Monoclonal Antibody Therapeutics – The Next Generation

Monoclonal antibodies have already transformed the treatment of many diseases; their future potential will depend on how successful new technologies are at addressing issues such as the need for parenteral administration and manufacturing costs.

By Dr David Milroy at ITI Life Sciences

David Milroy is Market Analyst Manager at ITI Life Sciences, the Scottish life sciences ‘innovation fund’. His role is to conduct ‘foresighting’ to identify commercially attractive areas that can be exploited through technical innovation and the generation of intellectual assets. The opportunities identified as a result of ITI’s foresight have culminated in the initiation of a number of programmes and a committed spend to date of almost £45m. Prior to joining ITI Life Sciences, Dr Milroy was a Senior Consultant with Wood Mackenzie, a global provider of research and consulting services to the pharmaceutical and biotechnology industries. He holds a degree in Pharmacy, a PhD in Molecular Biology (Bath University) and an MBA (University of Edinburgh Management School).

The discovery of monoclonal antibodies in the 1970s and the realisation of their therapeutic potential was a key milestone in the evolution of the biotech industry. Since then, a myriad of academic groups and companies have ploughed vast sums of money into refining antibody technologies and developing new generations of affinity molecules designed to more effectively diagnose, monitor and treat a wide range of diseases. This article looks at the current market for monoclonal antibodies and the technologies being developed to enhance their future therapeutic potential.

THE mAb SECTOR

The current biotech market consists of two major classes of biotherapeutics: the recombinant proteins, which includes blockbuster products such as EPO, and the monoclonal antibodies (mAbs). Over the next decade, growth of the latter class is expected to far outstrip that of the larger pharma market and will be a major driver for the biotech market. Most commentators agree that sales of monoclonal antibody therapeutics will exceed $30 billion in 2010, and if that weren’t sufficient to illustrate the importance of this sector, then one simply has to look at the explosion of deals within this space. Companies such as AstraZeneca and Pfizer, which were slower to enter the mAb field than some of their peers, have been much more bullish of late (for example, AstraZeneca’s proposed $1.3 billion acquisition of Cambridge Antibody Technology) and are now keen to stress their credentials in this area.

While the mAb sector can boast several blockbuster products and more than 200 mAb-based medicines in clinical trials, it is the impact that these products are having on disease management that is arguably more important and that will dictate the ultimate success of the sector. The treatment of conditions such as Non-Hodgkin’s Lymphoma (NHL) has been revolutionised by the emergence of mAbs. If the ability of a mAb therapeutic to secure a first-line treatment label can be used as a proxy for efficacy and/or meeting an unmet clinical need, then several antibodies have undoubtedly demonstrated their utility.

In addition to Rituximab’s approval as a first-line treatment for NHL, several other mAbs (for example, Herceptin and Avastin) are the first therapies to be deployed for specific types of cancer. Moreover, the anti-TNF mAbs, Humira and Remicade, have proved highly effective in improving the signs and symptoms of rheumatoid arthritis (RA), but also attenuating the

Table 1: Key challenges for those operating in the monoclonal antibody sector

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<tr>
<th>MAb Therapeutics are very expensive and therefore cost containment and reduction will be a major driver in this sector:</th>
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<tr>
<td>Their molecular complexity means they are comparatively expensive to manufacture</td>
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<td>The plasma concentration of mAbs required for therapeutic efficacy ranges from 100µg/L to 1-2mg/L for Rituxan, whereas Biologicals require much lower concentrations</td>
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<td>Many mAb drugs need to be used in combination with existing regimes such as chemotherapy (oncology settings), increasing the overall treatment cost</td>
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<th>Lack of novel, therapeutically relevant antibody targets:</th>
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<tr>
<td>“When it comes to antibody targets, the low hanging fruit is gone”</td>
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<tr>
<td>Companies are having to be more innovative in identifying novel targets for new antibody-based therapeutics</td>
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<tr>
<td>Technologies that can open up new classes of targets will be valuable</td>
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Despite possessing desirable properties such as high specificity and relatively low toxicity, mAbs have innate limitations:

- Bulky molecules – they can’t enter cells, penetrate deep into tissue or reach targets buried in other structures
- They can’t be administered orally
- Their binding to targets is reversible, so that they have to be given in concentrations on the order of 5,000-10,000 times the concentration of the target to be effective

Success has created an IP minefield
progression of joint damage, improving quality of life and preserving functional status.

