; ICH S6), is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope. Due to the specificity of mAbs the relevant species is in most cases a non-human primate species. The conduct of large comparative toxicity studies in non-human primate is not usually recommended. Also, the conduct of toxicity studies in non-relevant species (i.e. to assess unspecific toxicity only, based on impurities) is not recommended.

Quality differences due different production processes used by the biosimilar and reference medicinal product (RMP) manufacturers, may have an effect on immunogenic potential and potential to cause hypersensitivity. Immunogenicity assessment in animals is generally not predictive for immunogenicity in humans, but may be needed for interpretation of PK studies and toxicity findings (or lack thereof).

**Clinical Studies**

Clinical studies between the biosimilar and RMP mAb should always be conducted. The number and type of studies might vary according to the reference product and should be justified based on a sound scientific rationale. The guiding principle is to demonstrate similar clinical efficacy and safety compared to the RMP, not patient benefit per se, which has already been shown for the reference medicinal product. Extrapolation of clinical efficacy and safety data to other indications of the RMP (not specifically studied during the clinical development of the biosimilar mAb) is possible based on the results of the overall evidence of comparability provided from the comparability exercise and with adequate justification. A stepwise approach is normally recommended all through.

**Pharmacokinetics**

**Step 1**

A comparative PK study in a sufficiently sensitive and homogeneous study population (healthy volunteers or patients) normally forms an initial step of biosimilar
mAb development. PK data can be helpful to extrapolate data on efficacy and safety between different clinical indications of the reference mAb. It may, on a case-by-case basis, be necessary to undertake multidose PK studies in patients, or even to perform PK assessment as part of the clinical study designed to establish similar efficacy and safety.

If patient population is used, the choice should be justified. Factors that may influence the choice of the patient population are age of usual manifestation, age range, number of previous treatments, concomitant treatments, or expression of antigen. For mAbs licensed in several clinical indications, it is not generally required to investigate the pharmacokinetic profile in all of them. However, if distinct therapeutic areas are involved for one particular mAb (e.g., autoimmunity and oncology), separate PK studies may be needed if different target-mediated clearance exists for different therapeutic areas.

**Pharmacodynamics**

PK studies can be combined with PD endpoints, where available. PD parameters may contribute to the comparability exercise for certain mAbs and uncertain indications. Depending on the mAb and availability of PD endpoints the PD markers can act as either support to establish comparability or as pivotal proof for the efficacy comparability exercise. In case of latter, applicant will have to choose clinically relevant markers, justify these markers, and also provide sufficient reassurance of clinical safety, particularly immunogenicity. If PD markers cannot constitute the pivotal evidence of comparability, then sponsor needs to proceed to Step 2 (i.e., clinical efficacy).

**Clinical efficacy**

**Step 2**

If dose comparative and highly sensitive PD studies cannot be performed convincingly showing comparability in a clinically relevant manner, similar clinical efficacy between the similar and the RMP should be demonstrated in adequately powered, randomized, parallel group comparative CTs, preferably double-blinded and normally equivalence trials. The inclusion of patients from non-European countries is generally possible if there are no intrinsic differences, but it may increase heterogeneity.

**Clinical safety**

Clinical safety is important throughout the clinical development programme and is captured during initial PK and/or PD evaluations and also as part of the pivotal clinical study establishing comparability. Care should be given to compare the type, severity and frequency of the adverse reactions between the biosimilar mAb and the RMP, particularly those described for the reference product.

**Pharmacovigilance**

For the marketing authorization procedure the applicant should present a risk management plan/pharmacovigilance plan in accordance with current EU legislation and pharmacovigilance guidelines. The risk management plan should consider the probability of the biosimilar and RMP being used interchangeably or being switched in a clinical setting.

**United States**

For historical reasons, most biologic products marketed in the US have been approved under the Public Health Service (PHS) Act, which has no pathway similar to that described in Section 505(b)(2) of the amended Federal Food, Drug and Cosmetic (FFDC) Act. This applies for mAbs too (with the exception of gemtuzumabozogamicin). The US Senate passed an Act in March 2010 to create a pathway for approval of biosimilars in the country, as part of the Biologics Price Competition and Innovation (BPCI) Act of 2009. In Feb 2012, the US FDA issued three draft guidance documents on biosimilar product development to assist industry in developing such products in the US. These draft guidance’s cover scientific and quality considerations in demonstrating biosimilarity to reference product. Though not specific to mAbs, the principles described in these draft guidance will be applicable to biosimilar mAbs as well. Overall, these draft guidance’s describe a risk-based, totality-of-the-evidence, stepwise approach that the FDA intends to use in support of a determination of biosimilarity (Bobby, 2012).

