Proteomics International Laboratories Ltd

ASX Release/Technical Presentation

26 October 2015

ASX code: PIQ

Translating biomarker discovery into a diagnostic test for diabetic kidney disease

Life sciences company Proteomics International Laboratories Ltd (ASX: PIQ) is pleased to provide its latest technical presentation on its lead diagnostic test, PromarkerD.

The presentation was given as part of the 11th Australian Peptide Conference 2015, which is being held in Kingscliff, New South Wales from 25th-30th October 2015. The Company's Managing Director, Dr Richard Lipscombe, was invited to give the presentation at the conference's opening satellite meeting, titled 'The "Omics" Revolution: Uncovering Proteome Complexity' on Sunday 25th October.

The meeting attracted key opinion leaders from academia, research institutes, hospitals and industry, with delegates invited from around the world. The program covered many emerging areas of “omics” research with topics including Proteomics: Biomarker Discovery and Validation, and Big Data. Dr Lipscombe commented that an important take-home message was “the promise of personalised medicine will only be realised by integration of proteomics and metabolomics data into the genomics scaffold”.

The presentation covers the challenge presented by diabetes, and its complications, to global public health, and walks through the development of PromarkerD from the initial diagnostic study to the in-depth longitudinal analysis that produced the current predictive test.

Proteomics International is the wholly owned operating entity of the PILL Group.

ENDS

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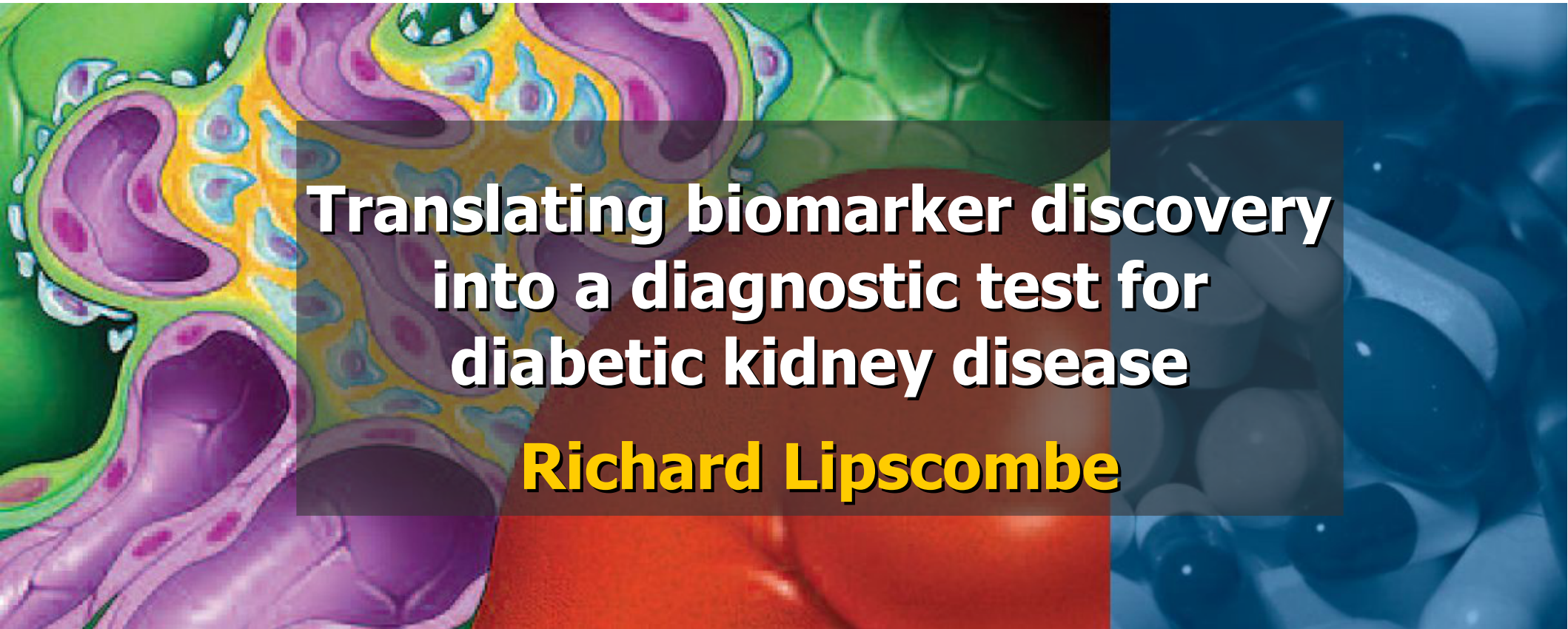
Proteomics International

The Omics Revolution | 25th October 2015

11th Australian Peptide Conference | Kingscliff, New South Wales



Translating biomarker discovery into a diagnostic test for diabetic kidney disease

Richard Lipscombe

Proteomics International

ISO 17025

Quality is Assured

This accreditation strengthens the Company's licensing position to deliver drug development data that is of the highest scientific integrity

Company:

- Founded 2001
- Listed on the Australian Stock Exchange April 2015 (Code: PIQ)
- Operates from specialist facilities in Perth, Western Australia

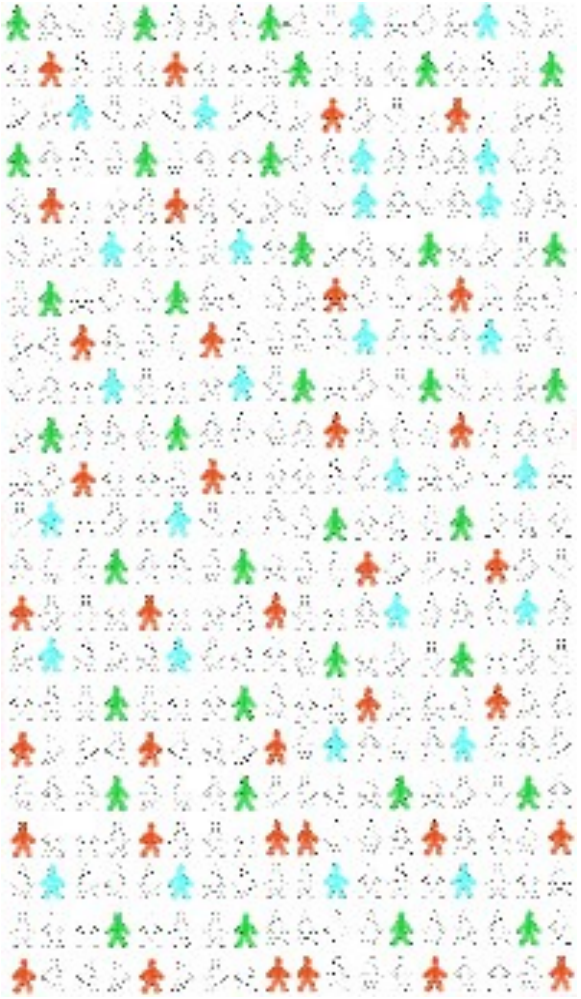
People:

