



Summary of the National Institute of Neurological Disorders and Stroke Parkinson's Disease Biomarkers Program Launch Meeting November 13th – 14th, 2012 Arlington VA

To address the needs of the PD community, the National Institute of Neurological Disorders and Stroke (NINDS) has established the Parkinson's Disease Biomarkers Program (PDBP) focused on promoting the discovery of biomarker candidates for early detection and measurement of disease progression. In September, 2012, nine research projects were awarded (under RFA NS-12-010 and RFA NS-12-011), and a meeting to officially launch the program was held on November 13th – 14th, 2012.

This meeting included the Principal Investigators (PIs) from each project, as well as the clinical and data management experts associated with the projects, representatives from non-government organizations, and staff from NINDS and other government agencies. This meeting focused on discussion and free flowing perspectives on the PD biomarker field, and as such, some of the meeting comments summarized here, do not necessarily reflect all views or a consensus. A summary of the meeting discussion is given below.

I. PDBP Overview

PDBP goals

- PDBP goals are focused on the discovery of biomarkers that can eventually be applied to improve the outcomes and shorten the duration of neuroprotective clinical trials (Phase II and III) for Parkinson's Disease.

PDBP structure and components

- **The PDBP Data Management Resource** (PDBP DMR) provides the infrastructure for PDBP data sharing and project management. The PDBP DMR, developed and maintained by the NIH Center for Information Technology (CIT), serves the stakeholders through support of electronic data capture, clinical site management, data quality assessment and data access for qualified academic and industry researchers.
- **Biorepository:** Biospecimens will be collected by several of the PDBP projects and these biospecimens will be banked by the NINDS DNA, Cell and Biospecimen Repository at Coriell. Academic and industry investigators interested in biomarker discovery can query and request PDBP biospecimens through the PDBP DMR. Requests will be reviewed by the PDBP Biospecimen Resource Access Committee (BRAC) and approved requests will be processed by the NINDS Repository.

- **PDBP Biospecimen Resource Committee (BRAC):** The BRAC will be composed of NINDS staff and subject matter experts. Requests for resources will be accepted on a regular basis through the PDBP DMR and reviewed in a timely fashion by the BRAC.
- **PDBP Steering Committee:** The PDBP Steering Committee is composed of NINDS staff and project PIs. A chair and vice-chair will be selected by the PDBP PIs and the positions will be rotated on an annual basis. The Steering Committee will have monthly calls and biannual in person meetings to assure the smooth running of the program.
- **PDBP Scientific Liaison Group:** The Scientific Liaison Group includes NIH, FDA, industry, academic, and non-government organization members who provide general input regarding the strategies and goals of the PDBP within the context of other PD biomarkers projects, and biomarkers research in general.

Lessons learned and suggestions from past and current biomarker efforts

- Milestones provide important tools for developing program metrics and timelines for enrollment and legacy data submission
- Early communication with the US Food and Drug Administration (FDA) is important for eventual biomarker qualification.
- Variability is a key issue that must be addressed early in biomarker development:
 - The signal to noise ratio of a biomarker readout in affected vs. control subjects is one example of this type of variability;
 - Standardization of data and biospecimen collection plays a key role in reducing variability across studies
 - Use of common calibration standards across sites enhances analysis of assay performance (robustness)
 - Replication vs. robustness: in general establishing a robust biomarker assay enables outcomes to be tested through replication.

PDBP Data and Resource Sharing policies

Sharing is a key component of this project and involves both sharing among the individual projects funded under the PDBP initiative, as well as sharing through controlled data access via the PDBP DMR. Investigators outside of the PDBP can access data through the PDBP DMR, once data access is approved by the PDBP Data Access Committee (DAC). Biospecimen requests and distribution will be adjudicated through the PDBP Biospecimen Resource Access Committee (BRAC) and biospecimens available can be accessed through the NINDS PDBP DMR and will be distributed via the NINDS Repository.

- For the first year of this initiative, there will be a one year embargo period on access to data submitted by PDBP funded investigators. Thereafter, aggregate data will be available to the public, while raw data will be available via controlled access to both academic and industry investigators.
- A record of data downloads will be available so that PDBP PIs and the NINDS can track data usage
- The PDBP database will be updated on a monthly basis, after the first year embargo period.
- The NINDS Repository Materials Transfer Agreement (MTA) for biospecimens will be used to govern use of biospecimens collected by PDBP projects.