**Canada**

The Biologics and Genetic Therapies Directorate (BGTD) within the Health Products and Food Branch of Health Canada is the regulator of biologic drugs for human use. On March 5, 2010, Health Canada released its finalized guidance document for Subsequent Entry Biologics (SEBs)/an alternate term for biosimilars used in Canada, titled “Guidance for Sponsors: Information and Submission Requirements for SEBs” creating a workable approval pathway for ‘highly similar’ versions of biologic drugs already approved for the Canadian market (Health Canada, 2010). Regulatory decisions regarding SEBs will be based on the Food and Drugs Act and Regulations. While the biologic drug authorized in Canada is preferable for comparative studies, it may accept use of non-Canadian reference biologic comparative studies with adequate justification provided the non-Canadian reference biologic drug must be from a jurisdiction that has an established relationship with Canada. Prior to receiving a notice of compliance (NOC) from Health Canada, which is required before marketing, SEBs drug manufacturers are required pursuant section 5 of the PM(NOC). Regulation 5 to address each patent listed on the Patent Register in respect of the innovator’s reference drug by either accepting that the NOC will not issue until the patent expires, or by sending a notice of allegation (NOA) alleging that the innovator has made a false statement, or that the patent has expired, will not be infringed or is invalid. The NOA triggers the provisions of the PM(NOC) Regulations and the issuance
of the NOC to the generic/SEBs is put on ‘patent hold’, until a proceeding resulting from the NOA is resolved.

World Health Organization (WHO) guidelines

WHO published guidelines on evaluation of similar biotherapeutic products (SBPs) with detailed recommendations on clinical development in October 2009 (WHO, 2009). It provides globally acceptable principles for licensing biotherapeutic products that are claimed to be similar to biotherapeutic products of assured quality, safety, and efficacy that have been licensed based on a full licensing dossier. This guideline applies to well-established and well-characterized biotherapeutic products such as recombinant DNA-derived therapeutic proteins. Again, there is no separate guideline from WHO for developing biosimilar mAbs.

India

Indian government has also taken several initiatives towards streamlining the way biosimilars will be regulated with the intention to harmonize as much as possible with other competent regulatory agencies and international organizations such as the WHO. In India, apart from Central Drugs Standard Control Organization (CDSCO), the office of Drug Controller General of India (DCGI), the Review Committee on Genetic Manipulation (RCGM), which works under Department of Biotechnology (DBT) are involved in the approval process of Similar Biologics products (SBPs)/an alternate term for biosimilars used in India (Bobby, 2012). The September 2011 draft “Indian guidelines on similar biologics: Regulatory requirements for marketing authorization”, after series of deliberations with key stakeholders has been refined further in May 2012. The final version is released by CDSCO and is effective from mid Sep 2012.

Other Regulatory Agencies

Some of the other countries who have come up with regulations/guidelines for governing biologics/biosimilar, drawing heavily from the EMA and WHO guidelines (with the corresponding period of issue) include Malaysia (July 2008), Turkey (Aug 2008), Australia (Aug 2008) Taiwan (Nov 2008), Japan (Mar 2009), Venezuela (Aug 2000), Brazil (Oct 2005), Argentina (Jul 2008), Mexico (Oct 2008), Saudi Arabia (Aug 2008) etc. The National Health and Medical Research Council (NHMRC, under Government of Australia) has also published guidelines for mAb production to assist institutional animal ethics committees in evaluation of applications involving mAb production (NHMRC, 2008). The key principles of regulating biosimilars have been the same across different agencies. They all emphasize the fact that the development of a biosimilar involves stepwise, risk based, comparability exercise(s). Demonstration of similarity in terms of quality is a prerequisite for the reduction of the non-clinical and clinical data set required for licensure. After each step of the comparability exercise, the decision to proceed further with the development of the biosimilar mAb should be evaluated. If relevant differences are found in the quality, non-clinical, or clinical studies, the product will not likely qualify as a biosimilar and a more extensive non-clinical and clinical data set will likely be required to support its application for licensure.

Business Prospects

The yearly sales of mAbs have galloped from $8–10bn in 2005 (Lawrence, 2007) to $27bn in 2007 (Andrew and Paul, 2010). In 2009, the global mAb market was worth an estimated $36 bn and between 2009 and 2015, the market is forecast to expand to $63 bn (Datamonitor). Currently quite a handful of biosimilar mAbs are in different phases of development. The vast majority of these mAbs are in preclinical space while others are in different phases of CTs. Most of these are targeted to oncology indications (e.g. alemtuzumab, bevacizumab, ofatumumab, panitumumab, rituximab, trastuzumab, etc.) while the remaining (e.g. ustekinumab, tocilizumab, omalizumab, natalizumab and infliximab, pavlivizumab, natalizumab and denosumab to name a few) are indicated for autoimmune/inflammation, metabolic, infectious disease and CNS disorders. While some companies have gone public in announcing their pipeline of drugs others have preferred to keep their development programme under wraps. Few of the companies actively working in the biosimilar mAb space are enlisted below (Table 5) (Fren, 2011).

