

# Biomarker panels by CE-MS

**Harald Mischak**



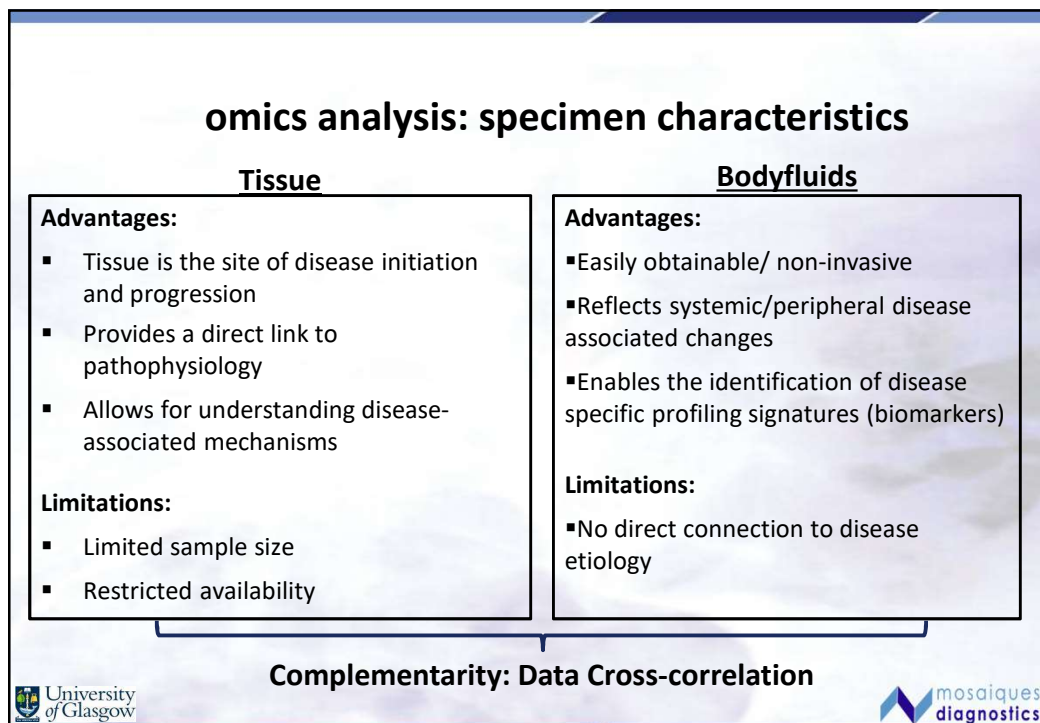
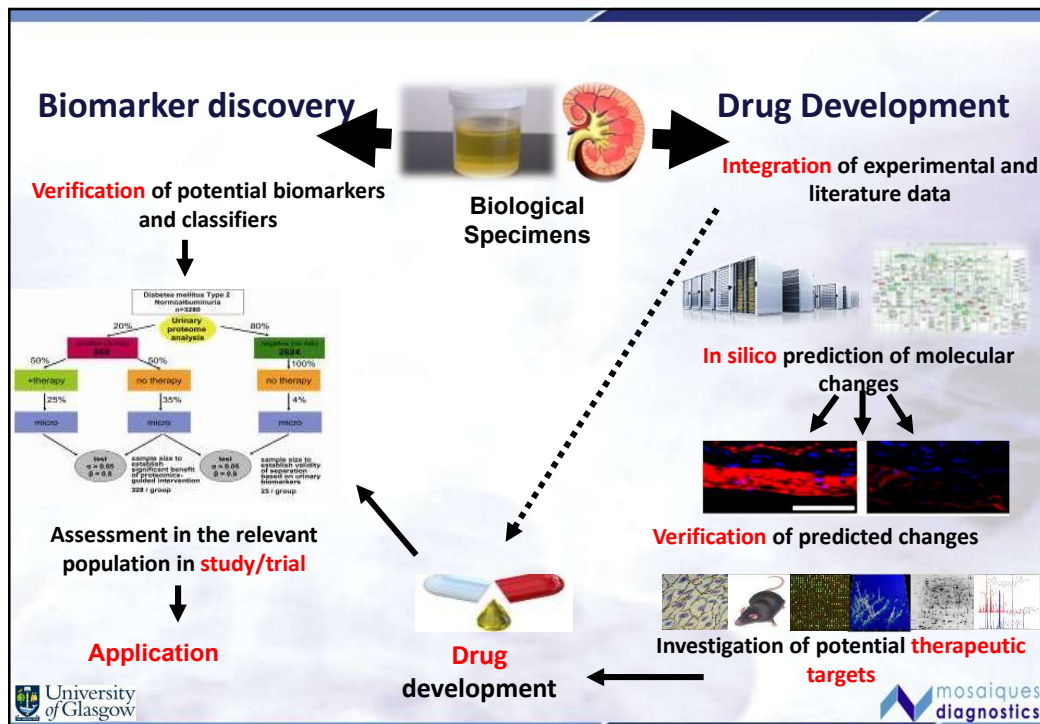
Conflict of Interest: Co-founder and co-owner of Mosaiques Diagnostics, DiaPat, Mosaiques Therapeutics



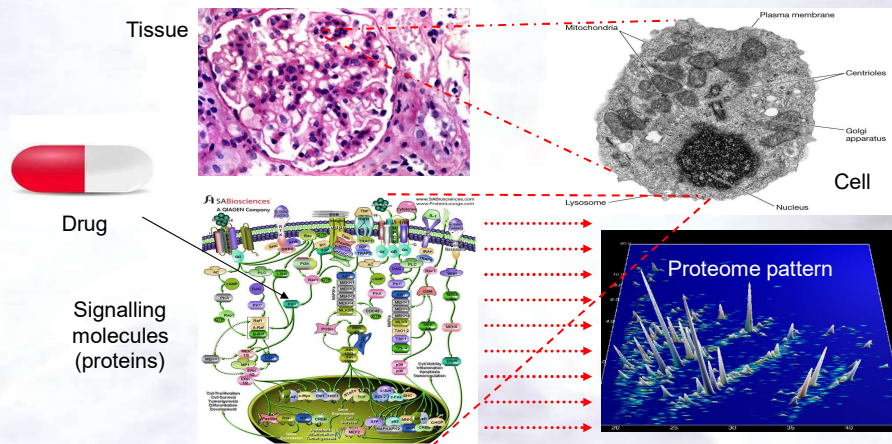
## Background

- **Proteins** are active key players in every organism that drive normal and pathological processes.
- **Proteins are responsible for disease-specific processes**, and can be targeted with drugs.
- **Proteome analysis enables** optimal diagnosis, prognosis, and selection of ideal therapeutic intervention.
- **Combinatorial Approach:** Rationale therapeutic targets, monitoring and stratification biomarkers and appropriate drugs, ultimately enable ideal and personalized patient management.

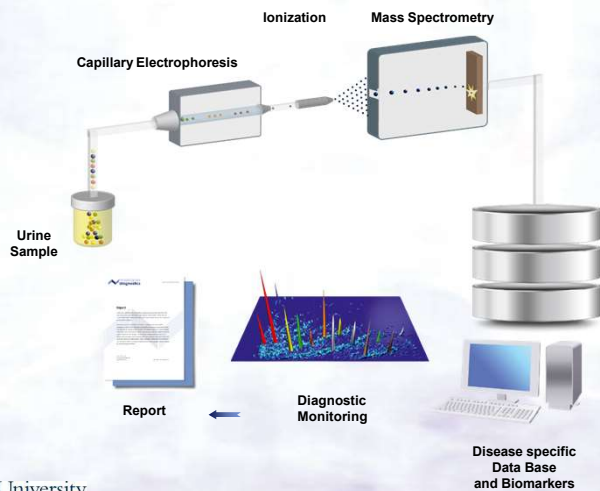




## Proteomic changes appear best suited to display drug effects



## Proteomics Technology platform: CE/MS Technology *Capillary Electrophoresis coupled to Mass Spectrometry to assess endogenous peptides w/o digest*



