

Third International Conference on Plant-Based Vaccines and Antibodies

Expert Rev. Vaccines 8(9), 1151–1155 (2009)

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Third International Conference on Plant-Based Vaccines and Antibodies

University of Verona, Italy, 12–17 June 2009

This relatively new biennial meeting – the first was in Prague in 2005 – was chaired by Julian Ma (Guy's Hospital, London, UK), with Mario Pezzotti (University of Verona, Italy) as local organizer, and attracted approximately 180 delegates from 25 countries. The theme was 'Plant Expression Systems for Recombinant Pharmacologics': there were 46 talks gathered into two plenaries, 12 themed sessions and 72 posters. Topics covered included publicly funded and commercial developments, innovation, regulation and commercialization, competition with conventional technology, manufacture and new products.

Plant production of biologics

The use of plants as an alternative means of production to mammalian and other cell culture-based systems for high-value biologics, such as reagents, diagnostics, therapeutics, and vaccines for humans and animals, is rapidly becoming an attractive option. In fact, the meeting seems to have outgrown its name: while antibodies remain a popular product, vaccines had a much lower profile than previously, and other therapeutic and reagent-oriented biologics were much more prominent.

Opening plenaries

Yuri Gleba of Icon Genetics GmbH (Halle, Germany) – recently acquired by Bayer Innovation – opened proceedings with an excellent overview of how plant-based antibodies and vaccines could contribute to improving human health. He commented that plants could produce complex proteins of pharmaceutical grade, and that products and processes could meet good manufacturing practice (GMP) and biosafety standards expected of biologics, and that there were now seven GMP facilities for plant products, up from two just 4 years ago.

An interesting observation was that the established monoclonal antibody (mAb) market had four blockbusters, which were going out of patent from 2016 or so, and could be good

candidates for plant production as biosimilars. In addition, engineering of glycan processing in plants could improve antibody-dependent cell-mediated cytotoxicity, which led to the concept of 'biobetters' rather than biosimilars, as the activity of well-established products could be improved by plant expression.

Charles Arntzen (BioDesign Institute, Arizona State University, AZ, USA) noted that mAbs were the greatest success story among protein pharmaceuticals, but that their very high prices (e.g., Avastin® costs US\$7700 per month for 400 mg) mean that new manufacturing methods may be attractive. The regulatory process required redoing the whole chain of preclinical/clinical work, but biosimilars – or even 'biobetters' – derisked the process.

The plant advantage over older technologies was speed of production, and platform flexibility in terms of several ways to produce antigen. For example, the tobacco mosaic virus (TMV)-based Icon vectors could be used to get gram quantities of protein quickly, and there are an increasing number of other useful virus-based transient systems being developed.

Publicly funded/commercial developments

Takeshi Matsumara (National Institute of Advanced Industrial Science and Technology, Japan) presented the surprise package of the

conference when he detailed developments in the €60 million 'METI Project' in Japan, which runs from 2006 to 2012: these constituted a closed GMP-compliant plant growth and processing factory for the transgenic expression of high-value proteins, molecular breeding for increased plant production including biomass and glycosylation, and development of transformation technology and plant viral vectors. The first products included a transgenic strawberry producing a cytokine (canine IFN- α) for the treatment of dog periodontal disease: 1.6 million doses (25 IU) could be produced in one 30 m² room per year.

Rainer Fischer (Fraunhofer Institut, Aachen, Germany) gave an account of the commercial-scale process development in their GMP facility for one of the EU's FP6 Pharma-Planta products – the anti-HIV-1 broadly neutralizing mAb 2G12, which has already passed regulatory hurdles when produced in Chinese hamster ovary (CHO) cells. They produced this as an apoplastically targeted protein in transgenic SR1 tobacco. They could produce 200 kg of leaves per batch, and recovery was 84% for extraction and clarification, with an overall recovery of 55%. The IC₅₀ values were equivalent to CHO cell-produced material. The value of this presentation was in the demonstration that a potentially very useful therapeutic antibody could be reliably produced at good yield in a defined process, to GMP requirements, in amounts suitable for large-scale clinical trials.