- Management – ASX-company, biotech trade sales, commercialisation, and marketing experience
- Team of 20 – R&D, protein chemistry, and industry experience

Business model:

- Biomarker and peptide drug discovery combined with established cash flow from global clients (proteomics & biosimilars)

Outline



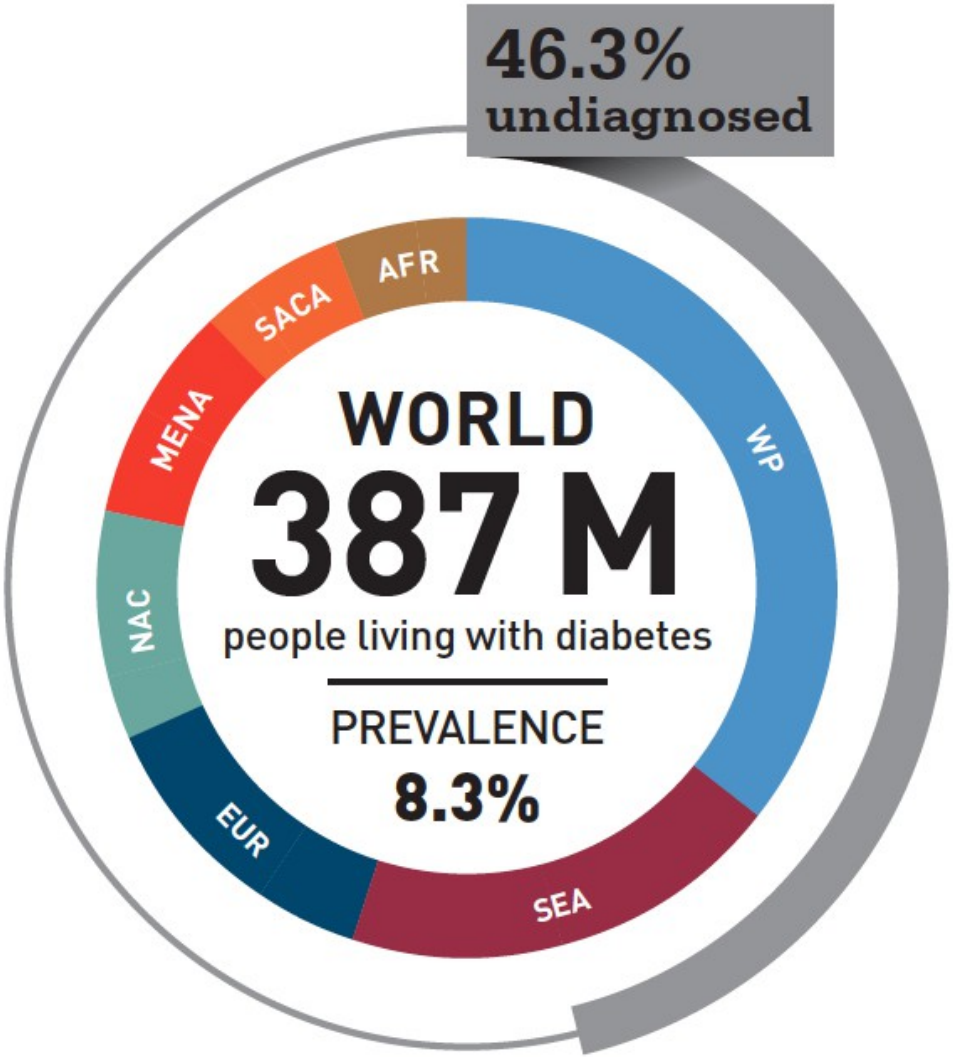
- PromarkerD test
- Clinical question
- Process
- Diagnostic
- Cross validation
- Prognostic
- Predictive Panel

The test - PromarkerD

Without PromarkerD

With PromarkerD

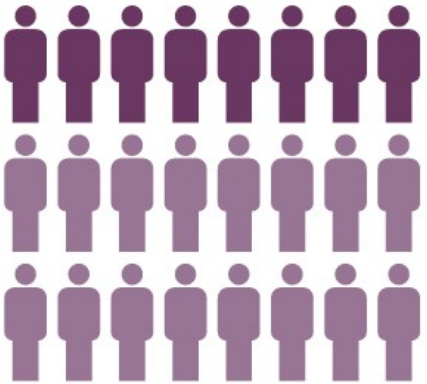
Diabetes



Africa
South & Central America
Middle East & North Africa
North America & Caribbean
Europe
South-East Asia
Western Pacific

2014

expected increase
+205
MILLION



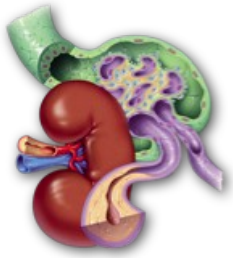
2035

- Total annual cost impact of diabetes in Australia - $14.6 billion

Clinical question

Phenotype:

Type 2 diabetes –
kidney disease (nephropathy)



- Glomerular filtration rate (eGFR)
- Albumin creatinine ratio (ACR)

(normo-albuminuria vs. micro- vs. macro)

	PROTEINURIA - assessed by ACR (mg/g)		
eGFR (mL/min/1.73m²)	NORMAL ACR <30 Dipstick: -ve	MILD ACR 30 – 300 Dipstick: Trace/1+	HEAVY ACR >300 Dipstick: ≥2+
≥90	Not CKD RISK CATEGORY 0	RISK CATEGORY 1	RISK CATEGORY 3
60–89		RISK CATEGORY 1	RISK CATEGORY 3
45-59	RISK CATEGORY 1	RISK CATEGORY 2	RISK CATEGORY 4
30-44	RISK CATEGORY 2	RISK CATEGORY 3	RISK CATEGORY 4
15–29	RISK CATEGORY 3	RISK CATEGORY 4	RISK CATEGORY 4

