Immunocapture based LC/MS investigation of different glycoforms clearance of a biotherapeutic Mab in human serum

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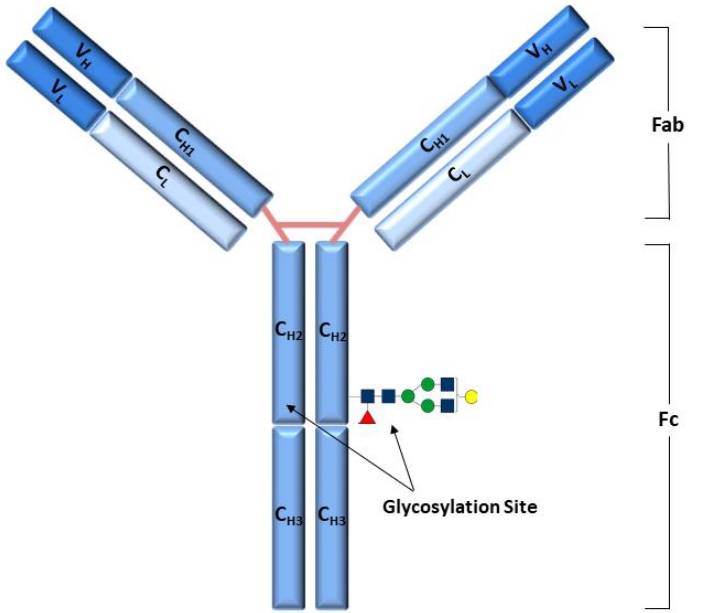
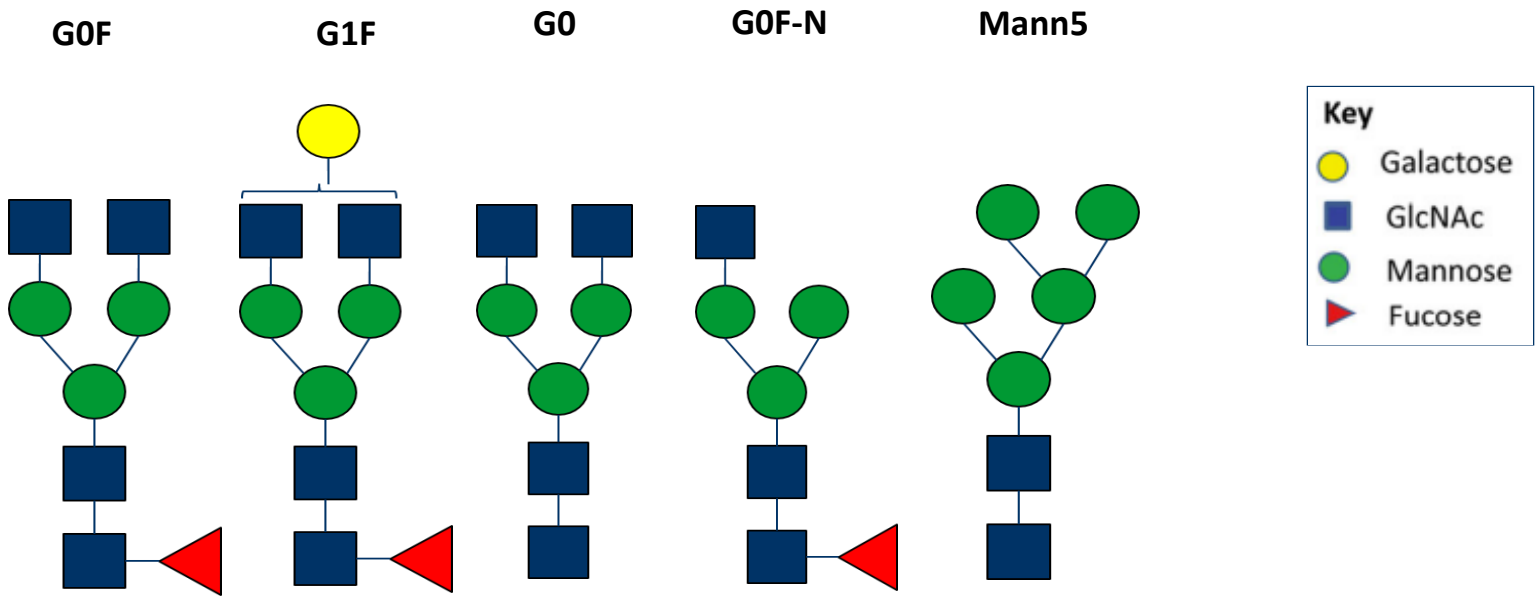
Background of N-glycosylation

