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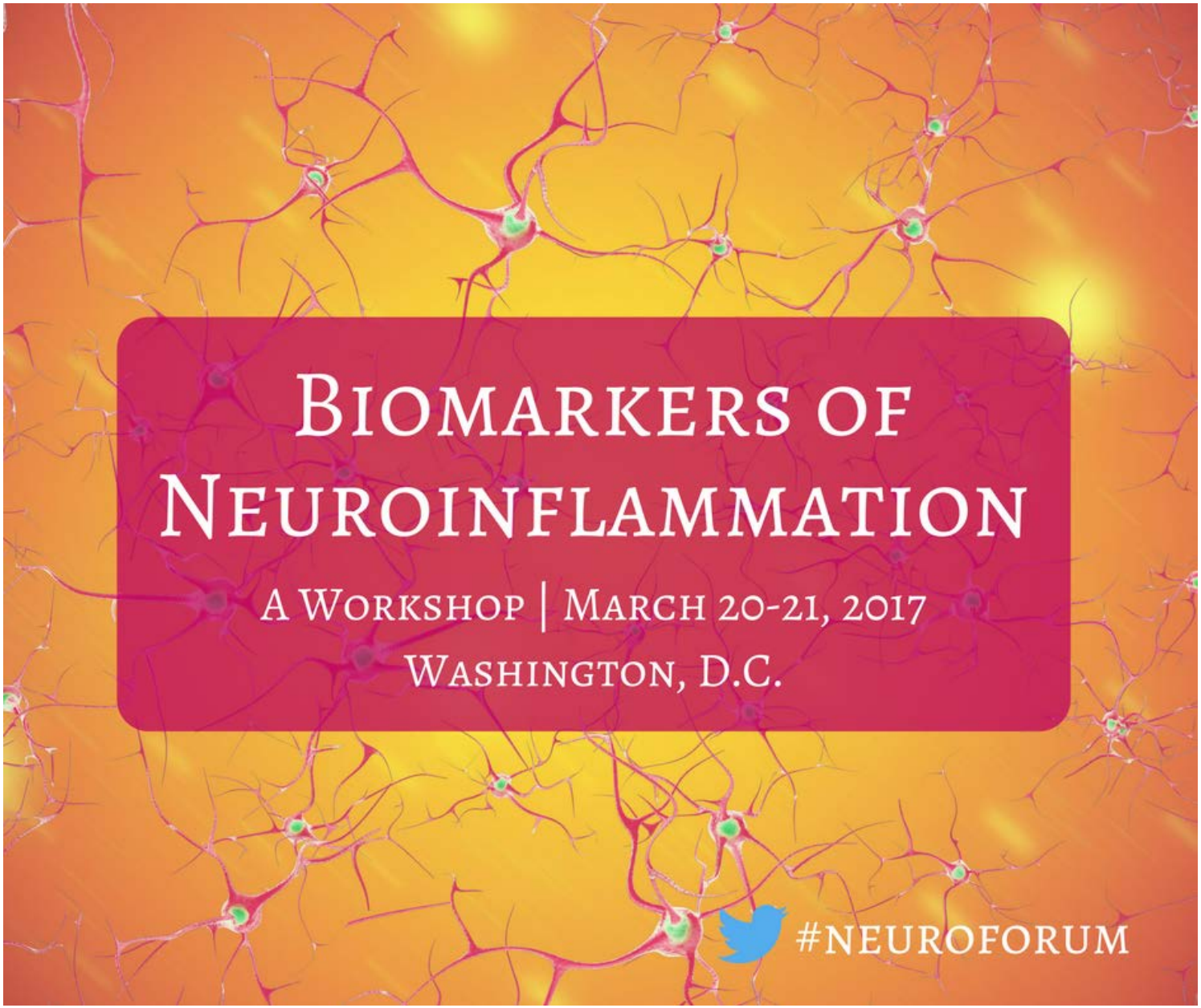
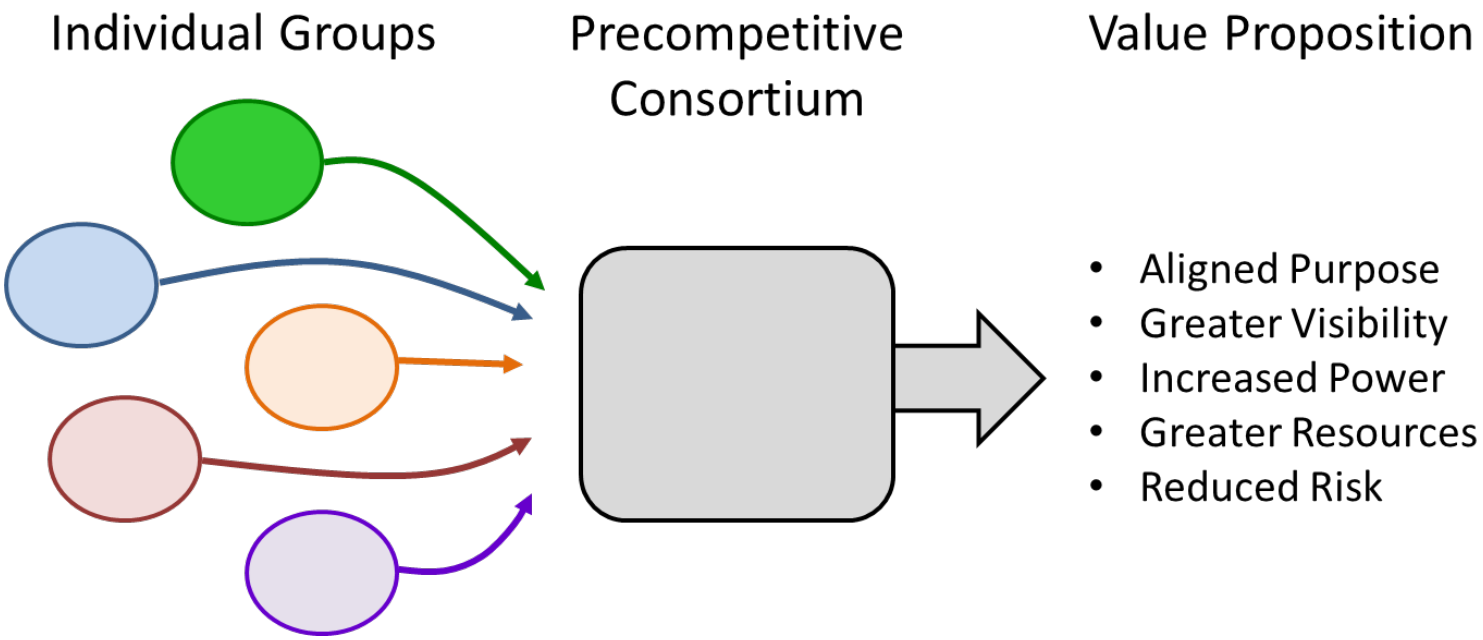


