advanced HTS systems in development and the opportunity to influence the direction of the programme.

Novo Nordisk was one of the first companies to buy into the system and they have been collaborating with APBiotech for six years. Like other companies, Novo Nordisk has found that the move towards increasingly focused libraries means that the throughput of screening programmes is often no longer the rate-limiting step. However, they are under increased pressure to develop better quality leads – the emphasis is now firmly on the identification of higher quality drug-like compounds to minimize later-phase attrition rates. They believe that such miniaturized screening technologies will enable them to do this more efficiently – both in terms of versatility, speed, overall cost and because of smaller amounts of target per well, minimization of waste and waste handling/disposal.

APBiotech claim that the new system could offer a fivefold increase in screening productivity, which could translate into an additional $40 million in sales for a major drug in terms of time gained in the screening cycle and hence reduced time to market.

Future prospects

Krogsgaard Thomsen believes that screening in the 1536 format on a regular basis is a realistic prospect within two to three years, taking into account advances in automation and integration. There is continuing debate over the requirements, changing platforms and costs associated with the trend for miniaturization of HTS (see July issue of *Drug Discovery Today*). It seems certain, however, that advanced imaging technology will provide impetus to drive screening further along this route.

Second acquisition

APBiotech appears to be in an acquisitive mood at present, as the company has recently acquired Molecular Dynamics (MD) in a deal worth $256 million. The deal will increase APBiotech's genomics capabilities and strengthen their position in this market. Having collaborated with MD on the development of its MegaBACE 1000 DNA sequencing system, launched last year, APBiotech has ~12 months to establish this technology in the marketplace before Perkin Elmer launches its PE 3700 DNA analyser, which is expected to be a strong competitor.

David Hughes

Therapeutic antibodies make a comeback

Since their discovery by Kohler and Milstein in 1975, the use of monoclonal antibodies (mAbs) in medicine has come a long way. There are now seven therapeutic mAbs approved in one or more major markets and a further 11 radiolabelled mAbs approved for *in vivo* imaging. A breakout session at the Bio '98 conference in New York, USA, on 14–18 June 1998, covered recent developments in the use of mAbs as biological response modifiers. The Chair of the session, David Glover (Cambridge Antibody Technology, Melbourn, UK) commented that the path to success for mAbs has been far from smooth and outlined some of the key difficulties that have had to be overcome in their therapeutic application. The conventional route to derive mAbs is to immunize mice. Such murine mAbs have widespread applications in research, but can trigger immune responses because of the foreign nature of the protein when introduced into humans. Several approaches have been taken in overcoming this problem, which has seen the development of chimaeric, humanized and now fully human mAbs (see Fig. 1). The speakers in the session concentrated on several antibody products that fall into the latter two categories, humanized and human mAbs, and their applications to modify biological responses.

Success of humanized antibodies

Jim Cornett of Protein Design Labs (PDL, Mountain View, CA, USA) gave a talk subtitled 'humanized antibodies succeed' focusing on PDL's product Zenapax®, the first approved humanized mAb. Before presenting details of Zenapax, Cornett indicated the importance of mAbs as biotechnological products. A recent survey suggested that over a quarter of all biotech drugs in development are mAbs. Within this group, humanized antibodies account for more than 30 products in the clinic for a wide variety of indications from autoimmune diseases to cancer.

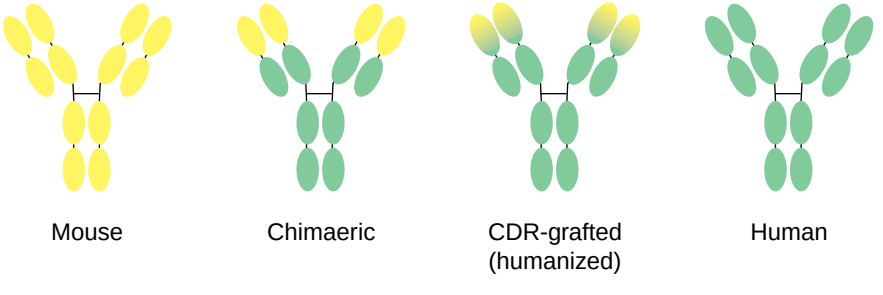

Figure 1. *Evolution of monoclonal antibodies.*

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Relative to murine mAbs, humanized antibodies minimize immune responses to the mAbs when they are introduced into humans. In addition, humanized antibodies will increase the persistence of the mAb in the human bloodstream – from two days with murine mAbs to over two weeks with humanized antibodies. Humanized mAbs are genetic combinations of a mouse antibody binding region with all the remaining portions of a human antibody, and this, Cornett argued, allows humanized mAbs to capture the advantages of murine mAbs without their disadvantages. Using PDL's SMART technology, it is possible to take a mouse mAb that may have undergone screening and early validation, and rapidly produce a humanized form suitable to enter the clinic.