### Advantages

Separation and analysis of more than **1,000** proteins and peptides

Run time **~60 min**

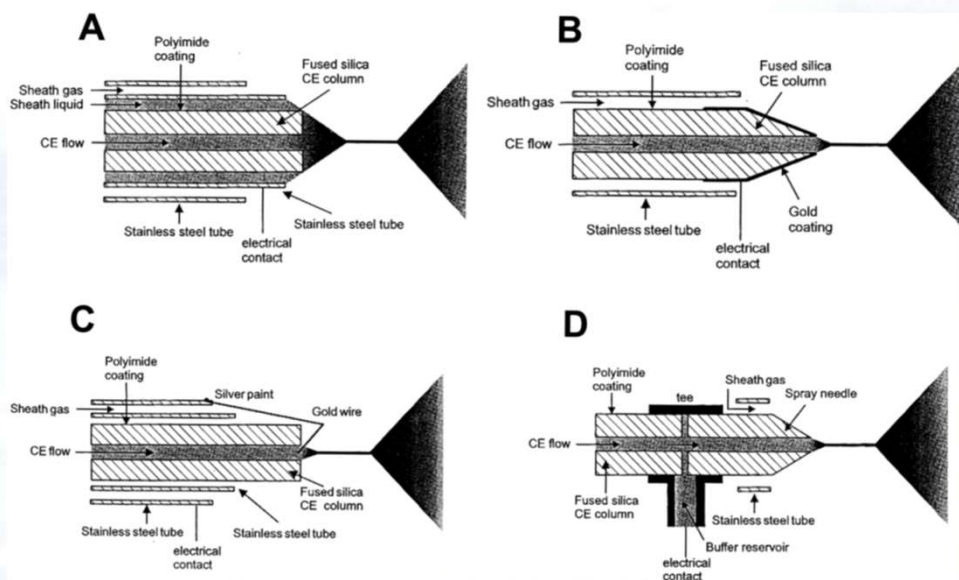
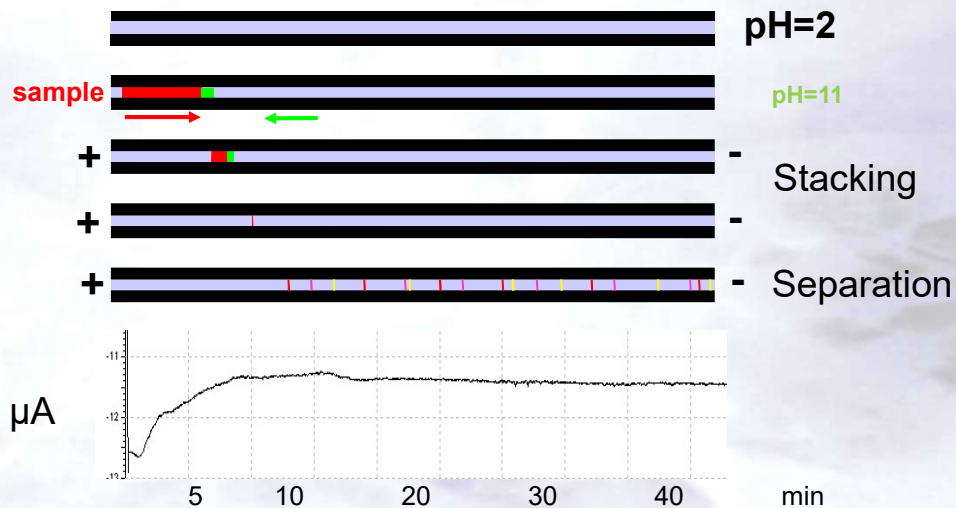
### CE

- fast
- robust
- inexpensive
- reproducible

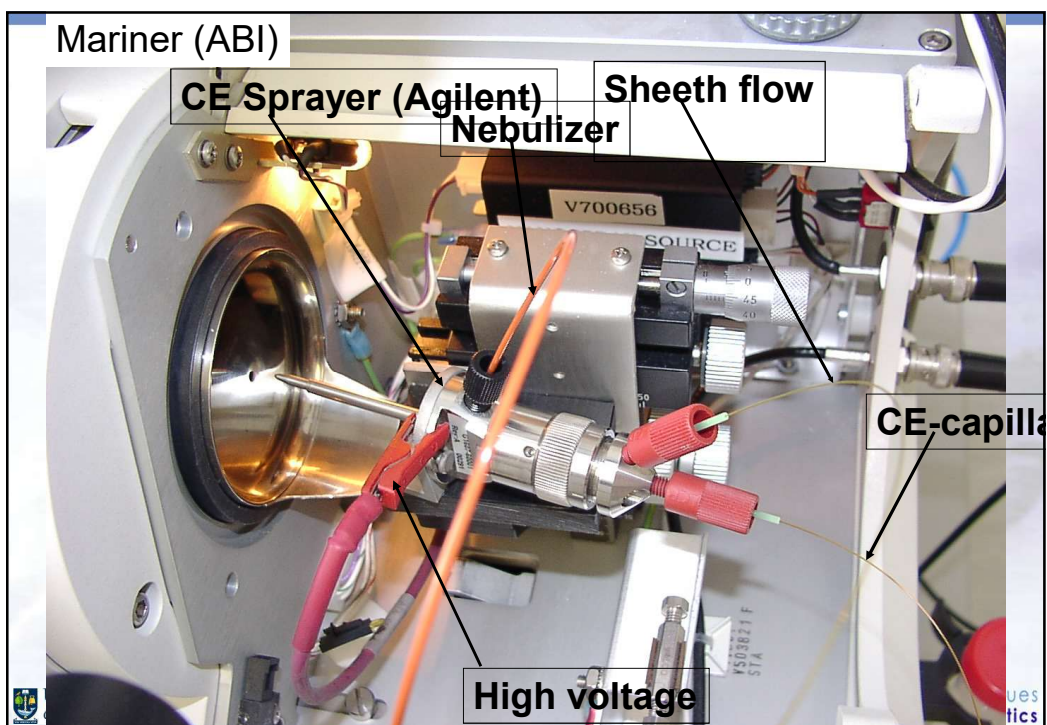
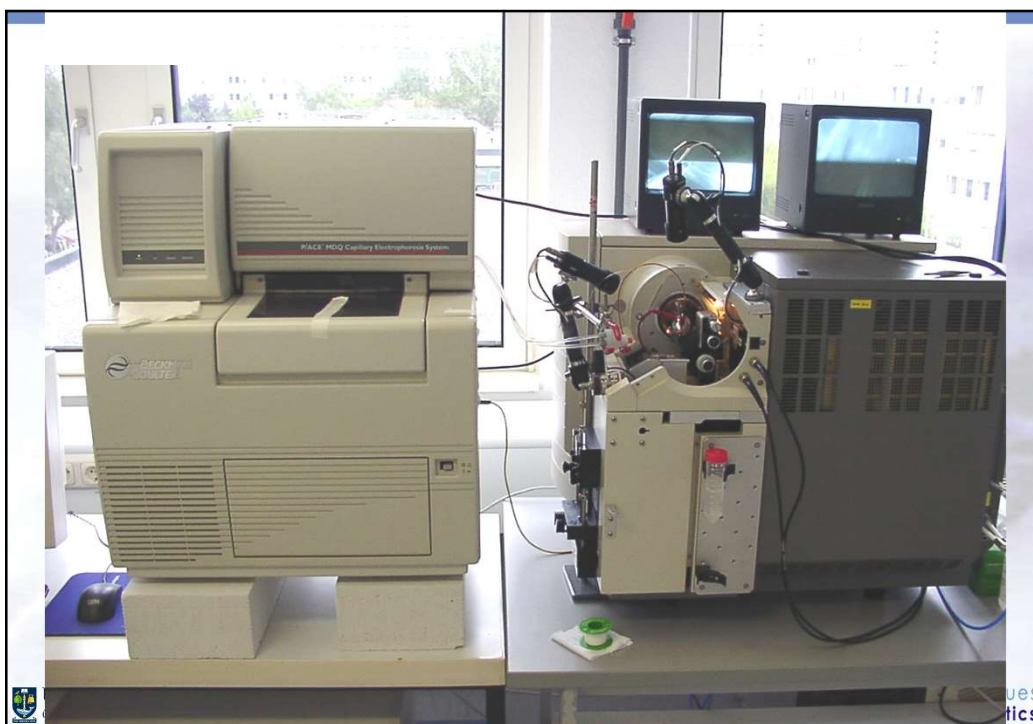
### MS

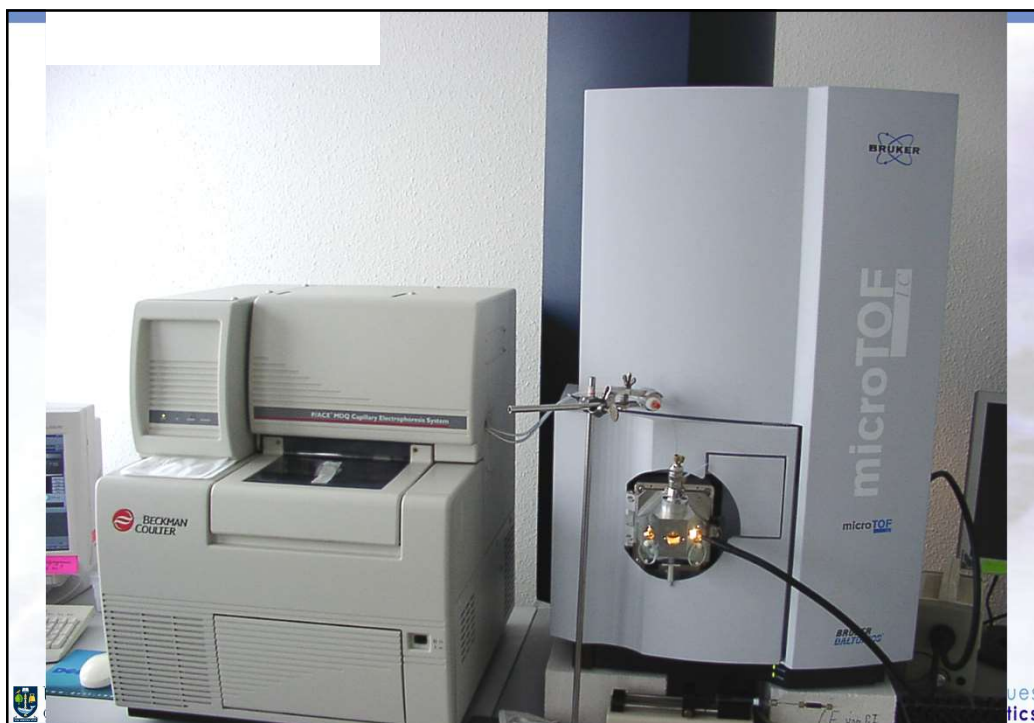
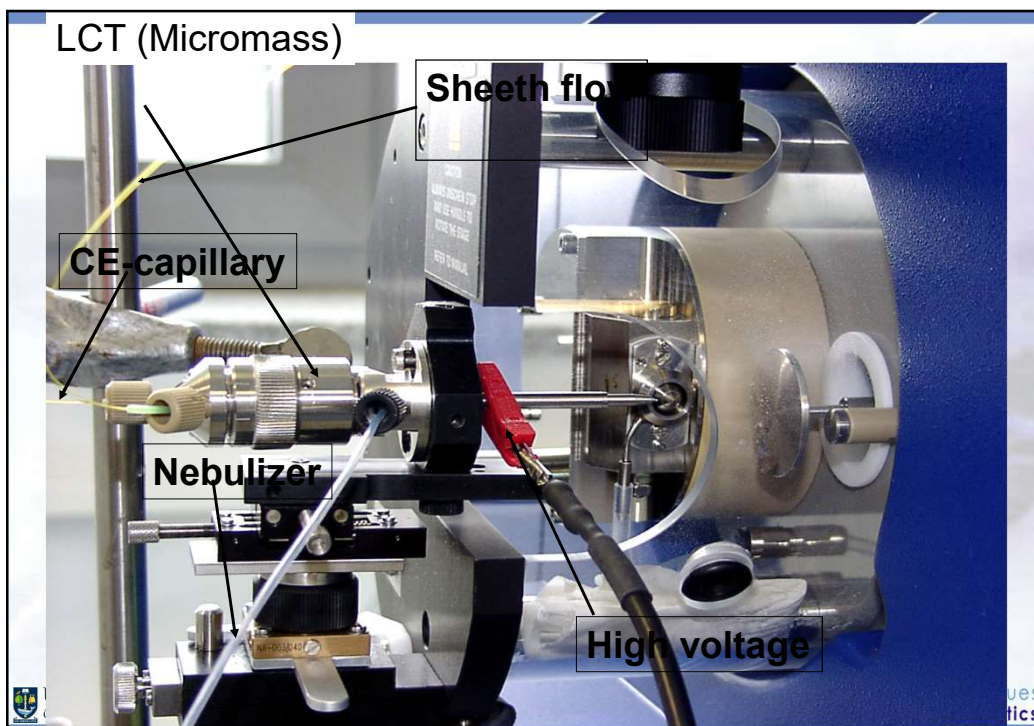
- resolution
- scan speed

