Fit-for-purpose inflammatory biomarker assay development and validation

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Novimmune – Company Profile

- Focused on advancing targeted medicines that address the causes rather than symptoms of disease
- Proprietary next-generation platforms for generation of monoclonal and bispecific antibody drugs
- Bench-to-bedside & pilot manufacturing capabilities
- Pipeline of differentiated antibody-based products
- Scientific excellence
  - Ten patent families
  - 60+ peer-review journal publications
  - 30+ collaborations with leading academic institutes
  - Partnerships with Genentech, Tiziana and Baxalta
Presentation Outline

- Background
  - Biomarker strategy
  - Bioanalytical assay strategy

- Verification of biomarker candidates
  - Qualified multiplex assay on the Luminex® platform

- Validation of a lead biomarker candidate
  - Validated singleplex assay on the MSD® platform

- Conclusions
Biomarker Strategy

- Why do we need one?
  - High attrition rate of new therapeutics
  - Model systems in discovery research are insufficiently predictive for efficacy and safety in man
  - Need better translational models and tools to determine drug exposure, efficacy and safety

  = Biomarkers

- What do we want?
  - Biomarker(s) of safety
  - Biomarker(s) of disease severity
  - Biomarker(s) of therapeutic efficacy
Biomarker Strategy

Discovery/Screening → Verification → Validation

Bioanalytical Assay Strategy?
Bioanalytical Assay Strategy?

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

Jean W. Lee,1,2,4 Viswanath Devanarayan,2 Yu Chen Barrett,2 Russel Weiner,2 John Allinson,4 Scott Fountain,3 Stephen Keller,3 Ira Weinstein,5 Marie Green,6 Larry Duan,7 James A. Rogers,8 Robert Millham,9 Peter J. O’Brien,3 Jeff Saltz,10 Masood Khan,11 Chad Ray,12 and John A. Wagner13

WHITE PAPER

SPECIAL FOCUS ISSUE: BIOANALYSIS OF BIOMARKERS

Recommendations on biomarker bioanalytical method validation by GCC

Richard Houghton1, Dominique Gouty1, John Allinson1, Rachel Green1, Mike Losaozo1, Steve Lowe1, Richard L. Lacheur2, Fabio Garofolo4, Philippe Courrèges1, Stéphane Bronner1, Petra Struve1, Christine Schiebel1, Timothy Sangster14, Colin Pattison15, Rafiq Islam16, Wei Garofolo12, Maria Pawula17, Mike Buonarati11, Roger Hayes11, Mark Cameron11, Robert Nicholson11, Jake Harman11, Jaap Wieling11, Theo De Boer11, Scott Reuschel11, Laura Cojocaru11, Tammy Harter11, Michele Malone11 & William Nowatzke11

White Paper

European Bioanalysis Forum recommendation on method establishment and bioanalysis of biomarkers in support of drug development

Philip Timmermans18, Christian Metzing19, Daniela Scillner12, Birgit Jahn12, Susanne Pihl20, Karen Elby12, Neil Henderson12, Begona Barreiro12, Stephen Fischmann12, Arjen Comanji12, Amands Versteijen12, Stewart Bate11, Clare Kingsley11 & Ulrich Kunz11

The AAPM Journal (© 2015)
DOI: 10.1289/ajr.2246-015-0030-y

White Paper

Recommendations for Use and Fit-for-Purpose Validation of Biomarker Multiplex Ligand Binding Assays in Drug Development

Darshana Jani1,4,13 John Allinson2, Flora Berisha2, Kyra J. Cowan2, Viswanath Devanarayan2, Carol Gleason4, Andreas Jeromin5, Steve Keller5, Masood U. Khan5, Bill Nowatzke16, Paul Rhyne11 and Laurie Stephen11
Bioanalytical Assay Strategy

- 3 tiers for biomarker assay method establishment
  - Screening
  - Qualified
  - Validated
Biomarker Strategy

Discovery/Screening → Verification → Validation

Bioanalytical Assay Strategy

Screening → Qualified → Validated
Biomarker Strategy: Discovery & Screening

Case Study

- *Monoclonal antibody (mAb) therapeutic* with target in inflammatory disease
  - Special patient population
  - Limited sample volume

- Biomarker candidate identification
  - Mechanism of action of therapeutic
  - Research and development data
  - Literature
Biomarker Strategy: Discovery & Screening

Case Study

- Observational samples collected from patient group of interest
  - *Screening* of 30 inflammatory biomarkers of interest

- Bioanalytical method strategy: *Screening*
  - Commercial multiplex assays
  - Commercial ELISA assays
Biomarker Strategy: Discovery & Screening

Conclusions

- Six inflammatory biomarkers identified as *potential* markers of disease severity and therapeutic efficacy

Biomarker *Verification*
Biomarker Strategy: Verification

- Bioanalytical Method Strategy: **Qualified**
  - Multiplex assay on Luminex® platform
Luminex® Platform

- Bead-based multiplex assay
Luminex® Assay Qualification

- Six analytes in parallel
  - Working range
  - Matrix effect/minimum required dilution
  - Precision & accuracy
  - Interference & cross-talk
  - Spiked sample dilutional linearity
  - Parallelism
  - Selectivity (spike recovery)

- Some limitations
Luminex® Assay Qualification
Inflammatory Biomarker 1

- Spiked matrix dilutional linearity

![Bar chart showing recovery percentage against dilution factor.](chart)

