Analytical Challenges Developing an Ultrasensitive Simoa Immunoassay for IL-1β as a Biomarker for NASH

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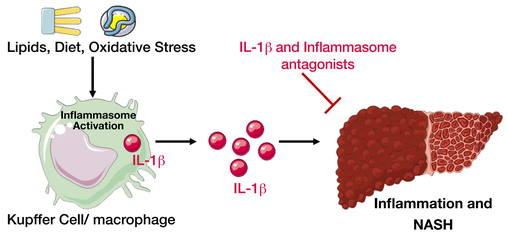
Introduction

There is imperative need for non-invasive biomarkers for non-alcoholic steatohepatitis (NASH). Proinflammatory cytokines promote progression of NASH and are potential biomarkers.

IL-1β, a cytokine produced by inflammasome mediated cell death, induces hepatic inflammation. Antagonists of IL-1β and inflammasome are under development. Thus, IL-1β could be a prognostic and target engagement biomarker for NASH drug development.

We hypothesized serum IL-1β increases with disease severity and developed an immunoassay for IL-1β for a **context-of-use (COU) of a prognostic biomarker for patient stratification during NASH trial enrollment.**



Methods

We developed a biomarker work plan for an IL-1β immunoassay for this COU.

Table 1. Biomarker Work Plan for IL-1β

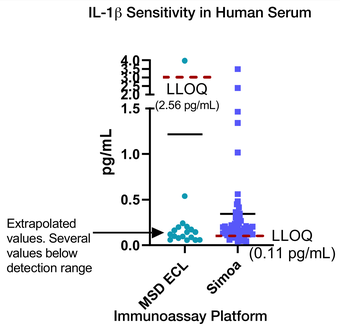
Parameter	Requirements
Analyte	IL-1β
Context of Use	Determine if **IL-1β is a biomarker of patient selection, I/E criteria, or prognostic enrichment** of steatosis and fibrosis and thus appropriate for inclusion in **NASH drug development clinical trials.**
De Novo or Commercial Kit	Kit preferred if enough sensitivity
Reference Material	Kit material
Concentration Range (Normal –> Disease)	Below 1 pg/mL expected based on extrapolated ECL values and literature with increase in NASH. **Ultrasensitive immunoassay needed.**
Sample Matrix and Volume	Serum
Sample Number and Study Length	100-200 samples over 2 years
Biological Considerations and Variability	Lipemia expected in NASH serum. **Measure incurred NASH samples in validation.**
Commercial Kit Adaptations	Test sensitivity, lipemia, endogenous lipemic QC

In our previous measurements of IL-1β in human serum with electrochemiluminescence (ECL), 69% of clinical samples were below the limit of quantification (LLOQ). The limited clinical studies which measured IL-1β reported values below the LLOQ. Therefore, we developed an **ultrasensitive immunoassay by converting an IL-1β commercial kit for the Quanterix Simoa SRX benchtop platform.** We optimized buffers to lower noise and determined a minimum required dilution (MRD) of 1:2 minimized matrix interference while maintaining assay sensitivity.

Since NASH patients typically have metabolic syndrome, we anticipated challenges with lipemia during sample collection and handling and challenges with reproducibility from experience with bead-based assays. Finally, we assayed incurred samples during validation from an **enrollment study which screened patients for NASH by non-invasive Fibroscan transient elastography imaging to determine if our assay fit the COU.**

Results

Challenge #1: Sensitivity



The Simoa IL-1β assay demonstrated low background and 25-fold increased sensitivity (LLOQ of 0.110 pg/mL vs. 2.56 pg/mL with ECL after accounting for MRD). 48 of 62 incurred samples were above the LLOQ compared to only 20 with ECL.

Challenge #2: Precision and Lipemic Sample Interference

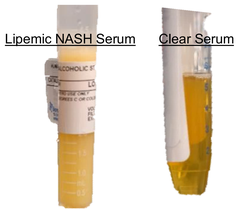


Table 2. Lipemic Sample Treatment Improves Precision with Simoa Platform

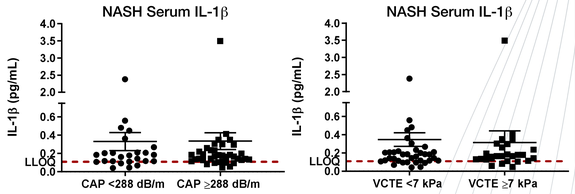
Sample	%CV Before Treatment	%CV After Treatment
1 (Non-Lipemic)	9.90	9.30
2 (Non-Lipemic)	5.88	4.78
3 (Lipemic)	39.9	15.2
4 (Lipemic)	57.4	10.2

Table 3. Lipemic Sample Treatment Maintains Analyte Recovery

% Recovery						
	High QC			Low QC		
Lipemia level	High	Mid	Low	High	Mid	Low
Sample 1	59	82	92	68	77	89
Sample 2	88	85	93	79	87	91

While Simoa improved sensitivity, most of our incurred samples were grossly lipemic which had higher coefficient of variation (%CV >50%) between replicates compared to non-lipemic serum. Sample dilution to overcome lipemia could not be used due to loss of sensitivity. We extracted lipids from NASH serum with LipoClear, a non-ionic polymer that binds and precipitates lipids. Addition of the buffer reduced %CV to less than 30% for incurred samples without impacting recovery. We also increased acceptance criteria for %CV to 30% which was acceptable for our COU.

Challenge #3: Performance of Biomarker for Patient Selection COU



IL-1β concentrations in 62 incurred samples measured by the Simoa assay did not increase with increased hepatic steatosis or fibrosis as measured by Fibroscan suggesting it cannot be used for trial inclusion/exclusion criteria.

Novel Aspect

This is the first reported use of an ultrasensitive technology to accurately measure IL-1β in NASH. While IL-1β did not correlate with NASH severity for trial inclusion, it may be a useful biomarker for other COUs like target engagement.

The challenges of sensitivity, lipemia, and incurred sample analysis are applicable to other NASH biomarker studies. For example, extraction of lipemia is necessary when using the Simoa platform to improve reproducibility and LipoClear did not decrease IL-1β recovery.

Finally, this study demonstrates the need for fit-for-purpose validation based on COU to drive biomarker bioanalytical validation. The decision to measure incurred NASH samples during bioanalytical validation allowed early recognition that IL-1β did not fit a prognostic biomarker COU before study sample analysis.

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