Quality Plant-Made mAbs with *FastPharming*®

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Introduction

The first therapeutic antibody received approval in the United States in 1986. Since then, technological advancements have resulted in antibody drug products, including monoclonal antibodies (mAbs), Fc-fusion proteins, antibody fragments, and antibody–drug conjugates (ADCs), becoming the largest class of biologic drugs. mAbs are used to treat an ever-growing array of conditions, including infectious diseases, heart disease, multiple sclerosis, asthma, autoimmune diseases, and many different cancers. In addition to therapeutic mAbs, bioprocess mAbs are critical tools in the production of more advanced biologics, including ADCs and cell therapies.

Chinese hamster ovary (CHO) cell culture–based systems are the predominant platform for the production of therapeutic mAbs. However, there are inherent shortcomings in CHO systems. Scaled, cost-effective recombinant antibody production requires extensive research and development to identify the optimal stable CHO cell line, cell culture conditions, and process scale-up protocols. Additional challenges may be faced regarding batch-to-batch consistency, quality, and heterogenous glycosylation.[1] Mid-scale production options may be limited and present a hurdle for some development programs. There is a need for the biopharma industry to look beyond this conventional system and toward alternative platforms that can circumvent or overcome these challenges. iBio's ***FastPharming* System®** is a platform that produces consistent, high-quality mAbs while offering advantages in terms of speed, scaling, and glycosylation control compared with CHO systems.

The data presented demonstrate the ability of the *FastPharming* System to produce consistent, high-quality antibodies. Antibodies produced using *FastPharming* exhibit qualities equivalent or superior to what is achievable using traditional CHO cell culture. In addition to the ability to produce high-quality mAbs, *FastPharming* offers a range of other benefits to developers.

FastPharming® as a Platform for the Production of mAbs

FastPharming uses a relative of the tobacco plant — *Nicotiana benthamiana* — as the bioreactor in the production process and transient transfection at scale to provide a range of benefits over traditional mammalian cell culture, not the least of which is avoiding the time-consuming requirements associated with developing stable producer cell lines and CHO master cell banks. Plants are infected with *Agrobacteria* as the "carrier" for the vector/gene that encodes the target recombinant protein, and translation of these recombinant viral vector mRNAs can result in the accumulation of gram quantities of target antibody in the plant leaves per batch of fresh plant tissue in less than a week **(FIGURE 1)**. Following extraction and clarification, proteins are purified using conventional separation and chromatography steps, generating consistent, high-quality, animal-free antibodies. *FastPharming* can produce recombinant proteins in a fraction of the time of traditional cell culture-based systems, while addressing the growing demand for animal-free protein products that reduce the risk of contamination by adventitious viral agents or other undesired pathogens.

The *FastPharming* System also provides tight control of antibody glycosylation, enabling the production of homogeneous mAbs with increased molecule quality and/or potency. Since *N*-glycosylation can modulate the therapeutic effector functions of antibodies, including antibody-dependent cellular cytotoxicity (ADCC), there can be significant variability in the safety and efficacy of a mixture of heterogeneous glycoforms. Homogenous production of specific glycoforms in CHO systems requires highly controlled growth conditions that must be optimized during extensive development.[1] Owing to the action of multiple overlapping, intrinsic glyco-related mechanisms in cell expression systems, this has been a historical challenge.

Using iBio's ***Glycaneering* Technology™**, transgenic (TG) plants produce antibodies with reliable, homogeneous glycan profiles, consistent structures, and — via afucosylation — enhanced ADCC activity.[2] This is an underexplored approach to date owing to the high cost of licensing afucosylation technology in CHO-based production platforms. The capability to develop innovative products with glycosylation patterns deliberately modified from typical human forms is highly relevant for both bioprocess and therapeutic antibody products.

iBio supports mAb and other recombinant protein programs via comprehensive onsite bioanalytical and protein characterization capabilities that leverage mass spectrometry and multi-attribute methodology (MAM) to identify and analyze the critical quality attributes (CQAs) of antibodies produced using *FastPharming*. In contrast to more conventional and orthogonal bioanalytical techniques, the MAM approach allows iBio to monitor multiple CQAs simultaneously, with consequent time and cost savings, and supports the quality-by-design (QbD) approach to antibody development endorsed by regulators. iBio's skilled internal team, supported by state-of-the-art instrumentation, can execute an abundant range of bioanalytical tests, including intact glycosylated or deglycosylated protein analysis, bottom-up proteomic analysis, subunit analysis, and *N*-glycan profiling, to fully characterize mAbs produced using *FastPharming*.

