Emerging antibody products and *Nicotiana* manufacturing

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Abbreviations: IVIG, intravenous immunoglobulin; Mab, monoclonal antibody; HIV, human immunodeficiency virus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; Δ XF, xylose and fucose knockout; CHO, chinese hamster ovary; gD, glycoprotein D

Antibody based products are not widely available to address multiple global health challenges due to high costs, limited manufacturing capacity and long manufacturing lead times. *Nicotiana*-based manufacturing of antibody products may now begin to address these challenges as a result of revolutionary advances in transient expression and altered glycosylation pathways. This review provides examples of emerging antibody-based products (mucosal and systemic) that could be competitive and commercially viable when the attributes of *Nicotiana*-based manufacturing (large scale, versatile, rapid, low cost) are utilized.

Introduction

Monoclonal antibody (mAb) based products have been therapeutically and financially successful in developed markets, but are not widely available to address multiple global health challenges due to high costs, limited manufacturing capacity and long manufacturing lead times. Now, rapid and versatile platform technologies, like *Nicotiana*-based manufacturing, may begin to address these challenges. *Nicotiana*-based manufacturing of antibody products has been revolutionized by two major technological advances: (a) viral based transient expression allowing high accumulation of antibody in days; (b) transgenic plants with altered glycosylation pathways that can produce antibody with mammalian glycoforms.

The abundant production of mAbs in plants has recently been reviewed,¹ but success is in the products and a major criticism addressed to plant biotechnologists is “after 20 years, where are the products?”² This review provides examples of emerging antibody-based products (mucosal and systemic) that could be competitive and commercially viable when the attributes (large scale, versatile, rapid, low cost, customizable glycosylation) of *Nicotiana*-based manufacturing are utilized.

Manufacturing mAbs in *Nicotiana benthamiana*

Transient expression. Proof of concept studies with plant-made mAbs^{3,4} and antigens⁵ stimulated significant interest in transgenic plant production. However, low expression levels, extended manufacturing timelines associated with generating stable transgenics, safety concerns regarding plant glycosylation and environmental concerns, hindered rapid development of products. Now, transient expression systems for *Nicotiana*, e.g., magnICON,⁶ Geneware,⁷ Gemini,⁸ pEAQ,⁹ and plastocyanin-based¹⁰ have been developed that alleviate many of these hurdles.

The first industrialized system to demonstrate the ability to produce antibodies “on demand” in large quantities, at low cost was magnICON.^{6,7} The technology is in essence an en masse infiltration of whole mature plants with a highly dilute *Agrobacterium* suspension carrying t-DNAs encoding viral replicons. The result is a high copy number of RNA molecules that encode the desired antibody, reducing the time needed to produce the protein to 6–8 days. The technique appears to be the most rapid route from genes to full-length, assembled mAb (i.e., 8 days from DNA delivery via *Agrobacterium tumefaciens* to harvested cells expressing mAbs). Moreover, increasing the volume of mAb-containing biomass does not require changes in growing conditions, infection procedures and is directly scalable. Unlike stable transgenic approaches, no genes are incorporated into the plant genome, and as a result, there is no risk of propagation of the transgene from pollen, seeds or other routes. Further, no intact and replication-competent virus is produced, eliminating the risk of virus-mediated spreading of the recombinant genes. Finally, the entire production system can be performed indoors in enclosed growth rooms, providing an additional layer of environmental security and quality control.

Until recently, most production of mAbs in plants had been at small scale in academic laboratories. Now however, a number of institutions are actively manufacturing molecules in *Nicotiana* for clinical studies under Good Manufacturing Practices (GMP). Contract GMP manufacturing in *Nicotiana* is currently offered by Kentucky BioProcessing (Owensboro, KY); Texas A&M (College Station, TX) has recently begun construction of a contract GMP manufacturing facility for *Nicotiana*. Icon Genetics (Bayer; Halle, Germany), Fraunhofer

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Figure 1. Distribution (%) of N-glycosylation patterns of two FDA approved Mabs produced in CHO (Rituxan) and NS0 (Synagis) compared to reported glycan patterns of anti-HIV mAb 2G12 produced in wild-type and transgenic *N. benthamiana* (Strasser et al. 2008). wt, wild-type *N. benthamiana*; ΔXF, transgenic *N. benthamiana* with native fucosyl- and xylosyl-transferase knocked-out via RNAi. Glycoforms representing less than 5% are not included in the table.