If this alone weren’t compelling enough, then one final characteristic should be decisive – namely that mAb therapeutics often secure multiple labels (that is, they are approved for more than one indication). Abbott has on several occasions referred to its mAb Humira (originally approved for RA) as a ‘pipeline in a drug’ – and Humira is not alone. Another approved anti-TNF antibody, Remicade, is approved for ulcerative colitis, Crohn’s disease, ankylosing spondylitis and psoriatic arthritis in addition to its approval for RA. Similarly, a number of mAbs that have secured approval for one type of cancer are being investigated in numerous other cancers, and the previously mentioned Rituximab (approved for various NHL indications) has also recently joined the growing list of mAbs approved for RA.

**KEY CHALLENGES**

It should be clear by this point that mAbs have become important weapons in the clinicians’ arsenal. What is less clear is what the future holds for these multi-faceted proteins. The antibody has been through a number of incarnations and is likely to undergo further engineering to address current challenges (see Table 1) and to broaden its utility. It is likely that the next chapter in the mAb story will be about addressing some of the drawbacks of mAbs, including the need for parenteral administration and the significant costs associated with manufacturing these protein therapeutics.

The manufacturing issue is critical, as many countries try to contain their drug spend by erecting a final hurdle in the form of health economics to look at cost as well as clinical effectiveness. As a result, health services and insurers may refuse to reimburse certain expensive antibodies based on a cost-benefit analysis. For example, Humira costs $16,000 per year whereas one of the drugs it often replaces, methotrexate, costs $500 for a year’s treatment. In Europe, although mAbs may possess clear advantages over conventional drugs, lower-priced small molecule drugs remain the first-line treatment choice in many instances, and the case for expensive mAbs is particularly weak where the condition is perhaps not immediately life-threatening.

Interestingly, those studies that attempt to factor in all the costs associated with treating a specific disease suggest that various mAb therapeutics such as those prescribed for RA are indeed cost-effective. Positive pharmaco-economic evidence is critical to securing reimbursement, and it remains to be seen whether other mAbs can surmount this final hurdle.

**ENHANCING THE POTENTIAL OF NEXT GENERATION TECHNOLOGIES**

**Improved or Alternative Expression Systems**

Due to the success of first-generation mAb technologies, there is now a plethora of companies offering technologies with the potential to deliver improved mAb therapeutics. These include the many companies – such as Biolex (plant-based system), GlycoFi (yeast system, currently being acquired by Merck & Co) and Viragen (avian transgenic system) – that are developing improved or alternative expression systems.

**Improved Manufacturing Processes**

In addition to developing very efficient expression systems, other companies are seeking to address manufacturing issues. For example, disposable plastic bioreactors may eliminate much of the expense and time associated with cleaning and revalidating steel reactors between production batches, and significant effort is being devoted to downstream process development, particularly purification – an area that companies such as GlaxoSmithKline believe is a substantial obstacle to bringing down the cost of goods.

**Increasing Potency**

Other players including BioWa (a wholly-owned subsidiary of Kyowa Hakko Kogyo), GlycArt (part of Roche), GlycoFi and Xencor are focusing on increasing the potency of antibodies by increasing their antibody-dependent-cell-mediated-cytotoxicity (ADCC). This can be achieved by engineering the molecule’s constant region (the region of the mAb that interacts with immune cells causing ADCC). ADCC activity is one of the major anticancer mechanisms of launched therapeutic mAbs such as Herceptin and Rituxan, and increasing the ADCC activity should lower costs and reduce side effects, as enhanced efficacy will enable low-dosage treatment.

