Global Biomarkers Standardization Consortium Face to Face
Minutes of meeting – July 14, 2012
Vancouver, Canada

Attendees:

Lisa Bain
Tobias Bittner
Marina Boccardi
Cindy Catry
Daniel Chelsky
Heather Darby
Willy Deleersnijder
Lurella DiDonato
Howard Fillit
George Green
Theresa Heath
June Kang
June Kaplow
Daniel Kidd
John Lawson
Robert Martone
Niklas Mattsson
Manuel Menendez
Pankaj Oberoi
Salvatore Salamone
Mary Savage
Les Shaw
Nina Silverberg
David Stewart
Charlotte Teunissen
Jane Timmer
Robert Umek
Manu Vandijck
Laura Vernoux
Henrik Zetterberg
Holly Soares
Maria Carrillo
Heather Snyder
Dean Hartley
William Thies
Piotr Lewczuk
Chris Spedaliere
Rand Jenkins
Erin Chambers
Diane Stephenson
Anthony Pacifico
Brad Navia
Dana Dornsife
Dave Dornsife
Annelys Farrell

I. Welcome and introduction – Maria Carrillo and Holly Soares
Consortium created by the Alzheimer's Association to bring together standardization efforts around the world. Although the field had been making progress with the current biomarkers, there was a clear need for a robust assay, reproducibility across sites. Also there needs to be a common reference standard and reference method and engagement of diagnostic companies.

II. Alzheimer's Association QC program overview – Niklas Mattsson
Biomarkers, especially reduced Aβ42 and elevated total tau and phospho-tau have become increasingly important in research, clinical trials, and clinical practice, particularly with new diagnostic criteria. However, variability across assays and across studies has been a problem. Thus Alzheimer's Association launched the Alz Association CSF QC program a few years ago to identify and monitor differences among labs, ultimately to facilitate standardization to support optimal management of patients.

a. QC samples prepared in MöIndal, Sweden. Shipped to participating labs who report data back. Labs then get report showing how they rank compared to others and longitudinal results.
b. Now includes 85 labs in over 20 countries. Labs are joining continuously. Three rounds each year, each one with 2 unique samples and one longitudinal sample in every round to track longitudinal stability. First 2 rounds published in 2011; now working on second publication.
c. Overall variability in program in first 7 rounds – ELISA and xMPA - overall CV of about 25% for Aβ42, a bit lower for t-tau and p-tau – between 15 and 20%. For xMAP in last round had high variability in total tau… found it was caused by one outlier – after removal, the CV for total tau is actually quite stable.
d. Using variance component analysis found contribution of between-lab and between-batch very different for different biomarkers. For Aβ42 there was more batch variability, whereas for t-tau and p-tau, more lab variability. Thus there are different confounding factors.
e. Experience matters – 3 labs with large experience and large turnover were designated as reference labs. These labs have small variability.
f. Using checklist to monitor lab performance. So far, no significant findings. May be too little data, too complex interactions, or problems with self-reporting.
g. Implementation of SOP – using certified reference materials and methods. Also need novel assays for fully automated analysis. Will continue to monitor and evaluate, and collaborate with other groups.

III. Industry updates
a. MSD – Pankaj Oberoi
Company serves as bridge between research assay and clinical diagnosis.
i. MSO is now ISO certified – will lead to better products and customer experience. ISO has encouraged company to characterize peptides to reduce variability.
1. Full analytical evaluation done on 3 independent raw material lots and 3 kit lots.
2. Major issue is addressing matrix interferences – at dilution factor of 8, most of interference is titrated out.
3. Also looking at stability of components.
ii. Clinical utility – did test with 49 normal, 50 MCI and 50 AD samples
iii. Dilution linearity. Kit now has sensitivity to 3 pg/ml, can be diluted 8- or 16-fold and still get good concordance. Diluent contains surfactants.
iv. Stability – would like to take it out to 30 months
v. Spiked recovery – 80-100%
vi. Multisite validation with release lots – ongoing. 4 labs are done.
vii. ADNI samples – have requested in order to conduct more analytical validation
viii. Multiplex assay – being validated. Includes plasma biomarkers for TBI and PD that are currently in research.