## CE Sample loading, stacking, and separation









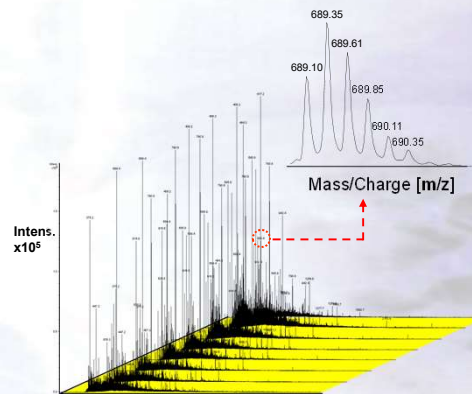
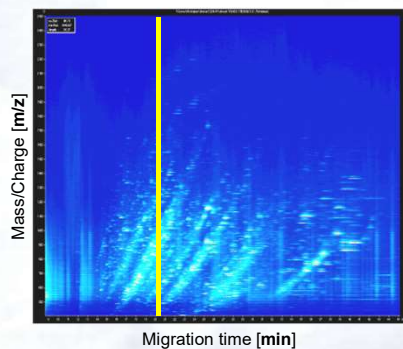




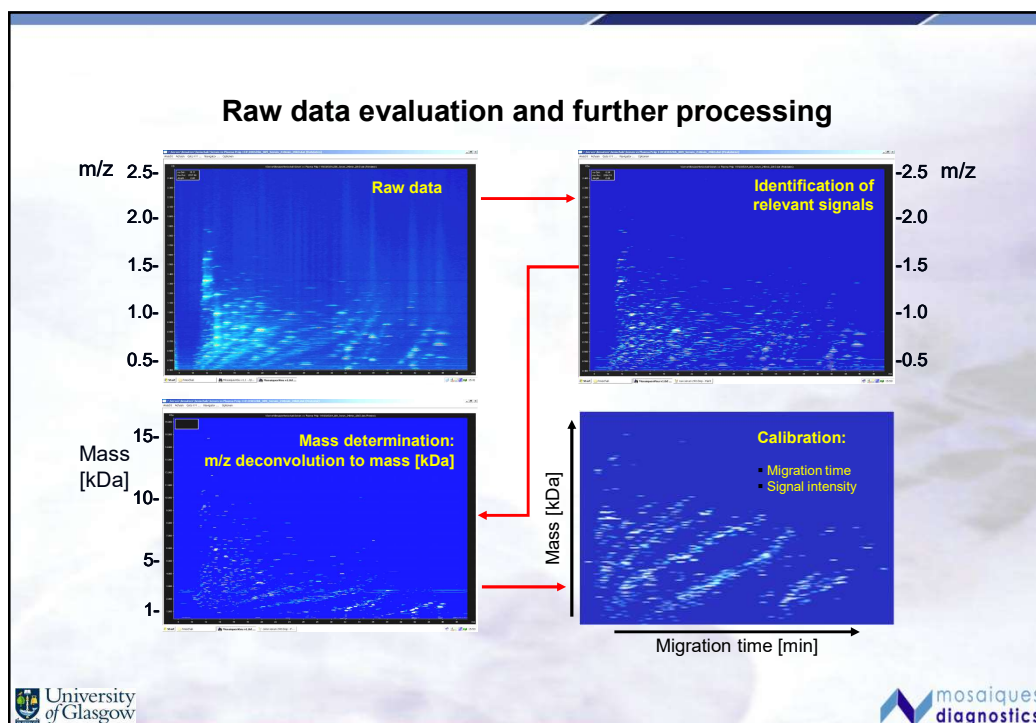
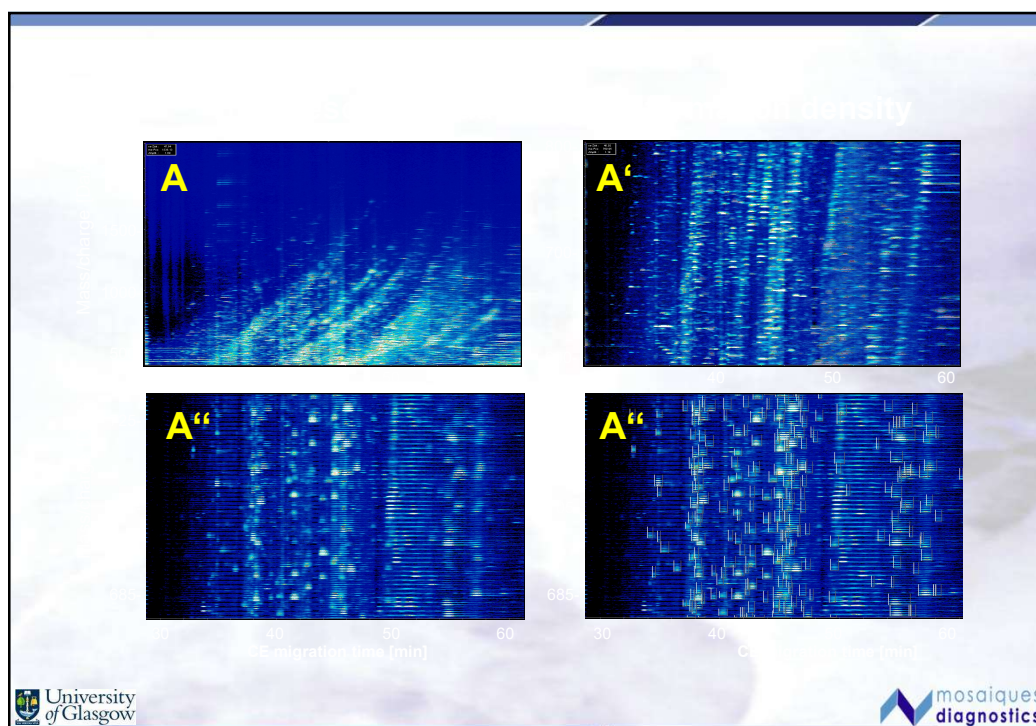
## Why urine

