CKD BioCon Phase II Protocols Summary				
Protocol	**Populations**	**Sample**	**Markers**	**Outcomes**
Metabolomics of CKD and Its Progression	AASK, MDRD, CRIC, CKiD, BWH Variability Cohort	Plasma	Non-targeted metabolomics (Broad Institute and Metabolon)	CKD progression (ESRD, halving of eGFR), Cardiovascular outcomes and mortality
Targeted blood biomarkers for diabetic nephropathy (DN) progression in subjects with reduced GFR	CRIC, REGARDS, VA NEPHRON D	Plasma	suPAR, TNFR1&2, KIM-1, MCP-1, BMP-7, YKL-40, endostatin	eGFR decline >40%, ESRD
Targeted urine biomarkers of incident CKD or ESRD in community-based cohorts	REGARDS, MESA, ARIC	Urine	α-1 microglobulin, PIII NP, uromodulin, MCP-1, EGF, endostatin, TIMP-2, YKL-40, IGFBP-7, phosphorus, IL-18	Incident CKD (eGFR decrease by >40% to a value <60 ml/min/1.73 m2)
Targeted blood biomarkers of incident CKD or ESRD in community-based cohorts	REGARDS, MESA, ARIC	Plasma	suPAR, TNFR1&2, KIM-1, MCP-1, BMP-7, YKL-40, endostatin	Incident CKD (eGFR decrease by >40% to a value <60 ml/min/1.73 m2)
Targeted urine biomarkers for diabetic nephropathy (DN) progression in subjects with reduced baseline GFR.	CRIC, REGARDS, VA NEPHRON D	Urine	α-1 microglobulin, PIII NP, uromodulin, MCP-1, EGF, endostatin, TIMP-2, YKL-40, IGFBP-7, phosphorus, IL-18	eGFR decline >40% or ESRD
Urine biomarkers predicting CKD progression in a pediatric CKD population	CKiD	Urine	α-1 microglobulin, PIII NP, uromodulin, MCP-1, EGF, endostatin, TIMP-2, YKL-40, IGFBP-7, phosphorus, IL-18	eGFR decline >50%, ESRD, slope of eGFR decline

CKD BioCon Phase II Protocols Summary (continued)

Protocol	Populations	Sample	Markers	Outcomes
Blood biomarkers predicting CKD progression in a pediatric CKD population	CKiD	Plasma	suPAR, TNFR1&2, KIM-1, MCP-1, BMP-7, YKL-40, endostatin	eGFR decline >50%, ESRD, slope of eGFR decline
Targeted blood biomarkers for diabetic nephropathy (DN) incidence and progression in subjects with preserved baseline GFR	ACCORD, REGARDS, CRIC	Plasma	suPAR, TNFR1&2, KIM-1, MCP-1, BMP-7, YKL-40, endostatin	eGFR decline >50%, ESRD
Targeted blood biomarkers for CVD in patients with prevalent DN	CRIC, REGARDS, VA NEPHRON D	Plasma	TMAO, SDMA, ADMA, hs-Troponin-T, p-cresol sulfate, indoxyl sulfate, galectin 3, sTNFR-1, sTNFR-2, TFG-β1, endostatin	CVD events (heart failure, MI, stroke, PAD), death
Biomarkers to Predict Therapeutic Response to Interventions	ACCORD, VA NEPHRON-D	Urine, plasma	Plasma: suPAR, TNFR1&2, KIM-1, MCP-1, BMP-7, YKL-40, endostatin Urine: α-1 microglobulin, PIII NP, uromodulin, MCP-1, EGF, endostatin, TIMP-2, YKL-40, IGFBP-7, phosphorus, IL-18	CKD progression
Proteomics of CKD: Cardiovascular Risk and Kidney Disease Progression	CRIC	plasma	Non-targeted Proteomics (SomaLogic)	MI, stroke, heart failure, CV death, CKD progression/ESRD