(Newark, DE) and Medicago (Quebec, Canada) are reported to have their own GMP facilities for Nicotiana production.

Glycan modification. Wild-type *N. benthamiana* glycosylates proteins differently than mammalian expression systems.¹¹ *N. benthamiana*, like other plants, produces the same core glycan as found in mammals, but uses xylose (which generally is not found in mammals) and fucose in a non-mammalian linkage (alpha 1,2). Because of the potential for the novel plant glycans to affect pharmacokinetics as well as immunogenicity in humans, it is highly desirable to produce mAbs in plants that have been modified to generate mammalian-like glycans. Now, with the development of transgenic strains of *N. benthamiana* with fucosyl- and xylosyl-transferase knocked down by RNAi,^{12,13} plants can produce mAbs with glycoforms that are essentially mammalian.

The resulting glycoforms in the double “knockout” (ΔXF, Fig. 1) are more homogeneous than FDA-approved mAbs produced in mammalian cell culture (top two rows); obtaining a consistent glycoform profile in production is desirable from a quality and regulatory perspective. Of particular note for the development of mAbs where Antibody Dependent Cellular Cytotoxicity (ADCC) is an important mechanism of action (e.g., anti-cancer antigen mAbs), the predominant glycoform is one that is ideal for ADCC activity—elimination of core fucose has been shown to increase ADCC activity dramatically.¹⁴

Knock-in strategies are now being used for mAbs that require galactosylated and sialylated N-glycans;^{10,11,15,16} current manufacturing methods based on mammalian cell culture allow only limited control of this important posttranslational modification.¹⁷ Galactosylated and sialylated HIV mAbs have been produced in Nicotiana.^{15,16}

Multipurpose Microbicides for Sexual and Reproductive Health

Unsafe sex is the second most important risk factor for disability and death in the world’s poorest communities and the ninth

most important in developed countries.¹⁸ Every year, more than 120 million couples have an unmet need for contraception (80 million women have an unintended pregnancy, half a million die from complications associated with pregnancy, childbirth and the postpartum period) and 340 million acquire a sexually transmitted infection (STI).

Improved reproductive health outcomes—lower fertility rates, improved pregnancy outcomes and lower sexually-transmitted infections (STIs)—have broad individual, family, societal and environmental benefits.^{19–22}

Although there are products for preventing pregnancy (e.g., the pill, IUDs, diaphragms) and STIs (male and female condoms), the epidemic incidence rates of both unintended pregnancy and STIs clearly illustrate the need for multipurpose prevention technologies with improved acceptability and access.²³ mAbs are highly specific, but in combination could yield a multipurpose microbicide active against a variety of STI pathogens with or without contraceptive activity.

HIV antibodies 2F5, 2G12, 4E10 combined as mAbGel are currently in early clinical trials as microbicides.²⁴ mAb 2G12 has been produced in transgenic maize plants,^{25,26} and the HIV-neutralization capability of the antibody is equal to or superior to that of the same antibody produced in CHO cells. The HIV mAb 2G12 has been produced in the ΔXF knockout Nicotiana line and was found to contain a relatively homogeneous N-glycan species without detectable xylose or α-1,3-fucose residues.¹³ Plant-derived mAbs were indistinguishable from Chinese hamster ovary (CHO)-derived 2G12 with respect to electrophoretic properties and exhibited functional properties (i.e., antigen binding and HIV neutralization activity) at least equivalent to those of the CHO counterpart. Fully galactosylated 4E10 and 2G12 were reported to be several fold higher in neutralization potency than CHO produced mAbs.¹⁵ Sialylated 2G12 exhibits similar in vitro HIV neutralization potency to other glycoforms derived from plants and CHO cells.¹⁶