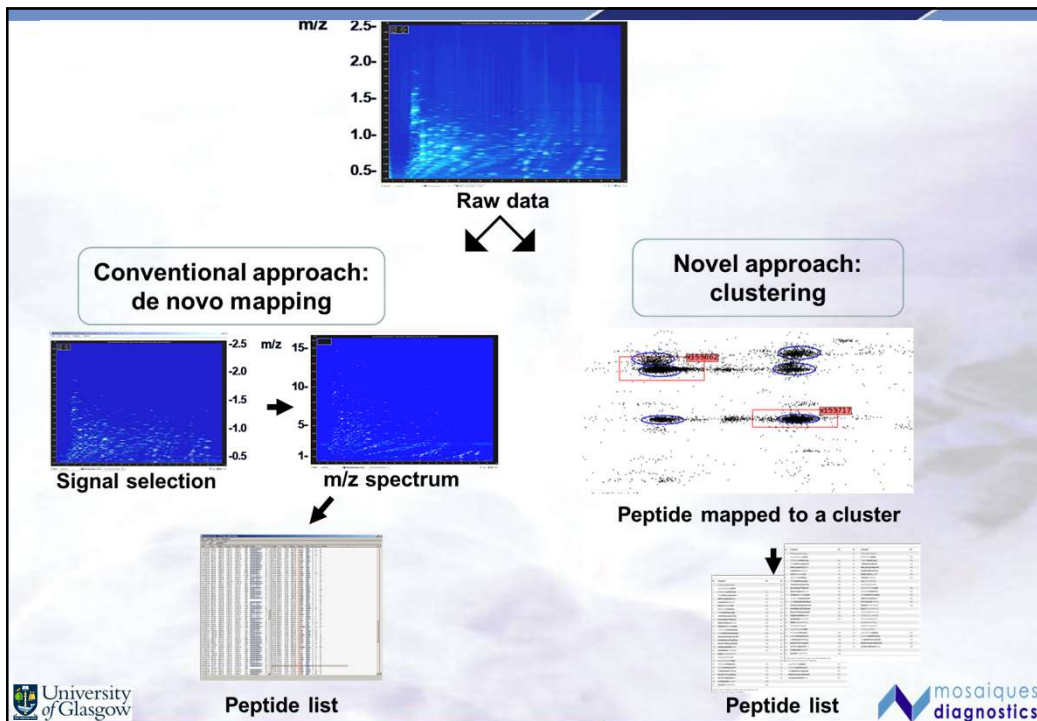
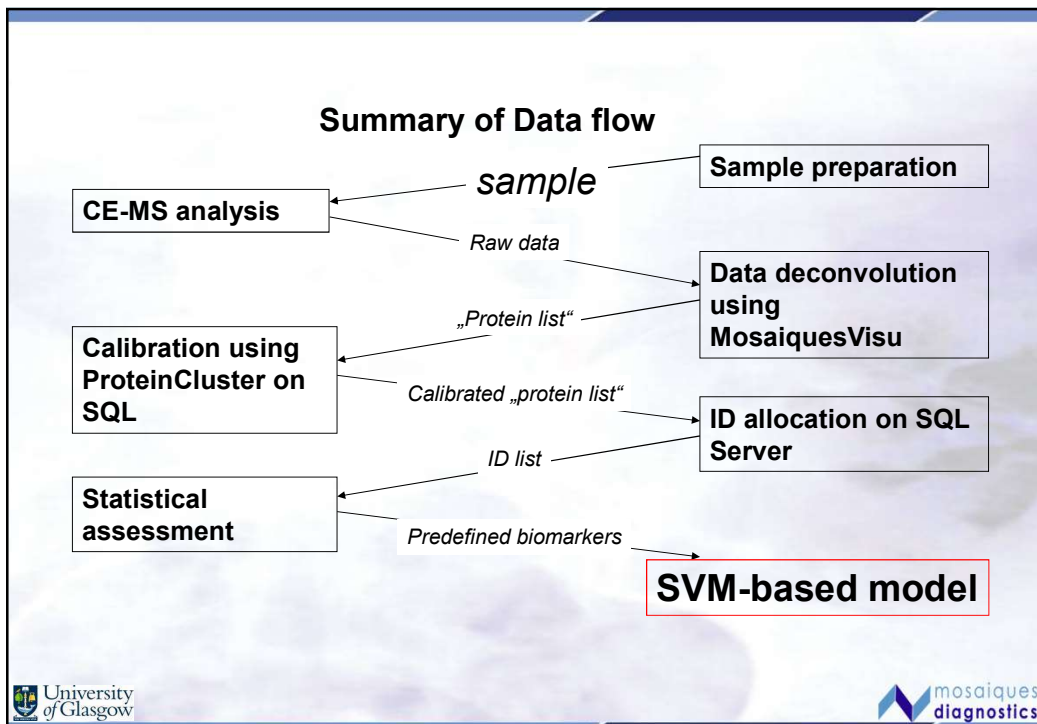


- Easily accessible
- Obtained non invasive
- Available in large quantities
- Urinary polypeptides are stable, yielding comparable datasets.
- Representing the filtrate of blood, urinary polypeptides display the “status” of the organism.







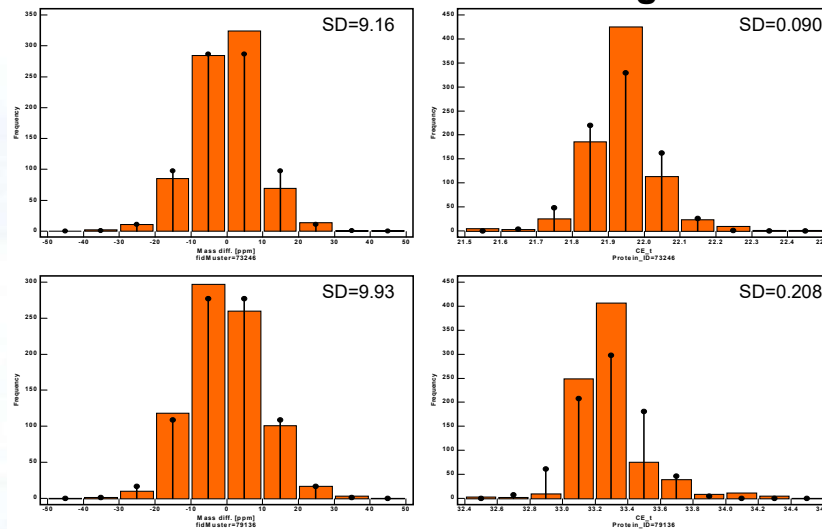


## Determinations of limit of detection for 5 peptides.

Pep11413 VLNLGPITR (Uromodulin 598-606), Pep49958 SGDSDDDEPPPLPRL (Membrane associated progesterone receptor component 1 153-67), Pep55143 PpGEAGKpGEQGVPGDLG (Collagen alpha-1 (I) 651-668), Pep59022 SVIDQSRVLNLGPITR (Uromodulin 591-606), Pep123969 GERGSpGGpGAAGFpGARGLpGpP-GSNGNPGpGp (Collagen alpha-1 (III) 861-895). p=Hydroxyproline.

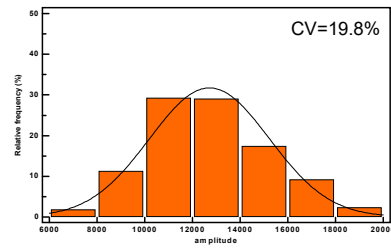
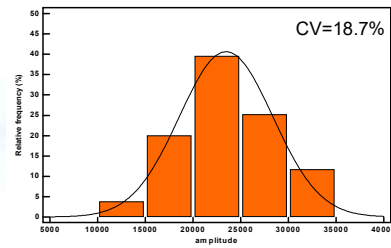
	Pep 11413	Pep 49958	Pep 55143	Pep 59022	Pep 123969
	amount by SNR 4 [pmol]	amount by SNR 4 [pmol]	amount by SNR 4 [pmol]	amount by SNR 4 [pmol]	amount by SNR 4 [pmol]
aLOD	0.00077	0.00040	0.0005	0.0025	0.00074
SD	0.00058	0.00015	0.0003	0.0004	0.00038

## Observed deviation in mass and migration time

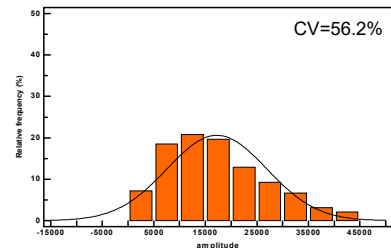
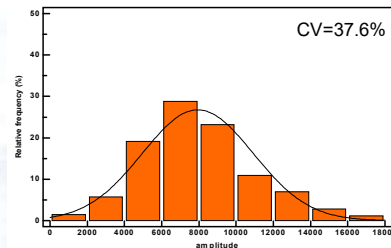


## Observed deviation in amplitude

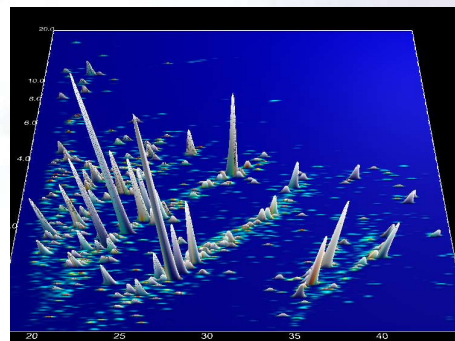
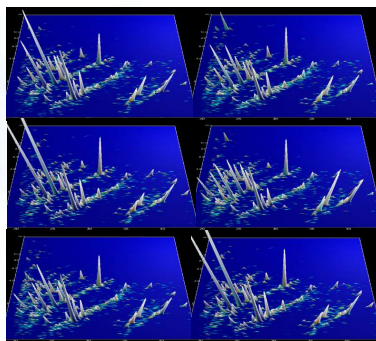
QC



