

GlycoTyper™ - a novel liquid-biopsy platform for high-throughput, high-sensitivity targeted glycoproteomics, exemplified by differentiating Systemic Lupus Erythematosus and Lupus Nephritis

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect many different tissues and organs. Lupus nephritis (LN) is a serious complication of SLE where autoantibodies form immune complexes that are deposited in the glomeruli, causing inflammation, kidney damage, and potentially kidney failure. It is estimated that up to 60% of adults and 80% of children with SLE will develop LN, and up to 30% of those who develop LN will progress to kidney failure. Because SLE is a heterogeneous disease with a highly variable course, early diagnosis and aggressive treatment are crucial to prevent progression to end-stage renal disease. Currently, in the absence of any liquid-biopsy-based tests, LN diagnosis still relies on renal biopsy as the standard of care. Alternative approaches are urgently needed. Here we introduce the GlycoTyper liquid biopsy platform, a MALDI-based-characterization (Bruker timsTOF flex) of enzymatically released N-glycans from affinity-captured target proteins.

Methods

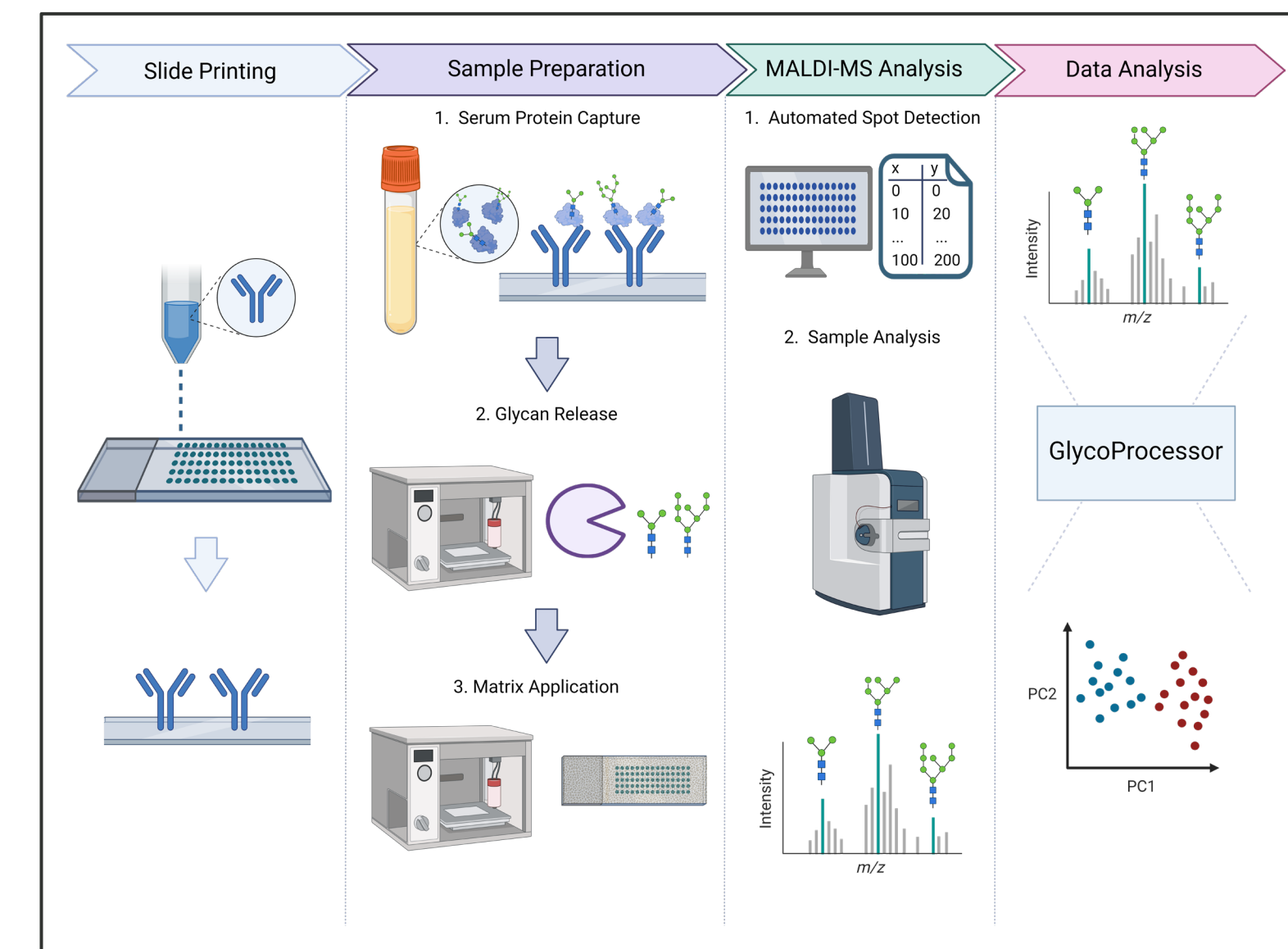


Fig. 1 The GlycoTyper workflow for the analysis of cleaved N-glycans derived from patient biofluid samples: Created in <https://BioRender.com>