- **Glycosylation is one of the most significant post-translational modifications (PTM) of therapeutic monoclonal Antibodies (mAbs) during production.**
- **Glycosylation pattern may affect the functional activity, safety, and efficacy of drug.**
- **N-glycosylation at Asn-297 on the C_H2 domain: G0F, G1F, G2F, G0, Mann5 *etc.***





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Liming Liu. *Journal of Pharmaceutical Sciences.* 2015, 104(6), 1866-1884.

Effects of N-glycosylation on PK and PD

Fc Glycans	Potential Effects
Fucose	Absence of core fucose enhances: • FcgRIIIa binding • ADCC activity
Galactose	Enhances antibody binding to C1q and CDC
Mannose 5	• Decreases half-life • Increases FcgRIIIa binding and ADCC activity • Decreases antibody binding to C1q and CDC
Bisecting GlcNAc	Increases FcgRIIIa binding and ADCC activity

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Challenges in the Comparability of Biologicals

- **The NBE manufacturing process changes during the drug development phase.**
- **Pre- and post-manufacturing product quality is maintained through comparability exercise**
- **Differences in high mannose content have been demonstrated to impact pharmacokinetics.**
- **In house-data/ tools are generated to understand impact on pharmacokinetics, however, uncertainty remains regarding acceptable clinical impact of smaller changes in mannose content**

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Study Overview

- **Goal**

  Develop an immunocapture based LC/MS assay to quantitatively measure the major glycoforms (G0F, G1F, G0, G0F-N and, Mann5) in human serum and assess relative clearance of mannose-5 compared to the major glycoforms as part of in vivo CQA.

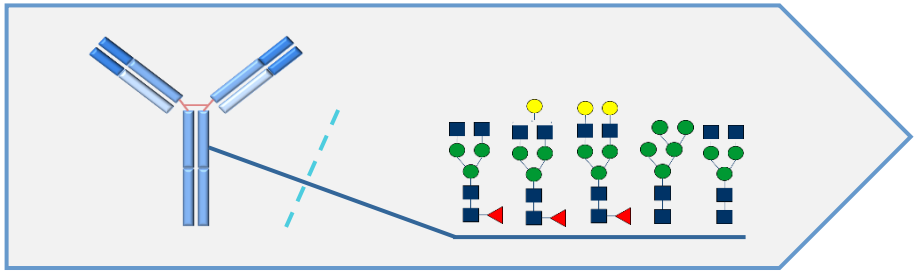
- **Methodology**

  Immunocapture-LC/MS/MS

- **Samples**

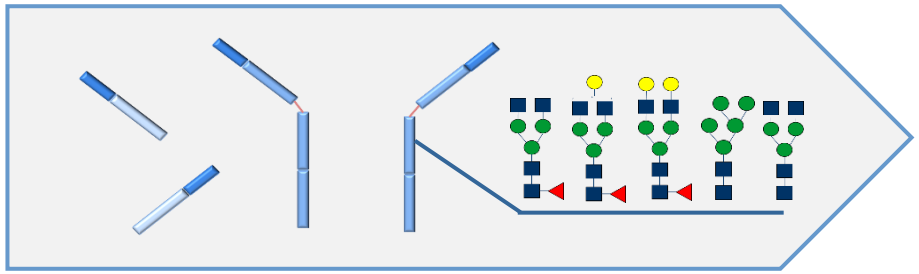
  Human plasma samples were from clinical trials