NC



## Establishment of a biomarker panel

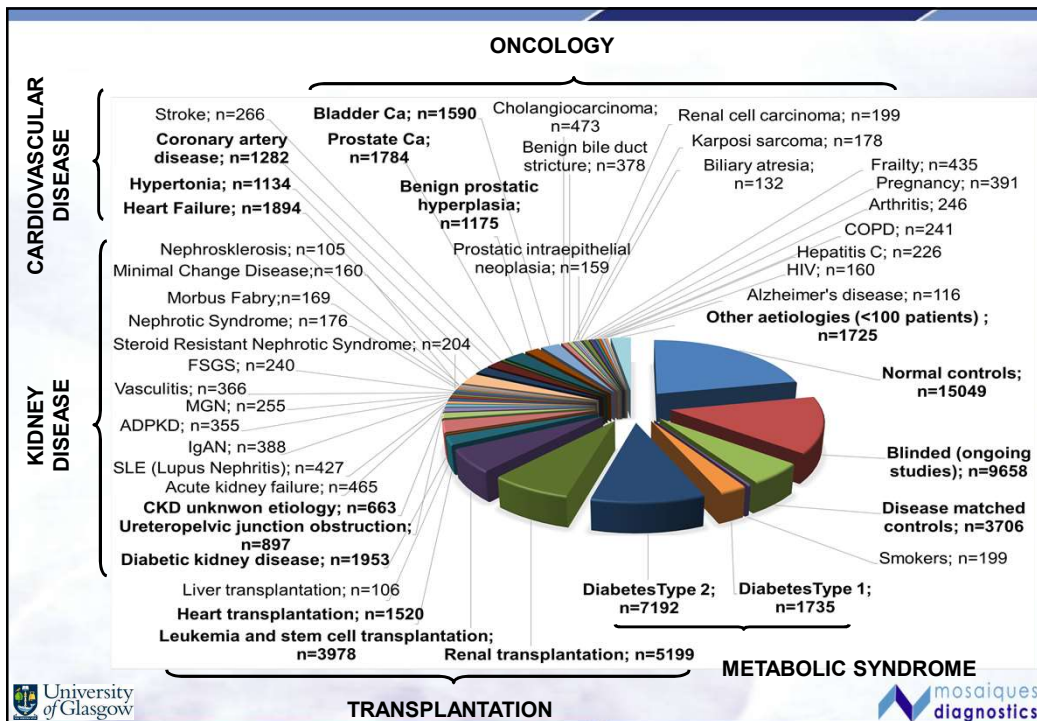
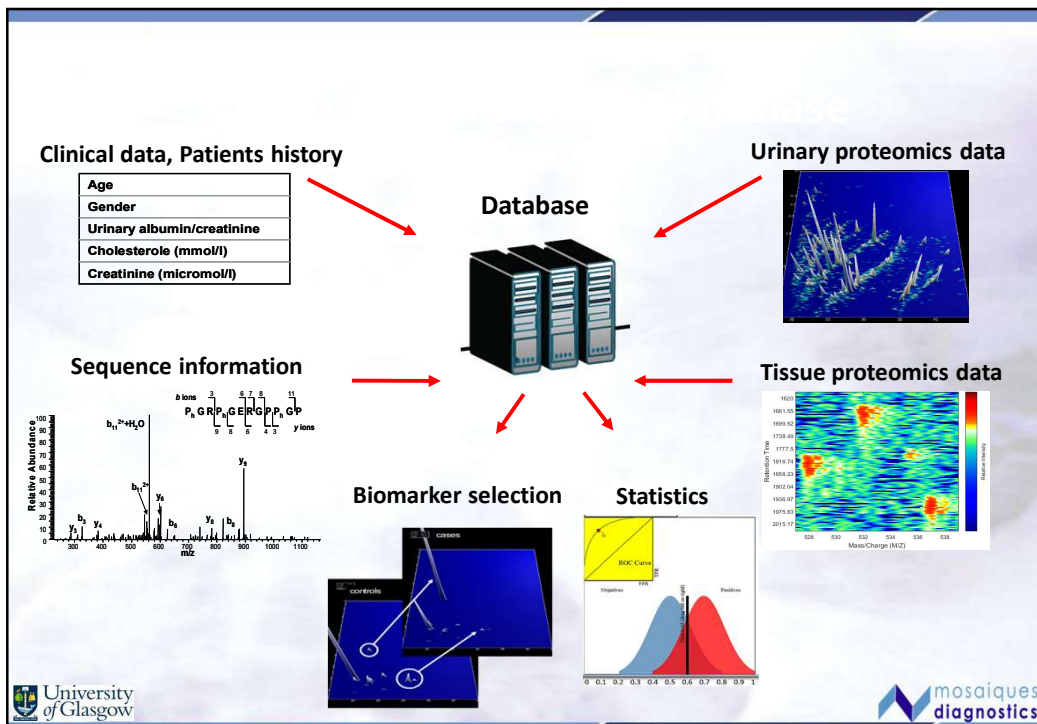


### Individual analyses

- Peptides and proteins are defined by mass and migration time, and identified based on its sequence
- Each peak represents a unique, specific peptide or protein, peak height is indicative of abundance
- Multiple housekeeping proteins/peptides enable compilation and comparison

### Compiled data

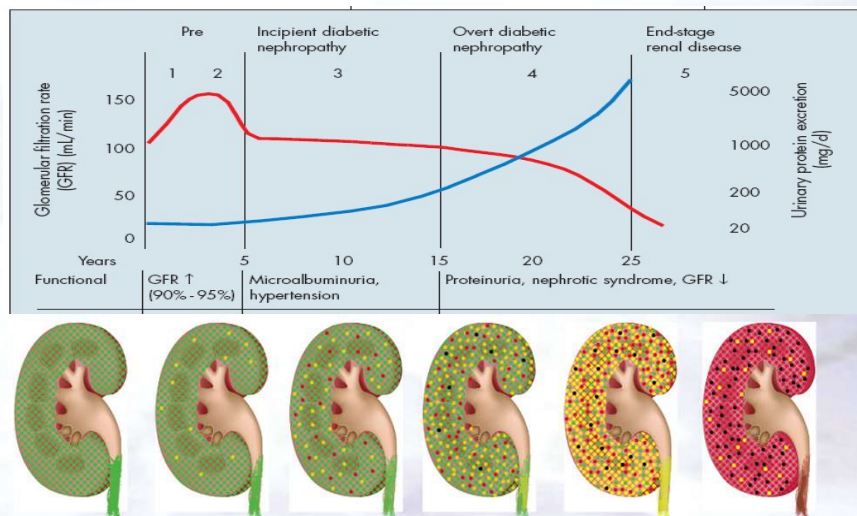




## Application in CKD, diabetic nephropathy

- Affects approximately 10% of the population
- Reduces life expectancy up to 20+ years
- Incurable
- Ultimately results in kidney failure, dialysis or transplantation
- One of the 10 major causes of death in developed countries

## Disease onset and progression in CKD



## Clinical diagnosis of Chronic Kidney Disease

### Urine albumin:

Urine albumin is commonly expressed as albumin/creatinine ratio.  
An increase  $\geq 30\text{mg/g}$  may be a sign of kidney damage.

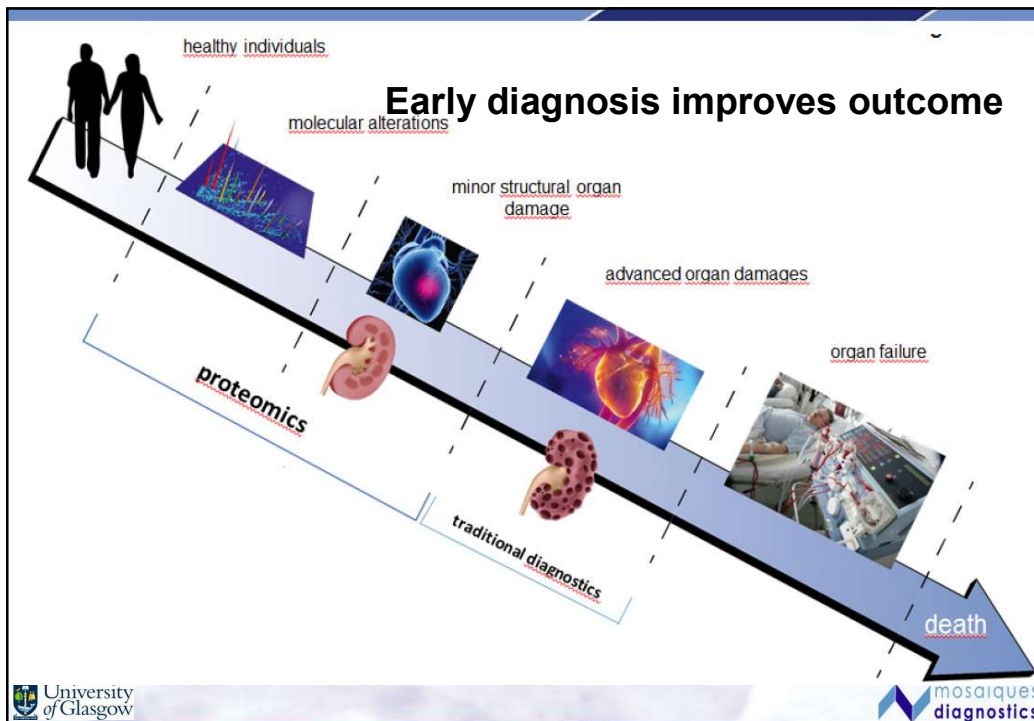
### eGFR (estimated glomerular filtration rate):

