Welcome and Introductions – William Thies and Philip Scheltens

- Bill: Global standardization is essential and we know this as a community. We need this in order to use biomarkers on a global basis. The Alzheimer’s Association is committed to the global standardization of biomarkers.
- Philip: Will lead the meeting. Maria Carrillo, Kaj Blennow, Philip Scheltens and Bill Potter organized this initiative.

ABSI Project on Pre-Analytical Aspects – Dirk Wouters, Innogenetics and Piotr Lewczuk, Erlangen

Dirk Wouters – ABSI project (Alzheimer’s Biomarker Standardization Initiative) is the Innogenetics sponsored platform for a KOL lead initiative for building consensus on standardization of procedures to collect and measure biomarkers. The goal of ABSI is to improve accuracy by looking at assay, lab and sample. The ABSI group submitted paper yesterday as result of ABSI project.

Piotr Lewczuk – When look at individual samples, there is significant inter-laboratory variation; although consistently see distinction between AD and control. ABSI organized consensus meetings to identify and
discuss issues with may influence variability in assays for AB1-42, t-tau and P-tau181p. List of key issues identified – assay production, sample collection, storage, laboratory SOPs to perform analyses.

Subsequent ABSI meetings focused on harmonization of results – data driven. Found that deep frozen samples can be reliably assayed within 2 years. No significant fluctuations of biomarkers in samples refrozen twice but on third freeze, had alterations. Paper summarizing the ABSI findings submitted yesterday.

**Alzheimer’s Association QC Program – Niklas Mattson, GOT**
Known biomarkers – AB42, t-tau, ptau may be beneficial in identifying diagnosis. Need for global standardization. Overview of the Alzheimer’s Association QC Program – first two rounds published. There have been six rounds completed to date – majority use INNOTEST, additional labs use Luminex. See on-going variability across the laboratories. Data suggests a potential decrease in variability with t-tau, but not AB42 or p-tau. Developed QC checklist to identify areas of process that may be an issue – have included this as a survey to participating laboratories. The next phase of this project is to identify confounding factors and develop certified reference materials and methods for CSF proteins.

**EMA Qualification of CSF Biomarkers - Maria Isaac, EMA**
Views presented are personal views and not reflective of EMA. The EMA is based in London, give scientific insight to the European Commission. Not equivalent to FDA – representatives from different countries (22 countries -- languages, politics, views, and clinical practices). This is essential to consider in regards to any statement made by the EMA. In 2009, the EMA developed guidelines that would be willing to consider disease modifying treatments if measurable by biomarkers. In April, 2011, the European Medicines Agency released the first qualification opinion for a clinical use. The biomarker (CSF) is intended to identify patients who can be recruited for clinical trials of treatments for predementia Alzheimer’s disease. Still requires standardization and agreement regarding the utility of biomarkers.

**QC efforts for Assay Vendors - Robert Umek, Meso Scale**
Across translational science, there is a lack of appreciation regarding the stability and feasibility of the kits and other products used in the measurements of biomarkers. Meso Scale is a dominant player in immune-testing. Meso Scale uses on-going QC tests throughout the development of the materials, including a intra-plate CV as component of QC, pattern efforts for the uniformity of rows, columns, rings, etc. When you understand variability, vendor can identify workable solutions and move progress forward. Discussion regarding the tau detection antibodies in the new kit and the need for mass spec to clean up; to date, these are not being shipped for use.

**Alzheimer’s Association LP Safety Study - Kaj Blennow, GOT**
LP Safety Study launched one year ago on the complications after LP. There is a small percentage of LP headaches and this seems to be correlated to age (less significant in elderly) and also more common in women and anxious patients. In addition, there is a higher incidence with larger needles. Research found that if person answered “yes” to “are you afraid to have LP”, then there was a 60% chance of LP headache. Enrollment nearing 400 patients, and are working to engage more centers (currently 21 clinical centers around the globe)

**ADC LP Experience LP Safety Study – Walter Kukull, NACC**
National Alzheimer’s Coordinating Center collects and standardizes data longitudinally. Effort is to take the current global AA LP Safety Study into the ADCs. Objectives are to describe current practice at ADC
as reported by ADC Directors (who, what, when, where, how ADC subjects are usually approached and informed about LP studies) and to describe characteristics and attitudes of persons requesting LPs at ADC (Self-report of each LP requestor at the ADC). Group has established surveys for the ADC centers – working toward enrollment of large number of individuals (estimated enrollment is TBD as there is no basis to estimate LP). This project will be launching in the next 6 weeks to 2 months. The data from this study will augment the AA LP Safety Study, and provide a larger set of data.

ADNI Studies Update – Les Shaw, Biomarker Core ADNI
Identification and control of pre-analytical and analytical factors that can contribute to variability in CSF biomarker concentrations is key to the qualification and the standardization of biomarker assays. In addition to identification and control of these factors is the importance of tracking important details associated with each biofluid sample collected and subsequently aliquoted, labeled and maintained in the ADNI biofluid biobank. Thus each sample has these very important collection details as part of its history. There are on-going efforts to improve standardization including global collaborations with colleagues in Japan.