Strategies for Glycoforms Analysis



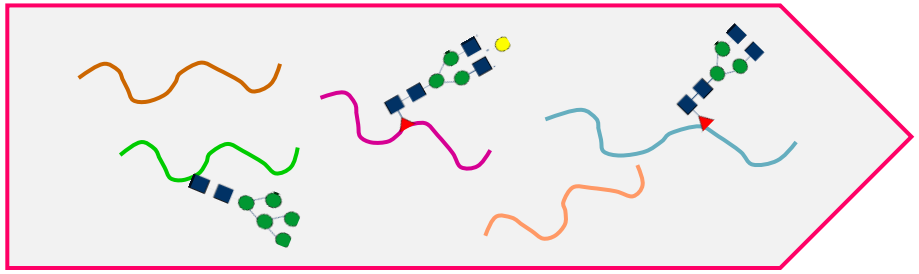
Release glycans from mAb then quantitate

- Interferences from endogenous proteins
- No information on the glycosylation sites



mAb intact/subunit analysis

- Information on the original protein
- No information about the glycosylation sites
- Low sensitivity



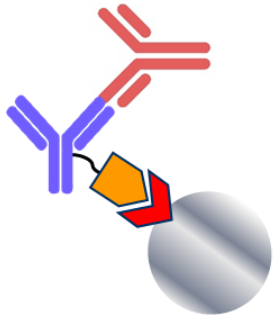
Digest mAb then quantitate glycopeptides

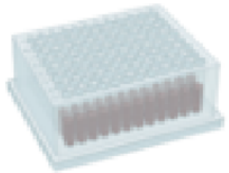
- High sensitivity
- High specificity

Immunocapture-LC/MS Assay Workflow

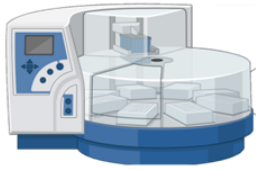
BI X (Drug) in Human Serum

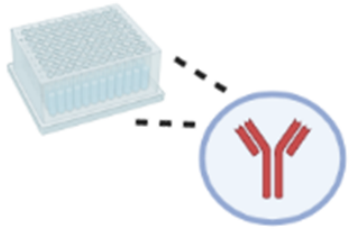
Immunocapture





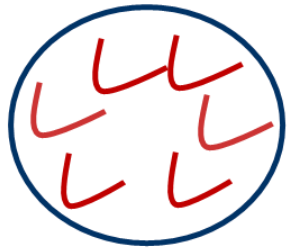
Elute

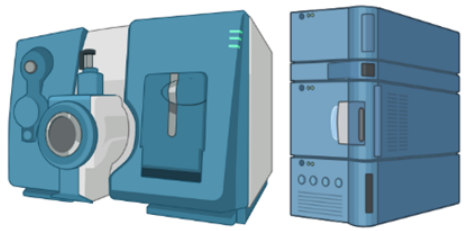




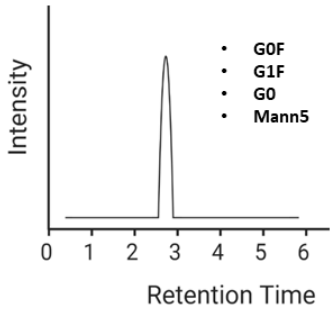
Elution plate

Trypsin Digestion

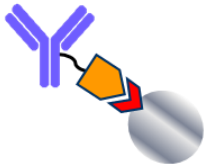




LC/MS-MRM



Glycopeptide



Capture Antibody & Streptavidin magnetic beads

Study Information

- We analyzed the 2 dose group patient samples

Dose Group	# of Patients	Sampling Timepoints
Low	3	Predose, 1h, 4h, 8h, 24h, 48h, 72h, 168h, 336 h
High	3	Predose, 1h, 4h, 8h, 24h, 48h, 72h, 168h, 336 h

LC/MS/MS Method

- Glycopeptides were analyzed on an AB Sciex QTrap 6500+ mass spectrometer coupled to a Waters Acquity UPLC system.

Glycoform	m/z of glycopeptide (+3)	MS/MS (MRM)
G0F	878.683	878.683 > 204.080
G1F	932.701	927.489 > 204.080
Mann5	802.646	802.646 > 204.080
G0	830.000	803.000 > 204.080
G0F	810.990	810.990 > 204.080