Consistent, High-Quality mAbs Produced Using *FastPharming*

Robust production of many antibodies that bind to different targets has been demonstrated using iBio's *FastPharming* technology. **FIGURE 2** shows SDS-PAGE evaluation of both intact and heated/reduced antibody samples of two well-characterized antibodies shown here as representative products, the NIST monoclonal antibody reference (NISTmAb) and rituximab. Both antibodies were

FIGURE 1.

FastPharming Schematic.

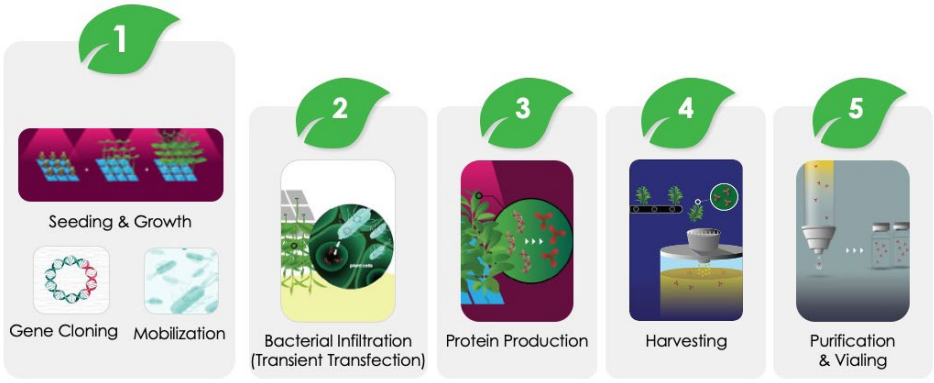


TABLE 1.

Mass of individual deglycosylated heavy (Hc) and light (Lc) chains in reduced antibody samples.

	NISTmAb		Rituximab	
	Hc Mass	Lc Mass	Hc Mass	Lc Mass
FastPharming	49461.5	23127.6	49098.7	23039.7
CHO	49461.9	23128.1	49068.2	23039.7

produced in three different *N. benthamiana* plant lines that support iBio's *FastPharming* System® and *Glycaneering*™ technologies. Non-heated/non-reduced (NHNR) samples show fully assembled, intact antibodies following recovery from plant biomass. Heated/reduced (HR) samples show independent heavy and light antibody chains. No degraded or truncated antibody products are observed in the antibody samples.

iBio's in-house advanced bioanalytical services are used to evaluate a number of CQAs of antibodies produced in the *FastPharming* System. Antibodies made in CHO cell culture and in *FastPharming* were evaluated using mass spectrometry. The mass of reduced, deglycosylated samples are presented in **TABLE 1**. The mass of individual heavy and light chains is comparable between the CHO-made and *FastPharming* antibodies.

Released glycans were evaluated by mass spectrometry to identify the glycans present in all four antibody samples. Mass spectrometry traces for NISTmAb and rituximab produced in CHO cells and in *FastPharming* are shown in **FIGURE 3**. The mass spectrometry traces of rituximab and NISTmAb samples produced in CHO cells exhibit three peaks, corresponding to a mixture of glycoforms present in these samples. Antibodies produced using the *FastPharming* System present a single peak, indicating that only a single glycoform is observed. The mass of the glycan correlates to a glycan without xylose and fucose modifications, as expected from the plant line used to produce these antibodies. Similar homogenous glycan profiles have been observed from many other antibodies produced using *FastPharming,* including commercially available antibodies and numerous proprietary antibody candidates. Production of antibodies with consistent glycan profiles is a hallmark characteristic of the *FastPharming* and *Glycaneering* technologies.

