PPMI Biochemical Biomarker Update

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May 5, 2011
Update on Alpha-synuclein ELISA

December 2008: Transfer of assay
- MJFF evaluated several CROs for ability to transfer Schlossmacher assay

February 2009-December 2009: Optimization of assay
- Improved incubation and blocking buffer conditions
- Switched detection antibody
- Improved sensitivity 20-fold

January 2010-July 2010: Generation of additional supply
- Additional polyclonal rabbit/goat antibody
- Rabbit monoclonal antibody generation

Current status: Optimized Assay with Rabbit Monoclonal
- Participant in Alpha-synuclein assay comparison study
- Developing commercialization plan for kit
Qualification Study

• Study Objectives
  – Determine diurnal fluctuations of DJ-1 and alpha-synuclein in healthy volunteer CSF
  – Determine within-subject variability and between subject variability
  – Establish a bank of biosamples (CSF and blood) to utilize for assay development and future biomarker validation

• Study Design
  – 13 healthy volunteers (ages 30-55): 9 male/4 female
  – CSF and serum/plasma collected at 11 time points over 26 hour time frame; protocol repeated two weeks later
  – CSF analyzed for DJ-1, alpha-synuclein, and hemoglobin levels
Assay Details

• **Alpha-synuclein ELISA**
  – Dynamic range: 4-2000pg/mL
  – Human specific alpha-synuclein
  – Polyclonal antibody capture, biotinylated-monoclonal antibody detection-appears to pick up all alpha-synuclein species present;

• **DJ-1 ELISA**
  – MBL commercial kit
  – Polyclonal capture, HRP-conjugated detection

• **Hemoglobin Detection**
  – Bethyl laboratories commercial ELISA kit
Alpha-synuclein levels over 26 hours

\( \alpha \text{-synuclein in CSF} \)

Time (hours)

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Period 1 — Blue
Period 2 — Red
Alpha-synuclein levels over 26 hours

- Mean observed concentrations: 800 (baseline)-1100 pg/mL (26 hours)
- Alpha-synuclein significantly changed over the 26 hour period (p<0.0001) but not within the two week time point
- Within subject variability: approximately 20%
- Between subject variability: 30%
DJ-1 levels over 26 hours

DJ-1 in CSF

![Bar chart showing DJ-1 levels over 26 hours](chart.png)
DJ-1 levels over 26 hours

- Mean observed concentrations: between 2400-2530 pg/mL
- No change over 26 hours
- No period effect on DJ-1 levels
- Within subject variability: approximately ~20%
- Between subject variability: ~26%
Acknowledgements

• California Clinical Trials/Parexel
• Covance/Schlossmacher Lab
• Chris Coffey/Chelsea Caspell (University of Iowa)