METHODS AND TOOLS

A large part of the success of the ADNI study rests on the consistency of data collection and processing. To guarantee this success, the cores and PIs have created uniform procedures and protocols to be used by researchers participating in the study. ADNI offers detailed information on the research process to ensure that all labs, centers and researchers participating in the study have the resources they need. To learn more about how ADNI data is collected, expand each section below.

Click each data type below for details

- Biomarker Analysis
- Genetic Data Methods
- MRI Analysis
- PET Analysis
- Proteomics Analysis

Results from the analysis of ADNI samples are summarized below. Anyone with an ADNI Data Archive account may view and download the analysis methods and the analyzed data. After logging in, click “Download” and “Study Data” to see all relevant ADNI documents available for download. See references here [Biomarker Core References]

Biomarker Core Aims and Progress for ADNI3

The Biomarker Core in ADNI3 is focused on 4 areas of activity and studies including:

- Biofluid banking (CSF, plasma and serum) management and pre-analytical assessments;
- standardization of CSF Aβ42, Aβ40, t-tau and p-tau181 measurement in ADNI patients using the highly validated Roche Elecsys cobas e 601 fully automated immunoassay platform and reference LCM/MSMS methodology for CSF Aβ42, Aβ40 and Aβ18;
- determination of cut-points for Aβ42, t-tau, p-tau181, and ratios using several approaches including ROC analyses using FBP amyloid PET imaging for disease detection and disease independent mixture modeling;
- collaborative studies on new biomarker development/validation/testing in CSF or plasma by immunobassay or rmm LCM/MSMS including NFL, total and phospho-α-synuclein, Vimentin, TDP-43, metabolomics/mass spectrometry biomarkers and proteomic quantitative assays.

Highlights of Biomarker Core activities include:

- **BIOFLUIDS**
  - Continue to receive, aliquot, store and curate all biofluid samples (CSF, plasma, serum) collected from subjects enrolled in ADNI3, including all who “carry over” from ADNI GO/2 and all newly enrolled individuals, with 24/7 surveillance in the ADNI freezers that are housed in secure, dedicated space at UPENN.
  - The updated (as of Feb 28, 2018) list of pristine aliquots of CSF, plasma and serum samples collected from ADNI subjects; “ALIQUOTS_LIST.csv” can be found on the ADNI LONI website under BIOSPECIMENS, but below in Table 1 is a brief summary of these samples.
  - **Table 1.** Summary of ADNI CSF, plasma and serum samples received and aliquots prepared as of 3/2/2018.

  ![Table 1. Summary of ADNI CSF, plasma and serum samples received and aliquots prepared as of 3/2/2018.](image)

Continue to monitor details involved in the preparation of Biofluid samples at study sites including time from obtaining each sample to the time of freezing on dry ice (a summary of this is provided in Figure 1 below). We continue to review details involved in the pre-analytical steps involved in biofluid samples. In the figure below a focus on sample preparation time shows that for CSF the mean, 95%CI and median values across 1,318 samples collected from ADNI3 and ADNI GO/2 phase participants are 44.8 min (41.7-47.8 min) and 28 min respectively. For 3,908 plasma samples the respective values are: 7.1 min, (70.0-73.4 min) and 55 min. This information is available for each ADNI sample. The handling at each ADNI site of these biofluid samples is very important to assure the quality of each sample. Avoidance of hemolysis and time-efficient sample preparation are essential to the goal of sample quality. For plasma, the recommended time from collection to freezing is no longer than 120 min; for CSF the recommended time is 60 min or less in order to minimize risk for biomarker degradation due to metabolic processes.

- **Figure 1.** Sample collection to freezing time for ADNI GO/2 plasma and CSF primary samples

Regularly communicate with Clinical Core staff regarding biofluid collections and any issues concerning sample quality, labeling discrepancies, and provision of updated samples-received summaries.

