Plant-Made Antibodies Show Superior Glycosylation Homogeneity While Retaining Pharmacokinetic Properties

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CREATED WITH FastPharming®

ABSTRACT

Monoclonal antibodies (mAbs) are a key class of biologic drugs. Their expression in alternative platforms, including plant-based systems, can provide a range of advantages. Two well-known mAbs, NISTmAb and rituximab, were produced in *Nicotiana benthamiana* and displayed increased homogeneity in their glycosylation pattern vs CHO-made analogs, while retaining similar pharmacokinetic properties. We believe these data support plant expression systems as promising alternatives for producing therapeutic mAbs for unmet medical needs.

INTRODUCTION

FastPharming uses a relative of the tobacco plant — *Nicotiana benthamiana* — as a bioreactor and a transient transfection system to produce recombinant proteins. Plants are infected with *Agrobacterium* as the "carrier" for the viral vector that encodes the target protein, and translation of the vector mRNAs results in the expression of the target antibody in the plant leaves in less than a week (**Figure 1**).

INTRODUCTION

Following extraction and clarification, mAbs are purified using conventional separation and chromatography steps. *N. benthamiana* can produce a variety of recombinant proteins, including mAbs, in a fraction of the time of traditional cell culture-based systems, while addressing the growing demand for protein products generated in an animal-free system which reduces the risk of contamination by adventitious viral agents or other undesired pathogens.

This plant-based expression system also provides tight control of antibody glycosylation, enabling the production of highly homogeneous mAb lots. Two well-characterized antibodies were used as representative products to evaluate the production of monoclonal antibodies in the *FastPharming* System: NIST monoclonal antibody reference (NISTmAb) and rituximab, a therapeutic monoclonal antibody widely-used to treat autoimmune diseases and cancer.

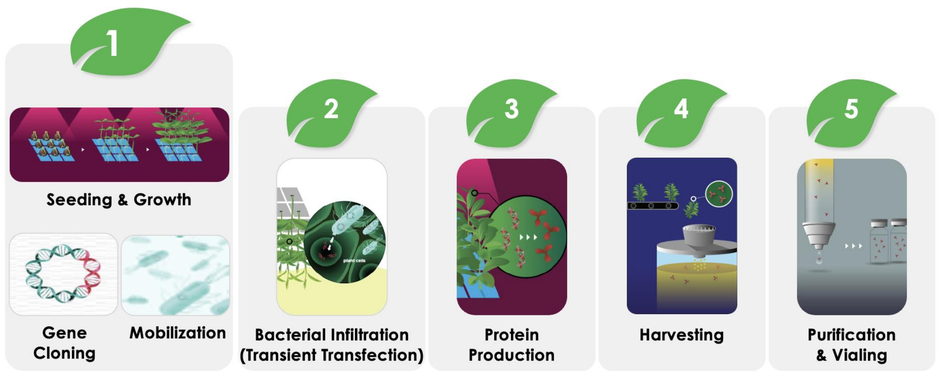


FIGURE 1.
The *FastPharming* process to produce recombinant proteins in *N. benthamiana*.

RESULTS and DISCUSSION

SDS-PAGE of intact and heated/ reduced NISTmAb and rituximab

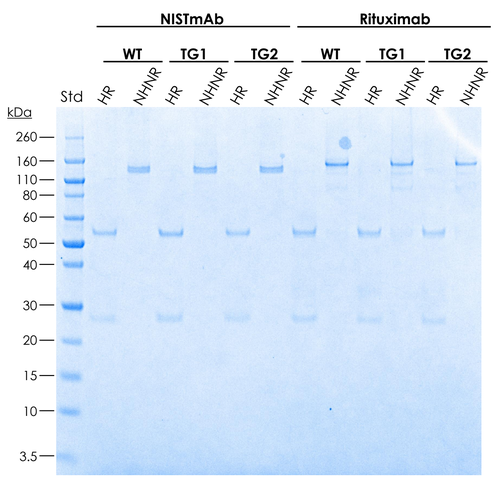


FIGURE 2.
NISTmAb and rituximab were produced in three different *N. benthamiana* plant lines: wild type (WT) and two transgenic (TG) plant lines. TG1 is a transgenic line (ΔXT/FT) with RNAi knockdown of β1,2-xylosyltransferase and a1,3-fucosyltransferase. TG2 is a ΔXT/FT plant line with added galactosyltransferase function. 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.

Mass Spectrometry Analysis of NISTmAb and Rituximab

iBio's advanced bioanalytical services were used to evaluate selected critical quality attributes (CQAs) of the mAbs. Deglycosylated intact mass and released glycan profiles of antibodies made in CHO cell culture and in the ΔXT/FT plant line were evaluated using multi attribute method (MAM) mass spectrometry.

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

TABLE 1:
Mass of individual deglycosylated heavy (Hc) and Light (Lc) chains in reduced antibody samples produced in CHO or plants are comparable.