Commercial developments

The three most important talks in this series covered the commercial-scale production of human therapeutics: this is a significant advance in the field, as the sentiment is that Big Pharma and other investors have been waiting for proof that plant-based manufacture is both feasible and advantageous before committing to the technology.

Einat Almon-Brill (Protalix Biotherapeutics, Israel) described the production of recombinant human glucocerebrosidase (rGCD) as a therapy for Gaucher's disease, which is caused by a hereditary mitochondrial defect. The team used a contained disposable bioreactor system with suspension-cultured carrot or tobacco cells, and claimed there were no mammalian cell culture risk factors; they obtained uniform glycosylation, and the exposed mannose allowed rapid macrophage uptake. The rGCD half-life was twice as long as that of commercial product, and had been trialed in Europe, Israel, South Africa, and north and south America.

Maurice Moloney (SemBioSys Genetics, Canada) covered their production of plant-made insulin. They used a field-grown seed system: this allowed product storage of up to 5 years as dry seed, which retained all activity. Their commercial vehicle was safflower: this is an oilseed, and there are only 100,000 acres grown in the whole of north America, making it easy to isolate. They target protein to oil bodies, which float well in purification. Insulin was targeted as 35% of potential demand uses 70% of supply, so it is a very good idea to bring the cost of the product down, in order to supply developing country demand. Moloney noted that 15,000 acres, or just three typical Canadian farms, could supply the whole world's demand. Their product was chemically

equivalent and folded identically, with identical mass spectrometry profile to the commercial product, and receptor binding was the same: this would clear the regulatory pathway for the US FDA and EMEA, as it was bioequivalent to Eli Lilly's Humulin R, the most widely used insulin in North America.

John Butler (Icon Genetics/Bayer, Germany) described their very welcome rescue of a personalized therapy product pioneered by the now defunct Large-Scale Biology Corporation (LSBC): the use of plants for the production of patient-specific or individualized idiotypic vaccines for non-Hodgkin's lymphoma. They could use their proprietary TMV-based expression technology and their GMP production facility to rapidly make single-chain antibody fragments for more than 95% of patient mAbs. No sample took longer than 12 weeks to produce, compared with a conventional production time of 6–9 months. Plant-produced idiotypes cross-reacted with hybridoma-derived products. In a clinical trial, 12 out of 20 patients were still tumor-free after 72 months, compared with none in the other treatment arm. He noted that one 5-kg batch of *Nicotiana benthamiana* per patient provided 400–500 mg of vaccine protein, which was a lifelong supply.

Daniel Tusé (VAXX Inc, AZ, USA) described their commercial-scale production of a norovirus virus-like particle (VLP)-based vaccine in plants, in competition with one other baculovirus-produced version. They used Icon vectors in *N. benthamiana*, and the GMP facility of Kentucky BioProcessing (KBP), to produce genotype 1 and the emerging genotype 2 proteins.

Innovation sessions

Two interesting talks in the first of these sessions both dealt with the use of fusion partners for the improved expression and purification of target proteins. Joensuu *et al.* (VTT Tech Research Centre, Finland) described the use of hydrophobin domain fusions – derived from a fungal protein that was the most surface-active protein known – to allow sequestration of recombinant proteins in endoplasmic reticulum-derived protein bodies, and accumulation up to 40% of total soluble protein, or approximately 6 g/kg, and an easy two-phase aqueous detergent/isobutanol purification that was easily scalable to 1200 l. Udo Conrad (IPK Gatersleben, Germany) and colleagues used elastin-like peptide–mAb fusion genes in transgenic tobacco to produce a number of pharma-plant therapeutic antibody candidates, such as the anti-HIV-1 gp41 mAb 2F5. This enhanced the production of several of these, and possibly helped with glycosylation as well, as this was more uniform. Elastin-like peptide fusion did not interfere with neutralization of HIV by 2F5.