Facilitating Biomarker Development Through Public-Private Consortia



- Why are precompetitive consortia needed for inflammation biomarker development?
  - Cost of well-powered studies are high
  - Duration of studies can take years
  - Lack of harmonization of preanalytical and analytical variables can make utility of datasets questionable

Recent Consortia Focused on Neuroinflammation Biomarkers in CNS Disease

- **Wellcome Trust**
  - *Consortium for Neuroimmunology of Mood Disorders and Alzheimer's Disease*
  - UK universities (Glasgow, Oxford, Cambridge, KCL, Southampton and Cardiff) and pharmaceutical partners (Janssen, Lundbeck, Pfizer, and GSK)
  - Blood and CSF biomarkers in MDD, and imaging in MDD and AD
  - 5 year project initiated in 2015
  - £5.5 million Wellcome Trust funding

- **FNIH Biomarkers**
  - *Inflammatory Markers for Early Detection and Subtyping of Neurodegenerative and Mood Disorders*
  - Blood and CSF biomarkers in AD and MDD
  - 4 year project proposed in begin in 2017

FNIH Biomarkers: Inflammatory Markers for Early Detection and Subtyping of Neurodegenerative and Mood Disorders

Hypothesis and basic premises:

- Aberrant immune function contributes to, or correlates with, the development and/or progression of a wide range of CNS disorders
- Measuring inflammatory markers in blood and/or CSF will yield biomarkers that will be useful in diagnosis and predicting treatment outcome of patients
- The lack of consensus and conflicting results stems largely from small sample sizes and preanalytical and analytical variability of biomarker measurements
  - Insufficient technical validation of assays between studies
  - Standardization of analyte panels examined
  - Differences in collection, handling, and storage of samples