Clinical studies:

Fremantle Hospital Diabetes Study (FDS)

- Longitudinal observational study of care, control and complications; with over 1,700 participants
- Participants had complete data for conventional variables: age, diabetes duration, blood pressure, anti-hypertensive treatment, diuretic treatment, diabetes medication, serum glucose, HbA1c, HDL-cholesterol, ACR, uric acid

Headed by Prof. Tim Davis, Medical School,
University of Western Australia

Study design

Proteomics International

Diabetic kidney disease cohorts

Discovery (iTRAQ MS)	**Total 3 pools (N = 60)**	**Pool 1 (N=20) Normo**	**Pool 2 (N=20) Micro**	**Pool 3 (N=20) Macro**
Analytical validation (targeted MS)	Total 3 pools (N = 60)	Pool 1 (N=20) Normo	Pool 2 (N=20) Micro	Pool 3 (N=20) Macro
	Total N=30 (individuals)	**N=10 Normo**	**N=10 Micro**	**N=10 Macro**
Diagnostic (targeted MS)	Total N=576 Year 0	N=311 Normo	N=191 Micro	N=74 Macro
(antibody)	Total N=549 Year 0	N=316 Normo	N=188 Micro	N=45 Macro

Mass spectrometry Cross validation

Antibody Cross validation

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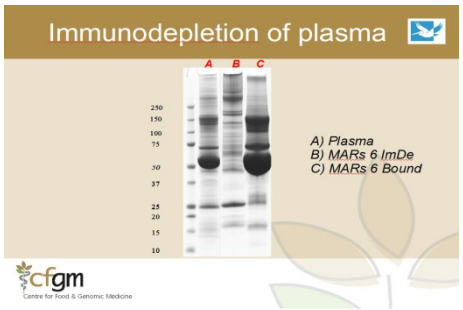
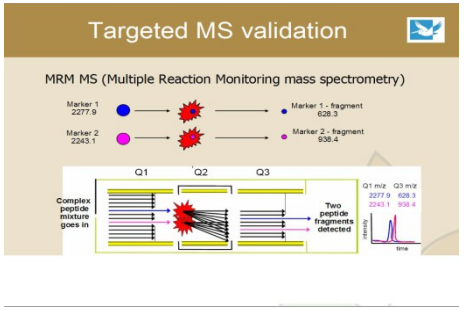
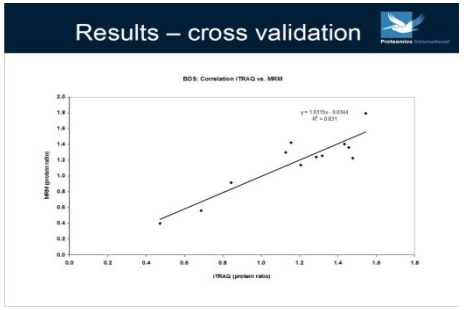
Process

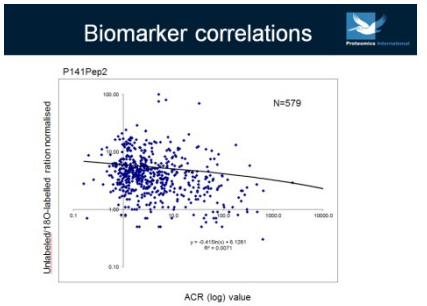
Platform Discovery Validation Clinical Diagnostic Prognostic

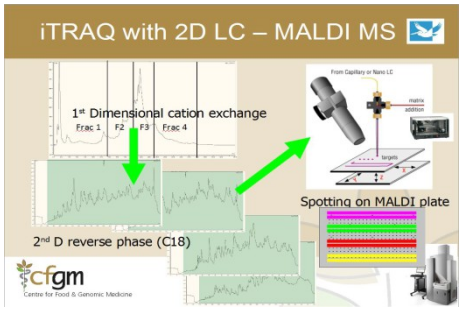
2008 → 2009 → 2010-11 → 2012-13 → 2014-15

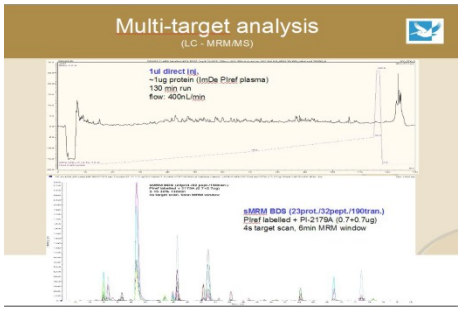


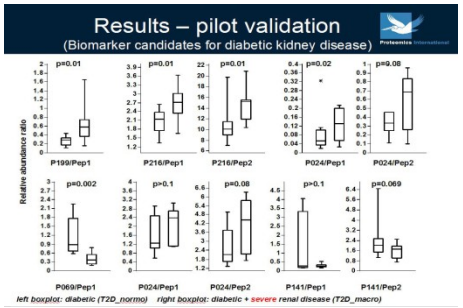


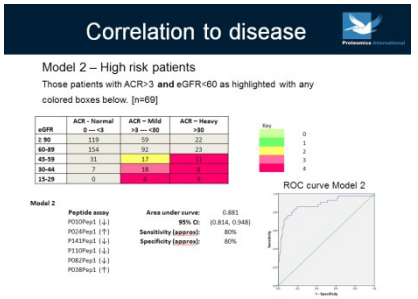












Targeted MS assay - design

Multiple reaction monitoring assays:

- Transitions developed for all potential biomarkers
- High stringency applied to peptide selections to eradicate false signals
- PeptideAtlas and MRMaid
- AB Sciex 4000 Q-trap
- ^{18}O-labelled reference plasma provided a common reference point
- Synthetic $^{13}C^{15}N$-labelled peptides used for absolute quantification