<table>
<thead>
<tr>
<th>No.</th>
<th>Country</th>
<th>Companies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Brazil</td>
<td>PharmaPraxis</td>
</tr>
<tr>
<td>2.</td>
<td>Canada</td>
<td>PlantForm</td>
</tr>
<tr>
<td>3.</td>
<td>China</td>
<td>SSBio</td>
</tr>
<tr>
<td>4.</td>
<td>Hungary</td>
<td>Gedeon Richter</td>
</tr>
<tr>
<td>5.</td>
<td>India</td>
<td>Biocon; Cipla; Dr Reddys Labs; Intas Biopharmaceuticals; Reliance Life Sciences; ZyusCadilla</td>
</tr>
<tr>
<td>6.</td>
<td>Israel</td>
<td>Teva</td>
</tr>
<tr>
<td>7.</td>
<td>Japan</td>
<td>Daiichi Sankyo ; Gene Techno Science</td>
</tr>
<tr>
<td>8.</td>
<td>Mexico</td>
<td>Probiomed</td>
</tr>
<tr>
<td>9.</td>
<td>Russia</td>
<td>BioCad</td>
</tr>
<tr>
<td>10.</td>
<td>South Korea</td>
<td>Celltrion; Hanwha Chemical; LG Life Sciences; Samsung Biologies</td>
</tr>
<tr>
<td>11.</td>
<td>Switzerland</td>
<td>BioXpress; Cerbios-Pharma; Novartis</td>
</tr>
<tr>
<td>12.</td>
<td>US</td>
<td>Biogen Idec; Hoeplira; Mylan; Spectrum</td>
</tr>
</tbody>
</table>

Being a capital intensive business, companies are using different strategies with some opting for collaboration so as to utilize each other’s complementary expertise for example in process development; characterization and analytical testing, patent search and filings; regulatory submissions, conducting PK/PD studies/CTs; marketing etc. The technologies enabling the generation of human Abs are also now accessible through partnerships or licensing from the companies.
that have developed these approaches. The acquisition of mAb technology companies, including Abgenix, Cambridge, Antibody Technology and Medarex, by major drug companies is an indication of the industry’s increasing interest in human mAb therapeutics (Aaron et al. 2010).

Unlike small molecule generics where the price drops by ~70%-75% one year post generic introduction, it is only about 25%-30% for biosimilars. With emerging markets such as India, China, Latin America and Eastern Europe being more cost effective, many of the biosimilar manufacturers are eyeing them. Apart from less stringent regulatory requirements, availability of substantial manufacturing capacities and substantial cost savings are the other principal factors driving companies towards these markets. According to EMA’s list of applications for new human medicines under evaluation by the CHMP released in April 2012, the agency will be reviewing a new drug application for a biosimilar version of infliximab (Remicade®). This is the first application for a biosimilar mAb submitted to EMA.

Not everyone is joining the biosimilars race. Some like BiOasis of Canada; ProtevoBio of US are choosing instead to focus on developing “biobetters”, targeting to improve upon the original molecule by increasing safety, half-life or overall efficacy of the drug to compete with the biosimilars. Again, the regulatory approval process for a “biobetter molecule” needs to be clearly defined.

**Future Outlook**

The concept of ‘magic bullet’ has translated to clinical reality thanks to Abs. The complexity of mAbs is a challenge (in terms of expertise, time and cost) for the development of new mAb products that are claimed to be similar to marketed mAbs as compared to that for generic small-molecule drugs or less complicated biosimilar products. The future promise of mAbs in different therapeutic indications is dependent on having a better understanding of the lessons learned from laboratory studies, on applying innovative approaches to target and Ab selection and on early phase CTs that will guide appropriate development strategies, leading to clinical benefit in the relevant patient population. It would not be surprising if we have in a few years’ mAbs having lower or less frequent dosing; having ability to have better penetration through blood brain barrier; being orally administered; having sustained release delivery; having enhanced effector functions etc. It is highly recommend that companies intending to develop biosimilar mAbs seek regulatory scientific advice early in the development process. The implementation of Quality by Design (QbD) and other new regulatory concepts would help in reducing the cost and development timelines. The initiatives been taken by different regulatory agencies would ensure more affordable biosimilar drugs including mAbs being manufactured and made available to the needy patients.

In the coming years, it will be interesting to watch the various strategies used by drug companies to enter into race for development of biosimilar mAbs and see which ones will prove the most successful for competing in the marketplace. The next generation of mAbs is poised to yield effective new treatments either alone or in combination based on the identification and validation of new targets, the manipulation of host microenvironment interactions, and the optimization of Ab structure. Modified Abs could dominate the new era of Ab therapy. The era of Ab-based therapy is here to stay!

**Acknowledgments**

I greatly acknowledge the encouragement and support of Reliance Life Sciences Pvt. Ltd. in carrying out this work (www.rellife.com).

**Disclosure of potential conflict of interest**

The author indicates no potential conflict of interest.

**References**


WHO Guideline on evaluation of similar biotherapeutic products (SBPs), Oct. 2009.


Address correspondence to: Dr. Bobby George, Assistant Vice President & Head Regulatory Affairs, Reliance Life Sciences Pvt. Ltd., Dhirubhai Ambani Life Sciences Centre, R-282, Thane Belapur Road, Rabale, Navi Mumbai – 400 701, Maharashtra, India.

E-mail: bobby.george@relbio.com, Tel: +91 22 40678770