To evaluate *in vivo* comparability, assessment of the pharmacokinetics of mAbs produced using iBio's *FastPharming* plant-based manufacturing technology and mAbs produced in CHO cells was performed. Rat pharmacokinetic (PK) data for NISTmAb and rituximab are shown in **FIGURE 4**. Rats were dosed with either *FastPharming*- or CHO-made antibody at 5 mg/kg. Human IgG1 was measured by MSD at the time points presented in the graphs shown. The resulting PK curves are fundamentally similar, and the half-

FIGURE 2.

SDS-PAGE with Coomassie stain of two different antibodies, NISTmAb and rituximab, produced in three different *N. benthamiana* plant line hosts: wild type (WT) and two transgenic plant lines (TG1 and TG2). Heated/reduced (HR) samples show independent heavy and light chain bands. Non-heated/non-reduced (NHNR) samples show fully assembled antibodies.

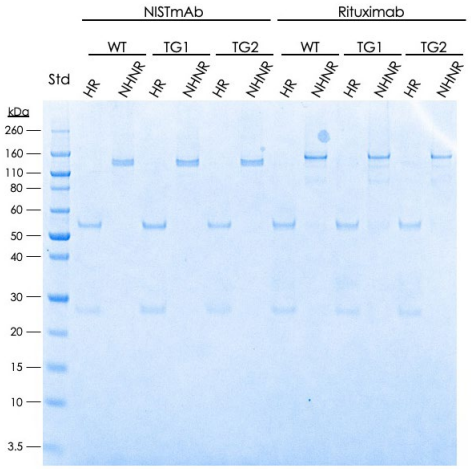
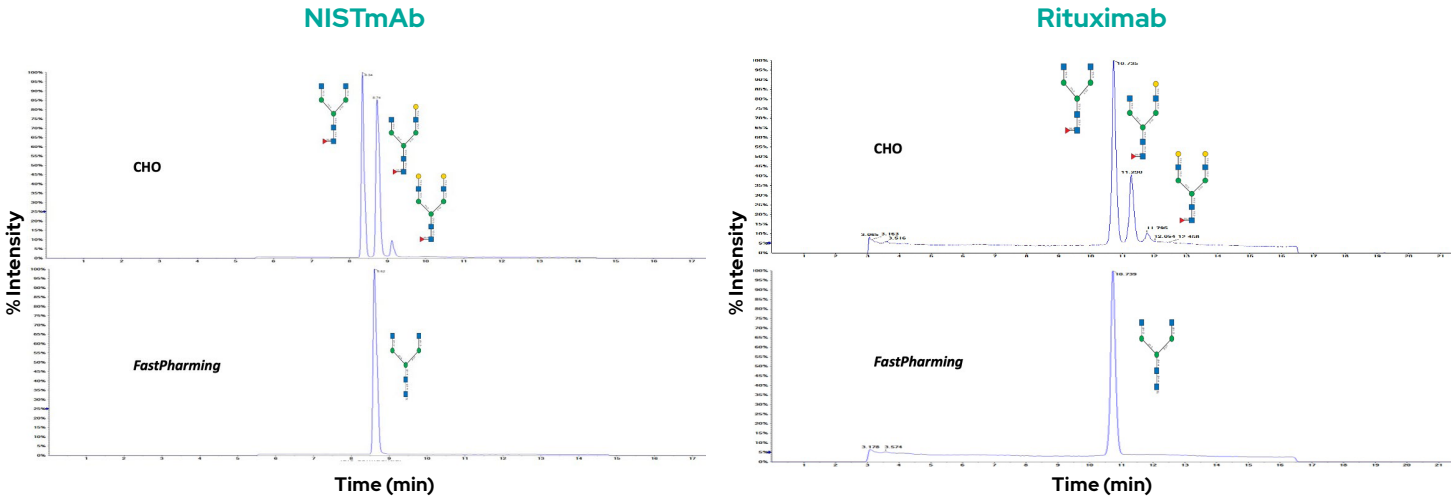


FIGURE 3. Mass spectrometry chromatographs of NISTmAb and rituximab produced in CHO cells and *FastPharming*. Identified glycans are labelled near the corresponding peaks.



life ($T_{1/2}$), maximum dosage (T_{max}), maximum observed concentration of antibody (C_{max}), and total dosage within time points (AUC_{last}) are comparable **(FIGURE 4)**.