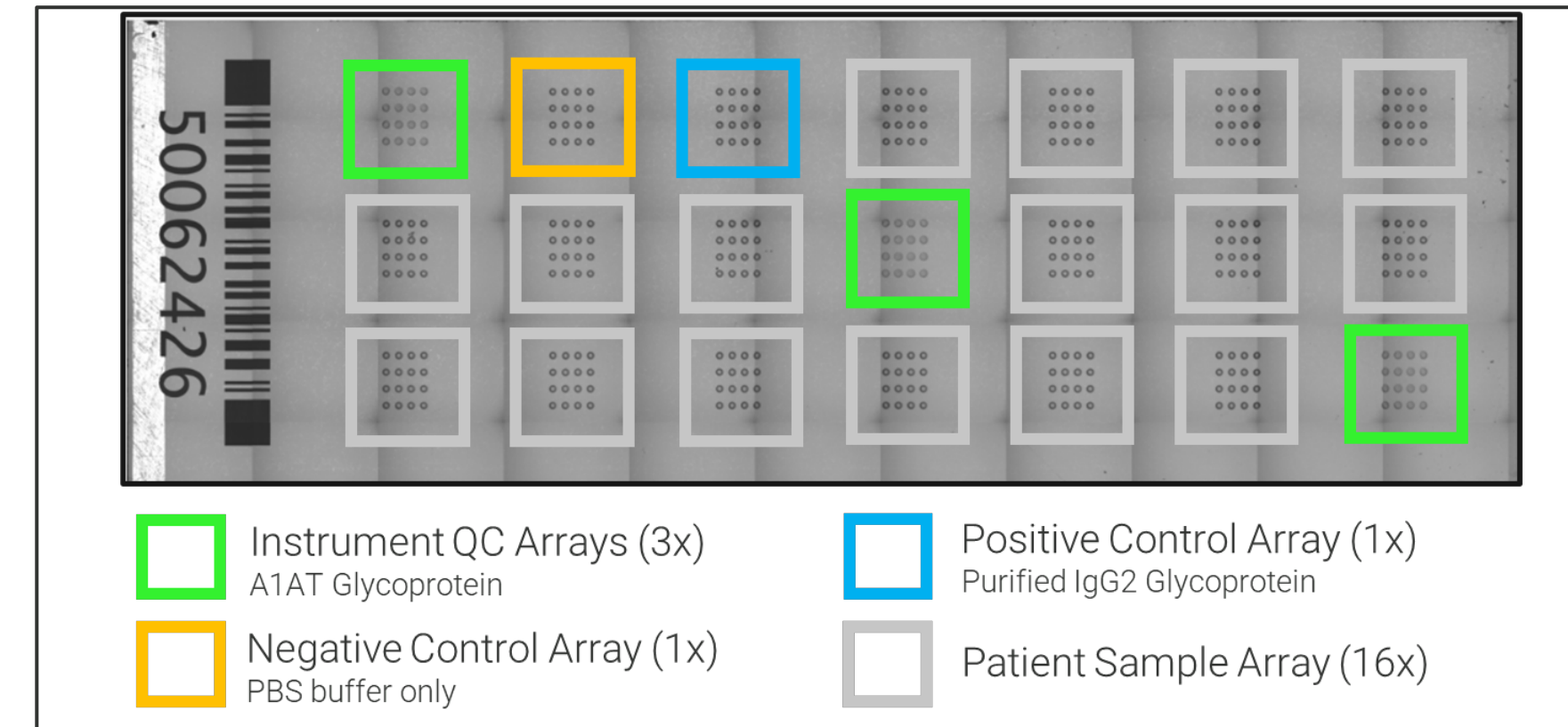


Fig. 2 The GlycoTyper Assay Slide

The GlycoTyper platform consisted of IgG capture antibody arrays printed on Nexterion-H-coated glass slides. The slides were modified to a custom multi-well format for the addition of patient samples. After IgG capture and washing steps, N-glycans were enzymatically released with PNGase F. The slides were then coated with matrix followed by N-glycan readout using MALDI mass spectrometry (Bruker timsTOF flex). The robustness of the platform was addressed by a quality strategy which included system suitability tests during sample preparation, incorporation of QC arrays throughout the slide, and both positive and negative control arrays.