Inflammatory Markers for Early Detection and Subtyping of Neurodegenerative and Mood Disorders

Goal:

- To identify and validate plasma- and/or CSF-based inflammatory biosignatures for CNS disorders
  - Develop best practices for sample collection and measurement
  - Assist in diagnosis and sub-typing of patients
  - Aid in appropriate therapeutic assignment and improve the tracking of disease progression
- The initial focus will be on Alzheimer's Disease (AD) as an example for a neurodegenerative disease, and Major Depressive Disorder (MDD) as an example for a psychiatric disorder

Prior Results Support the Need for Multicomponent Inflammatory Biomarker Signatures

- Significant differences in inflammatory markers between groups of healthy subjects versus psychiatric and neurodegenerative patients reported, however **very little of the literature attempts to use these measurements to predict diagnosis, disease stage, or treatment response**
- This is likely due to the very **modest differences in analyte levels** between the groups, which results in considerable overlap. Consequently, these studies may provide population-based insights, but do not support patient-based conclusions
- We **hypothesize that multi-biomarker panels**, measured using **harmonized techniques and technically well-validated assays** optimized for linearity with real-world analyte concentrations, **will provide more meaningful patient level insights**

References:

- Brietzke et al 2012, J Affect Dis 140(1), Towards a multifactorial approach for prediction of bipolar disorder in at risk population
- McIntyre et al 2014, Bipolar Disord 16(5), Advancing biomarker research: utilizing 'Big Data' approaches for the characterization and prevention of bipolar disorder

Scientific Strategy and Experimental Design

Overview:

- The project comprises **3 specific aims**
- Each aim requires a **decision process based on prior results** that determines whether or not further investment is warranted ("go/no-go"), or whether additional focus (assay platform, disease area, sample type) is needed
- In order to obtain meaningful results, the sample collection and handling **procedures will be harmonized** across sites, and state of the art assay platforms will be selected based on their performance

Aims:

- **In aim 1**, all assays will be validated with respect to sensitivity, linear range, reproducibility and other parameters comparing different assay platforms across multiple sites
- **In aim 2**, biosignatures will be developed based on existing samples (training set) that were collected, handled and stored using procedures that match the processes defined in this proposal as closely as possible
- **In aim 3**, the inflammatory biosignatures will be confirmed in a prospective study using a separate validation cohort of patients

Inflammatory Markers Project: Specific Aim 1

Methods development and assay validation

- **Assemble a standard panel of analytes** for blood and CSF
- **Assemble a well-characterized set of existing plasma and CSF samples** from AD and MDD patients (25 each) and healthy controls (50) for a total of 200 samples
- **Select and technically validate the assay platform(s)**
- **Decide which assay platform to employ** for aims 2 and 3

Aim 1: Methods Development & Assay Validation

Analyte	Assay Technology	Multiplex	Sample Volume (ml)
IL-1β	MSD-V-plex, SiMoA	No, lowest levels of all cytokines here so need BEST assay possible (SiMoA) and that uses higher volume sample than all the other single analytes.	0.25
IL-6	MSD-V-plex, SiMoA	Yes, SiMoA (IL-6, IL-10, TNF-α) or MSD V-plex (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α)	0.1
TNF-α	MSD-V-plex, SiMoA	Yes, SiMoA (IL-6, IL-10, TNF-α) or MSD V-plex (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α)	0
Trp	LC-MS/MS	YES	0.1
KYN	LC-MS/MS	YES	0
3-HK	LC-MS/MS	YES	0
3-HAA	LC-MS/MS	YES	0
QUIN	LC-MS/MS	YES	0.1
KYNA	LC-MS/MS	YES	0
IL-10	MSD-V-plex, SiMoA	Yes, SiMoA (IL-6, IL-10, TNF-α) or MSD V-plex (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α)	0
sIL-6R	MSD-V-plex, SiMoA	No	0.05
IL-1RA	MSD-V-plex, SiMoA	No	0.2
CD40		No	0.1
CRP	Siemens BNII Nephelometer	No	0.5
		TOTAL	**1.4**

- List of inflammation-related markers included in biomarker panel
- Analytes marked in green and orange indicate two separate multiplex sets