Kidney transplant rejection

Zenapax is the first such antibody to win FDA approval and is indicated for the prophylaxis of acute organ rejection in patients receiving renal transplants. Zenapax binds the interleukin 2 (IL-2) receptor on activated T cells and in effect shuts them down. The specificity of the mAb is such that Zenapax is the first immunosuppressive drug without significant side effects. The circulating half-life of the mAb has been shown to be 20 days and it exhibits no clinically significant immunogenicity. In clinical trials, patients receiving a renal transplant and treated with conventional immunosuppresive drugs showed a statistically significant reduction in acute rejection episodes when also given Zenapax. In Europe, using two conventional immunosuppressants, acute rejection was 28% on Zenapax and 47% in the control group. In the USA, where a triple combination of immunosuppressants was used, acute rejection was 22% on Zenapax compared with 35% in the control group. Clinical trials of Zenapax in other autoimmune indications are also proceeding.

SMART anti-CD3

Cornett proceeded to describe other humanized mAbs under clinical evaluation by PDL. SMART anti-CD3 is a humanized mAb which induced a profound, temporary T-cell depletion in Phase I trials (now nearing completion). A clinical trial in acute kidney transplant rejection is due to begin soon and PDL is also exploring the use of SMART anti-CD3 in a variety of autoimmune disorders.

SMART M195 is being developed for the treatment of leukaemia. A Phase II/III trial aimed at holding patients with acute myeloid leukaemia in remission following chemotherapy is under way. Other trials are aimed at acute promyelocytic leukaemia and the use of 213-bismuth-linked SMART M195 in the treatment of advanced myeloid leukaemia.

In addition to these humanized antibodies, PDL is developing OST577 (Ostavir™), a fully human mAb against hepatitis B surface antigen. The antibody has shown good results in neutralizing the virus in patients receiving liver transplants as a result of hepatitis B viral infection. A trial in Europe is under way to examine the use of OST577 as part of a combination therapy for chronic hepatitis B with the ultimate goal of curing the disease.

PDL also has several preclinical mAb product candidates including anti-selectin mAbs for the treatment of trauma, reperfusion injury, stroke and psoriasis; and anti-γ-interferon mAbs with potential indications in autoimmune disease. Other preclinical mAbs in the PDL pipeline are being developed for cancer and viral infections.

Humanized anti-leukointegrin for acute inflammatory disorders

Tom St John (ICOS, Bothell, WA, USA) presented information on two mAbs currently in clinical trials. LeukArrest™ (formerly known as Hu23F2G) is targeted towards cell adhesion molecules on the surface of white blood cells – the leukointegrins, which play a key role in mediating the adherence of white cells to the blood vessel endothelium in response to an inflammatory stimulus. By inhibiting adhesion, the passage of white cells through the endothelium is prevented. LeukArrest™ not only blocks the cell adhesive event but also prevents the activation of neutrophils and subsequent respiratory burst.

Application in multiple sclerosis

A potential indication for LeukArrest is in multiple sclerosis (MS) where white blood cells cross the blood–brain barrier and enter the CNS resulting in demyelination. In such a scenario, a patient presenting to a neurologist with clinical signs of MS would receive LeukArrest to prevent further infiltration of white blood cells into the nervous system.

Other indications for LeukArrest are in situations where tissue injury results following a clinical event where neutrophils infiltrate tissue. Such conditions are classed as neutrophil-mediated tissue injuries and occur in ischaemic stroke, myocardial infarction or haemorrhagic shock. Under such conditions, oxygen-free radical and protease release by neutrophils leads to tissue damage around the injury site.

The potential of LeukArrest to limit the passage of white blood cells has been evaluated using skin-scrape chamber studies, which measure the number of cells migrating into a chamber from a scrape on a patient's forearm. Data from such studies indicated that following dosing with LeukArrest cell adhesion was profoundly suppressed. Further studies in healthy volunteers showed the effect to be reversible.

The clinical development of LeukArrest involves Phase II trials in MS, for which enrolment is completed. Phase II trials in haemorrhagic shock, stroke and myocardial infarction are also under way.

A second humanized mAb put into the clinic by ICOS is ICM3 which recognizes another cell adhesion molecule, ICAM-3. In T-cell-mediated diseases, ICAM-3 plays a role in the activation of T cells. ICM3 is currently in a single dose Phase I trial in psoriasis, and multi-dose Phase I/II trials in psoriasis and another autoimmune indication are planned.