Mass spectrometry traces identifed released glycans from rituximab and NISTmAb samples produced in CHO cells as three main peaks, corresponding to a mixture of glycoforms present in these samples. Antibodies produced using the ΔXT/FT plant line contained a single main peak, indicating a single glycoform. The mass of the glycan in both ΔXT/FT plant-produced samples correlates to a glycan without xylose and fucose modifications, as expected from the ΔXT/FT plant line used to produce these antibodies.

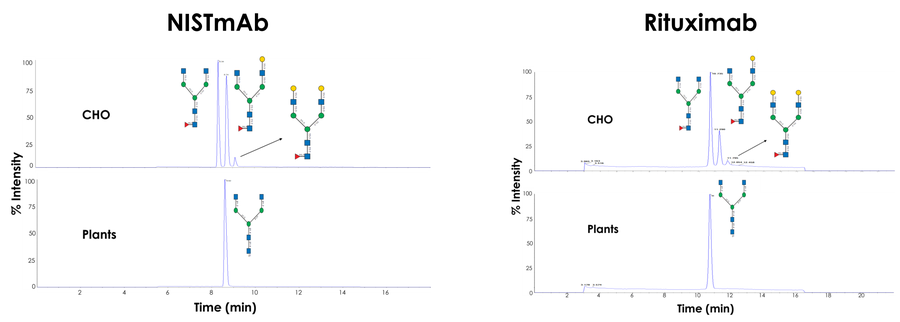
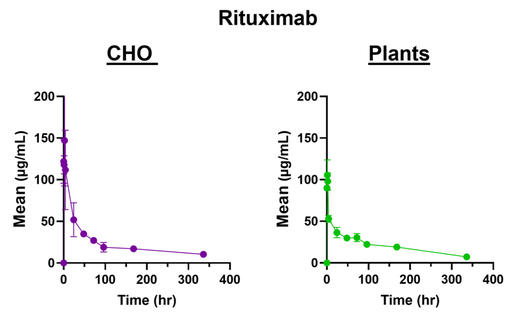
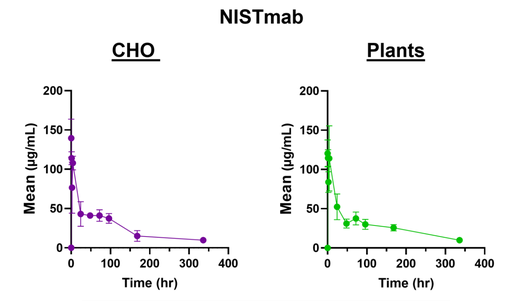


FIGURE 3.
Mass spectrometry chromatographs of NISTmAb and rituximab produced in CHO cells and ΔXT/FT *N. benthamiana*. Identified glycans are labelled near corresponding peaks.

Pharmacokinetics of CHO- and Plant-Made Antibodies



Expression	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ug/ml)	AUC (h*ug/ml)
CHO	194	1.5	154	6995
Plants	172	0.6	108	7104



Expression	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ug/ml)	AUC (h*ug/ml)
CHO	144	2.13	140	8831
Plants	98.5	2.5	130	9711

FIGURE 4.
NISTmAb and rituximab were evaluated in PK studies. Rats were dosed with either plant- or CHO-made mAb at 5 mg/kg. Human serum IgG1 was detected by MSD method. The resulting PK curves are fundamentally similar, and $T_{1/2}$, T_{max}, C_{max}, and AUC_{last} were comparable.

CONCLUSIONS

- Transgenic *N. benthamiana* plant line ΔXT/FT produces a single uniform G0 glycosylation pattern, lacking fucose and xylose, of NISTmAb and rituximab when compared to benchmark CHO-made reference material, which contain more heterogenous glycosylation patterns.
- Plant-made NISTmAb and rituximab demonstrate comparable *in vivo* rodent Pharmacokinetic profile to CHO-made references.
- The rapid and scalable transient expression of high quality mAbs for *in vivo* studies can significantly shorten the time to achieve *in vivo* proof-of-concept.
- iBio integrates plant-made mAb proprietary technology with rapid scalability to provide an advantageous platform for manufacturing biologic therapies.

REFERENCES

1. **Kipp, P, et al.** "Rapid, High-Throughput Screening to Maximize Protein Yields in the *FastPharming* System®. iBio. Nov 2021.
2. **Zhang, P, et al.** "Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs." *Drug Discovery Today*. 21 (5): 740-765 (2016). https://doi.org/10.1016/j.drudis.2016.01.006.
3. **Shields RL, et al.** "Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity." *J. Biol. Chem.* 277:26733–26740 (2002). doi:10.1074/jbc.M202069200.
4. **Strasser, R, et al.** "Generation of glyco-engineered *Nicotiana benthamiana* for the production of monoclonal antibodies with a homogeneous human-like *N*-glycan structure. *Plant Biotechnol. J*. 6:392-402 (2008). Doi: 10.1111/j.1467-7652.2008.00330.x.

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