Targeted MS assay - reproducibility

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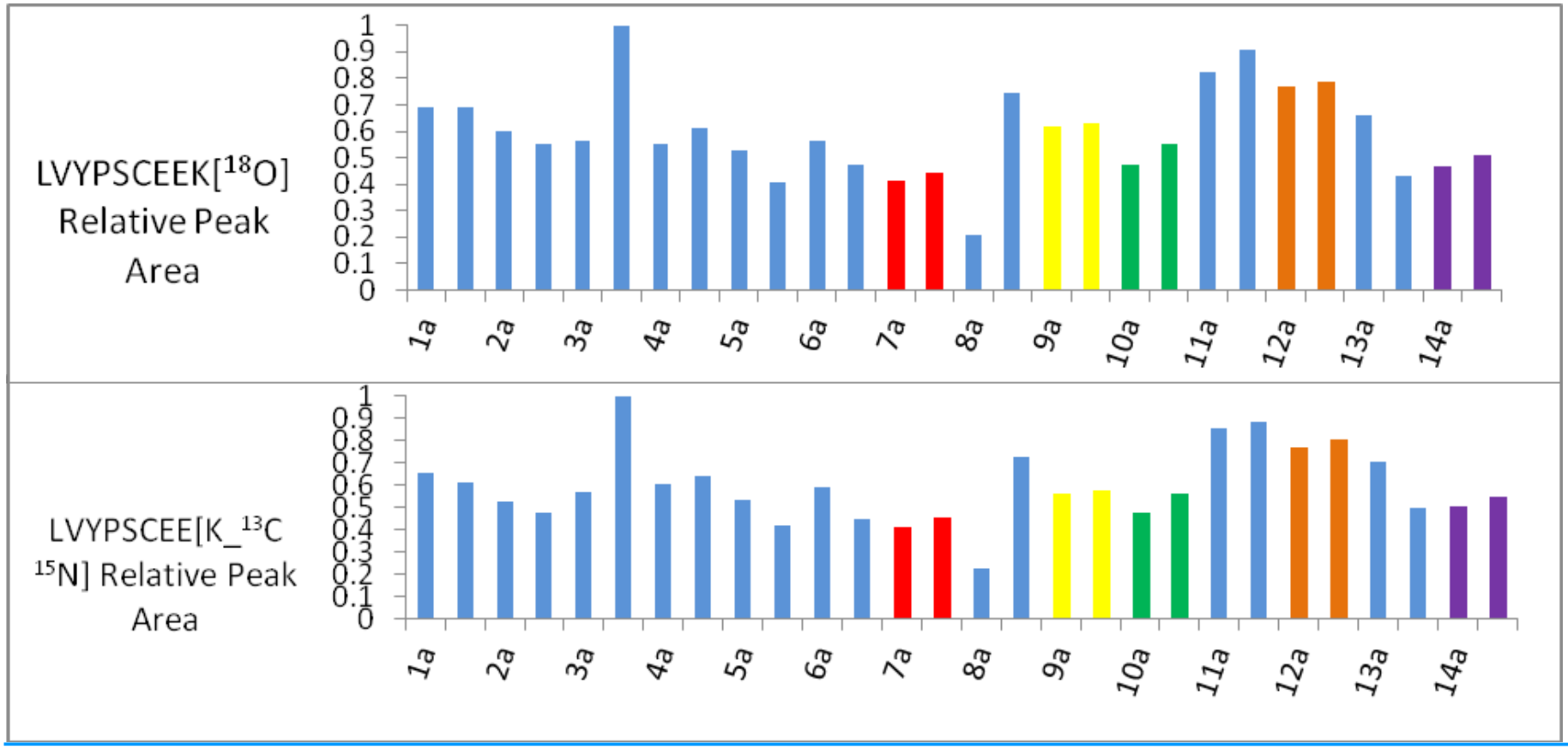
Intra- and inter-day peak area profiles (reference plasma)

- ^{18}O- versus $^{13}C^{15}N$-labelled
- example: FHR2 peptide LVYPSCEEK

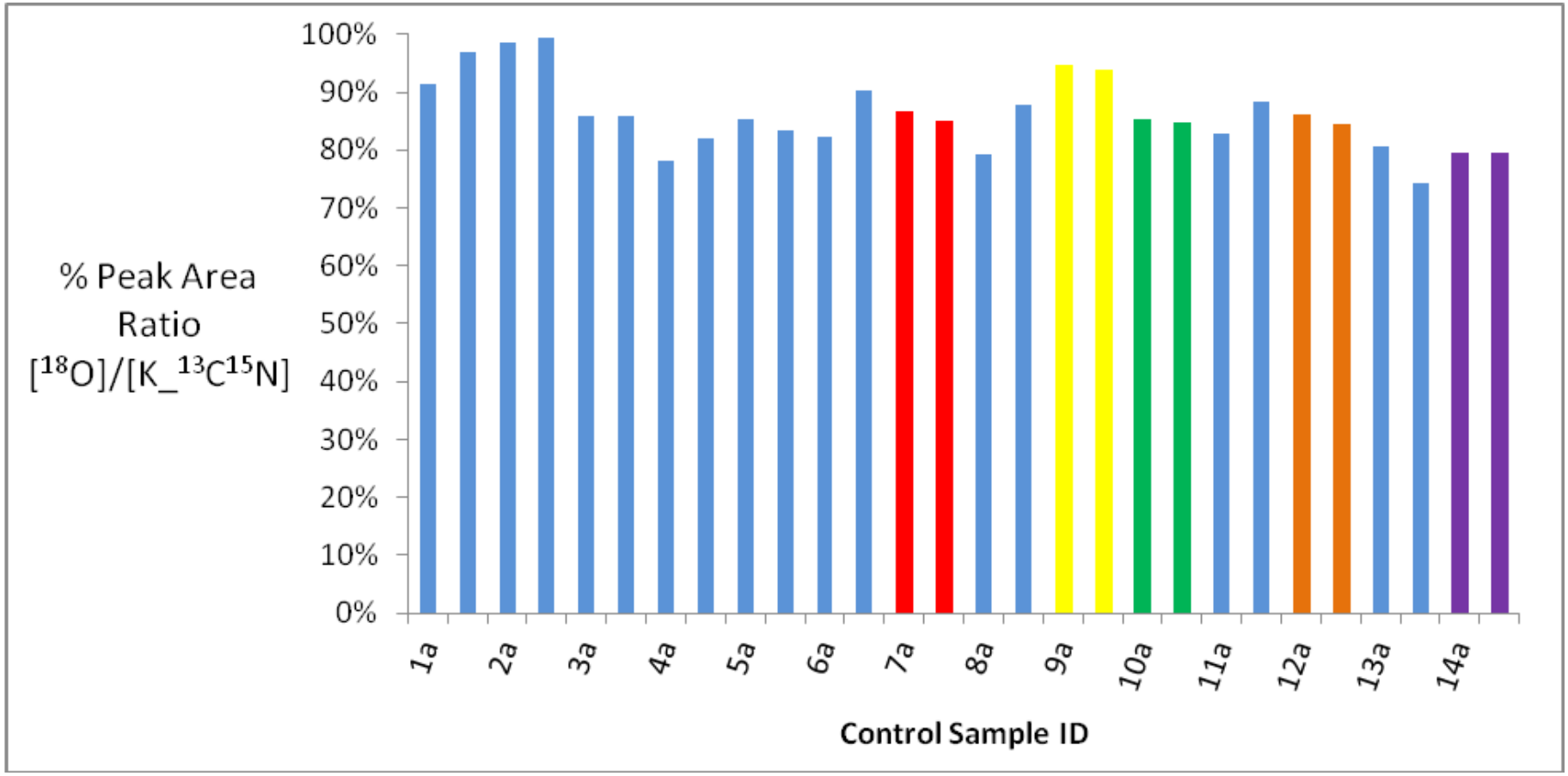


Targeted MS assay - reproducibility

Proteomics International

Intra- and inter-day peak area ratios (reference plasma)