Results

I. Baseline Sample Analysis

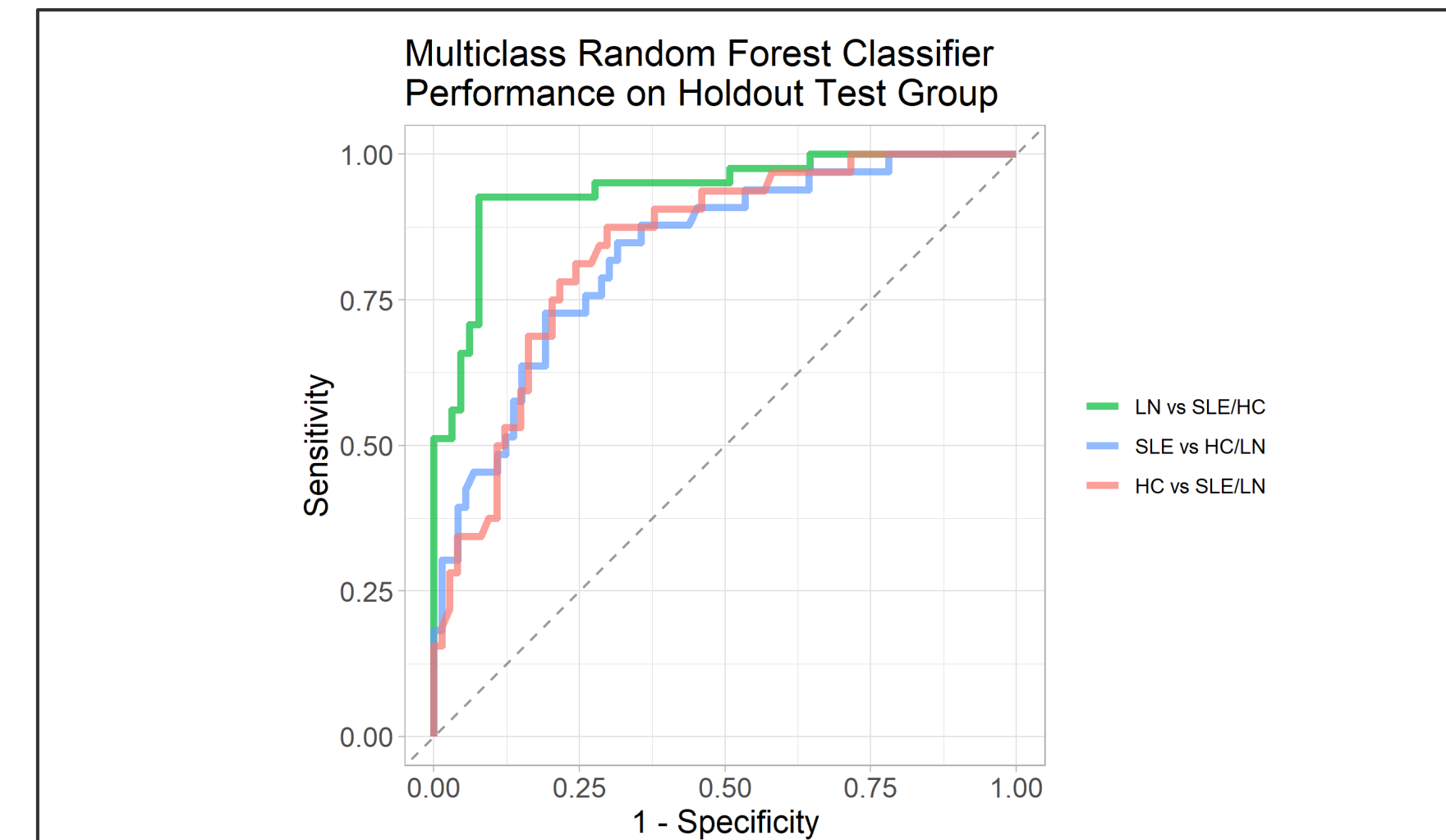


Fig. 3 Receiver Operator Characteristic Curves: Three one-versus-all performance estimates derived from the Random Forest classifier's application to the reserved test set : LN vs SLE/HC, SLE vs HC/LN, and HC vs SLE/LN. In the classification of test-set patients with LN vs SLE/HC, the random forest model had an AUC of 0.87, a sensitivity of 0.9, and a specificity of 0.88.