The 4E10 mAb has been produced in a transgenic tobacco rhizosection system.²⁷ A fusion protein of the HIV mAb b12 and cyanovirin produced in transgenic tobacco increased HIV potency compared to b12 or cyanovirin alone.²⁸ Several non-antibody microbicides, e.g., griffithsin and actinohivin have been transiently expressed in Nicotiana.^{29,30}

An immune infertile diagnosis can be determined by the presence of anti-sperm antibodies that agglutinate and trap sperm in cervical mucus (reviewed in ref. 31), suggesting that an anti-sperm mAb may add contraceptive activity to a microbicide. Indeed, an anti-rabbit sperm mAb has been shown to provide contraceptive activity in a rabbit model.³² A mAb (HC4) against a surface glycolipid present on human sperm has been produced in the ΔXF Nicotiana line and shown to co-agglutinate 100% of sperm and other seminal cells in less than thirty seconds at 100 ug/ml (Fig. 2). Testing the mucus trapping ability of this version of HC4 as well as aglycosylated, galactosylated and sialylated HC4 mAb is planned; the importance of mAb glycans for mucosal trapping is currently unknown—mAbs can trap sperm in mucus by making them “mucophilic”, i.e., the antibodies form adhesive interactions with the mucus

Figure 2. Agglutination of human sperm with mAb HC4 produced in *N. benthamiana*. Purified mAb was added to undiluted human semen and observed within 30 seconds via light microscopy.

gel that stops all forward motility (the “shaking phenomenon”); this is a primary mechanism that mediates immune infertility and appears to be associated with the Fc regions of antibodies.³³ A similar mechanism occurs with mucosal pathogens,³⁴ i.e., a sufficient number of low-affinity cross-linkages trap the pathogen in the mucus gel, thereby reducing the flux of pathogen that reach target cells.

A multipurpose microbicide that includes HSV and HIV mAbs (Fig. 3 and Table 1) has been produced under Good Manufacturing Practices (GMP) in *Nicotiana*.⁷ The cost and capacity challenges for an antibody-based microbicide are significant—a 10 mg multipurpose microbicide if used twice a week by ten million women would require ~10,000 kg of mAb per year. Coupled with conventional unit operations for very large scale mAb purification,³⁵ the price/dose could be less than one US dollar for *Nicotiana*-based manufacturing.

RSV Antibodies for Neonates and the Elderly

Globally, respiratory syncytial virus (RSV) is the most common cause of childhood acute lower respiratory infection (19.3–46.2 million new episodes per year), a major cause of admission to hospitals (2.8–4.3 million per year) and 66,000 to 199,000 deaths (99% in developing countries).³⁶ The impact of RSV in adults is less well characterized; however, it is estimated that approximately 2–9% of all community acquired pneumonia hospitalizations may be associated with RSV infection.³⁷ Additionally, 5–10% of elderly patients in long-term care facilities develop RSV infections per year with rates of pneumonia and death of 10–20% and 2–5% respectively.³⁸

A number of analyses have been performed on the pharmacoeconomics of RSV immunoprophylaxis. Although Synagis, an anti-RSV mAb administered prophylactically to high-risk infants, is an effective drug, most studies find that RSV immunoprophylaxis is not cost-effective. Indeed, some regions have begun to recommend more restrictive indications for the use of Synagis.³⁹ If the costs of the drug were lowered, the patient

populations in which Synagis would be cost-effective would be expanded.^{40,41} A second generation, affinity-improved version of Synagis, Motavizumab, was recently rejected by an FDA advisory panel due to safety considerations.

Since the quasispecies nature of RNA viruses such as RSV allows for rapid adaptation and selection of antiviral resistance, it is desirable to develop a mAb to a different epitope than Synagis as a second-defense if Synagis-resistant RSV strains become widespread. Production in plants offers a low-cost manufacturing option capable of significantly reducing costs and thereby enabling broader cost-effective use of an RSV mAb product for at-risk infant and adult populations. RSV25, a mAb to a different epitope on glycoprotein F has been transiently expressed in *Nicotiana* and has been evaluated for binding affinity (affinity for glycoprotein F as determined by surface plasmon resonance: Synagis = 2.2×10^{-10} ; *Nicotiana* derived mAb = 5.5×10^{-11} M) and neutralization (Fig. 4).