- intra-day CV = 5.9%
- inter-day CV = 8.1%



Analytically validated diagnostic biomarkers

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Proteins identified:

- Inflammation N=3

complement proteins C8, C1q, factor H related p2

- Metabolism N=4

adiponectin, apolipoproteins A-IV, B-100, C-III

- Oxidative stress N=2

peroxiredoxin-2, sulfhydryl oxidase 1

- Other N=4

protein AMBP, insulin-like gfbp3, CD5 antigen-like, hemoglobin subunit beta

Protein Biomarker Research Pipeline for Developing Protein Biomarkers for Diabetic Kidney Disease

Using AB SCIEX TOF/TOF™ and QTRAP® Systems

Scott Bringans[1], Thomas Stoll[1,2], Kaye Winfield[1,2], Tammy Casey[1,2], Tim Davis[3], Jenny Albanese[4] and Richard Lipscombe[1,2]

[1]*Proteomics International,* [2]*Centre for Food and Genomic Medicine, Australia,* [3]*University of Western Australia,* [4]*AB SCIEX, USA*

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International Bureau

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PCT

(10) International Publication Number
WO 2012/037603 A1

(54) Title: BIOMARKERS ASSOCIATED WITH PRE-DIABETES, DIABETES AND DIABETES RELATED CONDITIONS

Biomarker cross validation

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Individual diagnostic biomarker correlations:

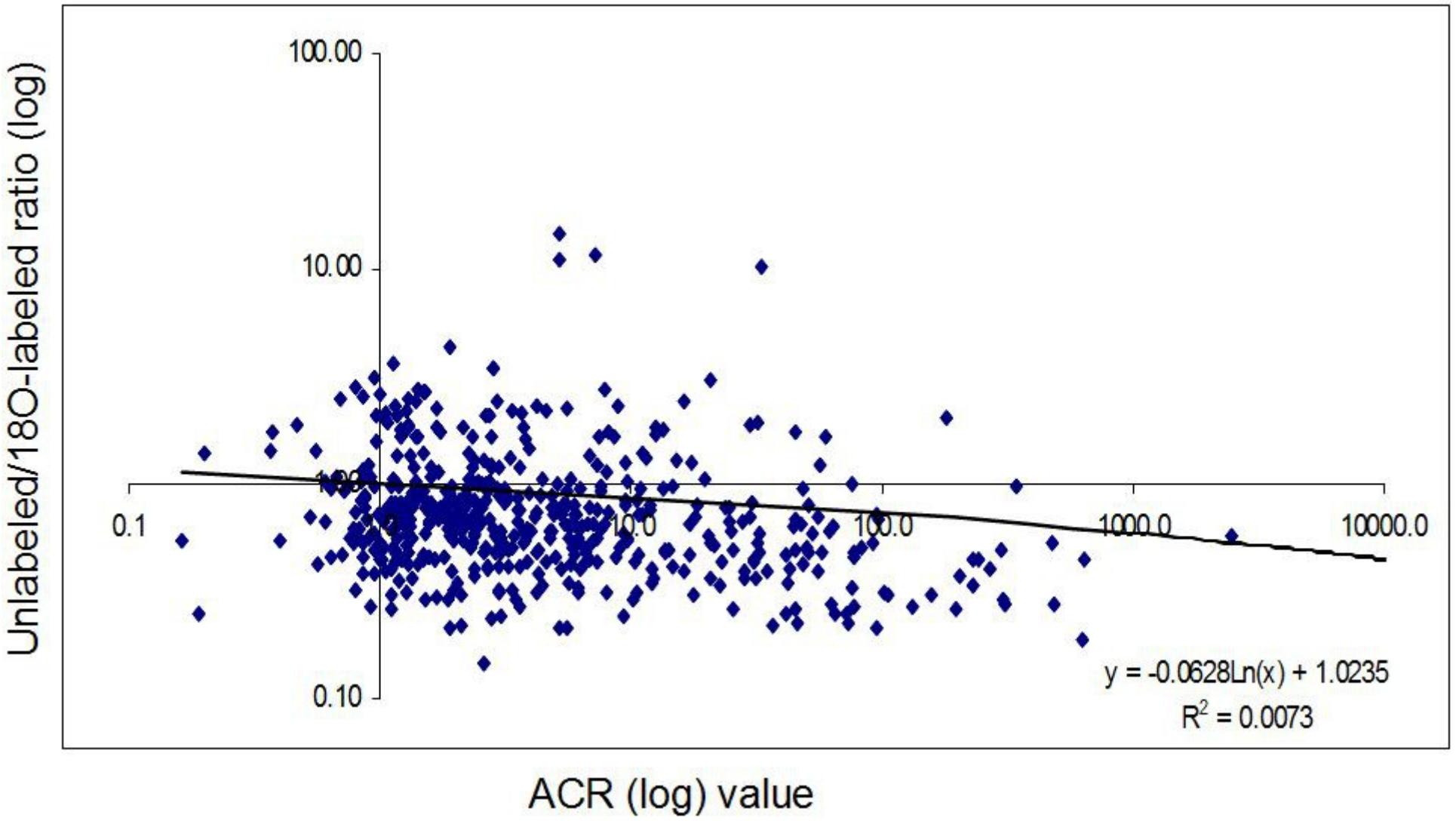
Plasma protein concentration **vs. ACR** **vs. eGFR**

Spearman's rho ($p < 0.05$ highlighted)

