Monoclonal antibodies (mAbs) have become a crucial tool, used for both diagnostic and medical applications. The technology was pioneered by César Milstein and Georges Köhler, who in 1975 successfully fused immortal myeloma cell lines with antibody-producing B-cells to produce hybridomas. In 1986, the first mAb therapy, Muromonab-CD3, was approved for use in humans. This is a complete, unmodified mouse antibody, and is one of only four fully murine therapeutic mAbs to be approved for clinical use. Murine antibodies have a short therapeutic half-life because they are recognised by the patient immune system as foreign proteins resulting in a human anti-mouse (HAMA) response.

**Sequence Modification**

Since the 1986 landmark, a variety of antibody engineering techniques have been employed to reduce immunogenicity and the HAMA response. A chimeric antibody, for instance, is one which has mouse variable domains, but human constant domains. As chimeric antibodies are about 70% human, they are not as readily cleared by the patient’s immune system. To date, seven chimeric antibodies have been approved, the most recent being Novartis’ Cosentyx® (Secukinumab), used to treat moderate to severe plaque psoriasis (raised, silvery flaking of the skin) in adults.

However, despite some specific-use cases, chimeric antibodies are generally still too immunogenic to be used as therapeutic antibodies; further modification of the antibody sequence is required to reduce patient immune response.

Humanisation is a process by which xenogeneic antibody sequences are modified to reduce this immunogenicity, and several approaches have been developed since the first approved.
humanised antibody, Daclizumab, in 1997. Since the 2002 approval of Adalimumab generated by phage display technology, and the 2006 approval of Panitumumab from transgenic mice, there have also been two other technologies capable of producing fully human antibodies.

This article discusses these three technologies – complementarity determining region (CDR) grafting, phage display, and approaches using transgenic mice – in an attempt to answer the question: why do we still need to humanise murine antibodies in the 21st century?

**CDR Grafting**

The CDRs are the hypervariable ‘ends’ of an antibody which are responsible for where antibodies bind to a specific antigen. CDR grafting is a humanisation technique whereby humanised antibody sequences are generated by carefully selecting the CDRs of the parental antibody (typically murine, but increasingly other species are being humanised, including rabbits) and grafting them into a human framework.

Some of the first humanisation strategies used a limited subset of well-characterised human mAbs and did not consider sequence similarity to the parental murine antibody (fixed frameworks approach). Modern approaches now use human variable regions with high amino acid similarity to the murine variable regions (homology matching or best-fit).

Although the molecular biology processes involved in CDR grafting are relatively straightforward, simply cutting and pasting CDR sequences from murine antibodies to a human backbone is not always sufficient to retain the binding strength and specificity of the parental antibody. Design is critical and, in recent years, has become an art form practised by a few key individuals around the world.

**Human Framework**

Design encompasses various choices, such as the boundaries of the CDRs, which human frameworks to use, and which residues, if any, to substitute from the murine mAb into the human framework regions (back mutations). CDR identification is critical to the humanisation process and, for the same antibody sequence, different numbering systems may differently define the CDR boundaries. In extreme cases, there could be as many as a 10 amino acid difference between two CDR definitions. Some companies have optimised this process and use a combination of CDR definitions to maintain only residues critical to binding.

Humanised antibodies developed by CDR grafting techniques cannot be classed as ‘human’ in origin because they are derived from a blend of several antibodies (murine antibody CDRs with human constant domains). It is theoretically possible for an antibody derived from humanisation technologies to have the exact same sequence as a ‘human’ antibody from phage display or transgenic mice but, due to their origin, they cannot be classed as ‘human’.

**Phage Display Technologies**

Originally described by George P Smith in 1985, generating antibodies by phage display is based on the process of genetically engineering bacteriophage and repeated rounds of antigen-guided selection. In 2002, Adalimumab (HUMIRA®) was the first therapeutic antibody produced by phage display technology to be approved for therapeutic use. It is also regarded as the world's first fully human
therapeutic antibody. A second phage display-generated therapeutic antibody, Belimumab from GlaxoSmithKline, was approved in 2011. However, with the exception of the pending anti-epidermal growth factor receptor therapeutic from Lilly Oncology (in Phase 3 trials at the time of writing), these have been the only two successfully approved therapeutics to emerge from a phage display approach.