Continue our collaborative studies on identifying and controlling pre-analytical factors that can contribute to variability in CSF or plasma biomarker measurements especially Aβ1-42 (Vandenberghe, et al. 2011; O’Bryant et al. 2015; Hansson, et al. 2018). An example of this is a world-wide collaborative effort under the auspices of the A4 Association Global Biomarker Standardization Consortium(GESC) whose members from industry and academic centers are defining a unified protocol for CSF sample collection for use in routine clinical practice and another for large multicenter studies such as ADNI. A new effort is the Biomarkers Consortium NSC - Plasma A4 Working Group that is just being organized to pursue various aspects of plasma A4 measurement. Although there have been mixed results for plasma A42/A40 for accurate detection of AD using a number of immunoassay approaches, it is fervently hoped that by identifying and controlling pre-analytical factors, improved analytical techniques, and controlling for concomitant disease factors(Rosman, et al. 2012) that progress can be made on improving the diagnostic utility of these measurements (Dovis V, et al. 2017).

RARC-approved studies using ADNI Biofluids

Prepare and ship biofluid samples(CSF, plasma or serum) to all investigators whose biomarker study proposals have been approved by the RARC (Resource Allocation Review Committee, appointed by the NIA) and following final review by NIA (see Table 2 for an up to date brief summary of these studies and status of results upload). Once completed the data from these studies, performed blinded, are uploaded on the LONI/ADNI website together with a Methods document that describes the methodology involved and quality control performance. Biomarker Core faculty, Drs Trojanowski and Shaw are happy to provide input on any study although this is not required but often we are asked. The procedure for making an application to the Resource Allocation Review Board(RARC) for ADNI biofluid aliquot samples can be found on the ADNI web site.

A study that builds upon the 2013-2014 study that used LCM/MSMS mmr mass spectrometry methodology will be conducted in 2018 to determine accurate concentration values for 5 candidate biomarkers that showed promise in the earlier semi-quantitative-based study(see Table 2 for a brief synopsis of all of these studies). An important characteristic of this study is its emphasis on measurements in longitudinal CSF samples across time from entry into the ADNI study (BASELINE) to at least 4 years later in order to assess these biomarker trajectories.

Some data can be informative to clinical trials that are seeking to use biomarkers as indices of drug engagement and drug effect.

- **Table 2.** A list of RARC-approved studies using ADNI Biofluids

<table>
<thead>
<tr>
<th>Study Number</th>
<th>Title</th>
<th>Investigators</th>
<th>Funding Source</th>
<th>Study Design</th>
<th>Study Period</th>
<th>Sample Type</th>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
<th>Study 4</th>
<th>Study 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Follow-up</td>
<td>Follow-up</td>
<td>Follow-up</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Plasma</td>
<td>Plasma</td>
<td>Plasma</td>
<td>Plasma</td>
<td>Plasma</td>
</tr>
<tr>
<td>CSF</td>
<td>CSF</td>
<td>CSF</td>
<td>CSF</td>
<td>CSF</td>
</tr>
<tr>
<td>Serum</td>
<td>Serum</td>
<td>Serum</td>
<td>Serum</td>
<td>Serum</td>
</tr>
</tbody>
</table>

A study with builds upon the 2013-2014 study that used LCM/MSMS mmr mass spectrometry methodology will be conducted in 2018 to determine accurate concentration values for 5 candidate biomarkers that showed promise in the earlier semi-quantitative-based study(see Table 2 for a brief synopsis of all of these studies). An important characteristic of this study is its emphasis on measurements in longitudinal CSF samples across time from entry into the ADNI study (BASELINE) to at least 4 years later in order to assess these biomarker trajectories.

Some data can be informative to clinical trials that are seeking to use biomarkers as indices of drug engagement and drug effect.