Streamlining mAb Development for Therapeutic Developers with *FastPharming*®

The data presented demonstrate the ability of the *FastPharming* System to produce consistent, high-quality antibodies. Antibodies produced using *FastPharming* exhibit qualities equivalent or superior to what is achievable using traditional CHO cell culture.

In addition to the ability to produce high-quality mAbs, *FastPharming* offers a range of other benefits to developers, including:

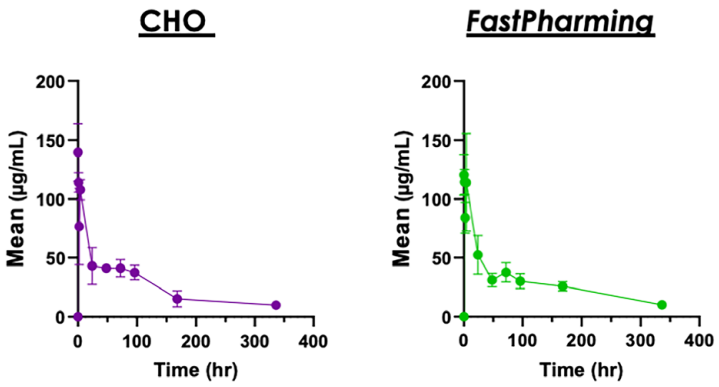
- Accelerated development that avoids time-consuming cell line development
- Rapid and simplified high-throughput candidate screening[3]
- Comprehensive glycosylation control
- Accessible production of afucosylated antibodies without high-cost licensing fees
- Ease of scale-up
- Versatility to express a wide range of recombinant proteins, including mAbs, growth factors, cytokines, lectins, and virus-like particles
- Internal supportive MAM-focused bioanalytical capabilities
- An animal-free process with reduced risk of contamination from mammalian viruses and prions
- Greater overall sustainability of the platform compared with conventional cell culture production

The *FastPharming* System is a compelling platform to spur innovation and accelerate the development and manufacture of therapeutic and bioprocess mAbs. The antibodies evaluated here demonstrate the capabilities of *FastPharming, Glycaneering*™, and iBio's in-house advanced bioanalytics. These capabilities are translatable to client-driven projects and support iBio's CDMO services.

FIGURE 4.

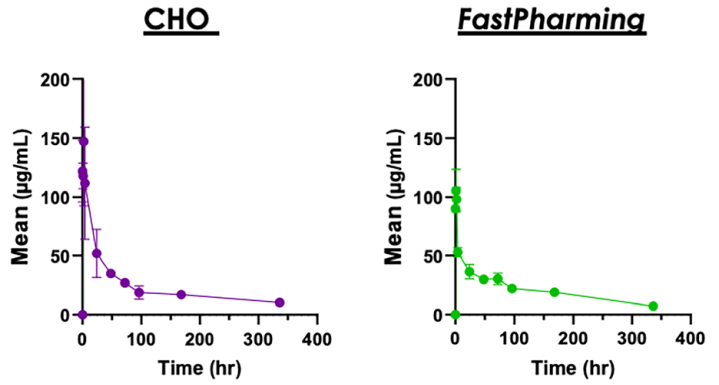
NISTmAb and rituximab produced in CHO cell culture and the *FastPharming* System have comparable rat pharmacokinetic (PK) properties. Following administration of NISTmAb or rituximab, blood samples were obtained at indicated timepoints. Human IgG1 (hIgG1) was measured in serum using the MSD method.

NISTmAb



NISTmAb – IV Dose (5 mg/kg)				
Expression System	$T_{1/2}$ (hr.)	T_{max} (hr.)	C_{max} (µg/mL)	AUC_{last} (hr*µg/mL)
CHO	144	2.13	140	8831
FastPharming	98.5	2.50	130	9711

Rituximab



Rituximab – IV Dose (5 mg/kg)				
Expression System	$T_{1/2}$ (hr.)	T_{max} (hr.)	C_{max} (µg/mL)	AUC_{last} (hr*µg/mL)
CHO	194	1.50	154	6995
FastPharming	172	0.625	108	7104

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