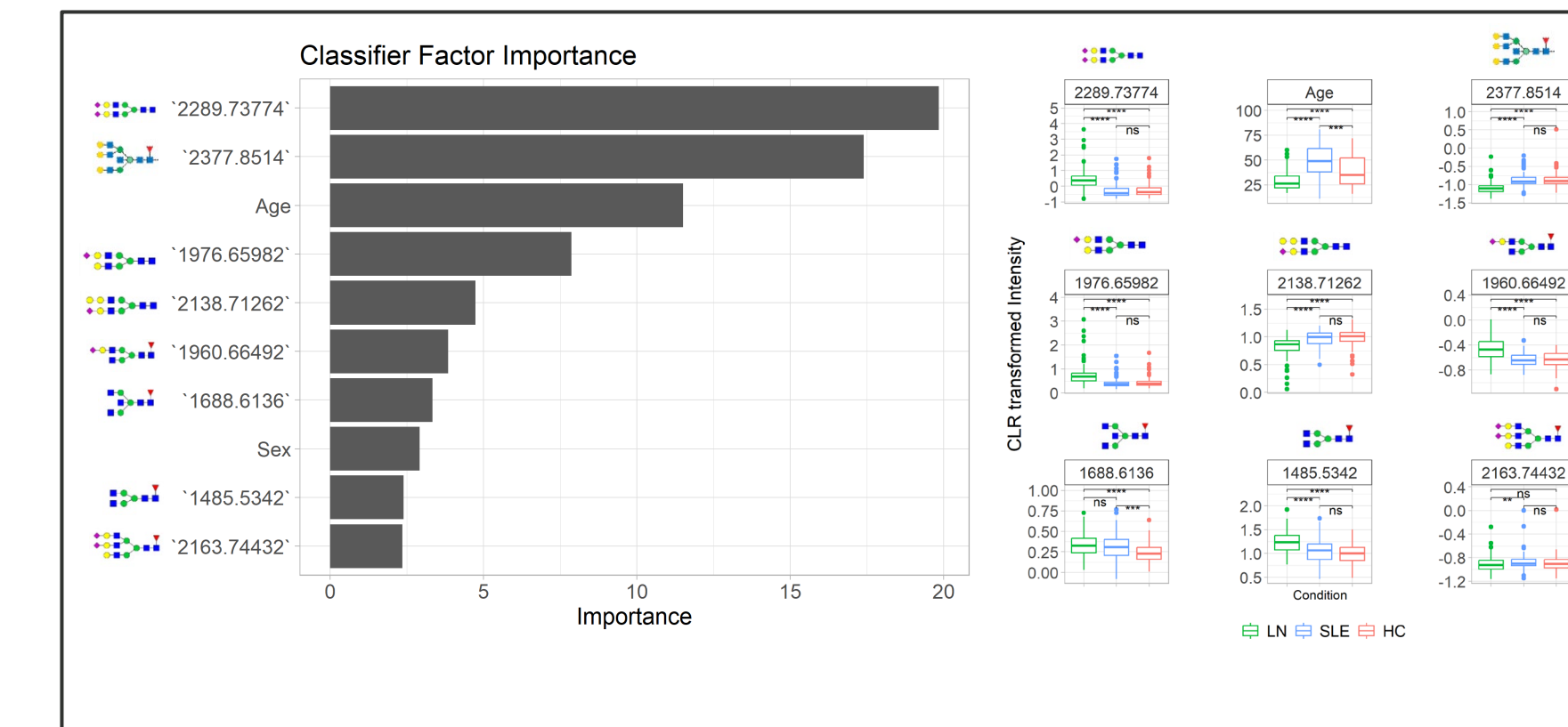