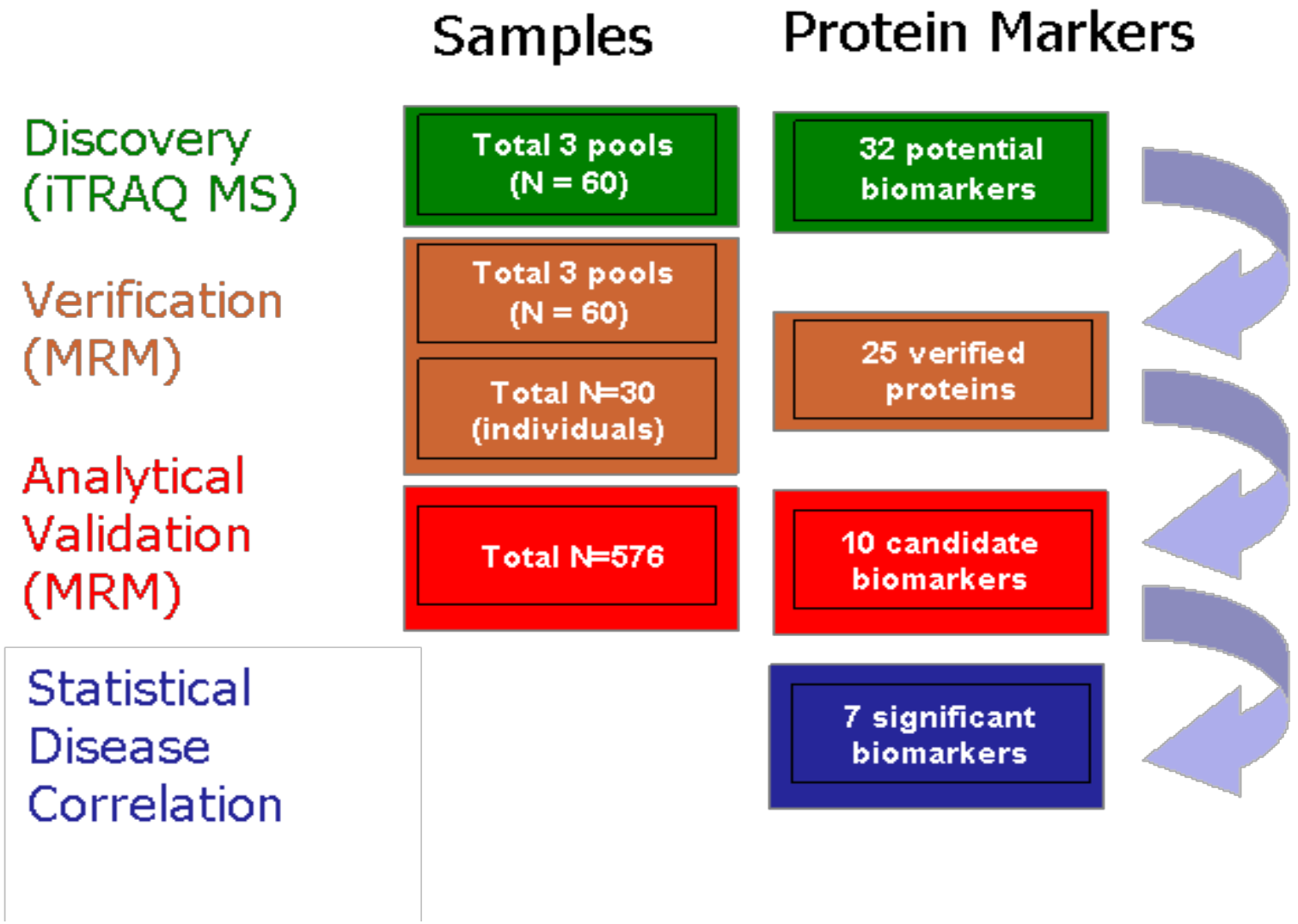
	targeted MS		antibody		targeted MS		antibody
PI-code	p for rho ACR correlation		p for rho ACR correlation		p for rho eGFR correlation		p for rho eGFR correlation
P010	0.550		0.966		0.431		0.305
P024	<0.001				<0.001		
P025	0.114		0.063		0.993		0.072
P027	0.500		<0.001		0.020		0.019
P054	<0.001		<0.001		0.041		<0.001
P069	0.208		0.664		0.296		0.230
P082	0.241		0.142		0.650		0.566
P089	0.002		0.857		<0.001		0.230
P125	0.001				0.408		
P141	<0.001		0.034		0.001		<0.001
P216	0.070		0.029		0.001		0.023
P038	0.000		<0.001		<0.001		<0.001

Biomarker correlations

P141



Study output

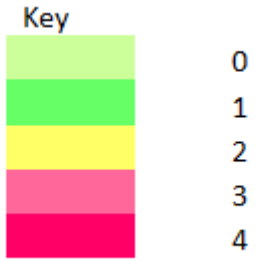


Correlation to disease - patient stratification

The table shows the distribution of patients when considering both ACR and eGFR measurements and the corresponding risk classification

Patient Risk Classification

eGFR	ACR - Normal 0 --- <3	ACR – Mild >3 --- <30	ACR – Heavy >30
≥ 90	119	59	22
60-89	154	92	23
45-59	31	17	11
30-44	7	18	8
15-29	0	6	9



Diagnostic models

- PromarkerD *(diagnostic)* compared with current commercial biomarker tests
- Different models define different risk categories (as defined by the ACR or eGFR)

PI Biomarker Panel Model	Type	AUC	Specificity	Sensitivity	PPV	NPV	DOR
ACR>30 mg/mmol	Diagnostic	0.75	70%	72%	26%	95%	6.0
eGFR<60 mL/min/1.73m²	Diagnostic	0.75	78%	68%	37%	93%	7.5
eGFR<30 mL/min/1.73m²	Diagnostic	0.83	89%	79%	16%	99%	30.4
Other Commercial Biomarker Tests	**Type**	**AUC**	**Specificity**	**Sensitivity**	**PPV**	**NPV**	**DOR**
PSA (Prostate Cancer)	Diagnostic	0.68*	21%	94%	30%	85%	8.4
CA-125** (Ovarian Cancer)	Diagnostic	0.89	80%	75%	58%	92%	21.2

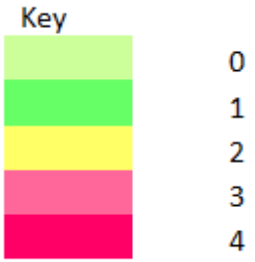
PPV, NPV = Proportion of positive and negative results that are true positive and negative. Dependant on prevalence of 'disease'.
DOR = The diagnostic odds ratio is a measure of the effectiveness of a diagnostic test. It is defined as the ratio of the odds of the test being positive if the subject has a disease relative to the odds of the test being positive if the subject does not have the disease. A larger DOR is better.
* Based on Thompson et al., 2005. (JAMA. 2005 Jul 6;294(1):66-70).
** CA-125 is the most frequently used biomarker for ovarian cancer detection. Around 90% of women with advanced ovarian cancer have elevated levels of CA-125 in their blood serum.