Aim 1: Assay Platform Selection and Validation

- **Assay platforms to be validated** chosen based on previous evaluations by members of the biomarkers working group
- **CROs selected** based on experience with platforms, the availability of validated assays for the analytes of interest, pricing/resource availability and timeline considerations
- **Inflammatory analytes**:
  - ELISA-based assays will be used for these analytes
  - The CROs of the assay technology platform companies will be engaged for assay validation, since they are most knowledgeable about the specific platforms
  - The subsequent aims will utilize the best-suited assay platform at the same CRO, since this will obviate the need for additional assay validation
- **TRP/KYN pathway metabolites**:
  - LC-MS/MS-based assays will be used to measure kynurenine metabolites
  - CROs chosen based on previous evaluations by members of the working group
  - The same CRO will be engaged throughout the entire project

Aim 1: Technical Validation of Assay Platforms

Comparison of 3 major high sensitivity platforms focusing on IL-6, IL-10, IL-1β, TNF-α

Dynamic Range

	pg/ml (LLOQ to ULOQ)		
	R&D	MSD	SiMoA
IL-1β	.13-8	.06-495	.02-120
IL-6	.63-10	.09-767	.04-30
IL-10	.78-50	.08-334	0.1-30
TNFα	1.56-50	.15-312	.03-50
R&D assay drops out based on poor dynamic range			

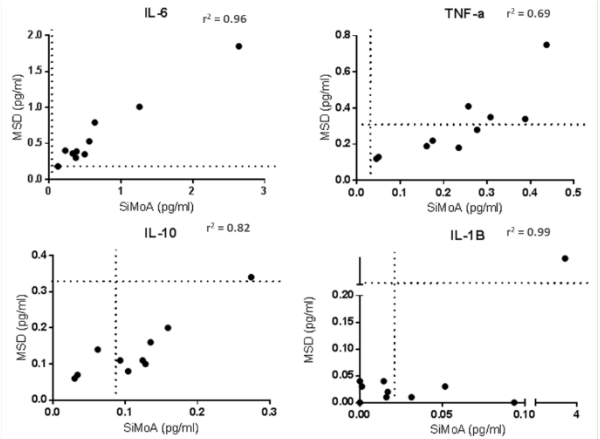
Precision

	Intra-run CV% (avg of quantifiable serums)		
	R&D	MSD	SiMoA
IL-1β	0.5	7.8	7.3
IL-6	2.9	3.1	4.9
IL-10	3	2.8	21.3
TNFα	14.6	11.8	8.6
All 3 platforms are good			

Sensitivity

	% serums quantifiable (>LLOQ)		
	R&D	MSD	SiMoA
IL-1β	10%	10%	40%
IL-6	90%	100%	100%
IL-10	10%	50%	>70%
TNFα	20%	70%	100%
R&D and MSD drop out based on poor sensitivity			

Concordance of SiMoA and MSD V-plex cytokine assays



- IL6, TNFa, IL10 exhibit concordance between assays.
- Difficult to say for IL1b since most <LLOQ on MSD platform.

Assay precision across platforms

Intra- and inter-assay precision was evaluated using pooled human serum samples spiked with supernatants from stimulated PBMCs

Technology (Vendor)	Intra-assay %CV				Inter-assay %CV			
	IL-2	IL-17a	IL-6	TNFα	IL-2	IL-17a	IL-6	TNFα
SiMoA™ HD-1 Analyzer (Quanterix)	10.1	10.2	3.4	10.0	13.9	10.2	14.2	10.7
Erenna® (Singulex)								
Milliplex® (EMD Millipore)	8.8	12.3	16.5	5.5	11.0	23.2	18.3	5.5
V-PLEX (MSD)	5.8	10.1	13.7	11.1	21.2	24.5		16.4
High Sensitivity ELISA (eBioscience or R&D Systems)		10.8	11.4	16.7		22.1	17.1	17.0
Biochip Array Technology (RANDOX)	17.8	6.6	17.1	15.7	22.9	6.6	17.1	17.6
Ella™ (ProteinSimple)	9.7	4.2	9.4	4.5	15.5	10.8	15.3	8.5
AMMP™ ViBE® (Bioscale)								
Imperacer® (Chimera Biotec GmbH)	12.4				17.4			

Assay precision was evaluated in 2 to 4 different runs by a single operator using at least 3 replicate sets of spiked samples on each run