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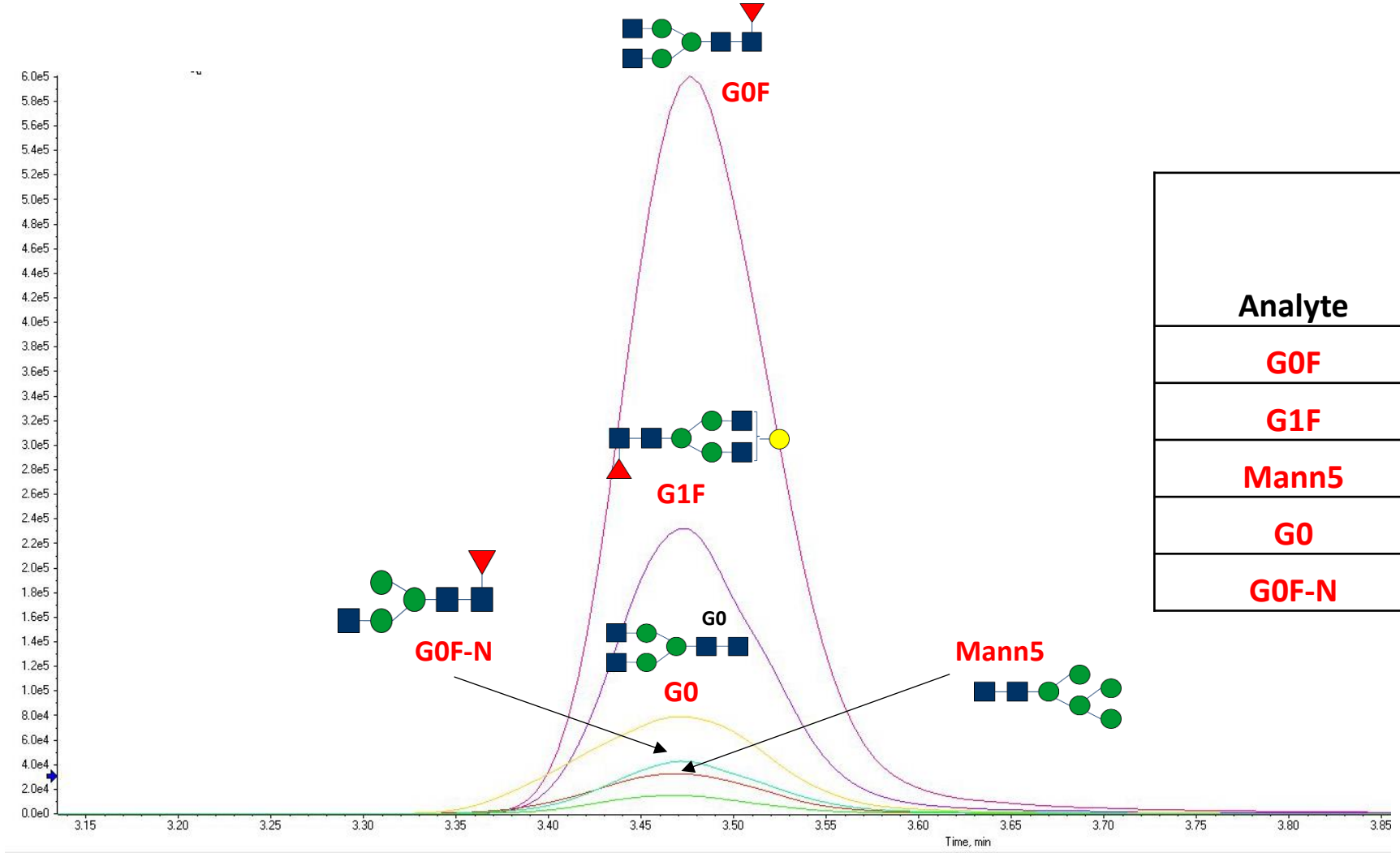
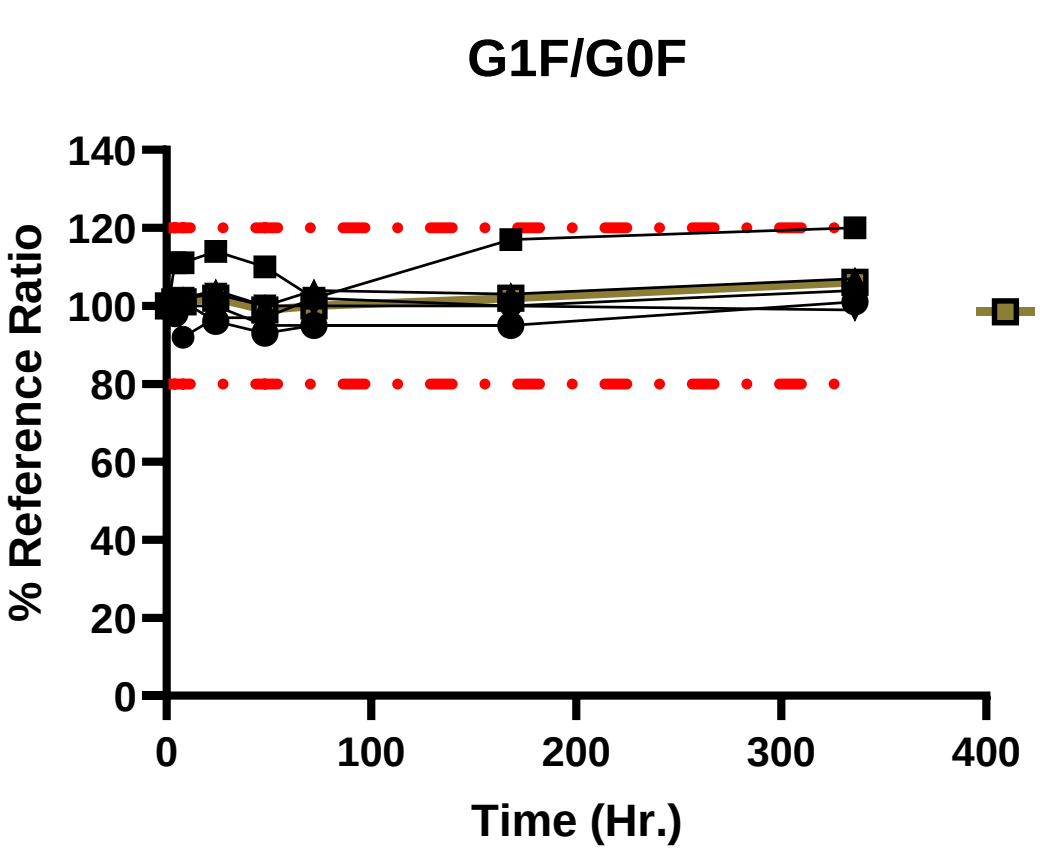