An excellent account of glycan engineering in plants was given by Herta Steinkellner (University of Natural Resources & Applied Life Sciences, Austria); while this is of more interest to a plant science audience, the salient features for vaccine relevance were that engineering of increased Gal incorporation in antibody glycans in Δ XT/FT plants led to some increases in IC₅₀. They were presently working to increase sialylation in glycoproteins, as this was important in inflammatory responses. Koen Weterings (Bayer Bioscience, Belgium) also dealt with 'humanization' of the *N*-glycosylation pathway in plants: he

noted that Δ XT/FT–Gal-positive plants that could make ‘multi-antennary’ glycans that contained sialic acid would increase the biotherapeutic range of products.

Ben Dugdale (Queensland University of Technology, Brisbane, Australia) described their proprietary geminivirus-based In-Plant Activation Technology inducible expression system as a means of achieving high-level transgenic expression, especially of difficult-to-produce or toxic proteins. A prime example was vitronectin, normally isolated from blood and selling at Aus\$300–500 per 100 μ g; another was the highly active and toxic RNase, barnase. The ethanol-inducible expression system was essentially host plant independent, and had been used in tobacco, banana and sugar cane.

Vidadi Yusibov (Fraunhofer, MI, USA) detailed a wide variety of products made in their GMP-rated facility using proprietary TMV-based ‘launch vectors’ via a contained hydroponic growth and high-throughput agroinfiltration system. Most important, however, was their production of grams of purified candidate influenza virus vaccines: these included H3, H5, H7 and H1 HAs of type A, and a type B HA. The impressive achievement was the production, in 21 days from obtaining the new swine flu H1 HA sequence, of grams of 95% pure HA. All HA proteins made produced hemagglutination inhibition (HI) titers of more than 1/40; with Quil A adjuvant, all were effective at dose levels of $2 \times 15 \mu$ g. Production levels were typically 0.1–1 g/kg, depending on the particular molecule. While trimeric HA was generally found, sometimes protein was only dimerized or nonpolymerized at all – however, this did not seem to affect its immunogenicity.

Regulation & commercialization

Highlights of this session were the description by Mike Udell (Medicines and Healthcare Products Regulatory Agency, UK) of regulatory requirements in Europe for plant-based vaccines and antibodies, and Hugh Haydon’s account of the business model of Kentucky BioProcessing (KY, USA), the only full-service GMP-compliant plant-based production facility. Udell pointed out differences between transgenic plants and other production platforms, chief among which were the problems of ‘banking’ (one generally could not bank cell lines, and sexual reproduction leads to genetic differences) and control of production conditions was far more difficult for field-produced than for greenhouse-produced plants, which were in turn more difficult than for fermenter-produced cells. Validation of product consistency was vital, and possible novel post-translational modifications and related impurities that could affect immunogenicity were a matter for concern. He stressed that GMP compliance was the gold standard, but that good agricultural and collection practice were a fallback if this was not possible – and that some quality systems needed to be adopted by producers.

Haydon detailed how KBP’s business had expanded from in-house LSBC production, to encompass production via transgenic tobacco plants, and via various TMV-based systems, for all-comers. They would also soon have commercial-scale agroinfiltration facilities. Their production model had quality assurance/quality control built in from the beginning, and had the

advantage that their pilot-scale production was easily scalable to commercial levels in-house, as all processes used the same compatible technologies. They had produced large batches of microbicide (griffithsin), lysosomal enzyme and 98% pure antibodies, among other products. Possibly most interesting, however, were their cost estimates: Haydon reckoned on US\$20,000–500,000 for bench-scale process, US\$100,000–500,000 for pilot scale and US\$500,000–3 million for cGMP production for a given protein. By comparison with CHO cell production, this is many times cheaper.

Competing against conventional technologies

This was an eagerly awaited session, given that plant-based production is touted as being much more cost effective and safer than mammalian cell culture for reasons of potential contamination, as well as being quicker to production. John Birch (Lonza Biologics, UK) gave a much-needed modern perspective on the mammalian cell culture production of antibodies. He made the point that a company would only change production platforms if there was a benefit to the end user or patient and if there was a significant improvement in the cost of goods. All in all, this was a good reality check for advocates of plant production as a competitor for mammalian cell-based systems!