Antibodies for the treatment of rheumatoid arthritis

Bob Kamen (BASF Bioresearch Corporation, MA, USA) described the development of a fully human mAb for treatment of rheumatoid arthritis (RA). This disease is a chronic, immune-mediated multisystem disease affecting some 5.5 million patients in the six major pharmaceutical territories. Of these, 20–25% exhibit moderate to severe RA that would be suitable for intravenous treatment. Current treatments include the nontoxic but relatively ineffective nonsteroidal anti-inflammatory drugs and analgesics; and also 'disease-modifying anti-rheumatic drugs' (DMARDs). Such drugs have no effect on the progression of the disease and are also of high toxicity. The goal is, therefore, to develop 'disease-controlling anti-rheumatic therapies' (DCARTs). Kamen suggested that anti-TNFα biologicals were among the most promising candidates as DCARTs.

BASF's D2E7 is a fully human anti-tumour necrosis factor (TNFα) mAb developed using Cambridge Antibody Technology's (CAT) phage-display technology. The aim was to produce a mAb with high affinity for TNFα, but not TNFβ, that was capable of completely blocking TNFα activity. In addition, the ideal antibody would require infrequent and convenient administration and be suitable for chronic use. This final requirement meant that any antibody should display minimal immunogenicity and thus BASF favoured a fully human primary sequence. Using a murine anti-TNFα antibody (MAK195) as a template, CAT's guided selection process was used to produce fully human light and heavy chains with high affinity for TNFα with no physical relationship to MAK195. The candidate monoclonal D2E7 shows high affinity for TNFα but no binding to other cytokines. Production capacity in Chinese hamster ovary (CHO) cells is now at 3000 litres and the mAb has been extensively characterized in terms of both protein and carbohydrate composition.

D2E7 has also been studied using an *in vivo* efficacy model – human TNFα transgenic mice, which is seen as a standard by the FDA for anti-TNFα drugs. Groups of Tg197 mice were dosed with D2E7 at doses between 0.01 and 10 $\mu g\ g^{-1}$. Histopathological and arthritic scores were evaluated and both showed a good dose-dependent response to D2E7.

D2E7 is currently under clinical development with a Phase I trial in patients in Europe completed. Phase II trials in Europe and Phase I trials in the US are also under way. At this stage, more than 1000 doses have been administered to patients.

A second human mAb being developed by BASF is human anti-IL-12. IL-12 regulates the production of Th1 T cells and is implicated in several autoimmune diseases including RA, Crohn's disease and MS (Box 1). BASF Pharma and Genetics Institute have collaborated with CAT to produce J695, a fully human, highly potent anti-IL-12 mAb. Phase I studies are planned for 1999.

Box 1. Potential indications for anti-IL-12

- Crohn's disease/inflammatory bowel disease
- Transplant rejection/graft-versus-host defence
- Multiple sclerosis
- Insulin-dependent diabetes mellitus
- Sarcoidosis
- Rheumatoid arthritis
- Other autoimmune diseases
- Septic shook

Anti-TGFβ mAbs to prevent fibrosis

In the final talk of the session, David Glover (CAT, Melbourn, UK) described the development of a human mAb to prevent fibrosis and scarring. This was developed from CAT's phage-display antibody library, which currently contains >67 billion specificities. Using the library, candidate antibodies can be isolated within a working week with sub or low nanomolar affinities for target antigens. These initial candidates are then studied and if necessary further engineering to increase affinity or other properties can be performed. Glover stated that CAT aims to progress from target antigen to clinical trials in around two years.

In fibrosis and scarring, most attention has been focussed on transforming growth factor β (TGFβ). TGFβ is a cytokine that induces its own production, antagonizes other cytokines and plays a central role in the wound healing process, possibly down regulating itself when healing is complete. It exists in three isoforms in man of which $TGF\beta_1$ and $TGF\beta_2$ are the most important in generating scar tissue or fibrosis.

CAT has developed a fully human anti-$TGF\beta_2$ monoclonal antibody using the activated form of human $TGF\beta_2$ as a target for its phage-display antibody library. While polyclonal antibodies specific to individual isoforms have been described, as has a murine mAb with broad specificity, CAT's anti-$TGF\beta_2$ is the first mAb specific to a single isoform and the first mAb with genuine potential as a human therapeutic. Evidence of biological activity *in vivo* of the anti-$TGF\beta_2$ mAb was initially obtained in a CNS-injury rat model where a reduction in scarring was observed.