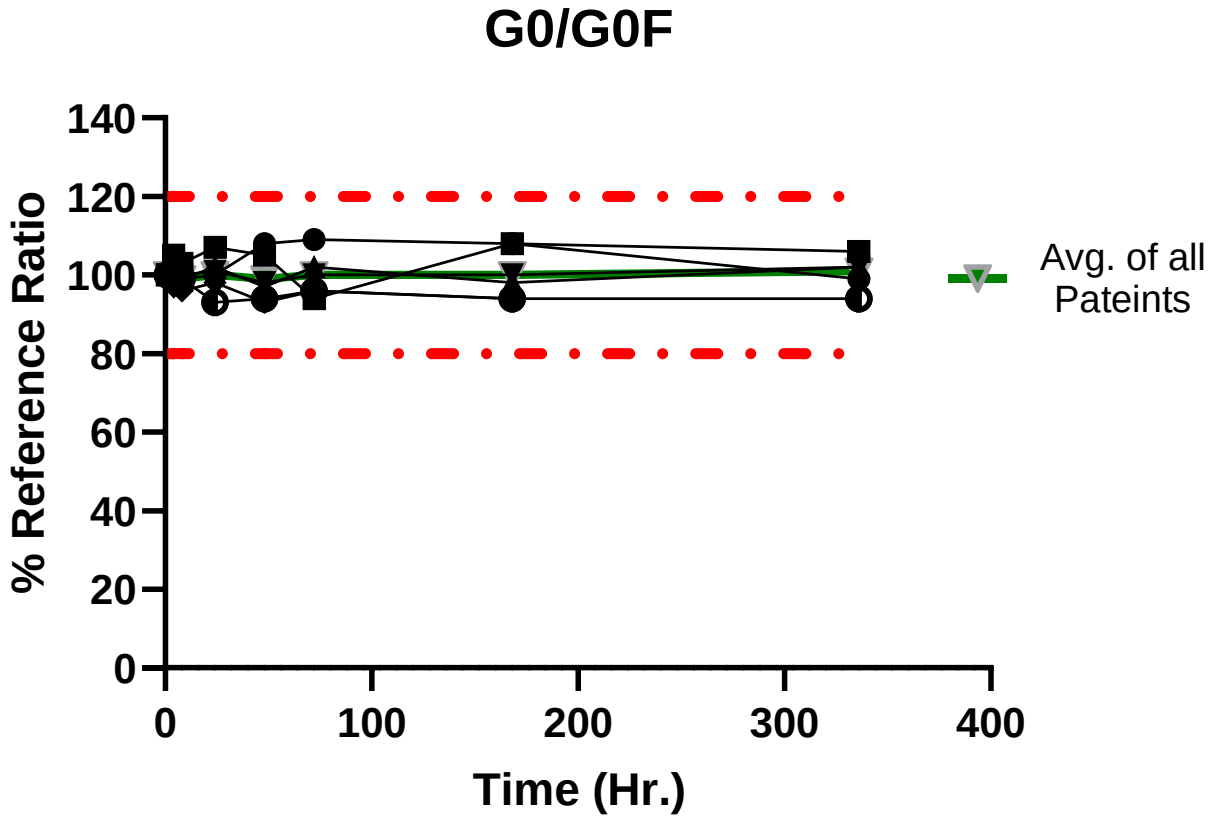
LC/MS Chromatogram for Glycoforms in Dosing Material (BI-X)