Orlando Chambers (University of Kentucky, KY, USA) gave some valuable insights into the realities of large-scale field growth of tobacco, the biopharmer’s crop of choice. Their work in developing new varieties, including those suited for biomass production, has led to more than 150 hybrids, some of which could produce up to 100 tons/hectare. He also gave a detailed cost breakdown for large-scale intensive tobacco production – including the fact that compliance costs for one herbicide-tolerant maize line were approximately US\$15 million, just to get it into the field.

Scott Deeter (Ventria Biosciences, CO, USA) gave another of the surprise talks of the conference, with his revelation that they had already placed plant-made lactoferrin and lysozyme into production and into registered rehydration products as ‘medical foods’. Their rehydration products had the potential to significantly improve child health in developing countries in particular, given that animal sources of both were potentially more allergenic and less effective. They had already proven efficacy in clinical trials, and were in field production in the USA and South America, with a production capacity of more than 1000 kg/year as rice seed-produced protein.

New products

Presenting on a vaccine topic that is probably the best suited to quick acceptance by regulatory bodies, but which was very under-represented at this meeting, was Andr s Wigdorovitz (CICVyA, Argentina). His talk on the successful immunization of cattle with a plant-derived bovine viral diarrhea virus vaccine was one of the success stories of the conference in terms of proof-of-efficacy in a developing country setting. The virus is a serious problem in Argentina, with a 70% incidence. The group made a soluble E2 envelope glycoprotein in transgenic alfalfa, albeit at low yield, and developed a two-phase purification regime that allowed ten-times

concentration in one step. They also compared their vaccine head-to-head with their own conventional cell culture-derived version, and determined that equivalent amounts (two doses of 1.5 µg) elicited the same serum response, and protected cattle better against viral challenge.

A novel strategy for protecting against inhalational anthrax was presented by Keith Wycoff (Planet Biotechnology Inc., CA, USA), whose CaroRx plant-made antibody (which blocks adhesion of *Streptococcus mutans*) is the first plant-made pharmaceutical approved as a Class I medical device in Europe. They used the extracellular domain of capillary morphogenesis protein 2 fused to the Fc domain of human IgG as a soluble blocker for *Bacillus anthracis* protective antigen (PA) binding to the native cell-linked protein. The Fc portion allowed dimerization, purification by protein A affinity columns, and conferred a half-life *in vivo* equivalent to IgG. The molecule protected against a wide range of anthrax-strain PA proteins.

One of the more interesting talks from a visual perspective was one on an antibody-based vaginal microbicide (Natasha Bohorova, Mapp Biopharma, USA). Their transiently expressed CD52g-specific mAb could be purified to approximately 99% purity, and 100 µg/ml agglutinated 100% of sperm and other seminal cells in sperm samples in seconds, and they had a movie to prove it!

The use of plants to address the looming novel antibiotic supply problem was the subject of Ralf Bock's talk in this session (Max Planck Institute, Germany). They used chloroplast expression of phage lysins to address the problem, with signal success: they could make a *Streptococcus* A+B-specific lysin to a yield of over 70% of total soluble protein (TSP), and a *Streptococcus pneumoniae*-specific lysin to 20% of TSP. The proteins were very stable in plastids, cannot be made successfully any other way, and can be injected directly into the bloodstream without obvious harm.

One of the more significant events in the conference – with the earlier paper by Vidadi Yusibov – was the presentation by Marc-André D'Aoust (Medicago Inc., Québec, Canada) on their production of pandemic influenza virus-relevant vaccines in tobacco. He commented that it took pharma companies 6 months to respond to a new strain challenge: by contrast, plant-based technology could respond in 1 month or less. They had shown that influenza A virus H5 HA could be produced very successfully in plants; that HA-only VLPs budded from plant cells. They could purify VLPs with a simple procedure to approximately 90% pure HA protein, and VLPs were more immunogenic than pure protein. They had also succeeded in producing swine flu H1 HA vaccine in less than 1 month after obtaining the sequence, indicating a significant advantage over conventional production modalities. They said regulatory agencies were 'comfortable' with their VLP product, and their target was a Phase I clinical trial in 2009, and getting to market in 2012 – with both pandemic and seasonal vaccines.