We continue to collaborate with biomarker scientists at UPenn and elsewhere (Irwin et al. 2017, 2018; Hu, 2010; 2015; Toledo et al. 2013, 2018; Mattsson, et al. 2013, 2016; Zetterberg and Blennow, 2016). New CSF biomarker-neuropathology correlations done with UPenn collaborators resulted in studies involving CSF tau and histochemical tau in FTLD and CSF tau and Aβ42 and synucleinopathy in autopsy Lewy Body disorders (Irwin et al, 2017, 2018). Such studies take advantage of the large set of CSF collected at UPenn from individuals prior to death and for whom an autopsy diagnosis provides accurate detection of not only AD neuropathology but concomitant pathologies such as Lewy Bodies, TDP-43 deposits, hippocampal sclerosis. A list of the publications describing the results of these studies thus far is included in References. Figure 2 illustrates the timing for onset and progression of AD, trajectories for amyloid and plaque biomarkers and some highlight characteristics of the mixed pathologies such as synucleinopathy and TDP-43 deposits that likely impact the timeline for clinical decline in individual patients.
Continue to review, evaluate, alter and update all biomarker studies (CSF, plasma, serum) collected from subjects enrolled in ADNI, including all sites, "core core" from ADNI2/GO and all newly enrolled individuals, with 10% in each ADNI center that are not required to recruit enrolled individuals in cohort studies. In the figure below a focus on sample preparation time shows that for CSF the mean, 95%CI and median values across 1,318 samples collected from ADNI2/GO participants is 44.8 min, (41.7-47.8 min) and 28 min respectively. For 3,908 plasma samples the respective values are: 71.7 min, (70.0-73.4 min) and 55 min. This information is available for each ADNI sample. The handling at each ADNI site of these biofluid samples is very important to assure the quality of each sample. Avoidance of hemolysis and time-efficient sample preparation are essential to the goal of sample quality. For plasma, the recommended time from collection to freezing is no longer than 120 min; for CSF the recommended time is 60 min or less in order to minimize risk for biomarker degradation due to metabolic processes.  

**Figure 1**. Sample collection to freezing time for ADNI GO/2 plasma and CSF primary samples

<table>
<thead>
<tr>
<th></th>
<th>CSF</th>
<th>Serum</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>3908</td>
<td>1318</td>
<td>3908</td>
</tr>
<tr>
<td>Average Time (min)</td>
<td>71.68</td>
<td>95.7%</td>
<td>71.68</td>
</tr>
<tr>
<td>Median Time (min)</td>
<td>55</td>
<td>95.0%</td>
<td>60</td>
</tr>
<tr>
<td>95% CI (min)</td>
<td>68.55-74.71</td>
<td>95.0%</td>
<td>60-71.68</td>
</tr>
<tr>
<td>Median Time (min)</td>
<td>28</td>
<td>95.0%</td>
<td>28</td>
</tr>
</tbody>
</table>

Regularly communicate with Clinical Core staff regarding biofluid collections and any issues concerning sample quality, labeling discrepancies, and provision of updated sample-received summaries. Continue our collaboration studies are identifying and characterizing key parameters that can contribute to variability in CSF or plasma biomarker measurements especially, Aβ40, Aβ42, t-tau, p-tau181, and BACE1. For example in a recent collaborative effort undertaken in response to the ACSA Associated Alzheimer Standardization Consortium’s (ASSC) request to analyze data from participating labs, we observed that Aβ42 levels obtained using four commercial ELISA kits were significantly different, ranging from 7.1 to 16.3 ng/ml. The ELISA’s kits were purchased from four different manufactures, which is the largest variation in our study. The handling at each ADNI site of these biofluid samples is very important to assure the quality of each sample. Avoidance of hemolysis and time-efficient sample preparation are essential to the goal of sample quality. Plasma, the recommended time from collection to freezing is no longer than 120 min; for CSF the recommended time is 60 min or less in order to minimize risk for biomarker degradation due to metabolic processes.
BIOMARKER ANALYSIS