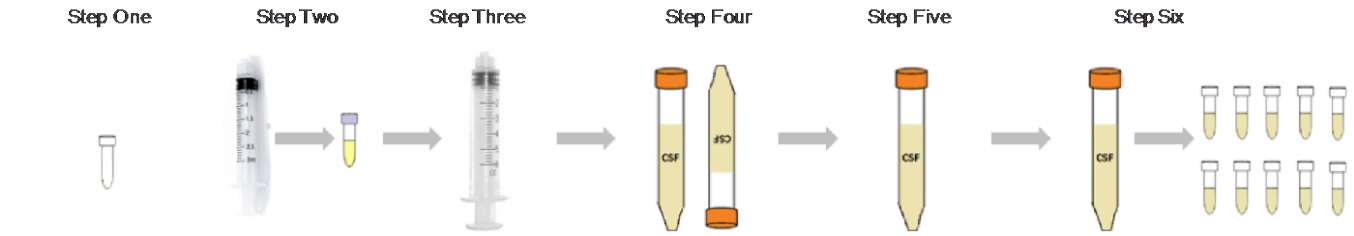
- Most evaluated assays had the intra- and inter-assay precision ~20%
- SimoA and Ella showed best and most consistent precision

- 4 assay platforms were evaluated
- 2 platforms were selected for platform validation):
  - **MSD V-plex**
  - **SiMoA HD-1 Analyzer**

Assay platform comparison data from Janssen NS Biomarkers, Biogen

Aims 1 through 3: Harmonization of Sample Collection SOPs

CSF Sample Collection



- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

- Collect CSF into the 3ml luer lock syringe.
- Dispense 1-2 ml into the purple cap cryovial.
- Send to local lab for testing.

- Collect CSF into the 6ml luer lock syringe.
- Collect 15-20 ml total, including the 1-2 ml sent to the local lab.

- Immediately after collection, invert tubes 3-4 times in conical tubes to mix samples.

Optional for Aims 1&2:

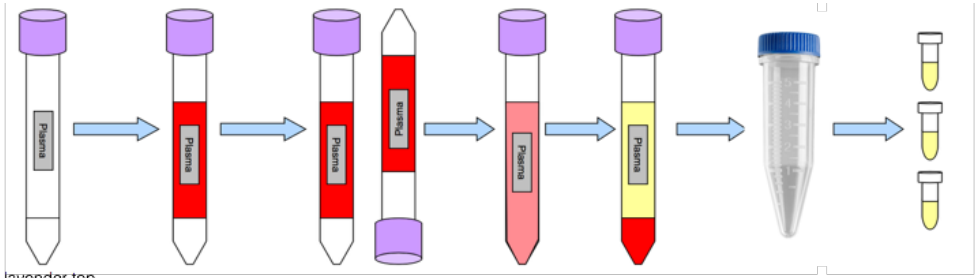
- Within 15 minutes of collection, centrifuge samples at RT at 2000 x g for 10 minutes.
- If centrifugation is not performed, please record this fact on the Lumbar Puncture data form.

For Aim 3:

- Subject one aliquot to centrifugation at RT at 2000 x g and collect a second aliquot without centrifugation.

- Aliquot 1.5 ml into clear cap cryovials, leaving the pellet in the bottom of the 15 ml conical tubes (if the sample was subjected to centrifugation).
- Store CSF aliquots at -80°C until until shipment.

Plasma Sample Collection



Store lavender top tubes at room temperature, label with pre-printed "Plasma" labels prior to blood draw.

Collect blood in Plasma Tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Immediately after blood draw, invert tubes 8-10 times to mix samples.

Within 30 minutes of blood draw, centrifuge samples at 4°C, 1300 x g for 15 minutes.

Immediately after centrifugation, transfer plasma to the 5 mL conical tube. Check for signs of hemolysis.

Label aliquot tubes with preprinted "Plasma" labels. Use transfer pipette to aliquot 1.5 ml samples of plasma. Store plasma aliquots at - 80°C until shipment.