Correlation to disease - model 1

CKD risk=4 High risk patients

Those patients with CKD risk=4 as highlighted with any colored boxes below [N=34]

eGFR	ACR - Normal 0 --- <3	ACR – Mild >3 --- <30	ACR – Heavy >30
≥ 90	119	59	22
60-89	154	92	23
45-59	31	17	11
30-44	7	18	8
15-29	0	6	9



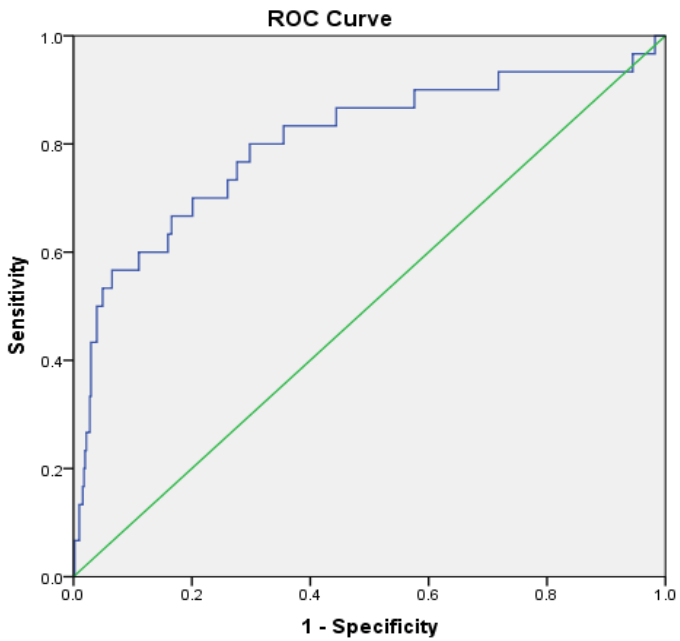
Model uses a panel of 5 biomarkers

Area under curve: 0.802

95% Confidence interval: (0.704, 0.901)

Sensitivity : 80%

Specificity : 70%

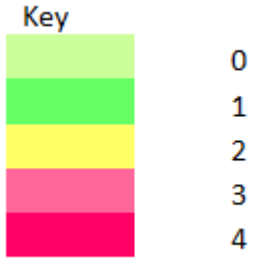


Correlation to disease - model 2

CKD risk≥2 Patients at risk

Those patients with CKD risk ≥2 as highlighted with any colored boxes below [N=121]

eGFR	ACR - Normal 0 --- <3	ACR – Mild >3 --- <30	ACR – Heavy >30
≥ 90	119	59	22
60-89	154	92	23
45-59	31	17	11
30-44	7	18	8
15-29	0	6	9



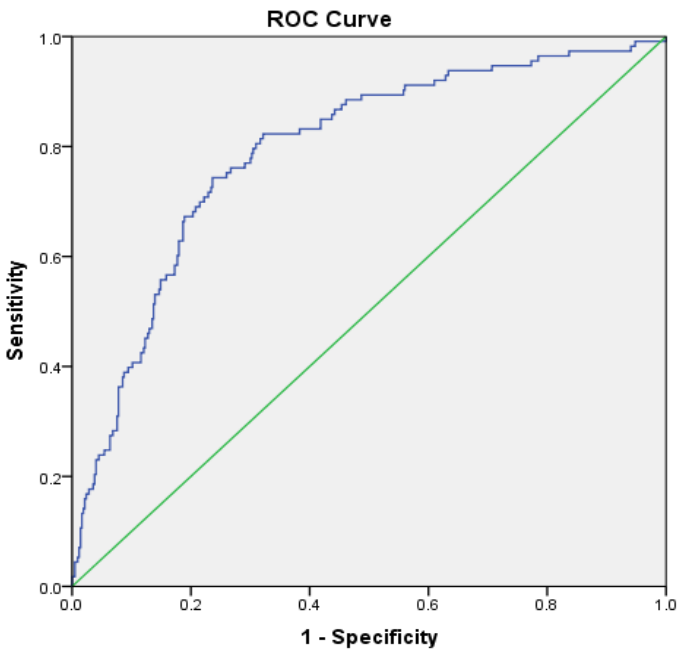
Model uses a panel of 5 biomarkers

Area under curve: 0.792

95% Confidence interval: (0.745, 0.839)

Sensitivity : 74%

Specificity : 76%



Study design - prognostic

Proteomics International

Diabetic kidney disease cohorts

Discovery (iTRAQ MS)	**Total 3 pools (N = 60)**	**Pool 1 (N=20) Normo**	**Pool 2 (N=20) Micro**	**Pool 3 (N=20) Macro**
Analytical validation (targeted MS)	Total 3 pools (N = 60)	Pool 1 (N=20) Normo	Pool 2 (N=20) Micro	Pool 3 (N=20) Macro
	Total N=30 (individuals)	**N=10 Normo**	**N=10 Micro**	**N=10 Macro**
Diagnostic (targeted MS)	Total N=576 Year 0	N=311 Normo	N=191 Micro	N=74 Macro
(antibody)	Total N=549 Year 0	N=316 Normo	N=188 Micro	N=45 Macro
Prognostic	Total N=545 Year 2	N=289 Normo	N=198 Micro	N=58 Macro
	Total N=434 Year 4	N=251 Normo	N=151 Micro	N=32 Macro

Mass spectrometry Cross validation

Antibody Cross validation

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Prognostic results

eGFR decliners – can the biomarkers predict who will develop diabetic kidney disease?