Analyte	Analyte Peak Area (counts)	R. Abundance
G0F	3.47E+06	58%
G1F	1.35E+06	23%
Mann5	2.22E+05	4%
G0	5.95E+05	10%
G0F-N	2.66E+05	4%

Relative abundance of each glycoform compared to G0F over time





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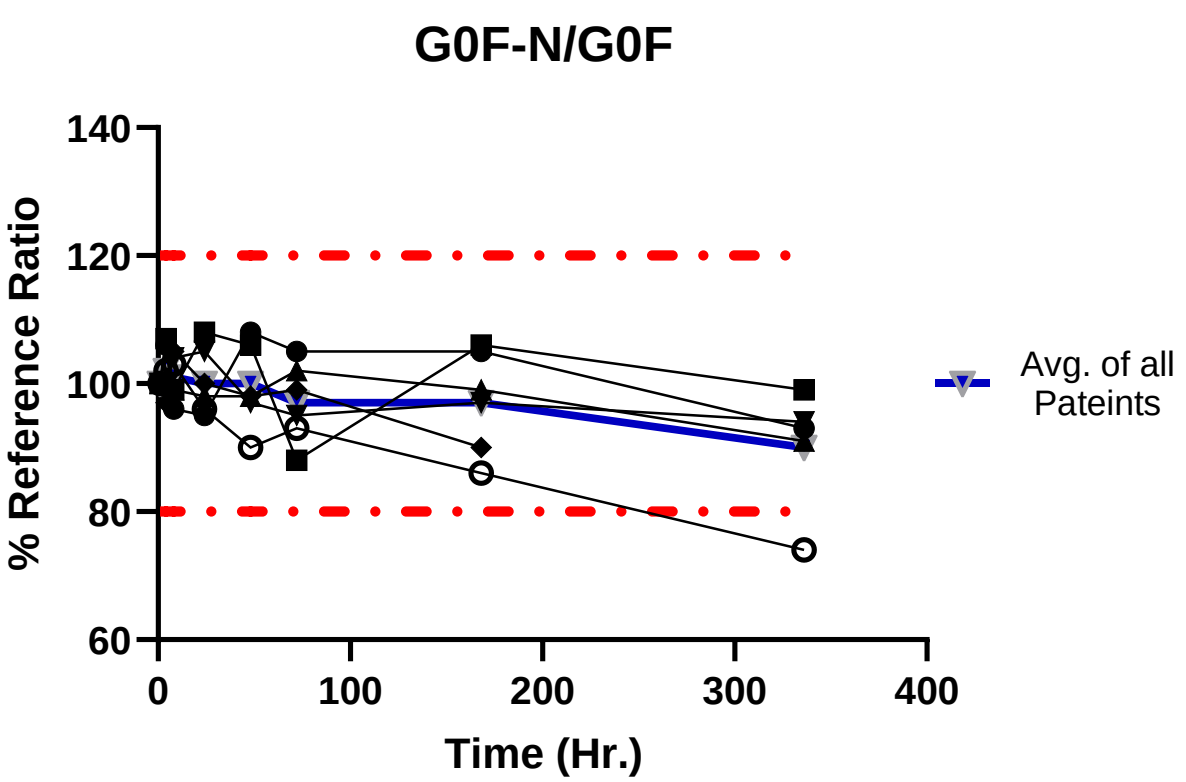
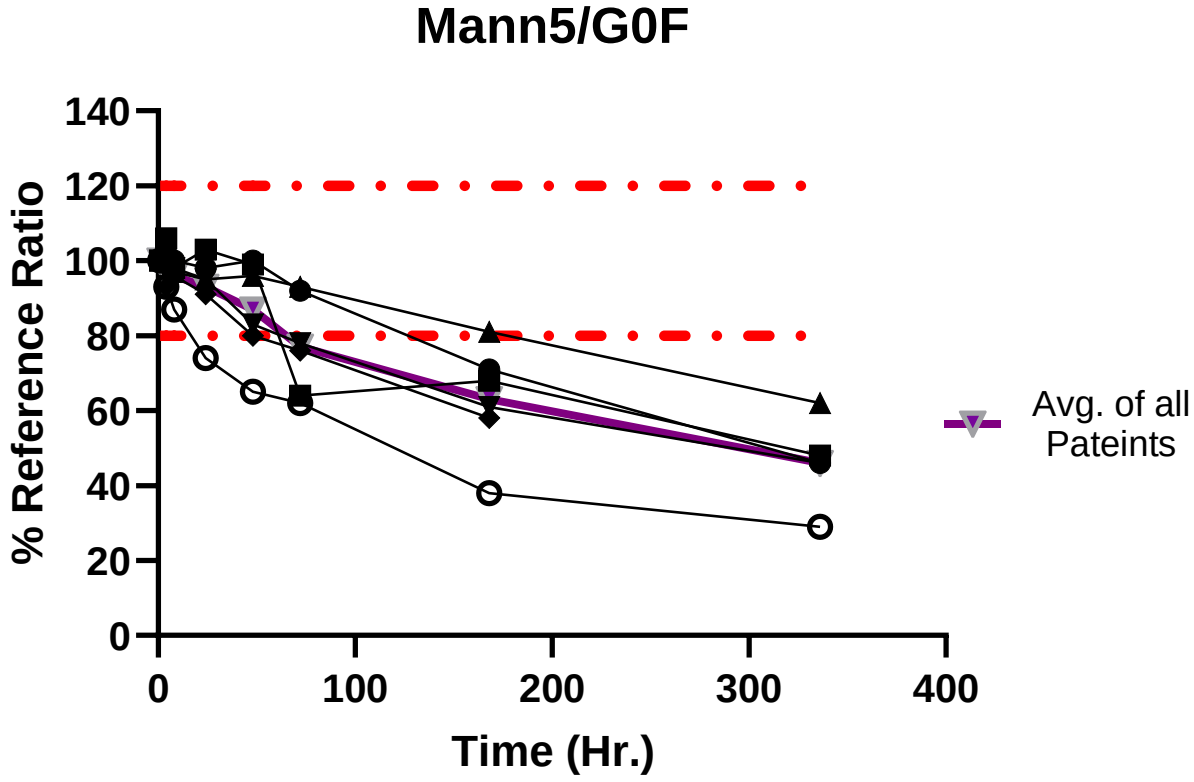
Relative abundance of each glycoform compared to G0F over time