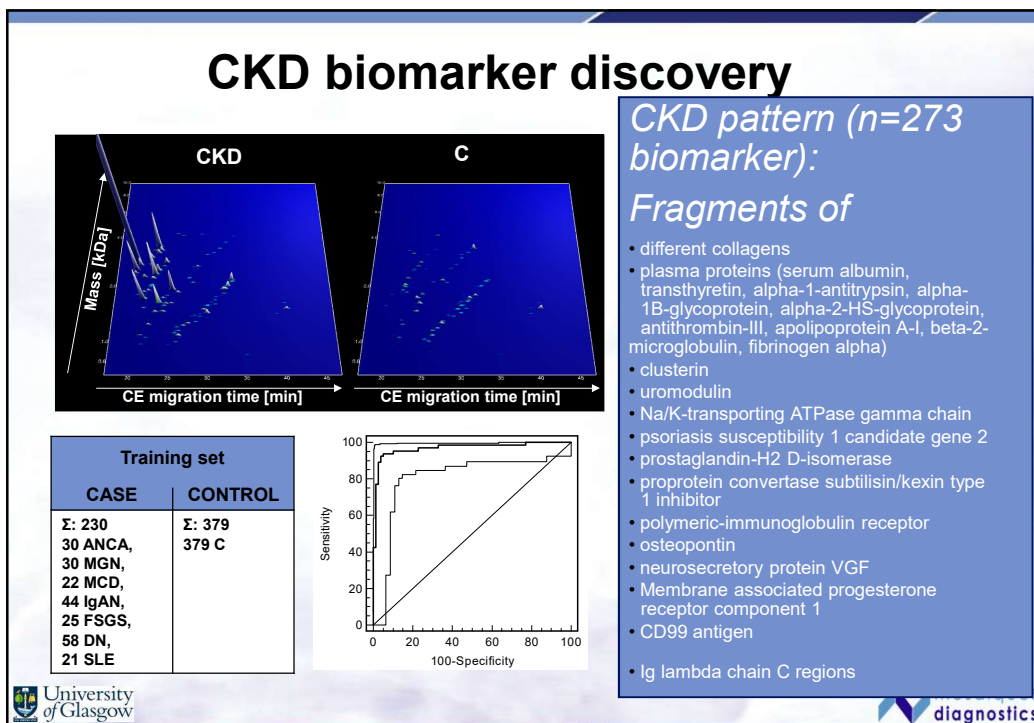
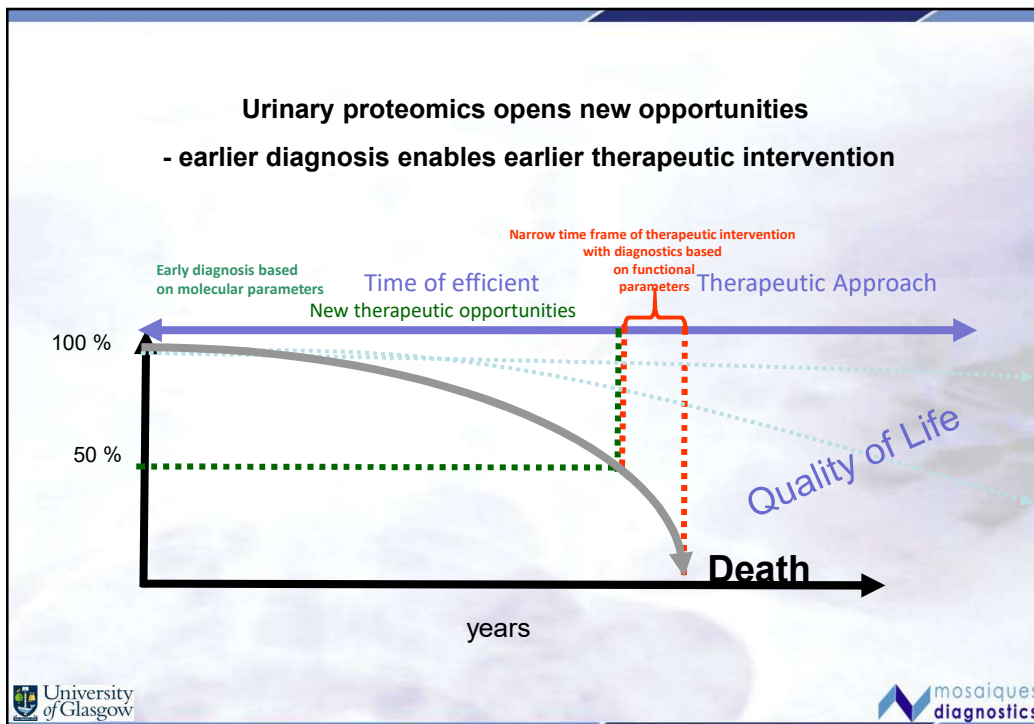
The calculation of eGFR is based on the amount of serum creatinine.

- Potentially beneficial drugs are available
- The early application of these drugs are desirable



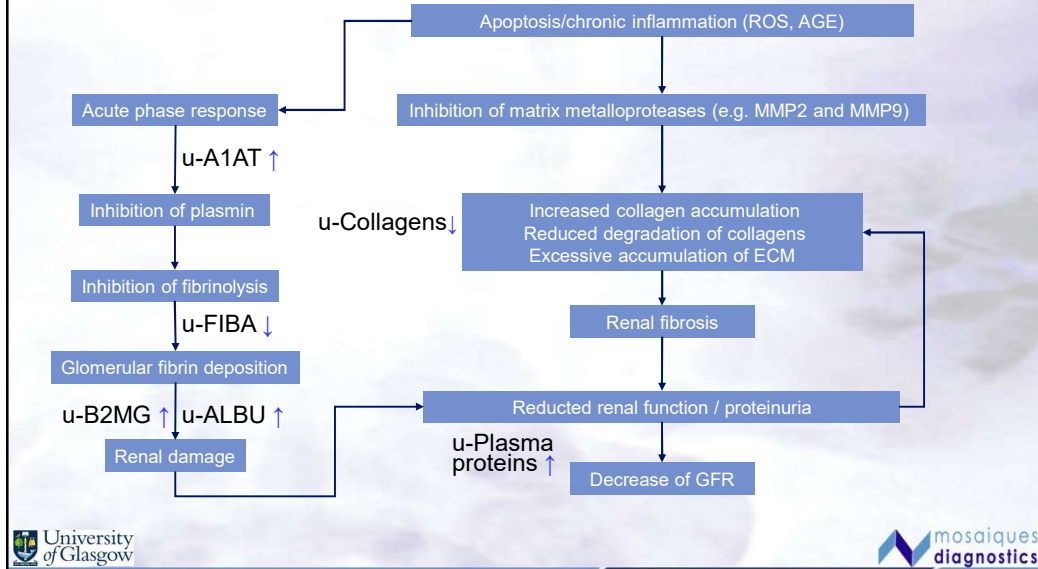
Markers of pathophysiological relevance are needed that are more likely to indicate early onset of chronic kidney diseases



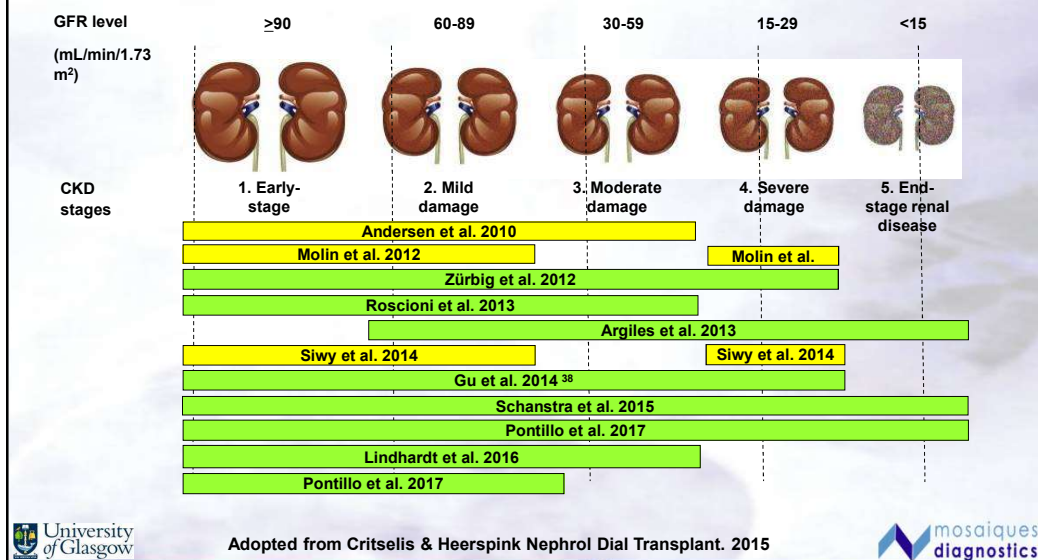




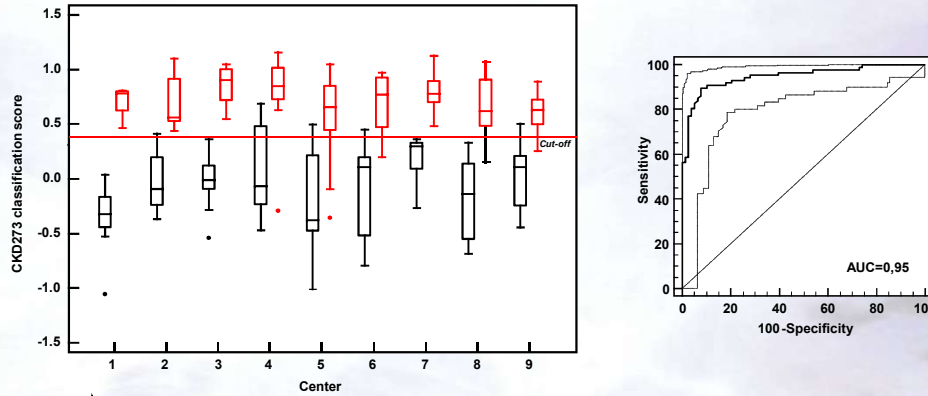
## Pathophysiological suggestions



## Application of CKD273 according to disease stage



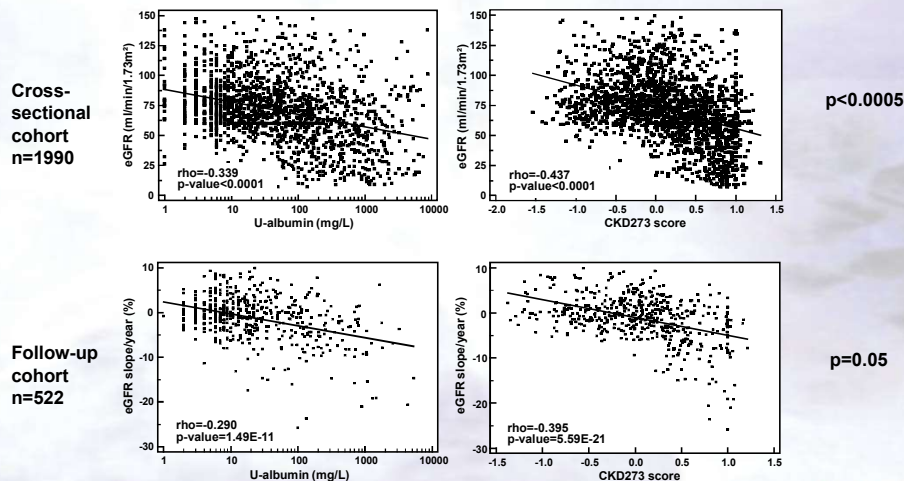
### Multicentre validation of CKD 273 (n=165)