Antibody Products to Reduce Inflammation Associated with Autoimmunity

High doses of human immunoglobulin G administered intravenously (IVIG) is capable of suppressing inflammation associated with a variety of autoimmune diseases.^{42,43} The precise mechanism by which IVIG alleviated autoimmune disease-related inflammation in humans remains unresolved. The drawbacks of IVIG treatment involve both the cost and the availability;⁴⁴ IVIG costs about \$100 per gram and a single treatment can require 20–30 g. For autoimmune diseases requiring high doses, treatment may involve up to five infusions.

The recent observation that IVIG activity in mouse models of autoimmune disease is caused by a limiting concentration of a sialic acid-bearing IgG glycoform^{45–47} provides the rationale that a fully recombinant product composed of hypersialylated IgG constant region could be a potent anti-inflammatory agent for use in autoimmune diseases in humans. It has recently been shown that the anti-inflammatory activity of IVIG in the

Figure 3. HSV neutralization testing of an anti-HSV mAb (αHSVgD) produced in *N. benthamiana*. Serial dilutions of acyclovir or αHSVgD were added to ME-180 cell monolayers followed by 2.5×10^3 pfu/ml of Herpes Simplex Virus (HSV)-2. After 5 days, the cytopathic effect was assessed. (Data courtesy of C. Lackman-Smith, Southern Research Institute, Fredrick, MD).

Table 1. Neutralization with anti-CCR5 Mab produced in *N. benthamiana*

Pseudovirus	Clade	IC ₅₀ ¹ (μg/ml) in TZM-bl cells ²
RHPA4259.7	B	0.19
Bal.26	B	0.30
6535.3	B	0.29
QH0692.42	B	5.87
PVO.4	B	0.50
TRO.11	B	2.06
SF162.LS	B	1.40
SHIV-SF162P3	B	1.33
SHIV-1157ipd3N4	C	0.42

¹Values are the sample concentration (μg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample). Neutralization testing was performed by Dr. David Montefiori (Duke University). ²TZM-bl cells are HeLa-cell derivatives that express relatively high levels of CD4 and co-receptors CXCR4 and CCR5.

K/BxN murine model of inflammatory arthritis can be attributed to a minor species of IgGs that is modified with terminal sialic acids on their Fc-linked glycans.^{48,49} Since this molecule does not involve antigen binding, it was demonstrated that a functional, recombinant substitute for IVIG could be provided by a sialylated IgG Fc constant region.⁵⁰ Although these results have yet to be extended to human autoimmune inflammation, there exists the possibility that a resolution to the problem of IVIG shortage and cost might ultimately be provided by a recombinant production system capable of producing completely human sialo-glycan mAbs. At the moment, only Nicotiana manufacturing has the capability of this type of mAb engineering coupled with scale-up capability to address demand of this magnitude.

A sialylated mAb was recently achieved by the introduction of an entire mammalian biosynthetic pathway into *N. benthamiana*, comprising the coordinated expression of the genes for (1) biosynthesis, (2) activation, (3) transport and (4) transfer of Neu5Ac to terminal galactose.¹⁶ The transient overexpression and functional integrity of six mammalian proteins that act at various stages of the biosynthetic pathway and correct subcellular localization was demonstrated. Sialylation was at great uniformity when glycosylation mutants that lack plant-specific N-glycan residues were used as expression hosts. Further, efficient neutralization activity of the sialylated monoclonal antibody was demonstrated, indicating full functional integrity. A hypersialylated mAb to a pro-inflammatory cytokine (e.g., TNF or IL-6) may result in superior autoimmune or anti-inflammatory products (see Biobetters section).

