assessed without the need to impose blocking doses of cold drugs, which are rarely specific at the doses needed to saturate the binding site. In addition, although the target has been genetically dissected out of the tissue, physical access of the tracer molecule to such binding sites is kept the same as in the wild type. Examples include the use of knockout models for the muscarinic M2 subtype receptor, glycogen storage disease and multidrug resistance.

The use of knockout technology, holds much promise with well-categorized tissues, but caution is advised for pathological tissues, which are inevitably in structural and functional disarray. For example, knockout technology might not be applicable or might cause distortions to function in infarcted, remodelled or neoplastic tissues. When considering the strategies reviewed by Eckelman, one must be aware of the major challenge that remains: *in vitro* screening of potential tracer molecules for their degree of nonspecific binding within the tissue of interest. This is a fundamental issue, because the kinetics of this binding need to be assayed to determine the time course of washout of the nonspecific component in comparison with that from the specific binding site [2]. An additional tool for this streamlining is the use of modelling techniques and bioinformatics, to help derive a library of molecular structures that make for tracers with good signal:background ratio for *in vivo* molecular imaging [3]

Eckelman points out that the main use of PET is for binding-site occupancy studies. However, in addition to this unique application, there are other areas where PET can assist in drug development. These include the *in vivo* measurements in diseased tissue of: (i) the level of expression of a given molecular therapeutic target, (ii) the pharmacodynamic response to a drug and (iii) drug pharmokinetics.

Finally, following validation from the knockout mouse, there is still the need to validate the new molecular probe and the method of analysis of the PET scan data in diseased human tissue. This stage continues to prove challenging in its demand for a multidisciplinary clinical research-led environment [2].

References

1 Eckelman, W.C. (2003) The use of PET and knockout mice in the drug discovery process. *Drug Discov. Today* 8, 404–410

2 Price, P. and Jones, T. (2002) Molecular imaging: what picture does it paint for future oncology? *Drug Discov. Today* 7, 741–743

3 Abrunhosa, A.J. *et al.* (2000) Preliminary studies of computer aided ligand design for PET. In *Physiological Imaging of the Brain with PET* (Gjedde, A. *et al.*, eds), pp. 51–56, Academic Press

Terry Jones
Wolfson Molecular Imaging Centre
University of Manchester
Christie Hospital
Wilmslow Road
Manchester, UK M20 4BX
e-mail: terry.jones@man.ac.uk

The future of drugs from plants

William F. Tissington Tatlow, Assistant Professor, Neurology, McGill University; Electroencephalographer, Montreal General Hospital; Biotechnology Analyst, 1650 Cedar Ave., Montreal, Quebec, Canada H3G 1A4; e-mail: tatlow@videotron.ca

Plant Made Pharmaceuticals was the subject of a three-day conference held in Quebec City, Quebec, Canada, 17–21 March 2003 (see http://www.cpmp2003.org/). Our world needs better production systems for protein-based drugs, especially edible vaccines to increase health, especially in the third world – pharmaceuticals from plants could be the answer.

The first speaker, Paul Arnison of the FAAR Biotechnology Group (http://infoweb.magi.com/~faarbio1/index.htm), pointed out that the use of 'plant factories' is called 'farming' to most people in the world! The DNA code was elucidated 50 years ago, hence the birth of genes, and the birth of plant molecules stemmed from the discovery of the causal agent of Crown Gall Disease in plants – *Agrobacterium tumefacianens*. This is contained in a large plasmid that was involved in the transfer of genes into plant cells, leading to the birth of plant molecules.

The crucial need for plant made pharmaceuticals

Halla Thorsteinsdttir from the University of Toronto (http://www.utoronto.ca) said that biotech products will have an impact on improving health in years to come, especially on communicable diseases in third world countries, but the drugs have to be robust, affordable and acceptable. Recombinant vaccines will be safer than those of present and could be produced from plants. Recombinant drugs are now

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synthesized in plants, milk, yeast and *Escherichia coli*; however, inserting the DNA of a drug into the genome of the organism is purer and potentially cheaper.