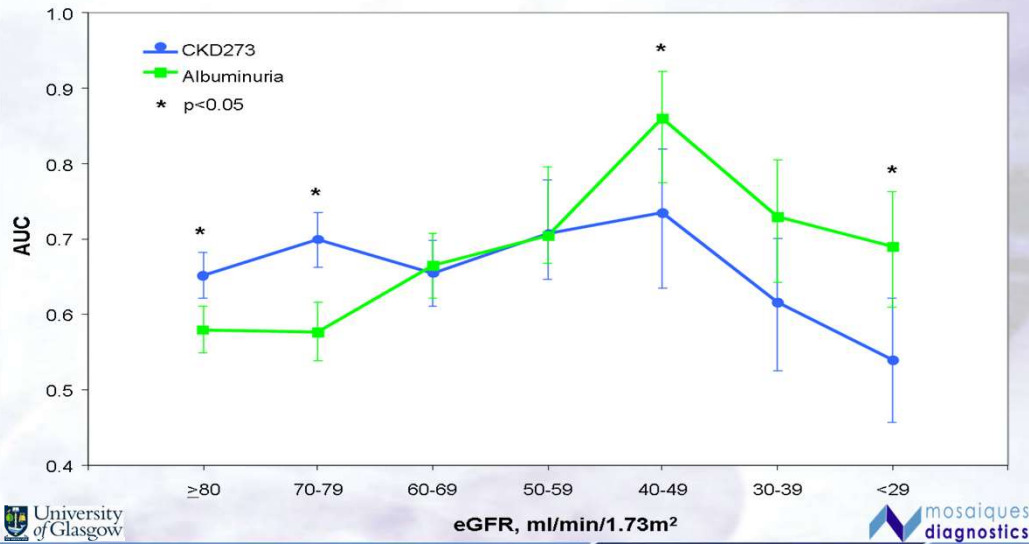
➡ **Origin of the urine sample has no impact on classification**

### CKD273 is more accurate diagnostic and prognostic biomarker relative to currently available gold standards

Association of CKD273 with eGFR and progression in CKD

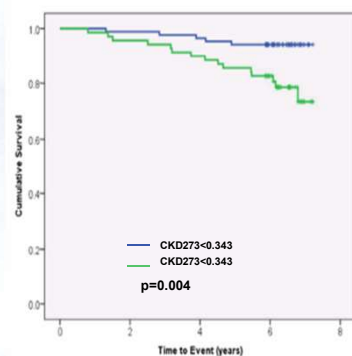


## Comparison of albuminuria and CKD273 in predicting CKD progression in 2672 patients



## Prediction of patient-relevant outcome by CKD273

Parameter	CKD273>0.343 (n=71)	CKD273<0.343 (n=86)	P-value
Age (years)	61 (59-71)	61 (60-70)	0.573
Gender (M/F)	61/10	59/27	*0.010
Diabetes duration (years)	10 (1-35)	13 (1-36)	0.153
Retinopathy (Y/N)	42/29	53/33	0.752
Smokers (Y/N)	27/44	16/70	*0.007
BMI (kg/m <sup>2</sup> )	31.3 (22.5-55.6)	31.7 (21.6-45.6)	0.662
SBP (mmHg)	130.4±17.4	128.8±15.3	0.547
DBP (mmHg)	75±11.2	73.8±11.3	0.543
HbA1c (mmol/mol)	59 (41-86)	59 (39-123)	0.118
Cholesterol (mmol/l)	3.9 (2-7)	3.8 (2.2-6.1)	0.549
UAE (mg/24hrs)	141 (9-1372)	57 (3-980)	*<0.001
eGFR (ml/min/1.73m <sup>2</sup> )	87.6±18	89.6±16	0.452
CKD273 score	0.527 (-1.078 - 1.231)	0.140 (-1.004 - 0.780)	*<0.001



All-cause mortality in patients with CKD273 score above and below threshold for diagnosis of DN

# CKD273 improves of patient stratification

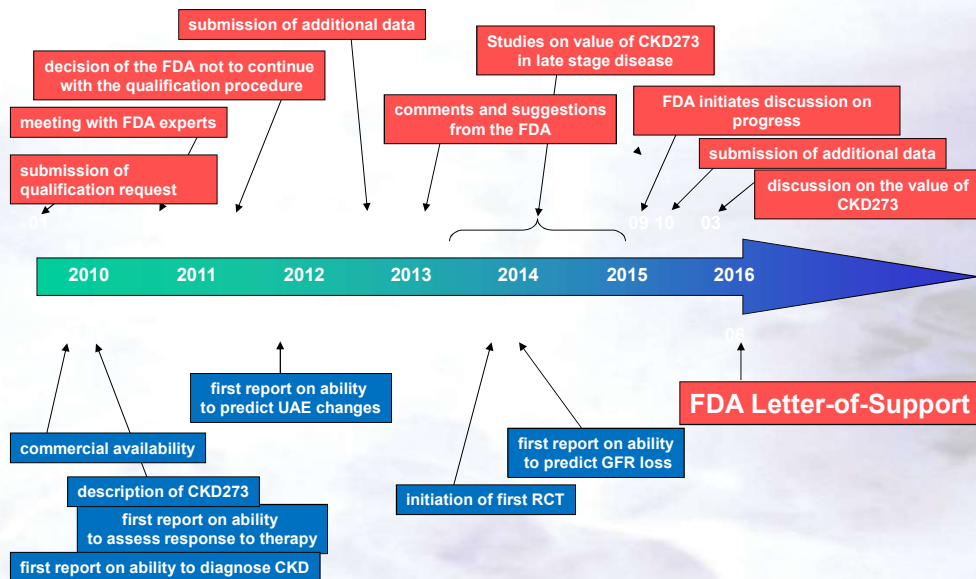
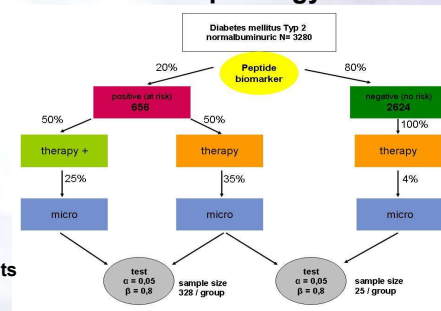


## RCT employing CKD273 for stratification Targeted therapy/personalized medicine in Nephrology

Early prediction of diabetic nephropathy  
through urinary proteome analysis

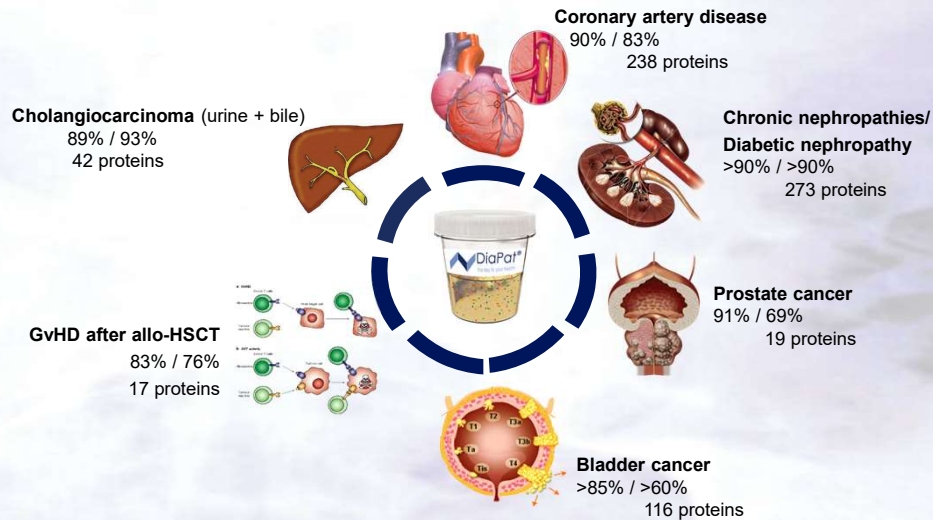
- Multicenter study
- 15 partners in Europe
- 6 years

- 1770 normoalbuminuric type 2 diabetic patients
- Stratification into low and high risk patients
- High risk patients will be randomly assigned to aldosterone blocker spironolactone 25 mg or placebo therapy on top of optimal standard therapy