Results from the analysis of ADNI samples are summarized below. Anyone with an ADNI Data Archive account may view and download the analysis methods and the analyzed data. After logging in, click “Download” and “Study Data” to see all relevant ADNI documents available for download. See references here:

Biomarker Core References

Biomarker Core Aims and Progress for ADNI3

The Biomarker Core in ADNI3 is focusing on 4 areas of activity and studies including:

- biofluid banking (CSF, plasma and serum) management and pre-analytical assessments;
- standardization of CSF Aβ42, Aβ40, t-tau and p-tau181 measurement in ADNI patients using the highly validated Roche Elecsys cobas e 601 fully automated immunoassay platform and reference LC/MSMS methodology for CSF Aβ42, Aβ40 and Aβ38;
- determination of cut-points for Aβ42, t-tau, p-tau181, and ratios using several approaches including ROC analyses using FBP amyloid PET imaging for disease detection and disease independent mixture modeling;
- collaborative studies on new biomarker development/validation/testing in CSF or plasma by immunoassay or mrm LC/MSMS including NFL, total and phospho-α-synuclein, Vilip-1, sTREM2, progranulin, TDP-43, metabolomics/lipidomic biomarkers and proteomic quantitative assays.

Highlights of Biomarker Core activities include:

STANDARDIZATION


Analyses of CSF for Aβ, t-tau and p-tau181 moved from the Research-Use-Only Fujirebio AlzBio3 xMAP bead-based immunoassay to the fully automated Roche Elecsys platform following extensive validation studies, and for Aβ42, comparisons with validated reference method LC/MSMS using the primary reference standard preparation of Aβ42, provided by the Institute for Reference Materials and Measurements (IRMM) following finalization of replicate amino acid analyses(Kuhlman et al, 2017, Certification Report).
METHODS AND TOOLS

A large part of the success of the ADNI study rests on the consistency of data collection and processing. To guarantee this success, the cores and PIs have created uniform procedures and protocols to be used by researchers participating in the study. ADNI offers detailed information on the research process to ensure that all labs, centers and researchers participating in the study have the resources they need. To learn more about how ADNI data is collected, expand each section below.

Click each data type below for details

Biomarker Analysis
Genetic Data Methods
MRI Analysis
PET Analysis
Proteomic Analysis

Results from the analysis of ADNI samples are summarized below. Anyone with an ADNI Data Archive account may view and download the analysis methods and the analyzed data. After logging in, click “Download” and “Study Data” to see all relevant ADNI documents available for download. See references here: Biomarker Core References

Biomarker Core Aims and Progress for ADNI3
The Biomarker Core in ADNI3 is focusing on 4 areas of activity and studies including:
biofluid banking (CSF, plasma and serum) management and pre-analytical assessments;
standardization of CSF Aβ42, Aβ40, t-tau and p-tau181 measurement in ADNI patients using the highly validated Roche Elecsys cobas e 601 fully automated immunoassay platform and reference LC/MSMS methodology for CSF Aβ42, Aβ40 and Aβ38;
determination of cut-points for Aβ42, t-tau, p-tau181, and ratios using several approaches including ROC analyses using FBP amyloid PET imaging for disease detection and disease independent mixture modeling;
collaborative studies on new biomarker development/validation/testing in CSF or plasma by immunoassay or mrm LC/MSMS including NFL, total and phospho-τ-synuclein, Vilip-1, sTREM2, progranulin, TDP-43, metabolomics/lipidomic biomarkers and proteomic quantitative assays.