AASK: African-American Study of Kidney Disease and Hypertension, ACCORD: Action to Control Cardiovascular Disease, ARIC: Atherosclerosis Risk in Communities Study, CKiD: Chronic Kidney Disease in Children , CRIC: Chronic Renal Insufficiency Cohort Study, MDRD: Modification of Diet in Renal Disease Study, MESA: Multi-Ethnic Study of Atherosclerosis, REGARDS: Reasons for Geographic and Racial Differences in Stroke , VA NEPHRON-D: Veterans Affairs Nephropathy in Diabetes, CVD: Cardiovascular disease; MI: myocardial infarction; PAD: peripheral arterial disease; ESRD: end-stage renal disease

Title and Aims	Populations studied*	Biomarkers studied*	Outcomes
Tubular injury biomarkers of and progression in established CKD Aim: To determine if levels of tubular injury biomarkers (NGAL, L-FABP, KIM-1, and NAG) are associated with subsequent loss of renal function, independent of known clinical risk factors for CKD progression in patients with established CKD	ARIC, CRIC, NIDDK American Indian Study	Urinary L-FABP, NGAL, NAG, KIM-1	CKD Progression
Assessing combinations of injury and filtration biomarkers for adverse outcomes in CKD Aims: To determine if combinations of blood filtration markers and urine tubular injury markers explain more of the observed variability in cardiovascular events, all-cause mortality and CKD progression than either type of biomarkers alone, independent of known clinical risk factors.	CRIC	Urine tubular injury biomarkers (L-FABP, NGAL, NAG, KIM-1) and blood filtration markers (B2M, BTP)	Cardiovascular events, all-cause mortality and CKD progression
Normal ranges and biological variability of CKD biomarkers Aims: 1) To determine reference values of CKD biomarkers in healthy individuals 2) To determine the short-term biological variability of CKD biomarkers in healthy individuals and in those with CKD	Healthy adult volunteers recruited from the community Adult patients with eGFR 15 to 60 ml/min/1.73m^2 or > 1gm proteinuria	Urinary KIM-1 Urinary NGAL Plasma KIM-1 Plasma creatinine and cystatin C	Reference ranges in healthy individuals Short-term biological variability
Prediction of Outcomes in CKD with Blood Biomarkers Aims: To evaluate blood biomarkers for their prediction of CKD outcomes	ARIC, NHANES NIDDK American Indian Study, AASK, MDRD, CRIC	BTP, B2M (compared to creatinine and cystatin C), FGF-23, vitamin D, vitamin D binding protein	Primary : ESRD and all-cause mortality; Secondary (where available): cardiovascular (CVD) mortality , non-fatal CVD events, heart failure, AKI, and progressive CKD
CKD Blood Biomarker Discovery Aims: To identify and verify novel blood biomarkers where altered levels precede or follow rapid CKD progression using state-of-the-art proteomic methods	CRIC	de novo proteomic discovery	CKD Progression defined as slope >5 ml/min/1.73m^2/year; total decline of at least 30 ml/min/1.73m^2; follow-up of at least 3 years

Title and Aims	Populations studied*	Biomarkers studied*	Outcomes
Prediction of Outcomes in CKD with Longitudinal Change in Blood Biomarkers Aims: To examine the longitudinal (one and two year) change in BTP, B2M, creatinine and cystatin C, alone and in combination, for enhanced risk prediction of ESRD, cardiovascular disease and mortality	MDRD, AASK	BTP, B2M (compared to creatinine and cystatin C)	Primary: ESRD, mortality; Secondary: CVD mortality and CKD progression defined in these clinical trials as a 50% reduction in measured GFR
Novel Blood Biomarkers for Chronic Kidney Disease: Verification and Validation of Initial Discovery Results Aims: 1. To identify existing assays or to develop assays which could be used for faster and more precise measurement of these novel biomarkers. 2. To verify and validate candidate blood markers identified in the discovery phase.	ARIC, AASK	Most promising markers that have been identified in the de novo proteomics discovery phase (CKD Blood Biomarker Discovery protocol)	CKD Progression
Cross-Species Targeted Marker Discovery (CSTD) Aims: 1. To screen kidney tissue damage markers, identified by cross-species (mouse-human) transcriptional profiling in human and murine kidney disease, using a step-wise approach of western blot and/or ELISA (or other suitable) methodology, in urine of non-renal controls, 'non-progressive' and 'rapidly progressive' CKD cases. 2. To advance prescreened candidate biomarkers for CKD progression to early and late BioCon validation protocols	Mount Sinai Clinical Cohort	Complement C3 fragments (C3a, iC3b and C5b-9), IGF-binding protein 3 (IGFBP3), Dickkopf 3 (DKK3)	CKD association, CKD progression
Biomarker Discovery and Analysis by Methods Appropriate to Urine Sample Storage **Aims:** 1. To perform proteomic analyses of NIDDK American Indian Study samples after long storage at -20°C. Compare proteomes to samples from the same individuals that have been stored at -80° C. 2. To identify markers of early stage CKD in persons with type 2 diabetes mellitus.	NIDDK American Indian Study	Promising urine proteome markers identified in discovery analysis.	Differences in promising markers identified in samples stored at different temperatures. Progression to macroalbuminuria, reduced GFR, ESRD, and death.