1/200 chosen to overcome matrix effects whilst maintaining an acceptable sensitivity

- Similar results for parallelism
  - limited number of samples
Luminex® Assay Qualification

Summary

- Development and qualification highlighted some limitations

- Considered *fit-for-purpose* for *verification* of biomarker candidates
Biomarker Strategy: Verification

- Small number of individuals to evaluate *efficacy* and *safety* of therapeutic in selected disease population
  - Special patient population
  - Limited sample volume

- Six lead biomarkers evaluated for *correlations* with *disease severity* and *therapeutic efficacy*
Biomarker Strategy: Verification

Inflammatory Biomarker 4

High levels pretreatment
Rapid drop during first days of treatment period
Level returns to healthy volunteer level

Very encouraging correlation to efficacy

Healthy volunteer levels <250 pg/mL
Biomarker Strategy: Verification
Inflammatory Biomarker 4

- In hindsight, a better representation of the data would be % change from baseline given the assay limitations.
Biomarker Strategy: Verification

Inflammatory Biomarker 5

High levels pretreatment
Drop during first days of treatment period
Level approaches healthy volunteer level
Encouraging correlation to efficacy

Healthy volunteer levels 30-500 pg/mL
Biomarker Strategy: Verification

Inflammatory Biomarker 1

- High levels pretreatment
- Rapid drop during first days of treatment period
- Rise in levels during periods of higher inflammatory response
- Possible correlation to disease severity

Healthy volunteer levels 50-200 pg/mL
Luminex® Assay: In Study Qualification
Inflammatory Biomarker 1

- Parallelism/matrix effect
Biomarker Strategy: Verification

Conclusions

- *Inflammatory Biomarkers 4 and 5* showed a *correlation* with *therapeutic efficacy*

- *Inflammatory Biomarker 1* showed a *possible correlation* with *disease severity*

Biomarker Validation
Biomarker Strategy: Validation

- Bioanalytical method strategy: **Validated**
  - Singleplex assay on MSD® platform
Meso Scale Discovery (MSD®) Platform

- Plate-based assay
- SulfoTAG label emits light when electrochemically stimulated
**MSD® Assay Validation**

**Method Establishment**

- Single analyte
  - *Recombinant protein standard*
  - *Substitute matrix*
- Working range
- Minimum required dilution / matrix effect
- Precision & accuracy
- Spiked sample dilutional linearity
- Selectivity (spike recovery)
- *Endogenous biomarker parallelism*
- *Endogenous biomarker stability*
- *Drug and target interference*
MSD® Assay Validation
Inflammatory Biomarker 1

- Robustness
  - inter-assay accuracy and precision of *recombinant biomarker* QCs in *surrogate matrix* to demonstrate appropriate range

![Graph showing accuracy, precision, and total error across different concentrations.](image-url)
MSD® Assay Validation
Inflammatory Biomarker 1

- Robustness
  - inter-assay assessment of *endogenous biomarker in serum matrix*

![Graph showing inter-assay data for MSD® Assay Validation](chart.png)

Inter-assay data used to set the assay acceptance criteria
MSD® Assay Validation
Inflammatory Biomarker 1

- Robustness
  - inter-assay assessment of *endogenous biomarker* in *serum matrix*

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<th>Mean Conc</th>
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<td>n</td>
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- Nominal value of **632.92 pg/mL** assigned
  - Target %RE criteria?
    - 2x inter-assay CV = 16.18 %
    - 3x inter-assay CV = 24.27 %
MSD® Assay Validation
Inflammatory Biomarker 1

- Parallelism (incurred samples)

Neat: analysed after 1/10 MRD
2, 5, 10, 50: additional dilution factors

Healthy volunteer levels 200-1000 pg/mL
MSD® Assay Validation
Inflammatory Biomarker 1

- Sample stability: 1 month freezer storage

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<th>Assay N°</th>
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<th>Concentration in pg/mL in Matrix</th>
<th>LoMatrixQC</th>
<th>Observed Conc</th>
<th>Accuracy (RE%)</th>
<th>Precision (CV%)</th>
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Long term stability study initiated
MSD® Assay Validation

Inflammatory Biomarker 1

- Drug and target interference

Recovery of LoMatrixQC with increasing spikes of Target (ng/mL) and fixed spike of Therapeutic (50 µg/mL)
MSD® Assay Validation

Summary

- All validation criteria passed
  - Assay robustness (accuracy & precision)
  - Selectivity (spike recovery, including haemolysed samples)
  - Spiked sample dilutional linearity
  - Parallelism
  - Stability
  - Drug & target interference

- *Fit-for-purpose for validation* of biomarker candidate
Biomarker Validation
Inflammatory Biomarker 1

- Different recombinant reference standards, different capture and detection antibodies, different technology platforms, different relative concentrations
Biomarker Validation
Inflammatory Biomarker 1

- Transform data to % change from baseline
- good agreement between the different assays
Conclusions 1

- **Biomarker strategy** followed a tiered approach:
  - Discovery/Screening, Verification and Validation

- Parallel tiered strategy for *bioanalytical assay*:
  - Screening, Qualified and Validated
Conclusions 2

- **Tiered approach** allows:
  - Check that *scientific assumptions* are correct before embarking on lengthy method establishment, validation and sample analysis
  - Ensure *bioanalytical assays* are *fit-for-purpose* for intended use

- **Bioanalytical strategy** for biomarkers should combine:
  - *Science, analytical performance* and *regulations/guidelines*
Thank you for your attention!

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