Antibody Products for Alzheimer Disease

There are currently four mAbs and two IVIG preparations being tested in human clinical trials: bapineuzumab (Wyeth), solanezumab (Eli Lilly), PF-04360365 (Pfizer), GSK933776 (GlaxSmithKline), Gammagard (Baxter) and Octagam (Octapharma). The mAbs are all directed against the prevalent amyloid peptide, Aβ, the accumulation of which in the brain is a hallmark of Alzheimer disease (AD). The IVIG preparations also contain antibodies against Aβ and the contribution of these polyclonal anti-Aβ antibodies to IVIG efficacy is a matter of continuing research since, as noted above, IVIG can function by a variety of mechanisms. Moreover, there are many additional mAbs that are in various stages of pre-clinical development and testing. The multiple unknowns of AD present challenges in mAb development and production. First, the specific epitope(s) on Aβ that is optimal for antibody binding resulting in clinical benefit has yet to be defined. Simple, high affinity binding of Aβ is not sufficient to elicit cognitive benefit in mouse models of AD (Mapp, unpublished observations). The epitopes that do result in cognitive amelioration are difficult to define since the Aβ amyloid peptide is continually aggregating until it is insoluble.⁵¹⁻⁵³ Consequently, anti-Aβ mAb discovery is a highly iterative process requiring evaluation of a large number of mAbs coupled with further optimization of isotype, serum half-life and, probably, glycan structure. Second, it has become increasingly clear that clinical intervention in AD needs to occur as early as possible. Where the results have been made available of the clinical trials mentioned above, significant cognitive benefit has been marginal. It may be that the inclusion criteria (generally mild-to-moderate AD) results in a clinical population that already has significant irreversible neuronal damage. The continuing validation of very early stage biomarkers for AD during the next decade will hopefully allow for clinical intervention in advance of extensive neuronal damage.⁵⁴ These factors will push passive immunotherapy farther into the realm of prophylaxis. Third, a vaccine for preventing AD is problematic in view of the complicating issues such as immune senescence in the elderly, inflammation resulting from auto-antigen immunization, and control of Th2 versus Th1 immune responses. The

Figure 4. RSV neutralization assay comparing Synagis (palivizumab; MedImmune) with mAb RSV25 produced in wild type (wt) or Δ XF (deltaXF) *N. benthamiana* (Synagis). The neutralizing activity of the Mabs was measured by a plaque reduction assay using HEP-2 cell culture and the A2 strain of RSV. Plaques for each Ab dilution were counted, triplicates averaged and Ab dilution vs. plaque number plotted. This study was performed by Dr. James Crowe (Vanderbilt University, Nashville, TN).

single vaccine clinical trial published to date (AN1792) failed for just these reasons resulting in a significant occurrence of meningoencephalitis.⁵⁵

As a result of these characteristics of AD, passive immunotherapy requires a production system that is highly versatile. A short expression cycle from DNA to protein as well as an ease of scalability to very large production runs is essential. If passive immunotherapy for AD is to find clinical application in the very early stages of the disease, the cost and production capacity will be critical elements of success.

Figure 5. Antigen binding ELISA with anti-Ebola mAb 13F6 produced in NS0 cells and in *N. benthamiana*. ELISA plates were coated with inactivated Ebola Zaire, serial dilutions of mAb were added and bound mAb detected with labeled anti-human lambda polyclonal antibody.

Anti-amyloid mAbs have been transiently expressed in *Nicotiana* (Mapp, unpublished data) and are being evaluated in a transgenic mouse AD model.

Antibody Products for Biodefense and Emerging Infections

Although some biodefense agents have been stockpiled, there is a need for rapid, versatile, low cost and large capacity manufacturing systems with “surge” capability to rapidly address a bioterror event as well as newly emergent and re-emergent pathogens. The surge capability of transient expression in *Nicotiana* was recently demonstrated with a project that went from gene to purified VLP vaccine in less than a month,⁵⁶ and with individualized full IgG idiotype vaccines in less than four months.⁵⁷ Cost and capacity is another vital component: one US funder has targeted GMP manufacturing of three million doses of vaccine (50 ug dose) at <\$1/dose and antibody (400 mg dose) at <\$10/dose in one to three months (DARPA BAA 06-31).

For anthrax therapy, a recombinant fusion protein comprised of a fusion of CMG2 (a human receptor for anthrax toxins) and the Fc of human IgG1, for long circulating half-life and immune effector cell interaction, has been expressed in stably transformed *Nicotiana* at high levels and processed to a high level of purity (Wycoff, PBVA 2009). A *Nicotiana*-based mAb has been shown to bind a highly conserved epitope on the envelope protein of the West Nile virus and protects mice in challenge studies.⁵⁸

Pathogen specific sialylated mAbs may also have additional therapeutic efficacy if they reduce inflammation (as described above), a common feature in the pathogenesis and morbidity of many pathogens (e.g., via a cytokine storm).