In the ADNI study for each run of CSF samples ~5% of the subjects a duplicate never before thawed aliquot is assayed to ensure process stability. The ADNI Biomarker Core laboratory is assessing the effect of potential variables for possible effects on CSF biomarker measurements including temperature of sample thawing, use of silanized pipette tips, vacuum pressure and others and will prepare a full report of the findings later this year.

CAMD CSF Biomarker Effort – Marc Cantillon, CAMD
Standardization is a timely issue and is a true pre-competitive space. In the past 18 months, the expectations of regulatory agencies require a qualified tool and an expectation that the population will be able to be identified as ‘at risk of conversion’ or to identify the enriched population. To date, there are really no cut offs and we need to continue to move this forward. In addition to kit production, there is the issue of how the materials are being used by the individual laboratories as well.

Industry/ Academic Role: Partnership – William Potter, FNIH
ADNI was not originally designed to qualify tools for early diagnosis or drive standardization to level required in clinical diagnosis. Standardization does not only apply to CSF. It is important that as a group we are aligned in standardization of CSF or other measures because the investment in standardization is high. There is a need for standardization of behavior/ cognitive measures as well.

Qualification of data going into the repositories is an essential issue; we will need to determine who or how alignment around data integration into larger repositories. There are different rules for industry and for academia:

- Intellectual property (e.g. freesurfer is free but no for industry)
  - Universities encourage NIH funded investigators to develop IP such that a method may be “protected”
  - Companies may not charge academia to do studies which help to establish value of a proprietary method
  - Industry – viewed as a paying customer for anything that can be protected

General Discussion – Moderator: Philip Scheltens, Amsterdam
There was little agreement on how the numbers and results were presented. This resulted in a loss of clarity for the effort and detracted from the important communication of what had been done and what
is now being proposed. As a consequence, I suggest that an academic site be given the responsibility for the data analyses and the integrity of the data input. (Mony de Leon)

It is essential to identify variables throughout checklist, bring them together in different groups and standardize each specific issue. (Hugo)

The QC program is effort to monitor variation to help inform what we will do – one suggestion is to test and retest to document performance including equipment, processes, staffing, etc. (Kaj Blennnow)

Validation of assays to do pharmacokinetics – dilution linearity assessment that is not linear. Concern that we are dealing with background issues of matrix effects based on temperature, storage, age of assay, etc. Suggests developing solution to dealing with matrix effects (Bob Dean) – we know you can identify sub-populations. The AD analytes are difficult to work with and this may be our issue.

If it is a matrix issue, then vendors should modify product to make product applicable to these environmental issues. This will be necessary for regulatory consideration. There is a solution but it will be a new version of the product. Example is cholesterol has significant matrix interactions and these interactions are essential in the measuring of cholesterol. (Hugo/Bob)

Workshop on practical issues of AB and tau measures. When everyone brought their individual pipettes to the workshop, there was decreased variability. All laboratories work in affiliation with clinical measures so many are already certified. (Charlotte). German law requires multi-annual calibrations of pipettes and other equipment annually (Piotr).

Issues related to the kits and the matrix issues must be sorted out initially – need development of a reference method. This is independent of antibody. Need to be collaborative, across manufacturers.

What is the matrix for the calibrators and standards – all running in aqueous environment that has innate bias to CSF. As long as we use aqueous buffers, this will be an issue. (Adam)

Need for artificial CSF – need to consider that will need to have same characteristics. Need to use mass spec to link all buffers from all vendors to develop consensus. (Hugo) Why cannot CSF be natural? Difficulty between CSF sample in a commercial kit and the regulatory requirements around that and also analytes must be stable over time. Also aspects of cost, collection and other issues.

The discussion of CSF used as artificial or not to dilute antibody may reduce substantial amount of “noise”. One instance is rat CSF at 5% to dilute – and it is working. What is our respective timeline? Formulate timeline to identify what we want accomplished in one year or two years. There must be some aspect of sample handling, matrix effects, etc. because we have protocol and we are still seeing some variability. (Les)

We need the cholesterol of CSF and make it a reliable measure. The criteria are out and diagnosis is based on biomarkers so we need to figure this issue out. (Philip)

There may be need for two AB42 assays – the distinction of the matrix between AD and control is part of the assay. (Hennrik)
See variation of ApoE allele in 30 and 40 years old in the CSF – CSF is complex substrate and we need to develop standard to work here and now. Any new comer will need to prove they are “better”.

Advances of the mass spectrometer to help increase sensitivity – need to incorporate data-driven debate (Adam).

Issues of screening drugs for matrix effect is not going to impact drug pharmacokinetic (group has evaluated already) but will impact the distinction between levels of AD and control.

How can we proceed with this? How can we move this forward? (Kaj led discussion)
- Don’t try to solve all the issues in one discussion, break down the specific points and discuss individually.
- What are issues of matrix or not? What are issues of mass spec or not? Other issues.
- Break down smaller issues and have small group session (working group) to solve individual issues associated with each segment
- Many small groups already going on – need to
- No consensus – need to assign “best value” in order to make assignments. We have 5 liters and hope for 10 liters in next 6 months. This will help identify “gold standard” for now …
- ABI goal was to standardize the kits however then there was a goal to incorporate data
- There will be future discussion for forum appropriate to continue these discussions and we will communicate with the larger audience.