Highlights of Biomarker Core activities include:

DETERMINATION OF CUT-POINTS FOR Aβ42, t-tau, p-tau181, AND RATIOS USING SEVERAL APPROACHES
Completion and upload to LONI the Methods Report, “ADNI3: Batch analyses of Aβ42, t-tau and p-tau181 in ADNI1, GO, 2 CSF samples using the fully automated Roche Elecsys cobas e immunoassay analyzer system”. This dataset, uploaded April, 2017, includes a total of 2,401 never-before-thawed aliquots of ADNI1, GO and 2 CSF samples that had been collected between 9/7/2005 and 7/25/2016. See PPT set #101 for a description of major parts of the method validation for Aβ42, and some cut-point estimations and see PPT set #102 for further analyses, determinations of cut-points and relationships of “abnormal” and “normal” biomarker results to cognitive decline and progression from MCI to AD dementia done so far and working toward:
• definition of cut-points for Aβ1-42, t-tau, p-tau181 and the ratios, Aβ1-42/t-tau and Aβ1-42/p-tau181 using Mixture Modeling and ROC analyses;
• an understanding of the predictive performance for cognitive decline and progression of MCI participants to a clinical diagnosis of AD dementia using these cut-points;
• assessments of the comparisons between Aβ1-42, t-tau, p-tau181 and the ratios, Aβ1-42/t-tau and Aβ1-42/p-tau181 for predictive performance of each pair [Aβ1-42 is below CSF cut-point value; Aβ1+ is at or above CSF cut-point value; t-tau181 is below or (at or above, respectively) the cut-point value for CSF tau; analogous pairs for Aβ1 and p-tau181] for cognitive, memory and functional decline and progression from MCI to a clinical diagnosis of AD.
• assessments of concordance between CSF Aβ1-42, t-tau, p-tau181, the ratios, Aβ1-42/t-tau and Aβ1-42/p-tau181 and Florbetapir PET imaging-based plaque burden assessments.
• Inclusion of validated CSF Aβ40 to Aβ1-42, t-tau and p-tau181 in ADNI3 will permit evaluation for the Aβ1-42/Aβ40 ratio for possible improvement over Aβ1-42 alone for clinical utility.

A manuscript describing the concordance performance of ADNI/GO2 Roche Elecsys CSF Aβ42, t-tau and p-tau181 biomarker data and that from the Swedish BioFINDER study with either Florbetapir PET or Flutematomol amyloid PET imaging, respectively, in the respective study cohorts, has been accepted for publication (Hansson etal, 2018b).

Provided support for development of new immunoassays for CSF Aβ42, t-tau, p-tau181 by providing residual CSF aliquot samples to 3 vendor laboratories (see Table 2 for more information).

Figure 2. Timing for the onset and progression of AD
METHODS AND TOOLS

A large part of the success of the ADNI study rests on the consistency of data collection and processing. To guarantee this success, the cores and PIs have created uniform procedures and protocols to be used by researchers participating in the study. ADNI offers detailed information on the research process to ensure that all labs, centers and researchers participating in the study have the resources they need. To learn more about how ADNI data is collected, expand each section below.

Click each data type below for details:

BIOMARKER ANALYSIS
GENETIC DATA METHODS
MRI ANALYSIS
PET ANALYSIS
PROTEOMIC ANALYSIS

Results from the analysis of ADNI samples are summarized below. Anyone with an ADNI Data Archive account may view and download the analysis methods and the analyzed data. After logging in, click “Download” and “Study Data” to see all relevant ADNI documents available for download. See references here: Biomarker Core References

Biomarker Core Aims and Progress for ADNI3

The Biomarker Core in ADNI3 is focusing on 4 areas of activity and studies including:

- biofluid banking (CSF, plasma and serum) management and pre-analytical assessments;
- standardization of CSF Aβ 40, Aβ 42, t-tau and p-tau 181 measurement in ADNI patients using the highly validated Roche Elecsys cobas e 601 fully automated immunoassay platform and reference LCM/MSS methodology for CSF Aβ 42, Aβ 40 and Aβ 38;
- determination of cut-points for Aβ 42, t-tau, p-tau 181, and ratios using several approaches including ROC analyses using FBP amyloid PET imaging for disease detection and disease independent mixture modeling;
- collaborative studies on new biomarker development/validation/testing in CSF or plasma by immunoassay or mrm LC/MSMS including NFL, total and phospho-α-synuclein, Vild-1, sTREM2, progranulin, TDP-43, metabolomics/lipidomic biomarkers and proteomic quantitative assays.