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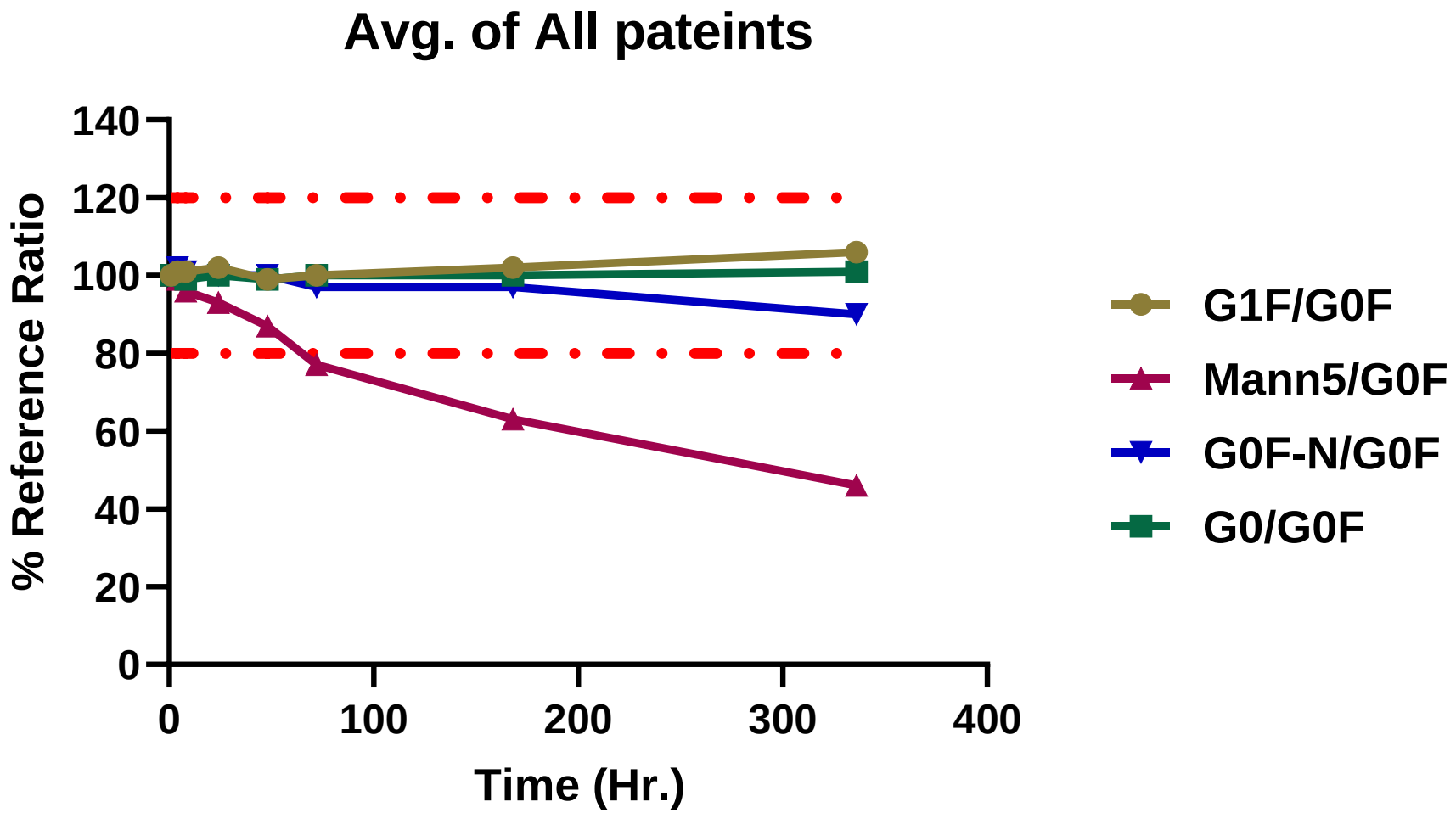
Relative abundance of each glycoform compared to G0F over time



Avg. T1/2 (hr.) of different glycoforms

Glycoform	T 1/2 (hr.)
G0F	205
G1F	231
G0	223
G0F-N	206
Mann5	129

Summary

- An LC/MS assay was developed to quantitatively measure glycoforms of BI X. The assay consisted of immunocapture purification, tryptic digestion and LC-MS/MS analysis.
- The assay was successfully applied to BI X to determine differences in clearance among G0F, G1F, G0, G0F-N and Mann5 in human Serum.
- Compared to G0F, G1F, and G0 ($t_{1/2}$ were 205, 231, 223 and 206 respectively), Mann5 had a shorter $t_{1/2}$ (129 h).
- The assay provides a generic approach for in vivo critical quality attribute assessment; can be used for any IgG-based NBEs, different species and different matrices.

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Thank you

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