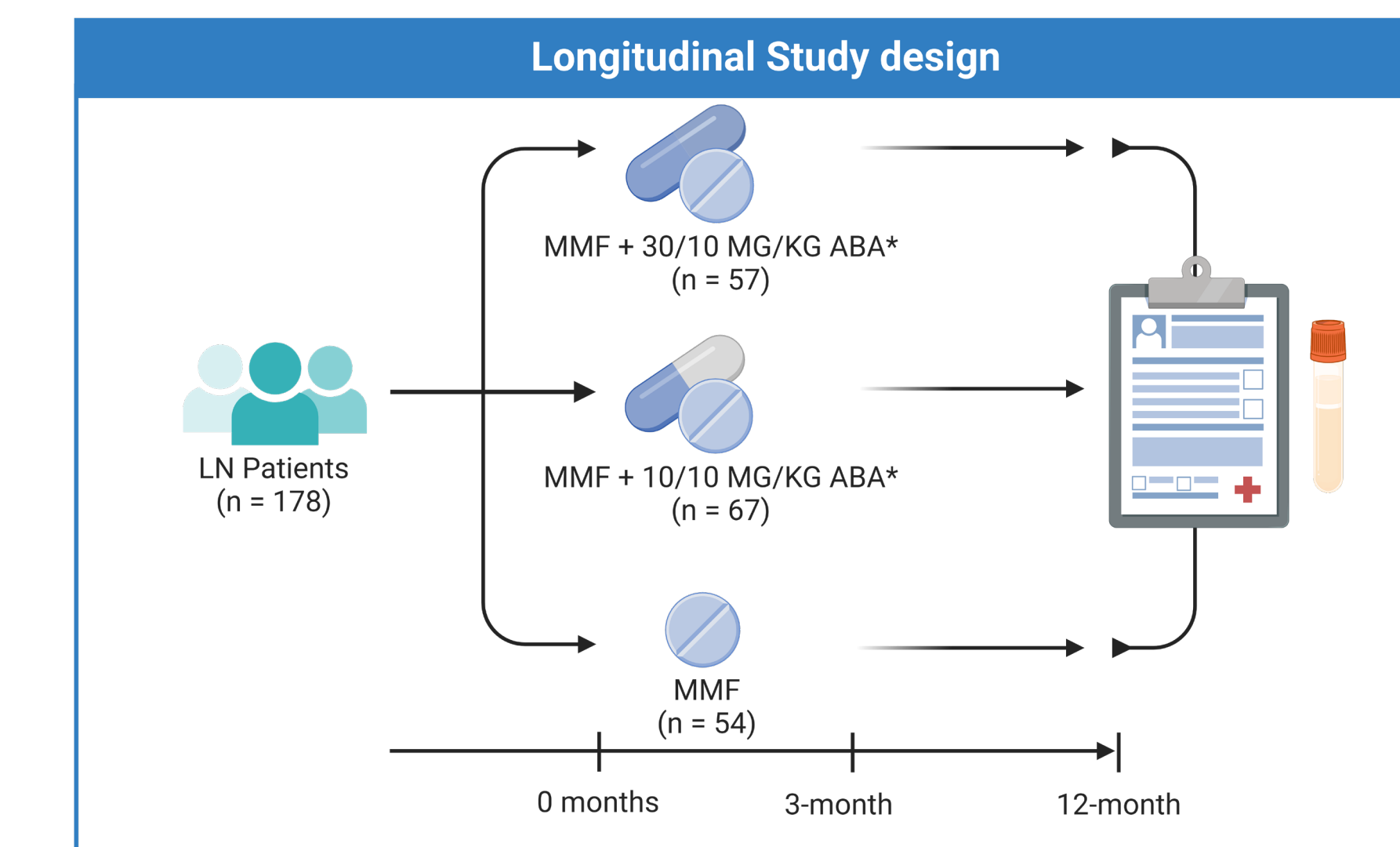