**Polyclonal Antibodies**

There has also been something of a renaissance in developing technologies for generating better-defined polyclonal antibodies. Widespread use of polyclonals from blood has been limited due to constrained supply, as well as problems with batch-to-batch variations and also safety issues. However, despite these drawbacks, polyclonals do have attractive features – such as effective target clearance, as they can work through a combination of actions (neutralisation, improved phagocytosis or ADCC, and increased complement activation), and infectious agents and cancer cells are less likely to escape immune recognition by polyclonals through mutation.
This has resulted in a number of companies taking a fresh look at polyclonals. For example, Hematech (part of Kirin Brewery) is developing technologies to produce human-like polyclonal antibodies in transgenic cows. However, these transgenic animal technologies have limitations, such as the need for purification (to remove unwanted animal proteins), and the fact that the transgenic hyperimmune immunoglobulins (Igs) will contain a significant fraction of irrelevant, normal non-specific Ig.

Companies such as Symphogen and Merus are taking a different tack, and have developed technologies for identifying and reproducibly manufacturing recombinant polyclonals. These polyclonals should be active against complex antigens and retain activity in the event of antigen mutation (potentially useful therapeutics for infectious diseases). They will also have a better safety profile than plasma-derived products.

**mAb-Based Targeting**
Since virtually all anticancer agents are associated with dose-limiting toxicities, significant interest has surrounded the possibility of improving drug/radiation efficacy while minimising systemic toxicity through mAb-based targeting strategies. Consequently, antibodies have been conjugated to a variety of molecules including cytotoxic drugs, toxins, radionuclides and a variety of other molecules (cytokines/chemokines, peptides, proteins, enzymes, liposomes and viruses).

However, it has only been in the past few years that the critical parameters for optimisation have been identified and begun to be addressed. Several promising new agents comprising potent anticancer drugs attached to mAbs through optimised linker technologies are showing unprecedented activities in preclinical models, and many of these agents have entered into clinical trials.

**Intrabodies**
Yet another area of development is the functional expression of intracellular mAb fragments (termed intrabodies). This area offers the potential to bring many more targets within the reach of mAbs. For example, intrabodies could be used to ablate or modify crucial transcriptional and translational regulators. While significant progress has been made (for example, achieving functional folding of the mAb scaffolds in the reducing intracellular environment), as with gene therapy strategies, the main obstacle is likely to be delivery of the expression vector. An alternative to gene delivery could involve targeting intracellular antigens directly by fusion of the intrabody to a membrane translocator sequence, a naturally internalising mAb or by direct selection for internalisation.

**Bispecific Antibodies**
Bispecific antibodies (bisAbs) comprise two different binding specificities fused into a single molecule, and are being refined by companies such as Micromet. They can be designed either to bind two adjacent epitopes on a single antigen, thereby increasing both avidity and specificity, or to bind two different antigens for numerous applications – but particularly for recruitment of cytotoxic T-cells and natural killer (NK) cells, or re-targeting of toxins, radionuclides or cytotoxics. The ability to design multivalent specificity into mAb fragments is also enabling the re-emergence of pre-targeting for cancer therapy. A mAb fragment bispecific for a tumour marker and a hapten are first infused to specifically localise the bisAb fragment at the tumour site; subsequently, a drug-hapten conjugate or radiolabeled hapten is introduced, which then binds to the bisAb.

**Fragment Technologies**
One of the most exciting trends within the mAb sector is the refinement of existing, and development of new, mAb fragment technologies. Antibody fragments come in a variety of shapes and sizes (see Figure 1) and, depending on the characteristics sought, one can choose the most appropriate format.

Fragments have a number of attractive features; for example, many pathogenic viruses have evolved narrow cavities in their surface antigens that bind their target receptor but are poorly accessible to intact antibodies, and are therefore largely immunosilent. Other targets inaccessible to conventional antibodies include G protein-coupled receptors and enzyme active sites. Single variable domains offer a potential strategy for these blind-spots or what are called ‘cryptic’ epitopes.

Unfortunately, such fragments don’t normally retain the affinity of the parent antibody, and suffer from poor solubility and a tendency to aggregate – although these issues are starting to be addressed. However, the camelids (camels, llamas) and cartilaginous fish are known to have evolved high-affinity single variable domains mounted on constant domain frameworks as a crucial component of their immune system. The variable domains of these...
molecules can penetrate cavities in target antigens, such as enzyme active sites. Moreover, the molecules are in general soluble and stable.