Downstream processing & manufacture

Potential problems – and their solution – in the growth and maintenance of transgenic plants, and the optimization of yields, were explored in this session. Julian Ma (St George's Hospital, UK)

explored the issue of intraplant and interplant variability in transgenic production levels of various proteins. IgG yield was three-times higher in upper (younger) leaves, while cyanovirin yield was not different, possibly due to the specific proteolysis of IgG. Temperature was important, with higher yields at higher temperatures, while day length and N₂ concentration did not affect yield. IgG yield fell with increasing plant age, with a significant decrease at flowering, which could be prevented by 'topping' IgG yield, and was also negatively affected by wounding and ethylene exposure.

This theme was carried forward by Markus Sack (Fraunhofer Institute, Germany), who detailed their experience with large-scale transgenic tobacco production of the 2G12 mAb. There was a clear daily variation in yield, with the best yield at 18:00 and a dip at 20:00; there was also a clear difference between younger and older leaves, the leaf tip and base, bottom and top leaves, and between leaves and stems. Accordingly, they only harvested leaves above the bottom two-thirds, when plants were 90–100 cm high. There was only approximately 12% variation in yield in a batch, with purified product variation being approximately 1%. They had defined basic guidelines in the absence of regulatory guidelines on what was acceptable.

Victor Klimyuk (Icon Genetics, Germany) described their clinical manufacturing facility, which is based on their proprietary magnICON[®] TMV-based transient expression technology. Using their improved TMV, turnip vein-clearing virus and potato virus X-based vectors, they were able to obtain 0.5–4.8 g/kg yields of mAbs in infiltrated leaves – or up to 50% of the biomass. Engineering *Agrobacterium tumefaciens* to be auxotrophic, as well as engineering a 'suicide' vector, were being investigated, as was use of transgenic plants for virus movement genes in order to prevent the spread of vector viruses.

Special discussion session: future prospects

This was a novel feature of the conference, primed by a few chosen informal speakers giving opinions on a list of questions, and brought a good deal of interesting speculation to light. The question "What would persuade major pharma to embrace molecular pharming?" was possibly the best discussed, unsurprisingly, given the stake many of the audience have in the technology. The point was made that there were no representatives of major pharma present, despite their having been invited – and that this was possibly indicative of their interest. Charles Arntzen said we especially needed big pharma for vaccines, given it took approximately US\$300 million just to get one human product through licensure – and that we possibly needed large charitable foundations and governments to guarantee or buy products to make them viable.

An interesting comment from Mike Udell was that if medical maggots could receive regulatory approval, then so could plant products, and that what was needed was some changing of concepts of biopharmaceuticals, and education of both industry and regulators.

Addressing the question "How can the potential of molecular pharming be realised in under-developed countries?", Ed Rybicki (University of Cape Town, South Africa) decried the fact that so few delegates were present from developing countries – just 16

out of over 180 – when the stated aim of many presentations was the provision of low-cost vaccines and therapeutics specifically for them.

Conclusion

This was an excellent meeting, in terms of content, contacts, host city venue and conference dinner. However, it was not so good in terms of price – it was much more expensive than recent similar-sized meetings in Europe, while offering less – and in terms of speakers canceling talks that were not replaced. All in all, though, it was an excellent means of staying in touch with a rapidly moving field. While antibodies remain central to the endeavors reported, sadly, vaccines are taking a back seat: this is probably due to regulatory and funding problems, and the long path to commercialization compared with reagents, diagnostics and even therapeutics.

Financial & competing interests disclosure

Edward Rybicki is partially funded by Era Biotech (Spain) for work related to the subject matter of this conference. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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