A human phage display library is constructed by first isolating antibody RNA from a given source – for example, sequencing from human peripheral blood mononuclear cells – followed by ligation into a phage display vector. These vectors can then be used for expression of human Immunoglobulin G (IgG) on bacteriophage hosts to represent the entire immune repertoire from which the RNA was isolated. It is then possible to screen (or ‘pan’) a phage library for those which bind to a particular antigen and isolate the original IgG sequence.

**Transgenic Mice**

In 1994, two papers described the production of genetically engineered mice which were capable of expression of full human antibody repertoires. Since then, the field has taken off. The first human antibody from a transgenic source, Panitumumab, was approved in 2006 and, from 2009, there have been a further seven, including the 2014 approval of Nivolumab for treating melanoma and squamous cell carcinoma.

Transgenic mice are generated by targeted modification of the endogenous mouse antibody genes to suppress their expression, combined with the introduction of human antibody heavy and light chain gene sequences. The result is a mouse which expresses fully human antibodies.

One of the major advantages of the transgenic mouse approach is that antibodies are generated by the same methods developed in 1975 by Milstein and Köhler. As a result, techniques are well-established, optimised and understood, which facilitates an easy path to clinical trials and market approval. This has likely contributed to the rapid growth in the number of antibodies, with 10 out of 32 antibodies approved since 2002 being from either the Medarex, Abgenix or Regeneron transgenic platforms.

**Discussing the Techniques**

So why is CDR grafting so popular, what are the issues with phage display technologies and, despite their potential, why have we not seen as many antibodies from transgenic mice as we might have expected?

Since 1985, four murine antibodies have been approved for clinical use, with long gaps between cases. In the 1990s and early 2000s, there was a rise of chimeric antibody approvals, but this was quickly superseded by the development of humanised antibodies from 1997 onwards, the first phage display antibodies from 2002, and transgenic antibodies from 2006. Also, in the past five years, an almost equal number of ‘human’ and ‘humanised’ antibodies have been developed. With technologies available to produce human antibodies, why are we not seeing more?

Although human antibodies from transgenic mice have increased rapidly, the number of platforms has remained limited and exclusive. All eight approved antibodies derive from three platforms: Abgenix (purchased by Amgen in 2005 for $2.2 billion), Medarex (purchased by Bristol Myers Squibb in 2009 for $2.4 billion), and the more recent VelociMouse® (Alirocumab) developed by Sanofi/Regeneron. These large pharmaceutical partnerships and acquisitions leave transgenic mouse platforms far out of reach of the average small/mid-sized biotech, and even further from the likes of academics and virtual companies. Even before the likes of Bristol Myers Squibb or Amgen went behind closed doors, generating antibodies with transgenic mice was not only incredibly expensive but also came loaded with significant royalties.

There has also been some debate over the limited germline repertoire which was engineered into the mouse immune system – and therefore the mouse’s ability to produce a diverse range of human antibodies. In addition, there has been some concern that the human antibodies from transgenic mice are essentially hybrids of mouse and human components – for example, human immunoglobulin sequences and mouse signalling molecules. Although they develop into

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**Figure 1: Antibodies approved for therapeutic use, 1985-2015**
what appear to be ‘normal’ human antibodies, some academics initially expressed concern. Further development of the transgenic mouse platforms has reduced these doubts in recent years.

So what about human antibodies from phage display technologies? The main difficulty with such antibodies is expression. Proteins such as antibodies, or antibody fragments, that are derived from eukaryotic organisms are often difficult to express within a prokaryotic cell. The ‘unnatural’ human sequences which result from phage display have proven difficult to develop and express in sufficient quantities, and may explain why only two antibodies are currently available on the market.

Looking Ahead

CDR grafting was the original process developed by Greg Winter in 1986, and still remains one of the most popular techniques for the production of therapeutic antibodies. It has been stated that humanised antibodies do suffer from one disadvantage when compared to human antibodies, in that they are slightly more immunogenic. However, when the alternatives are considered (difficult to express phage display antibodies, or inaccessible/expensive transgenic mice technologies), the small amount of potential additional immunogenicity presented by humanisation methods has proved to be an acceptable compromise.

Moreover, modernised versions of the CDR grafting technique now include T cell epitope avoidance technologies, which further reduces the potential immunogenicity of humanised antibodies. There has also been a growth in companies using powerful systems to screen the full B cell repertoire of a wide range of host species, including rabbit, avian and llama, to select the optimum mAbs with the correct characteristics.

On this evidence, we will see a lot more specific antibodies being developed to a wider range of precision targets in unmet medical needs going forward, whereby transgenic animals and CDR grafting humanisation will lead the approvals for the next five years.

About the author

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