Two companies are leading the development of these domain antibodies, namely Ablynx (camelid domains, better known as nanobodies) and Domantis. Not only are these domains able to target cryptic epitopes but they also exhibit high stability – offering the potential for oral administration. Both Ablynx and Domantis are advancing programmes examining oral and inhaled domains, and while major questions remain – such as the likely dose required – if either company could demonstrate proof of concept, then this would be a major milestone for the mAb sector and one that could drastically expand the market opportunity.

A number of the drawbacks to using fragments (see Table 2) have been addressed. For example PEGylation (that is, conjugating in a highly controlled fashion the polymer PEG to a fragment) can be used to modify the half-life of the fragment. MAbs mediate their therapeutic effect either by blocking a target or by exerting effector functions residing in the Fc region to activate complement or cytotoxic cells. However, fragments do not have effector functions and therefore much of the focus has been on target neutralisation.

While there are a number of therapeutic fragments on the market – including ReoPro (GPIIb/IIIa), DigiFab, Digibind and CroFab – we have seen some time since the launch of a new therapeutic fragment. Moreover, the revenue generated by the launched fragments has been modest. However, this is likely to change with the recent approval of Genentech’s and Novartis’ Lucentis (ranibizumab) for the treatment of age-related macular degeneration (AMD).

During 2006, approval is also likely for UCB’s PEGylated humanised antibody fragment against TNF-alpha, Cimzia (certolizumab), to treat Crohn’s disease. If both products are successfully approved, then the antibody fragment market will grow significantly – fuelling further interest in the area.

### Table 2: Some of the potential advantages and disadvantages of fragments (depends on the exact fragment format chosen)

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<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>High solubility and stability</td>
<td>Reduced affinity compared to IgGs (poor target retention time)</td>
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<tr>
<td>Accessible to pulmonary and oral administration</td>
<td>Plasma half lives of &lt;1hr (2-3 weeks for an IgG) as a result of size reduction (&lt;70kD glomerular filtration cutoff) and removal of Fc region (mediates recycling through FcγR receptor)</td>
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<tr>
<td>Reduced costs of goods through manufacture in yeast and bacteria</td>
<td>No effector function (complement dependent cytotoxicity (CDC) or antibody cellular cytotoxicity (ADCC))</td>
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<tr>
<td>Small size leads to better tissue penetration and allows access to cavities on receptor targets</td>
<td>Potential immunogenicity</td>
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<tr>
<td>Freedom to operate</td>
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### Non-Antibody Affinity Ligands

In parallel with work on fragments, several companies have been developing molecular scaffolds that are not Igs, but can bind with similar high affinity and specificity to targets. These molecular scaffolds can be roughly divided into the immunoglobulin-like scaffolds (proteins with a conserved framework region and a highly variable antigen-binding site, some of which are from non-immunoglobulin adaptive immune systems) and other frameworks (not involved in immunity).

Companies such as Pieris (developing lipocasins) and Compound Therapeutics (fibronectin) are developing immunoglobulin-like scaffolds, while Affibody (protein A), Biorexis (transferrin), Archemix (aptamers), Borean (tetranectin) and several other companies are developing alternative scaffolds. These scaffolds may start to bridge the gap between mAbs and small molecules, combining the most desirable characteristics from each group.

The challenge lies in determining what is possible using these scaffolds, and for which applications they may prove to be superior to antibodies. Several scaffolds are now in development, including Affibody® molecules (derived from an Ig-binding domain of Staphylococcus aureus protein A) and those of Dyax, which has DX-88, a selective and high affinity inhibitor of human plasma kallikrein – in clinical trials.

### EVOLVING THE NEXT GENERATION

It is clearly going to take time for many of the companies developing next-generation technologies to demonstrate proof-of-concept, and longer still to gain access to targets and initiate candidate development programmes. However, there is increasing interest in these areas from large pharmaceutical companies and from investors who recognise the enormous potential of next generation technologies in enhancing the diagnosis, treatment and management of many diseases. At this stage, it is impossible to say whether it will be enhanced antibodies, antibody fragments or small-molecule-like affinity molecules that will emerge as the leading technology; it is likely that different technologies will be best suited for different applications. The overriding message is that if these next-generation technologies can reduce the cost of mAb therapeutics or reach previously untargetable targets, then they will surely have a bright future.

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