- A Data Use Certification Agreement (DUC) is required for the use of data and must be signed by the investigator (requestor) and their institutional official.
- There will be different criteria for approval based on renewable and non-renewable resources and the necessity for post-lab analysis data. Examples requests based on these different types of resources and data include, but are not limited to: 1) requests for biospecimens (which are not renewable); 2) requests for controlled access to clinical data in the PDBP database; and 3) requests for post-lab analysis data (such as proteomic data, imaging data) in the PDBP database.
- PDBP PIs using advanced technology (e.g., mass-spectrometry, imaging) will work with other PIs in the PDBP to develop standards and reasonable sharing parameters for their technologies. These will be discussed by the steering committee and a policy developed for sharing of advanced technology data. A subcommittee, led by Dr. Babcock (NINDS), will be formed to discuss imaging issues specific to data sharing and these issues will be presented to the steering committee with policy recommendations.

PDBP Publication Policies

It is expected that investigators with access to NINDS Parkinson's Disease Biomarker Program (PDBP) data will seek to publish and present study related scientific reports. If PDBP data and/or biospecimens are used in the analyses or publications, as part of the Data Use Certification Agreement, investigators will be asked to agree to a Publication Policy as follows:

- All manuscripts will acknowledge the NINDS PDBP Biomarkers Discovery Cohort data as specified in the PDBP Data Use Agreement, and PDBP publication policy.
- All manuscripts will acknowledge support of the National Institute of Neurological Disorders and Stroke (NINDS) in the collection of data and (if relevant) biospecimens used in the study.

In addition to the named authors, all manuscripts will be submitted with an acknowledgement of the PDBP initiative on the author line followed by an asterisk (see Data Use Certification Agreement). The asterisk will point to a printed listing (if allowed by the journal) or a web site listing of the names of the PDBP cohort investigators.

Full citation of all published manuscripts will be provided to the PDBP cohort investigators upon acceptance of manuscripts. A copy of the manuscript will be provided to the PDBP Data Management Resource (DMR) upon publication of the manuscript.

Suggestions for Subject Recruitment and Retention

- When considering enrollment and recruitment, it is important to understand the subjects' motivation to participate. Understanding those reasons can benefit the study as well as the subject.
- Research should be relevant to the people who are its subjects: treatments, health care practices matter to them.
- Lack of minority subjects is a historical weakness of PD research. This should be taken into consideration when developing recruitment strategies. Specific subpopulations including but not limited to ethnic minorities may be important for biomarker discovery (they may show an effect that may not be seen in a more general population).
- Cohorts of long-term local residents are important for long-term follow-up.
- Useful tools:
 - Use of mobile apps to help disseminate information regarding the PDBP program to a broad audience.

Discussion regarding sample collection

- Cerebrospinal Fluid (CSF) is a precious resource – care must be taken to ensure that sample collection and handling is managed appropriately.
- Two CSF protocol issues were discussed: (1) centrifuge of samples and (2) maintenance of caudo-rostral gradient:
 - Within the PDBP initiative, CSF is pooled and centrifuged.
- Hemoglobin (Hg) measurement and contamination of CSF by blood:
 - Contamination of CSF by blood is believed to have a tremendous effect on alpha-synuclein measurements by currently available methods.
 - Hemoglobin (Hg) measurement provides an accurate estimate of how much red blood cell (RBC) contamination there is in a CSF preparation; WBCs in CSF may be an indication of a recent infection.
 - Hg central measures – measurements to be performed by the NINDS repository on all CSF samples: to be discussed by the steering committee and recommendations developed.
- Dietary impact on CSF collection: while collecting CSF, a note should be made of the subject's fasting state vs low fat diet state. Apolipoproteins, which are a component of some studies in PDBP, can be affected by fasting. An electronic data form (part of the eforms available in the ProFoRMS module of the PDBP DMR) has been developed to capture this information for CSF collection.
- There may be a diurnal variation in CSF which may also impact certain analytes.
- The issue of subjects being on/off meds is important for the biomarker studies.
- RNA stability in CSF is something that is not well understood, and could be further studied to help determine best practices.
- Standard measures for genetic polymorphisms of interest and known gene mutations across the biospecimens collection would be of value.
- It is important to use polypropylene tubes for spinal tap (they don't adsorb most known biomarker analytes unlike some other types of plastic). Kits provided by the NINDS Repository include polypropylene collection tubes.

Considerations for the PDBP Data Management Resource (DMR): NINDS Clinical Data Elements (CDEs) and Data Collection

- PDBP PIs are required to use NINDS CDEs. This is accomplished through incorporation of NINDS CDEs into the electronic forms that are part of the ProFoRMS module of the PDBP DMR
- Only de-identified data will be stored in the DMR.
- Issue of tracking diagnosis change over time:
 - What if the physician changes the diagnosis? There should be a way to update the diagnosis in the system. The Neurological Exam form captures diagnosis information
 - Not knowing how the diagnosis has changed means loss of data. It should be possible to lock the diagnosis, then to add another data point if the diagnosis needs to be changed. The Neurological Exam will be completed each year and thus changes in diagnosis can be recorded by using this form and linking the form to visit type in the database.