Although recent successes with an Ebola vaccine and siRNA technology are encouraging, because of the excellent safety and efficacy profile mAbs are being evaluated as Ebola therapeutics. Three mAbs (individually and in combination) have been shown to provide significant prophylactic and therapeutic protection in mice.⁵⁹ Humanized versions of these mAbs have been expressed in *N. benthamiana* (Fig. 5), tested in mice and GMP *Nicotiana* manufacturing is underway to provide supply for evaluation in primate efficacy studies. Similar earlier stage projects are underway for Marburg and Lujov viruses, as well as a set of potential biowarfare toxins (*C. perfringens* epsilon toxin, ricin, Staphylococcal enterotoxin B).

Antibodies and Vaccines

There are a number of synergies between recombinant mAbs and vaccines: (a) blood from vaccinees can be used as a source for

Table 2. Manufacturing capacity requirements: scenarios for subunit vaccines*

Immunization protocol	Antigen requirements (per person lifetime)	Kilograms for 1 million people	Kilograms for 1 billion people
Single systemic	0.001 to 0.1 mg ^(a)	0.001 to 0.1 kg	
Systemic prime and boost (2X)	0.003 to 0.3 mg	0.003 to 0.3 kg	
Systemic prime and mucosal boost (2)	0.02 to 2.1 mg ^(b)	0.02 to 2 kg	20 kg to 2,000 kg
Monthly mucosal boosts	0.25 to 25 mg ^(c)	0.25 to 25 kg	25 to 25,000 kg
Multiple antigens (4X) and monthly mucosal boosts	1 to 100 mg	1 to 100 kg	1,000 to 100,000 kg

*Assumptions: (a) 1.0 to 100 ug systemic prime dose; (b) 10X the systemic dose required on mucosal surfaces; (c) monthly mucosal boosting to model a replicating organism.

mAb discovery; (b) mAbs can be used in challenge models to predict the neutralizing titers required for protection by vaccine candidates; (c) mAbs can be used as a method of purifying antigens; (d) mAbs can be used as anti-idiotypic vaccines. Further, for some diseases, the use of vaccines coupled with passive immunotherapy is routine (e.g., Hepatitis B, rabies).

Purification of antigens. The large-scale production of scFv in transgenic *Nicotiana* and its use for immunopurification of Hepatitis B surface antigen for a vaccine has been described.⁶⁰ Lab scale purification of *Nicotiana*-derived HSV glycoprotein D (gD)—a potential vaccine antigen—has been used with transiently expressed full length anti-gD mAb from *Nicotiana* (personal communication, Hugh Mason). Although economically desirable, the use of mAbs for industrial purification of target antigens is not currently routine.

IgG idiotype vaccines. Individualized vaccines require very small scale manufacturing runs but are highly dependent on rapid manufacturing capability. The cost, production time and availability of tailor-made, patient- and tumor-specific antigens (idiotypes) have been significant hurdles to previous developments in this field. An scFv idiotype vaccine for non-Hodgkins Lymphoma (NHL) has been transiently expressed in *Nicotiana* and successfully completed a phase I clinical study for safety and immunogenicity in NHL patients.⁶¹ To improve expression and streamline purification (via Protein A), whole IgG idiotype NHL vaccines were transiently expressed (0.5 to 4.8 g/kg of leaf biomass) in *Nicotiana*;⁵⁷ clinical trials with this strategy are expected to yield early results in 2011.

IgG idiotype vaccines can utilize standard mAb purification technology, including protein A. Downstream processing of mAbs are complex and costly (accounting for as much as 50–80% of total manufacturing costs); the use of a *Nicotiana*-derived immunoabsorbent (based on a tobamovirus displaying protein A) may be an especially attractive tool for the purification of *Nicotiana*-made mAbs,⁶² including anti-idiotypic vaccines.