Brandon Price of Goodwin Biotech (http://www.goodwinbio.com) said, 'the capacity crunch is already here and world capacity constraints are growing for bioreactors – 18 biopharm products are waiting at the FDA to be approved' (Food and Drug Administration; http://www.fda.gov). He told us to look at fears of transgenics and regulatory unknowns, including timelines, costs, technology, intellectual property and the public perception of animal rights.

The Lemna System™ of Biolex (http://www.biolex.com) makes therapeutic proteins from plants; David Spencer showed a biomass that doubles every 36 hours, with the timeframe from gene to tested product of about 1 year. Biolex have 12 human proteins expressed with interferon currently in development and have also produced colony stimulating factor (CSF), human growth factor (HGF) and monoclonal antibodies.

Ulrich Steiner from Bayer (http://www.bayer.com) commented on the business case for plant made pharmaceuticals (PMPs) as being low cost and large scale compared with *E. coli*, yeast or mammalian cells. Insulin, recombinant factor VIII and recombinant factor IX from goats and sheep is less safe than that produced from plants. He forecast that therapeutic antibodies will be selling for US$20 billion within the next 10 years but significant savings with PMPs will require large-scale production to decrease costs.

Protein production using transgenic tobacco

David Williams from CropTech Corporation (http://www.croptech.com) described the primary processing of host plant materials used for the production of human recombinant proteins as generally based on green plant tissue or seed. Processing includes harvesting, shipping, storage, extraction, clarification, volume reduction and purification. Equipment for communication and extraction have been available for many years in the food industry, but their application has not been generally applied to the recovery of human recombinant proteins that could be used in parenteral therapies.

New designs and materials of construction will be required for the production of those proteins that will be regulated by the FDA. Along with innovative equipment design and application, innovation regarding primary processing and downstream purification should be incorporated to ensure a balanced cost approach to facility capital expenditures and operating expenses.

Roger Wyse from Burrill & Co. (http://www.burrillandco.com) explained it is difficult to find the winners because there is a shortfall in capacity. The production of proteins from plants might be possible but purification is important and there is tremendous diversity among plants. He commented that 80% of proteins will ultimately fail and challenges include pollen drift, food supply contamination and glycosylation. His conclusions were that no current company has a broad competitive advantage over another and it is a case of wait and see.

Plant derived pharmaceuticals must have stable expression in transgenic plants but they must be fully inactivated to eliminate disease spread. It is important to choose the plant with a full sequence of inserted genes and consistency in codon maintenance, but it is also important to detect potential weeds and pests.

PMPs and human disease

The French company Meristem Therapeutics (http://www.meristem-therapeutics.com), which has an office in Cambridge (MA, USA), have lead products in clinical trials, in particular, gastric lipase in Phase III. This will target patients who have pancreatic insufficiency as a result of cystic fibrosis, pancreatitis or pancreatic surgery. The company also has a vaccine therapy for non-Hodgkin lymphoma, which is currently in Phase I. Meristem also has some interesting antibodies, including Carorex, which kills bacteria on the teeth, and also those from tobacco, pneumococcus and botulinus.

Large Scale Biology (http://www.lsbc.com) has molecular biology, proteomics and manufacturing facilities with bioreactors from tobacco; this is harvested after 14 days, having been grown from a virus targeting for non-Hodgkin lymphoma. Barry Holtz explained that the viral vector is expressed in the tobacco plant, is gene mediated either by *in vitro* transcription or innoculation into the tobacco leaves (the RNA and protein expressed in the infected plant).

Croptech Corporation is the leader in tobacco-derived pharmaceuticals but there are capacity issues and plants might moreeffective, particularly tobacco, which is the easiest crop to genetically engineer. This company has 3000 lines and a unique, flexible manufacturing system that wounds the plant, then injects the gene of choice and, after harvesting, extracts from the biomass by shredding. The company has the capacity to produce 1 million pounds (~450,000 Kg) of protein per day.