b. Innogenetics – Manu Vandijck
    i. Innogenetics is part of Fujirebio group and committed to continue with neurology products.
    ii. Product portfolio includes single analyte ELISA (INNOTEST®), Multi-anayltye- xMAP® (INNO-BIA), and Genetics (LiPA). All are sold for RUO – research use only – in U.S.
    iii. Working on registration strategy for regulatory agencies.
    iv. New initiatives – Innotest for Aβ40, improvements to INNO-BIA and INNOTEST; also investigating Lumipulse platform
    v. Internal QC to guarantee quality and consistency and monitor how products are performing.
    vi. INNOTEST lot consistency – using longitudinal sample, 7 rounds over 2.5 years. Variability below 10%
    vii. Run validation control (RVC). Two samples in each kit, single analyte in CSF-like matrix. Will enable customers to validate test runs and calibrate; also should enable alignment with country-specific requirements for lab accreditation.
        1. Have assessed impact of stabilizing proteins and detergents
        2. Over 12 runs, different recoveries of Aβ
        3. Use of increased protein concentrations and addition of detergent had positive impact for Aβ, t-tau, and p-tau
        4. Stability demonstrated over 6 months a -80°C; studies ongoing. Also evaluating stability at elevated temperatures and with freezing and thawing.
        5. Addition of GuHCl to samples negatively impacted recovery at evaluation (INNOTEST).
    viii. Ready-to-Use Calibrators (RTU). Should improve ease of use and reduce variability. Feasibility study ongoing.

c. Saladax – Salvatore Salamone
    i. Developing an Aβ42 test for the VITROS® ECIQ immunodiagnostic system. Goal is a test that is easy to use with performance characteristics of a cholesterol test.
    ii. Demonstrated good calibration curve across full range including lower concentrations
    iii. Stability of reagents – up to 7 months at 2-8°C. Now have data for up to 32 weeks on several lots.
    iv. Calibrator – synthetic Aβ42 calibrator gets away from stickiness of native. Stable up to 40 weeks at 2-8°C.
    v. Precision – in 5-day study, intra-instrument precision <2%, inter-instrument precision <5%.
    vi. Shows linearity upon dilution with no high-dose hook effect
    vii. Tau program not as advanced at Aβ42.
        1. Full and lower concentrations look linear up to 50 pg/ml
        2. Synthetic calibrator – good precision and stability
        3. Dilution linearity up to 8X

4. No high-dose hook effect.

d. Roche Diagnostics – Tobias Bittner
    i. Immunoassay for CSF Aβ42 and tau to run on Cobas platform, which has >25,000 placements worldwide.
    ii. Developed as a companion diagnostic to enable patient selection for trials of gantenerumab, which is currently in phase 2.
    iii. Secondary objective to develop stand-alone assay for early AD.
    iv. Precision: within run CV <2%, between run CV <3%
    v. Linearity: CV <4%
    vi. Cross reactivity – none with Aβ40, slight with Aβ43 (comparable to Innogenetics)
    vii. Comparison to Innogenetics ELISA – r=0.97
    viii. Calibrator – synthetic peptide
    ix. Antibodies – similar epitopes as Innogenetics

IV. Developing candidate reference method for direct measurement of Aβ42 in CSF – Les Shaw
    a. Alzheimer's Association/GSBC sponsored effort – a work in progress.
    b. Collaborative project of four groups: Erin Chambers (Waters Co), Rand Jenkins (PPD), Les Shaw (UPenn), and Kaj Blennow (U Goteborg). Each lab will validate their methodology; then will participate in an inter-lab round robin study.
    c. Sample preparation is hugely important – Use GuHCl to release Aβ into monomeric forms.
    d. Calibrators and calibrator diluent also very important – 3 different matrices across 4 labs
        i. 5% rat plasma in aCSF
        ii. 4 mg/ml BSA in aCSF
        iii. hCSF
    e. Assessing linearity and reproducibility. So far reproducibility is acceptable but not as good as we expect.