- Forms for diagnostic data from instrumented tests and mobile apps (e.g., iTUG, iSWAY) could be uploaded into the DMR at a defined time point in the future.
- DICOM standards for imaging data in DMR are available.
- On January 15th, 2013, the ProFoRMS module will go live with the required or “core “ electronic clinical data forms (PIs will be able to create a study, manage/schedule subjects, collect data and view reports).

II. PDBP Technology, Science, and Clinical Resources

The following technologies, science, and resources were discussed at the meeting as components of currently funded PDBP projects or ideas which might be included in projects in the future:

Technology

- Mobile applications (apps) for diagnosis (e.g., APDM's Mobility Lab gait analysis - a new device that can perform a very detailed analysis of gait);
- Multiplex bead-based high throughput immunoassay;
- Multiple Reaction Monitoring for mass-spec;
- Aptamer technology (Somalogic) – a new platform permitting simultaneous highly sensitive detection of large numbers of proteins (this approach is relatively untested);
- RNA-Seq in dopaminergic neurons;
- Peptoid libraries: these are used to look for antibodies that increase in PD. Peptoids are protease resistant peptidomimetics (N-substituted glycines), which can be synthesized in many different versions and assembled into large libraries;
- Imaging:
 - Multimodal imaging and statistical analysis: analysis of a multimodal dataset permits a much better disease classification than each biomarker taken individually;
 - DTI FA (early changes in SN), R2* (iron is a perfect progression marker).

Basic laboratory discovery science

- Exosomes, RNA in CSF:
 - Red Blood Cells secrete exosomes which get degraded: there is renewed interest in collecting blood for exosomal study.
 - Lrrk2 is in exosomes (Lrrk2 is also found in non-brain organs, e.g., kidney). Therefore urine is a source of exosomes where LRRK2 can be measured.
- “Alzheimerization” (ie, Tau, and Abeta protein function and dysfunction) is thought to be important for PD progression:
 - Tau is important for the risk of PD, there is a decrease in Abeta in CSF in PD subjects (albeit to a much smaller extent than in AD) and a decrease in phospho-Tau (this is different from AD);
- Abeta, phospho-Tau levels appear to correlate with pre-symptomatic stage in AD (alpha-synuclein does not appear to follow this pattern based on data available to date).
- EGF:

- Reduction of EGF levels in plasma predicts cognitive decline in PD;
- EGF in cell culture is trophic for neurons, particularly for midbrain dopamine neurons.
- Imaging:
 - Resistant residual Dopamine neurons (DAN) in PD: most cells are lost before the symptoms present, but 5-10 years after the disease onset, cell loss becomes halted, residual cells are highly resistant (Lang 2007);
 - Reduction of DTI FA in caudal SN (not in rostral SN) is an early PD marker;
 - Changes in iron concentrations are a potential progression marker. Differences in biomarkers indicate that progression and cause of the disease are different.

Clinical science resources

- Johns Hopkins/Udall Center PD progression cohort;
- The University of Texas (CSF + brain donation);
- Harvard BioDiscovery cohort consists of 2000 subjects with PD (blood, CSF, brain donation);
- Kaiser Permanente (KP) Georgia patient database contains information from over 235,000 patients (530 subjects diagnosed with PD have more than one year of observation);
- Special populations of subjects may be important for biomarker discovery as they may show trends that are not seen in larger/more diverse populations.

Conclusions

- Cohort projects funded under the NINDS PDBP initiative will contribute to a standardized longitudinal collection of biospecimens including serum, plasma, RNA, DNA and CSF from PD participants that span the disease spectrum, as well as age and gender matched controls. Investigators from both academia and industry can access these resources through the PDBP data management resource. Requests for biospecimen resources will be adjudicated by the PDBP Biospecimen Resource Access Committee and approved requests will be processed by the NINDS Repository. Clinical data can be queried through the controlled access PDBP data management system. All cohort studies will collect a core set of clinical data, as well as other data related to their research interests.
- PD biomarker discovery in PDBP is supported through several novel platforms including aptamer and peptoid library screening, RNA sequencing/transcriptome analysis, exome-based screening, multiple reaction monitoring for mass spectrometry and multi-modal imaging approaches.
- The PDBP initiative unites ongoing efforts in PD biomarker discovery through integration with the Harvard Biomarker Neurodiscovery Initiative, and NINDS-funded Morris K Udall Center PD Biomarker efforts. Statistical modeling of PD disease onset and progression is supported through the Kaiser Permanente Georgia patient database.
- Considerations for standardization and quality assurance measures for CSF biospecimen collection include, but are not limited to: 1) standardized methodology for centrifugation of samples; 2) collection of CSF to control for dietary and circadian effects on biomarker discovery; 3) quality measures to ascertain blood contamination in CSF collection; and 4) accurate reporting of medications to minimize variation in biospecimen set analyzed.
- PDBP policies for publication and data use have been established and are available on the <http://pdbp.ninds.nih> website.