In a second presentation by Dave Williams, he commented again on the prolific seeds from tobacco, a cheap biomass and spoke at length about transforming growth factor-beta (TGFB). TGFB molecules are drugs for proliferation, migration, differentiation and apoptosis. Müllerian Inhibitor Substance (MIS) is a member of the TGFB family, which produces regression of the Müllerian Duct in male infants,

leading to gender separation. MIS has potential therapy for ovarian, uterine, breast and prostate cancers. Note that it has high specificity with no *N*-linked glycan problems and no toxicity issues.

Oral vaccines

Arizona Biodesign (http://www.azbio.org) is focusing on oral vaccines and is in Phase I trials for a vaccine for Norwalk Virus and Hepatitis C using tomatoes. The public perception is addressed with 3600 square feet of white seedless tomatoes, with 120 acres under glass.

Dow Agro Sciences (http://www.dowagro.com) is making oral antigens from plants. The advantages, according to the presenter, are that PMPs show decreased costs with antigen stability and the company has been doing this now for three years with no immunological responses at all. The company has many collaborations and has also shown use in the nasal delivery of antibodies.

It was interesting to hear NeoRx (http://www.neorx.com; speaker Becky Bottino), who were the first to produce monoclonal antibodies from corn but stopped because of the cost, manufacturing capacity and high investment for little return, which is a lesson for all small biotech companies. The problems included 30 months to clinical manufacture, 36 months to commercial production and only two years in stability of the corn seed itself.

Conclusions and future directions

In summing up the conference, Richard V. McCloskey of Centocor (http://www.centocor.com) explained that an antibody needs to produce at least one kilogram with no change in the production process between Phases II and III and cost should be US$10 per gram for non-purified bulk. He warned to 'watch out for the cost to the patient', have fewer production sites with efficient and rapid scale-up and multiple systems for flexibility, timing and purification. He concluded by advising that, 'you are going to want tons of the stuff!'

The future of PMPs, according to François Arcand of Medicago (St-Foy, Quebec; http://www.medicago.com), changes all the time. He forecast that, for PMPs to prosper, we are going to have to show that mammalian cells are finished and we will have to see that in next years conference program.

In conclusion, this conference, set in such a wonderful city, had presentations of fantastic and interesting science. However, let us be realistic; the actual drugs themselves are many, many moons away for those patients that will use them. It is an exciting road ahead but we are all still learning.

scFvs and beyond

Andrew Bradbury, Los Alamos National Laboratory, Los Alamos, New Mexico; e-mail: amb@lanl.gov

'I don't want to knock single chains, but…' was one of the leitmotifs at the *Recombinant Antibodies* and *Molecular Display* meetings (12–15 May 2003, Hyatt Regency, Boston, MA) organized by Cambridge Healthtech Institute (CHI). This statement was invariably followed by lists of problems regarding single chain antibody variable region fragments (scFv) – poor expression, aggregation, instability, difficult storage, a tendency to dimerize and a variable unknown fraction of functional antibody – and a solution to the problem. Although, there is little doubt that scFvs can have these problems, their strengths as tools for discovery should also not be underestimated. These are perhaps best represented by the seven antibody drugs Cambridge Antibody Technology (http://www.cambridgeantibody.com) currently has in clinical trials – all developed using scFv phage display.

Yeast display

Another discovery platform based on scFvs was described by Michael Feldhaus (Pacific Northwest National Lab; http://www.pnl.gov) who has created a naïve scFv yeast display library. This represents a considerable *tour de force*, particularly when one considers the low transformation efficiency of yeast, which is approximately 10,000 times less than *Escherichia coli*. The library has a diversity of just over a billion clones and has been used to select binders against a number of different targets, with affinities in the low nanomolar range.

Although one could be forgiven for thinking 'yet another display platform', yeast offer a couple of significant advantages to phage display. First, and perhaps most important, is the relative ease with which affinities can be measured. In fact, Jim Marks (UCSF; http://www.ucsf.edu) now routinely clones scFvs that have been isolated

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