Predicting Treatment-Response for HCV Therapy: Successful Translation from Discovery LC/MSE to Verification LC/MRM

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BACKGROUND

Translation of candidate biomarkers from discovery technologies to verification technologies is an integral component of any biomarker pipeline. While modern high resolution, accurate mass tandem mass spectrometers are quite suitable for discovery proteomics, they do not have the requisite sample throughput, ruggedness, reliability and simplicity of operation required for verification, validation, and clinical implementation protocols. Fortunately, triple quadrupole mass spectrometers operated under MRM have a proven record of success in "post-discovery" analyses.

The standard-of-care therapy for HCV results in sustained virologic response in only half of patients, and the costs of this therapy, in terms of both adverse side effects and in dollars (~ $30,000/year) has led to a need for predictive biomarkers. An expression pattern predictive of response to the therapy from treatment naive patients was successfully translated. The response signature was initially identified in serum from the analysis of 96 patient samples by nano-LC coupled with high resolution, accurate mass tandem MS. Sparse latent factor metaprotein expression modeling yielded three metaprotein predictors comprised of 521 peptides, which were then curated based on set selection criteria, generating a list of 87 peptides for MRM analysis.

Subsequent MRM analysis of the original 96 samples yielded a final group of 10 peptides which maintained a Bonferroni-corrected statistical significance for predicting treatment response. The assay has been verified by a blinded analysis of a 51 patient all-comers clinical trial, which yielded an AUROC of 0.91 with a sensitivity of 0.828 and specificity of 0.786. The analysis of an additional clinical trial cohort (n=243) is ongoing, including the use of stable-labeled peptides for the most promising candidates.

STEP 2: Sample Preparation and Data Collection Quality Control

LC-MS data collection Quality Control
- Internal Standard (ADH1_YEAST) at 50fmol/ug in every sample
- Daily QC Injection (pooled plasma standard) book-ending and within cohort
- Retention Time and Principal Components Analysis

Internal Standard 
KIGYNGKIE W from ADH1_YEAST

Retention Time Shift 
Required shift for alignment

Principal Components Analysis

STEP 3: Statistical Analysis Using Bayesian Factor Regression Modeling

"Metaprotein" statistical analysis groups peptides across the analysis cohort based on two factors, expression pattern and parent protein, without regard to clinical phenotype. Importantly, it allows the following:
- Casts a relatively wide net for potential biomarker peptides
- Discordant peptides are not required to be in the "parent" metaprotein
- Grouping of large numbers of co-expressing peptides to improve S/N
- Direct implementation in to a (predictive) regression model

Metaprotein Regression Model, including Clinical Interprets
Probit (p) = 1.22 - .90 (VTDB) – 0.54(AHSG) + .53 (CS) -0.02 (female) -1.5 (AA) -0.95 (log HCV RNA)

Model Fitting Results (n=55)

Performance of the Metaprotein Model for Blinded Prediction of 2 Verification Cohorts

Discovery (n=55) 
Matched Cohort 

Verification 1 (n=41) 
Matched Cohort 

Verification 2 (n=51) 
All-Comers Trial

REFERENCES

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