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Advances in molecular farming: key technologies, scaled up production and lead targets

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The field of molecular farming has experienced something of a rollercoaster ride since its inception two and a half decades ago. Realization of the potential for molecular farming followed quickly after the development of technologies in the 1980s to introduce recombinant DNA into plants, with a patent application describing edible vaccines (Curtiss and Cardineau, 1988), and the literature describing the potential for plant-produced antibodies and vaccines (Haq *et al.*, 1995; Hiatt *et al.*, 1989; Usha *et al.*, 1993). The development and refinement of expression technologies for transgenic and transient systems, along with application of these approaches to a wide variety of target molecules produced in a range of model and crop species, were reflected in a flurry of literature and patents over the next decade. Early reports were followed by technology and product development efforts in established agricultural biotechnology companies and newly founded companies focused on molecular farming, with some lead candidates entering early phase clinical trials, recalled in this issue by Arntzen (2015). In parallel, the first patent on chloroplast genetic engineering (Daniell and McFadden, 1988) demonstrated the system's potential advantages for high-level leaf-based expression and transgene containment via maternal inheritance, with the first vaccine antigen expressed over a decade later (Daniell *et al.*, 2001).

However, despite these advances, a combination of non-competitive yields in transgenic plants engineered via the nuclear genome compared to other established recombinant technologies, limited capital for scale-up and clinical product development, regulatory concerns over field production and the need to adapt the technology to conform to the rigorous reproducibility and standardization requirements for vaccines and therapeutics, resulted in most commercial efforts retrenching or terminating. Nevertheless, several significant programmes remained afloat both in academic laboratories and in industry, largely supported by government and charitable foundation grants, due to their long-term potential to combat diseases at reduced cost in developing countries and to react rapidly to emerging bioterror threats. Together with unmet healthcare needs and the high price of biopharmaceuticals, this maintained interest in the field and allowed for the honing of expression technologies, scaling up of processes and exploration of new approaches to improve efficacy.

As a result, the last few years have seen some lead candidate molecules progress into later stage clinical trials, pilot and commercial-scale facilities come online and perhaps most significantly, pharmaceutical companies begin to invest significantly in plant-based technologies thorough buyouts and partnerships. The recent application of plant-produced antibodies to treat patients that had contracted Ebola virus disease has also highlighted the

technology to the general public. This special issue reviews many of the most important recent advances in the field, together with key products and expression technologies. With one plant-produced therapeutic on the market and several other vaccine and therapeutic candidates in later stage clinical trials, there is considerable optimism that the field is starting to give returns on its early promise.

To kick off this special edition, one of the pioneers in the field, Charles Arntzen, provides a historical perspective of plant-made vaccines and therapeutics and relays his experiences with plant-based products developed for oral delivery (Arntzen, 2015). Because of uncertain funding for clinical development of recombinant plant-made vaccine antigens against infectious diseases, it is no surprise that the first FDA approved plant-made biopharmaceutical (PMB) treats a genetic/metabolic disorder (Fox, 2012). Therefore, Kwon and Daniell in this issue provide a brief overview of recent advances in oral delivery of several PMBs to treat major metabolic/genetic disorders including Alzheimer's disease, diabetes, hypertension and retinopathy. Yet another major advancement in this field is the use of biopharmaceuticals made in chloroplasts of edible plant cells for suppression of toxic antibodies that are produced in response to injected protein drugs, with industrial production supported by a major pharmaceutical company (Su *et al.*, 2015a). In addition, Su *et al.* (2015b) report significant suppression of GAA-specific inhibitory antibody in Pompe mice, even with a very low oral dose of GAA bioencapsulated in plant cells, further demonstrating the importance of oral tolerance induction. Although FDA approved glucocerebrosidase made in carrot cells is an injectable product, Shaaltiel *et al.* (2015) in this issue demonstrate the concept of oral delivery of this PMB. Takaiwa *et al.* (2015) then review recent advances in the expression of several antigens in rice seeds for immunotherapy against infectious, allergic and autoimmune diseases. Chan and Daniell (2015) in the next review point out the challenges in advancing vaccine antigens made in plant cells towards clinical development; they emphasize mechanistic aspects of immunity versus tolerance and provide several examples for combining the use of highly expressing chloroplast technology with carriers that bind receptors in the gastrointestinal tract to more precisely deliver target molecules. Plant-based oral vaccines are particularly attractive for veterinary applications, where there is considerable pressure to keep costs low, but regulatory hurdles are less stringent than those for human products. To this end, Ruiz *et al.* (2015) review recent advances with plant-made bovine vaccines.

Since the inception of molecular farming in the early 1990s, antibodies and engineered fragments and fusions thereof have constituted one of the lead product areas, and plant-produced antibodies have received particular attention for engineering post-translational modifications (Schähs *et al.*, 2007). To reflect the application of novel engineering technologies in plant systems, Wycoff *et al.* (2015) recount the engineering and expression of immunoadhesins in plants. The maturation of antibody production technology in transgenic plants is addressed by Sack *et al.* (2015), who describe the development of good manufacturing practices (GMP) for the production of an anti-human immunodeficiency virus monoclonal antibody, and by Ma *et al.* (2015), who recount the regulatory approval path and clinical testing of this antibody.

The most striking improvements in target expression levels and associated product yields over the last fifteen years have come from the development and widespread application of transient expression technologies, some of the most effective of which combine aspects of binary and virus-derived vectors, as reviewed here by Peyret and Lomonossoff (2015). Such transient technologies have been particularly attractive in developing targets against emerging and re-emerging infections and bioterror threats, reviewed by Streatfield *et al.* (2015). A transient virus vector-based expression approach has also been applied to the anti-HIV microbicide griffithsin, and Fuqua *et al.* (2015) review progress with this particularly cost-sensitive molecule in the light of alternative expression technologies. Robert *et al.* (2015) then report on leaf proteome rebalancing to enrich for a transiently expressed recombinant target *in vivo*, and Holtz *et al.* (2015) provide an overview of the construction and application of a commercial-scale production facility developed for plant-based transient expression systems.

Although whole plant systems have received the most attention, they are also the most distinct from established microbial and mammalian cell production technologies. It is therefore not surprising that the first plant-based human therapeutic to get to market was produced in cell culture. In this issue, Reski *et al.* (2015) provide an overview of a moss bioreactor system, and Tekoah *et al.* (2015) recount directing taliglucerase alfa to market and the development of further products in plant cell culture. Finally, Paul *et al.* (2015) provide an overview of product development experiences of several of the major commercial ventures in the field, drawn from interviews with principal players.

It is hoped that this special issue will provide both historical context and spotlight important new developments in the field of molecular farming as it progresses further products to market and gains more widespread acceptance in the biopharmaceutical industry.

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