This prediction is concerned with the trajectory of the patients' eGFR - of the current cohort of 349 patients 10% were eGFR decliners

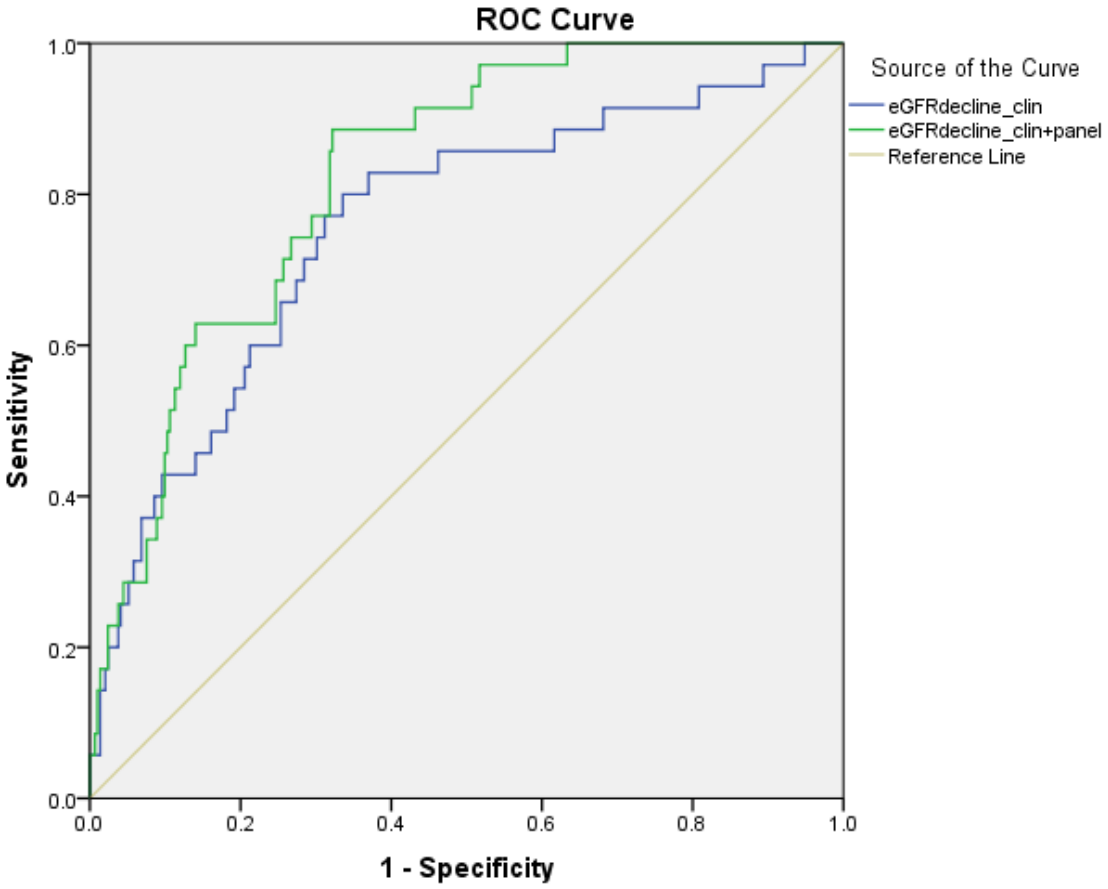
A rapidly declining eGFR is one of the strongest indicators of significant renal impairment and a steady progression of diabetic kidney disease

Statistical tools

- Performance assessed by measures of calibration, discrimination & reclassification
- Hosmer-Lemeshow test; DeLong's method
- AUC corrected for statistical overfitting using cross-validation and bootstrapping
- Optimism corrected AUC provides a more approximate estimate of model performance

Prognostic model

Trajectories – does the panel predict who will decline rapidly?



ROC curves for models predicting eGFR decline using 3 biomarkers

Clinical predictors:

- AUC (95% CI) = 0.75 (0.66-0.84)
- Optimised corrected AUC = 0.73

Clinical predictors + biomarkers:

- AUC (95% CI) = 0.83 (0.77-0.89)
- Optimised corrected AUC = 0.79
- *Improvement P-value 0.027*

- 89% sensitivity
- 68% specificity

Clinical predictors are age, HDL cholesterol and diuretic use

Summary of results

The clinical study examined over 500 individuals using two technology platforms; targeted mass spectrometry and antibody systems

PromarkerD as a Diagnostic

- 7 biomarkers were individually **validated at high stringency** using the mass spectrometry platform, 4 using antibody systems (some were unavailable)
- Mass spectrometry data showed **almost complete correlation** with the antibody platform
- The protein biomarker panel can discriminate **different risk categories** of diabetic kidney disease

PromarkerD as a Prognostic

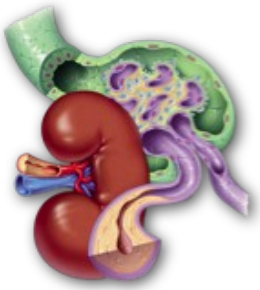
- Predicts which patients are at risk of a **significant & rapid decline** in kidney function, **better than any other known measure**
- The preferred model of 3 biomarkers as a predictor of eGFR decline had an AUC of **0.83** with **89%** sensitivity, **68%** specificity
- People who have altered levels of protein from the biomarker panel are up to **7 times more likely** to be in the eGFR decliner trajectory group

PromarkerD - where to next?

Multiple routes to market:

- **Specialist diagnostic test** run by clinical laboratories (laboratory developed test – LDT)
- **Standard clinical pathology assay** produced by diagnostic companies (in vitro diagnostic – IVD)
- Next generation test to monitor a patient's response to drug therapy and enable personalised medicine – **companion diagnostic test** (CDx)

Timeline:



Patent in national phase examination:
Australia, Brazil, Canada, China, Europe, India, Indonesia, Japan, Russia, Singapore, USA

Proteomics International

Thank you!

Core partners

Harry Perkins Institute of Medical Research
Centre for Food and Genomic Medicine (WA)
BioPlatforms Australia

Richard Lipscombe
Bill Parker
John Dunlop
Terry Sweet
Andreja Livk
Scott Bringans
Kaye Winfield
Tammy Casey
Fran Jones
Amber Boyatzis
Jason Ito
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Roop Judge
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Kirsten Peters
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& Thomas Stoll

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Peter Nilsson (KTH Royal Inst Tech)
Peter Leedman (Harry Perkins)
Michael Phillips (Harry Perkins)
Satvindar Dahliwal (Curtin)

Tim Davis (UWA/Fremantle)
Kirsten Peters (UWA/Fremantle)
Wendy Davis (UWA/Fremantle)

NATA

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An Australian Government Initiative

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