Fig. 4 Random forest classifier feature importances and relative abundances in baseline patient samples: Feature importance calculated using the mean decrease in Gini, a method of estimating a feature's ability to sort a heterogenous input into more homogenous outputs. Among the top-4 most important features are two sialylated biantennary afucosylated N-glycans whose relative abundances are higher at statistically significant levels in LN samples than either SLE or HC samples. These glycans also play a role in treatment outcome when LN patients are placed on immunosuppressant therapy.



*ABA administered via IV infusion on days 1, 15, 29, 57, 113, 141, 169, 197, 225, 253, 281, 309, and 337. Patients on 30/10 MG/KG ABA regimen received the 10/10 MG/KG dosage from day 85 onward

Fig. 5 Immunosuppressant therapy schema for Lupus Nephritis patient cohort: LN patients were randomly partitioned into three immunosuppressant therapy groups: mycophenolate mofetil (MMF), MMF + Abatacept (ABA) 10/10 MG/KG, or MMF + Abatacept 30/10 MG/KG. Spot urine samples were collected prior to treatment, after 3 months of treatment, and after 1 year of treatment. Created in <https://BioRender.com>

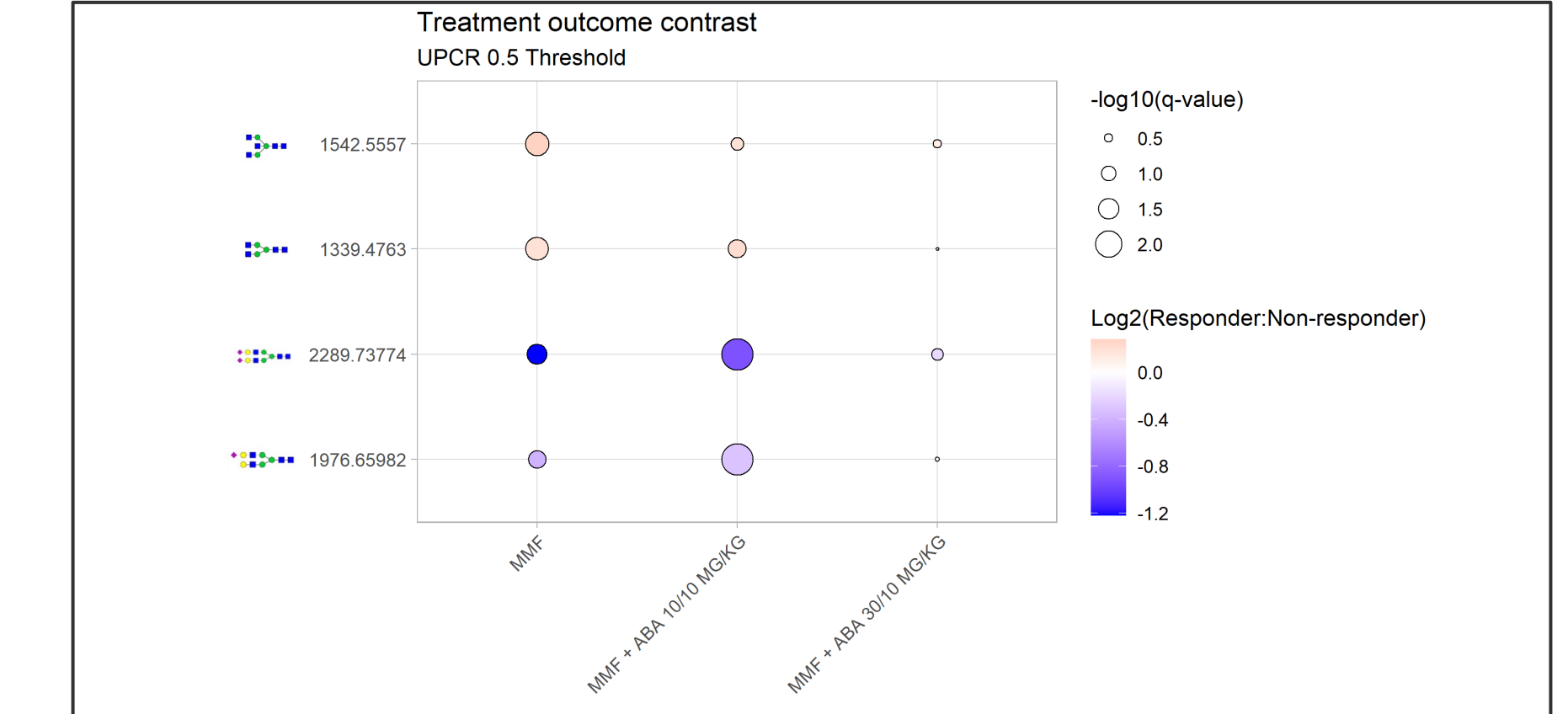


Fig. 6 Statistically significant results from a Kruskal-Wallis-based comparison of IgG N-glycans between treatment outcome groups, as determined by a UPCR threshold of 0.5 g/g : Two sialylated and afucosylated biantennary glycans were found to be associated with immunosuppressant responder/non-responder outcome as determined by the urine protein-to-creatinine ratio (UPCR).