Ophthalmological applications

The major clinical indication for anti-$TGF\beta_2$ is in conditions involving fibrosis/scarring in the eye. Human retinal pigment epithelial cells have been shown to respond to the anti-$TGF\beta_2$ mAb by reduced contraction of retinal explants. Work in this *in vitro* model of the contractile phase of proliferative vitreoretinopathy is now being followed by a Phase I/IIa trial in patients undergoing victrectomy surgery for retinal detachment. The first dose level in the trials has been completed and initial results are expected in late 1998.

A second clinical trial is also under way in glaucoma filtration surgery (trabeculectomy) wherein the surgeon creates a new drainage channel in an attempt to reduce pressure and preserve eyesight. The major reason for failure of this surgery is the development of scar tissue that closes off the channel.

Scarring following glaucoma surgery may currently be treated with potent anticancer drugs such as mitomycin C, which are applied topically. Such treatments are difficult to administer safely. Their mode of action appears to be to kill fibroblasts and their toxic effects can be sight threatening in themselves. Pre-clinical studies examined the effects of anti-TGFβ_2 mAb on human Tenon's capsular fibroblasts. Subnanomolar concentrations of anti-TGFβ_2 mAb were able to inhibit proliferation, migration and contraction of these cells. In an *in vivo* model of trabeculectomy the anti-TGFβ_2 mAb was successful in preventing surgical failure. In clinical trials, the mAb is being administered at the site of operation pre- and post-operatively with the aim of controlling the development of scar tissue. The Phase I/IIa trial of anti-TGFβ_2 in this indication was started in June 1998 and is expected to be complete in the first quarter of 1999.

David Hughes

Viral intelligence on the Web

Important chemical intelligence on the influenza virus was made available to medical researchers worldwide by Los Alamos National Laboratory (LANL) in New Mexico with the launch of a comprehensive database on the WorldWide Web. The database contains a collection of genetic information about the influenza virus, which scientists will be able to use to better understand how the influenza virus mutates. The obvious impact will be in the development of more-effective new vaccines and putative drugs to combat the disease as well as allowing researchers to track the disease more effectively.

Cohesive research

Los Alamos National Laboratory's Influenza Sequence Database was introduced to prospective users at the American Society for Virology meeting in July in Vancouver, British Columbia. The manager of the database Catherine Macken said that having a central collection point for all the published and unpublished genetic sequences for the virus is the only viable way to get all the information out to the research community. 'With an international repository, we can conduct cohesive analysis rather than patchwork research around the world,' she said.

The new database contains viral sequence data, results from immunological studies, and information on viral protein structures – all crucial components of an investigation into the virus. Importantly, the database will hold unpublished, but nevertheless invaluable, sequence data. Researchers can now add their own information to the database as well as use it to make comparisons of species and strains, that are currently infecting people, with older influenza viruses.

LANL is also working with scientists from the University of California and the Centers for Disease Control and Prevention to expand the database still further. At the moment, the database holds the total influenza sequences held in GenBank, a database managed by the National Institutes of Health. GenBank, however, only collects sequences that have been published in scientific journals. LANL and its institutional collaborators will verify and annotate unpublished genetic data collected around the world and add this to the database.

Working model

It is hoped that the LANL database will act as a working model for similar tools that will be used to track the spread of other deadly diseases, including those that may have been intentionally released. Alan Perelson, head of Los Alamos' Theoretical Biology and Biophysics Group, adds that 'Los Alamos is actively involved in developing new, cutting-edge capabilities to reduce threats to our national security'. He adds that while the influenza database is not directly part of that effort, it does show the kind of expertise Los Alamos can bring to problems of national and global importance.

If the database were just a library of gene fragments it might not have much impact on research, but because it is annotated with background information on how a sequenced virus was grown, for instance, researchers using the information can be more critical of the relevance of particular mutations. This should allow them to assess whether they were the result of problems with culture technique or wild mutations.

Macken and her team are in the process of developing software tools to allow browsers to recognize and analyse characteristic patterns in the sequences. These tools will allow visual and statistical assessments of variations to be made more readily than is possible now. Such developments will arm researchers with information about which strains of influenza are appearing or moving around the world so that health officials can be advised on where to deploy resources. Access to the >3000 influenza sequences in the Los Alamos' Influenza Sequence Database is available on the World Wide Web at: http://www-influenza. lanl.gov/

In this issue, John Oxford and Robert Lambkin (Retroscreen, London, UK) focus on the concept of neuraminidase inhibition in the fight against influenza.

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