Highlights of Biomarker Core activities include:

- Determination of cut-points for Aβ 42, t-tau, p-tau 181, and ratios using several approaches.
- Completion and upload to LONI the Methods Report, “ADNI3: Batch analyses of Aβ 42, t-tau and p-tau 181 in ADNI3, GO and 2 CSF samples that had been collected between 9/7/2005 and 7/25/2016. See PPT set #101 for a description of major parts of the method validation for Aβ 42, and some cut-point estimations and see PPT set #102 for further analyses, determinations of cut-points and relationships of “abnormal” and “normal” biomarker results to cognitive decline and progression from MCI to AD dementia done so far and working toward:
  - definition of cut-points for Aβ 1-42, t-tau, p-tau 181 and the ratios, Aβ 1-42/t-tau and Aβ 1-42/p-tau 181 using Mixture Modeling and ROC analyses;
  - an understanding of the predictive performance for cognitive decline and progression of MCI participants to a clinical diagnosis of AD dementia using these cut-points;
  - assessments of the comparisons between Aβ 1-42, Aβ 40, Aβ 42, t-tau, Aβ 38, and Aβ 38 for predictive performance of each pair: Aβ 42 below CSF cut-point value; Aβ 40 is at or above CSF cut-point value; t-tau- is below or (at or above, respectively) the cut-point value for CSF t-tau; analogous pairs for Aβ 1-42/p-tau 181 for cognitive, memory and functional decline and progression from MCI to a clinical diagnosis of AD.
  - assessments of concordance between CSF Aβ 1-42, t-tau, p-tau 181, the ratios, Aβ 1-42/t-tau and Aβ 1-42/p-tau 181 and Florbetapir PET imaging-based plaque burden assessments.
  - Inclusion of validated CSF Aβ 40 to Aβ 42, t-tau and p-tau 181 in ADNI3 will permit evaluation for the Aβ 40/Aβ 42 ratio for possible improvement over Aβ 42 alone for clinical utility.

A manuscript describing the concordance performance of ADNI5G02 Roche Elecsys CSF Aβ 42, t-tau and p-tau 181 biomarker data and that from the Swedish BioFINDER study with either Florbetapir PET or Flutemetamol amyloid PET imaging, respectively, in the respective study cohorts, has been accepted for publication (Hansson et al, 2018b).

Provided support for development of new immunoassays for CSF Aβ 42, t-tau, p-tau 181 by providing residual CSF aliquot samples to 3 vendor laboratories (see Table 2 for more information).

- Figure 2. Timing for the onset and progression of AD

The figure below illustrates the timing for the onset and progression of AD in the upper right panel with examples of mixed pathologies found in AD brains in the upper left panel while the lower panel summarizes the timing of pathology deposition and neuron death as well as current considerations for the treatment of AD. This figure is from a recently published update of earlier ADNI reviews from Kang et al, 2015, that provides our current understanding of the hypothetical timeline for the onset and progression of Alzheimer’s Disease neurodegeneration and cognitive impairments progressing from normal to mild cognitive impairment and then to Alzheimer’s disease dementia.

![Image of Risk Factors, Plaques & Tangles, and Cognitive Impairment]

Risk Factors
Plaques & Tangles
Cognitive Impairment
Preventive
Modifying
Symptomatic

Valvignet and aggregation of Aβ plaques and tangles followed by neuronal atrophy and neurodegeneration leading to plaque samples, and tangle samples, and cognitive function loss.

Preventive strategies include lifestyle changes and drug therapies that may delay or prevent the onset of Alzheimer’s disease.

Modifying strategies include medications that may slow the progression of Alzheimer’s disease after it has been diagnosed.

Symptomatic strategies are treatments for symptoms of Alzheimer’s disease, such as memory loss and other cognitive impairments.