Title and Aims	Populations studied*	Biomarkers studied*	Outcomes
Development of IPP2K and Bradykinin Assays Aim: To develop and optimize a quantitative mass spectrometry assay for urine IPP2K and plasma bradykinin.	Joslin clinic cohort	Urine IPP2K and plasma bradykinin peptides	Renal function decline in type 1 diabetes
Markers of Early Diabetic Kidney Disease Aim: To identify markers of early stage CKD in persons with diabetes mellitus.	NHS, RASS, NIDDK American Indian Study	Urine MCP-1, Hepcidin, IPP2K, uromodulin, transferrin, albumin, and plasma bradykinin peptides	Changes in kidney histology and morphometry over 5 years (NHS and RASS). Cross-sectional associations of histology and morphometry (NIDDK American Indian Study)
Biomarkers of Interstitial Kidney Pathology Aim: To test combinations of biomarkers for detection of interstitial inflammation or fibrosis in lupus and other glomerular diseases.	Ohio State University, Cincinnati Children's Hospital, and the Brigham and Women's cohorts	Osteopontin, hemopexin, endothelial protein C receptor, urine MCP-1, serum creatinine, proteinuria	Interstitial inflammation or fibrosis identified on kidney biopsy
Markers of Early Lupus Nephritis Aim: To identify markers of early stage CKD in persons with lupus nephritis and diabetes mellitus.	Ohio SLE Study, Children's Lupus Cohort, NHS, RASS	Urine MCP-1, Hepcidin, transferrin, uromodulin, LFABP, IPP2K and plasma bradykinin peptides	Lupus Nephritis: Incident CKD Diabetes Mellitus: Changes in kidney histology and morphometry over 5 years
Plasma Proteome Biomarkers of Chronic Kidney Disease Progression in Type 1 Diabetes Aim: To study the plasma proteome to identify candidate biomarkers of CKD progression in subjects with type 1 diabetes.	DCCT/EDIC	Plasma proteome	Progression to advanced CKD, as defined by eGFR < 30ml/min/1.73m^2 with or without associated albuminuria

Title and Aims	Populations studied*	Biomarkers studied*	Outcomes
Plasma KIM-1 and Chronic Kidney Disease Aims: 1) To test plasma KIM-1 as a predictor of CKD progression 2) To test plasma KIM-1 as a predictor of cardiovascular disease events	CRIC	Plasma KIM-1	CKD progression, Atherosclerotic disease events, Congestive heart failure events

***Abbreviations**

AASK: African-American Study of Kidney Disease and Hypertension
ARIC: Atherosclerosis Risk in Communities Study
BTP: beta-trace protein
B2M: beta-2 microglobulin
CRIC: Chronic Renal Insufficiency Cohort Study
DCCT: Diabetes Control and Complications Trial
EDIC: Epidemiology of Diabetes Interventions and Complications
FGF-23: fibroblast growth factor 23
IGF: insulin-like growth factor
IPP2K: inositol pentakisphosphate 2-kinase
KIM-1: kidney injury molecule-1
L-FABP: liver fatty acid binding protein
MCP-1: monocyte chemoattractant protein-1
MDRD: Modification of Diet in Renal Disease Study
NAG: N-acetyl-β-D-glucosaminidase
NGAL: neutrophil gelatinase associated lipocalin
NHS: Natural History Study
RASS: Renin Angiotensin System Study
SLE: Systemic Lupus Erythematosus