V. CAMD FDA Qualification Update – Diane Stephenson
    a. Coalition Against Major Diseases (CAMD) is one of 5 consortia at the Critical Path Institute (CPI). Theme across Institute is to develop pre-competitive ways to work together by sharing data, developing data standards, pooling data across diverse sources, qualify biomarkers, and develop "accepted for use" quantitative disease models.
    b. Qualifying biomarkers – qualification is a regulatory conclusion that, within a specific context of use, the results of biomarker assessments can be relied on to have a specific interpretation and application in drug development
    c. Two teams – one aimed at structural/volumetric MRI for enriching trials; the second is CSF biomarker team. Hope to use CSF biomarkers to identify people in predementia stage who will progress to AD
    d. Our strategy – evaluate one biomarker at a time. Eventually hope to combine genetics and multiple biomarkers to define patients better.

e. Briefing document submitted to FDA, followed by face-to-face meeting. Themes discussed:
    i. How do biomarkers relate to cognitive impairment
    ii. Precision-based vs. accuracy-based assays (eventually want to get to accuracy-based). Precision-based performance:
        1. INNO BIA AlzBio3 immunoassay – 3 independent studies in 3 cohorts
        2. Comparable clinical accuracy
    iii. Data analysis and interpretation
    iv. Creating SOPs
    v. Crucial factors in advancing CSF assays to qualification:
        1. Run validation
        2. Lot consistency
        3. Stability
        4. Characteristics of raw materials
        5. Robustness (need regulatory guidance)
        6. Reference materials and methods
        7. SOPs
    vi. FDA recommended that CAMD look into possibility of obtaining the data from companies who have conducted relevant trials. If at least the placebo group data could be obtained it may both strengthen the evidence regarding the biomarkers and address the concern about potential differences in patients in a natural history study vs. drug intervention trials.

VI. IRMM/IFCC Project – Henrik Zetterberg

a. Institute for Reference Materials and Methods (IRMM), one of seven institutes of the Joint Research Centre (JRC), a directorate of the European Commission (EC), is one of the world's leading reference material producers as well as a provider of reference measurement data.

   IRMM made a formal decision to assist in the development of reference materials for CSF Aβ42, t-tau, and p-tau. They will produce and provide the final materials if we can provide information.

b. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) is a worldwide, non-political organization. In May, 2012, IFCC formed a working group on CSF biomarkers. This is a global consortium with Kaj Blennow serving as chair. Companies can be represented in WG if they are corporate members of IFCC, so membership in the WG may change.
    i. Objectives –
        1. Develop international reference material for CSF with a focus on AD biomarkers.
        2. Establish reference methods
    ii. Methods –

1. Candidate reference methods will be developed by GSBC members, the IFCC WG, and other research teams
2. Protocols can be published and also submitted to IFCC WG for evaluation
3. Scientific division of IFCC gives advice on methods and can submit nominations for IFCC certification. Independent validation is essential
4. IRMM will do feasibility analysis.

c. Current status:
   i. Candidate reference methods in place for Aβ42
   ii. Round robin and further harmonization efforts in progress
   iii. Tau approaches need to start
   iv. Candidate reference materials will be produced and evaluated this fall
   v. Should have a version to submit by end of 2012
   vi. Themed issue of *Biomarkers in Medicine* in August, 2012
d. Outstanding issues
   i. Not even the gold standard is gold
   ii. Amino acid analysis of the same internal Aβ42 standard at two independent laboratories varied by 20%.
   iii. Reconstitution of reference materials may be a challenge – proposal will be to evaluate many different reference materials; then will store in liquid nitrogen in solution
   iv. Long term stability may be difficult
   v. Reference material in a certain matrix may not react the same in all assays (commutability)
e. IFCC is keen on independent evaluation – different methods must give same results.

VII. Conclusion (Holly Soares) – We have been struggling with issue of biomarker standardization for a long time. Without the support of the Alzheimer's Association, we probably would probably not have come together and moved this forward as much as we have. The goal is to make certain we have a test that accurately defines who is on track to develop AD.