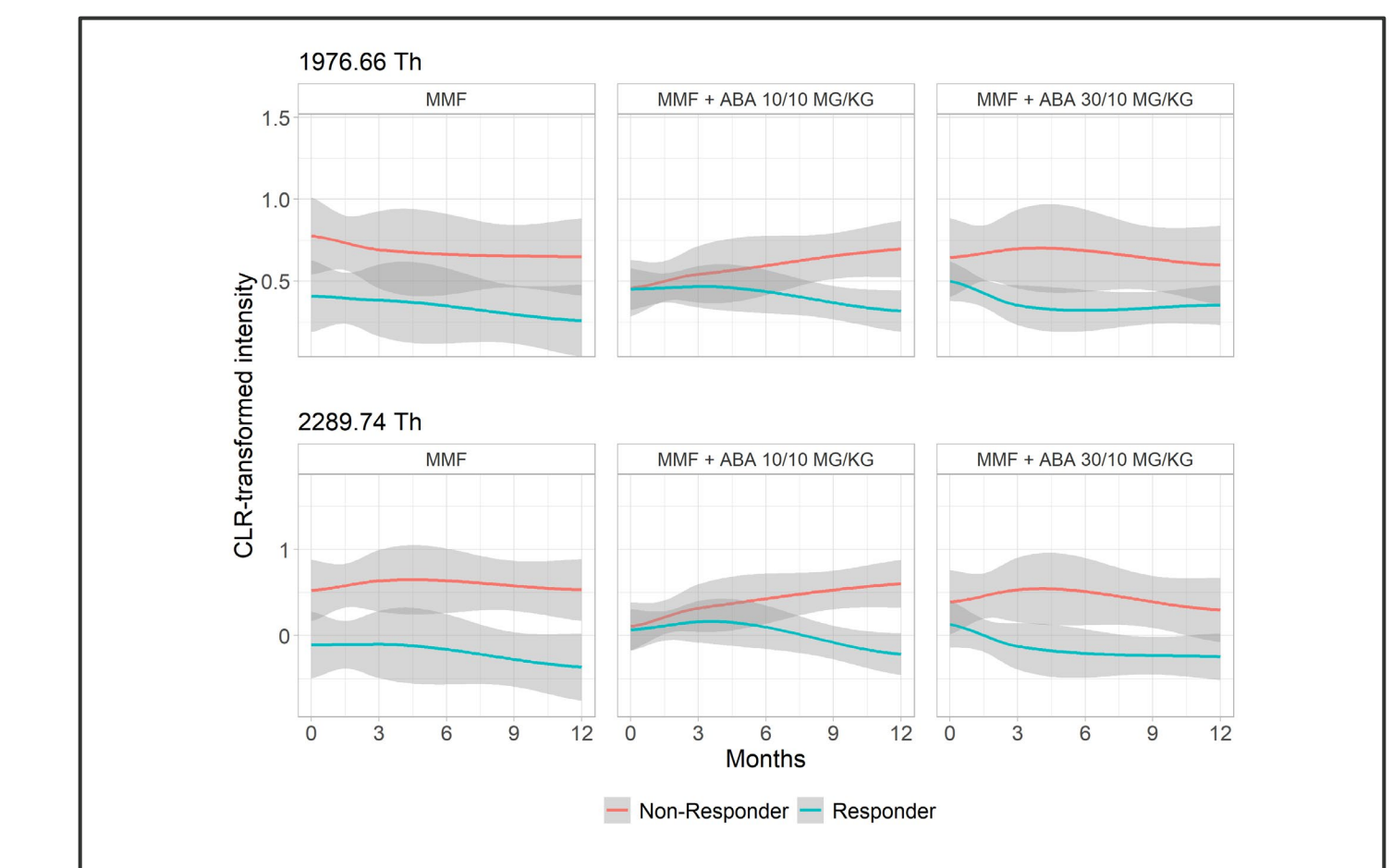


Fig. 7 Relative abundances for two sialylated and afucosylated biantennary N-glycans across the 0th, 3-month, and 12-month time points associated with immunosuppressant therapy study: Higher relative baseline abundance or increasing abundance of biantennary, sialylated, afucosylated glycans was associated with adverse renal disease outcome in LN patients placed on immunosuppressant therapy.

- Random forest classifier distinguishes LN from SLE/HC samples at 0.87 AUC
- Biantennary, sialylated, afucosylated glycans rank among the top-5 most important features determining classifier performance
- Higher baseline relative abundance or increasing abundance of biantennary, sialylated, afucosylated glycans was associated with adverse renal disease outcome in LN patients placed on immunosuppressant therapy.
- Glycan profiles, independent of cognate protein abundance, are a potentially important source of biomarkers

Conclusions