- Generation of standardized SOPs critical for reproducibility of results
- SOPs prepared based on insights from ADNI, PPMI, and Janssen
- Assay collection procedures used to help prioritize sample providers for Aims 1 and 2, and for sample collection procedures in Aim 3

kers CONSORTIUM | FNIH Foundation for the National Institutes of Health

Aim 2: Biosignature Development

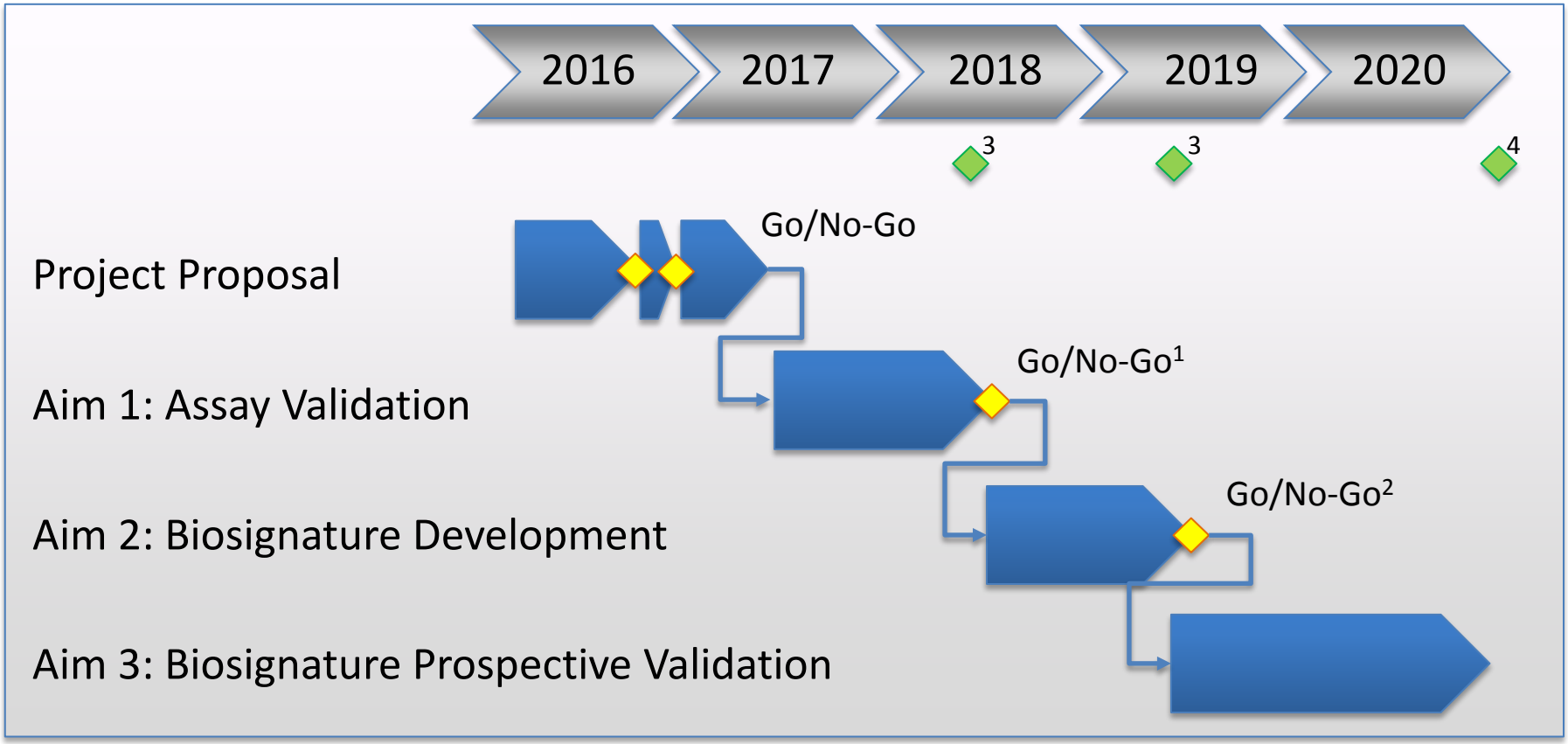
Biosignature development with existing samples

- **Assemble a training set**: Existing plasma and CSF samples from 100 healthy controls, 100 patients with AD, 100 MDD subjects for a total of 300 plasma and 300 CSF samples. Approximate the proposal's harmonized sample collection processes as closely as possible
- **Measure the levels of inflammatory markers** in CSF and plasma in the training set using the assay platform that was selected and validated in aim 1
- **Obtain existing clinical rating data** for each subject based on standard disease area-specific rating scales
- **Perform univariate and multivariate data analysis** of these measurements related to clinical observations at the time of sample collection (Biosignature Development)
- **Decide whether the results are sufficiently strong** to warrant further investment (go/no-go) and whether further disease area and sample type prioritization is needed based on the strength of the Biosignature results