Although subunit,^{63,64} VLP,⁵⁶ and full IgG idiotype⁵⁷ vaccines have been expressed in *Nicotiana*, a unique opportunity

with anti-idiotypic mAbs is the use of recombinant technology as mimics of vaccine antigens that are difficult to manufacture (e.g., carbohydrate antigens).

Overall, the worldwide vaccine market has registered revenue of US \$21 Billion in 2008. However, new vaccines are often not globally accessible due to cost.⁶⁵ In addition, new subunit vaccines may require significantly larger quantities if used without adjuvant, are mucosally boosted or have multiple antigens. Several scenarios for subunits and the required manufacturing capacity are shown in Table 2.

Biobetter Antibodies

A significant number of mAb products will be coming off patent over the next five years. To achieve sales in the biosimilars sector, companies will have to invest significant capital while risking product failure during development stages. Due to recent data exclusivity laws in the US, some developers may be better off pursuing a BLA with a superior product and better pricing. The barrier of entry to this market is substantial, but may be mitigated by the low capital costs and the speed to phase 1 cGMP supply with transient *Nicotiana* manufacturing (Table 3).

Inflammatory diseases. TNF is a major pro-inflammatory cytokine involved in multiple inflammatory diseases (e.g., rheumatoid arthritis, Crohn's disease, psoriasis, septic shock). Single-domain antibodies to TNF have been produced in *Nicotiana* and prevented septic shock in mice.⁶⁶ For commercially available TNF antibodies, the cost to a US consumer is ~\$50,000/year.

Cancer. If long-term (years) cancer therapy with mAbs becomes routine, the pharmacoeconomics and supply requirements may change substantially. For example the mAb Herceptin (Genentech) can cost a breast cancer patient more than \$37,000 for a year of treatment. A HER-2 mAb has been transiently expressed, purified from *N. benthamiana* and evaluated in a mouse cancer model.⁶⁷

Infectious disease. Specific immunoglobulin products are commercially available as therapeutics and prophylactics for a

Table 3. Cost and time comparisons for mAb manufacturing systems

Manufacturing system	Time to Phase 1 cGMP supply	Cost to Phase 1 cGMP supply
Mammalian cell culture (CHO, NS0, PER.C6)	18 months	\$5–6 M
Transient <i>Nicotiana</i> (magnICON, Geneware)	6–12 months	\$0.5–0.8 M

Based upon quotes from Lonza (Slough, UK) and Kentucky BioProcessing (Owensboro, KY).

Table 4. Attributes of Nicotiana-manufacturing and the importance* for each attribute to successfully commercialize the product

Product	Attributes of Nicotiana-manufacturing				
	Lower cost	Large capacity	Versatility**	Speed	Glycans
STI Microbicide	High	High	High	Low	Low to Medium
<i>C. difficile</i>	Medium	Medium	Low	Low	Low to Medium
RSV	Medium	Medium	Low	Low	High
Biodefense	Medium	Low to High	High	High	High
Personalized vaccines	Medium	Low	High	High	Low to Medium
Global health vaccines	High	Low to High	High	Low to High	Medium to High
Alzheimer Disease	Medium	Medium	High	Low	High
Anti-inflammatory	Medium	Medium	Low	Low	High
Biobetters	Medium	Medium	Medium	Medium	High

*The requirement for each attribute is rated by the authors as low, medium and high. **Flexibility to address unique challenges by the product.

broad range of pathogens (Tetanus, Varicella/Zoster, Hepatitis B, Rabies, Rubella, Vaccinia, Staphylococcus, Cytomegalovirus, Hepatitis A, Pertussis). At present, mAbs against RSV, HIV, HSV, HBV, *C. difficile*, have been transiently expressed in Nicotiana.

Summary

A new generation of mAb-based products have the potential to alter the pharmacoeconomic landscape due to Nicotiana manufacturing that is versatile, rapid, scalable, lower in cost and glycan modifiable. These Nicotiana manufacturing attributes (Table 4) may have a significant impact on the development of mucosal

and systemic antibody products that require one of the attributes for commercial viability. With the growing number of GMP Nicotiana manufacturers available to facilitate development of quality products at commercial scale, the plant biotechnology field may soon be able to answer the question, “where are the products?”²²

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