Aim 3: Confirmation of Biosignature

- **Prospective Biosignature Validation**
  - **Collect fresh plasma and CSF samples** from patients (100 AD, 100 MDD) and healthy controls (100) for a total of up to 300 CSF and 300 plasma samples
    - 400, if only one disease area is carried forward
    - 300 and 200, respectively, if only one sample type (plasma or CSF) is carried forward
  - **Determine clinical ratings for each subject** based on the same disease area-specific rating scales that were employed in aim 2
  - **Confirm the biosignatures** in the prospective, blinded validation cohort

FNIH Biomarkers Project Timeline



Footnotes:

[1] Determination of final biomarker panel
[2] Determination of most promising indication
[3] Interim report – 2Q/2018; 2Q/2019
[4] Final report- 4Q/2020

Complementary Rather Than Competitive with Other Neuroinflammation Biomarker Consortia

Comparison: Wellcome Trust biomarkers consortium

- Both efforts evaluate plasma and CSF samples from major depressive disorder (MDD) patients
- Overlap in some MDD inflammation biomarker panel endpoints (in red)
- MSD platform will be evaluated in both consortia

Key differences

- Technical validation of assay platforms
- Evaluation of SiMoA platform performance included in FNIH Biomarkers
- Evaluation and comparison of AD plasma and CSF samples

Inflammation Biomarkers

	FNIH Biomarkers	Wellcome Trust
AD	CRP, TNF-a, IL-1b, IL-6, IL-10, sIL-6R, IL-1RA, CD40 KP metabolites	TSPO imaging
MDD	CRP, TNF-α, IL-1b, IL-6, IL-10, sIL-6R, IL-1RA, CD40 KP metabolites	CRP, IFN-g, TNF-α, IL-1β, IL-6, IL-10 KP metabolites TSPO imaging

Opportunity to capitalize on synergies

- Opportunity exists to include biosamples from Wellcome Trust consortium in FNIH Biomarkers consortium assays to compare outcomes

Summary

- Strong interest in CSF and fluid inflammation biomarkers related to CNS disease to assist in diagnosis and to track treatment responses
  - Understanding relationship of inflammation markers in peripheral and central compartments
  - Analysis of relationship between biomarkers and clinical ratings scales
- Consortia playing a key role in evaluating neuroinflammation biomarkers
  - Fully aligned objectives across multiple organizations in precompetitive space
  - Cost sharing to reduce risk
- Alignment of preanalytical and analytical protocols essential for reproducibility and broad utility of measurements

Project Concept Team Members

Project ConceptTeam Members	Affiliation	Role
(Co-Chair) Hartmuth Kolb, PhD	Head of Neuroscience Biomarkers, Janssen R&D, US	Project Oversight Team Co-Chair
(Co-chair) Brian Campbell, PhD	Vice President, MindImmune Therapeutics; Ryan Research Professor of Neurosciene, University of Rhode Island	Project Oversight Team Co-Chair
Linda Brady, PhD	Director, Division of Neuroscience and Basic Behavioral Science, NIMH	NIH Representative, Project Team Member
Susan Croll, PhD	Director, Neuroscience, Regeneron Pharmaceuticals	Project Team Member
Nancy Desmond, PhD	Associate Director, Division of Neuroscience & Basic Behavioral Science	NIH Representative, Project Team Member
Danielle Graham, PhD	Director, Neurodegenerative Disease, Biomarker Discovery and Development, Biogen	Project Team Member
James Hendrix, PhD	Director, Global Science Initiatives, Alzheimer's Association	Project Team Member
John Hsiao, MD	Health Science Administrator, NIA	NIH Representative, Project Team Member
Andreas Jeromin, PhD	Chief Medical Officer, Quanterix	Project Team Member
Richard Margolin, MD	Vice President, Clinical Development, CereSpir	Project Team Member
Terina Martinez, PhD	Senior Associate Director, Research Programs, Michael J. Fox Foundation	Project Team Member
Niels Plath, PhD	Vice President, Synaptic Transmission, Lundbeck	Project Team Member
Bill Potter, MD, PhD	Senior Advisor to the Director , NIMH	NIH Representative, Project Team Member
Robert Umek, PhD	Director of External Scientific Affairs, Meso Scale Discovery	Project Team Member
Hong Wang, PhD	Senior Research Scientist, Eli Lilly and Company	Project Team Member