## Areas of Application (Sensitivity / Specificity)

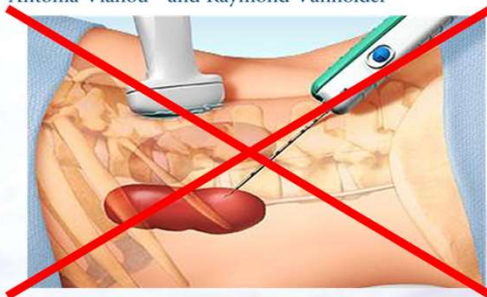


## Original Article

Nephrology Dialysis Transplantation

### Noninvasive diagnosis of chronic kidney diseases using urinary proteome analysis

Justyna Siwy<sup>1</sup>, Petra Zürlbig<sup>1</sup>, Angel Argiles<sup>2</sup>, Joachim Beige<sup>3</sup>, Marion Haubitz<sup>4</sup>, Joachim Jankowski<sup>5,6</sup>, Bruce A. Julian<sup>7</sup>, Peter G. Linde<sup>8</sup>, David Marx<sup>9</sup>, Harald Mischak<sup>1,10</sup>, William Mullen<sup>10</sup>, Jan Novak<sup>7</sup>, Alberto Ortiz<sup>11</sup>, Frederik Persson<sup>12</sup>, Claudia Pontillo<sup>1,13</sup>, Peter Rossing<sup>12,14,15</sup>, Harald Rupperecht<sup>16</sup>, Joost P. Schanstra<sup>17,18</sup>, Antonia Vlahou<sup>19</sup> and Raymond Vanholder<sup>20</sup>



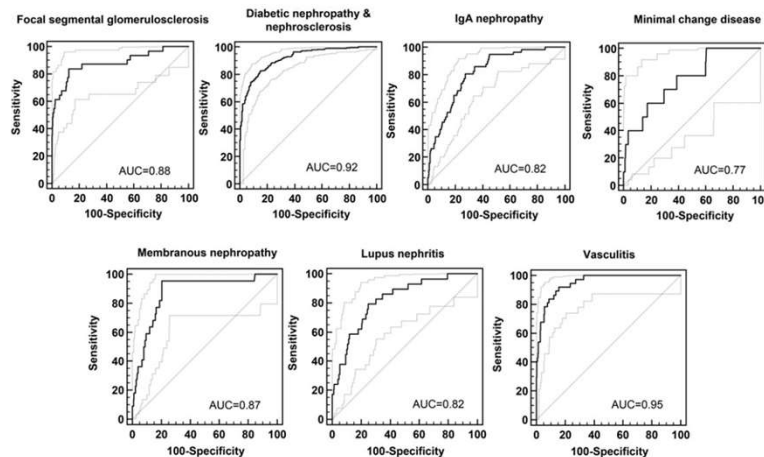
## Differential diagnosis based on urinary peptides

### Subjects and design of the study

Disease	Discovery set (n=706)				Validation set (n=474)			
	Sample number	Gender (%male)	Age	eGFR (mL/min/1.73m <sup>2</sup> )	Sample number	Gender (%male)	Age	eGFR (mL/min/1.73m <sup>2</sup> )
Focal segmental glomerulosclerosis	79	62	41.3 ± 21.8	45.1 ± 26.7	31	55	29.1 ± 23.2	46.9 ± 32.7
Diabetic nephropathy & nephrosclerosis	288	66	65.4 ± 13.8	40.0 ± 22.9	288	57	64.7 ± 10.7	55.6 ± 22.8
IgA nephropathy	122	65	42.6 ± 16.0	50.8 ± 29.8	57	63	37.0 ± 14.2	94.7 ± 30.0
Minimal change disease	25	72	35.1 ± 15.2	85.8 ± 35.9	10	40	45.7 ± 23.2	103.4 ± 53.9
Membranous nephropathy	55	74	52.0 ± 15.2	68.5 ± 32.4	22	67	50.9 ± 16.4	89.6 ± 22.3
Lupus nephritis	63	17	39.8 ± 12.6	57.1 ± 23.5	29	13	35.6 ± 13.4	99.3 ± 17.6
Vasculitis induced kidney disease	74	58	64.5 ± 10.3	41.3 ± 22.4	37	44	58.8 ± 14.6	70.2 ± 13.7

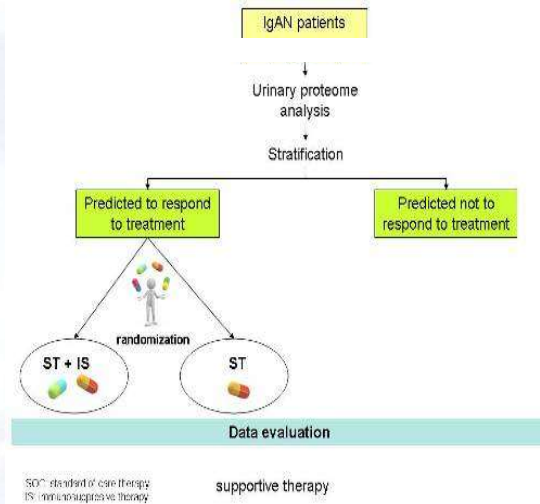
Siwy, et al. Noninvasive diagnosis of chronic kidney diseases using urinary proteome analysis NDT, (2016)

## Differential diagnosis based on urinary peptides



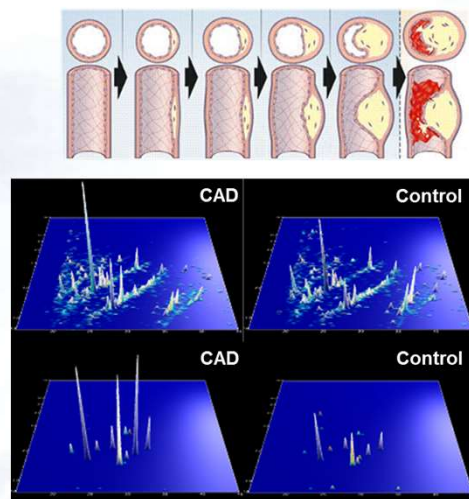
Siwy, et al. Noninvasive diagnosis of chronic kidney diseases using urinary proteome analysis NDT 2016

## PersTlgAN: Proteomics-guided personalized Treatment in IgA Nephropathy



Recruited patients will be stratified using urinary peptide classifiers to treatment response and non response group. The predicted "responder group" will be randomized to the supportive therapy (ST) plus immunosuppressive (IS) therapy or to ST without IS. After 6 months the study data will be evaluated and follow-up will be extended outside the current project frame.

## Assessment of coronary artery disease (CAD)



International multi-centre study cohort:

Biomarker discovery / SVM modelling:

CAD / unstable angina pectoris / MI cases: N = 204  
Controls: N = 382

Validation:

CAD cases: N = 71  
Controls: N = 67

Peptide profile

CAD peptide biomarkers

Delles et al., J. Hypertens., 2010, 28:2316-22

# Atherosclerosis - Prediction of outcome

International multi-centre study cohort:

*Biomarker discovery:*

ACS cases: N = 72

Controls: N = 72

*Validation:*

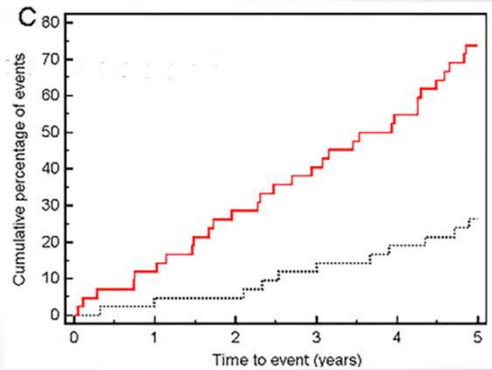
ACS cases: N = 42

Controls: N = 42

**Added prognostic value of ACSP75 over FRS**

NRI =  $0.405 \pm 0.113$ ,  $P = 0.0007$

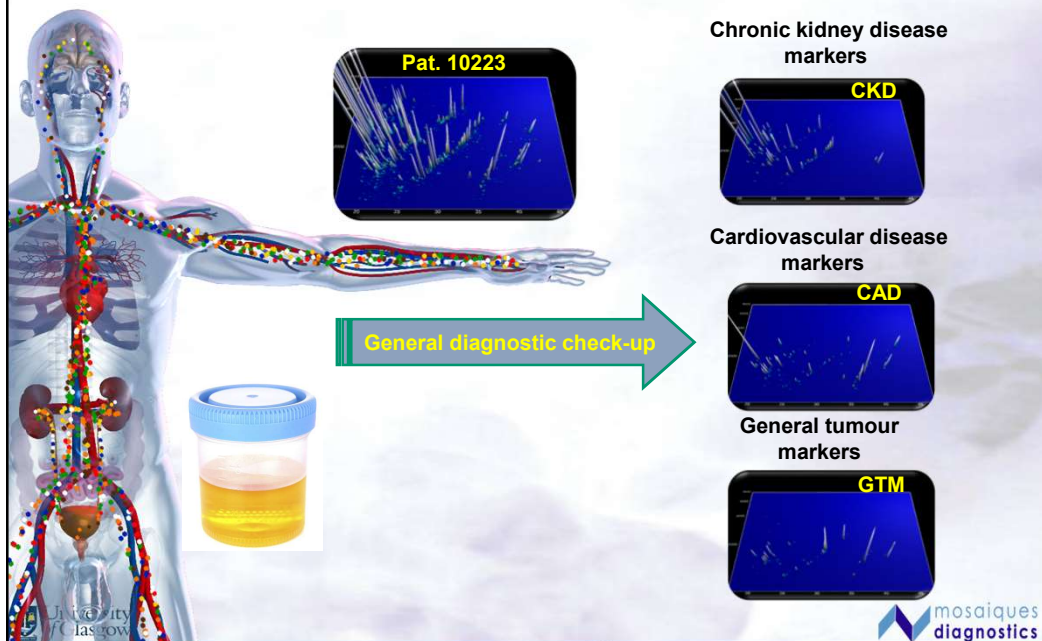
IDI =  $0.273 \pm 0.048$ ,  $P < 0.0001$



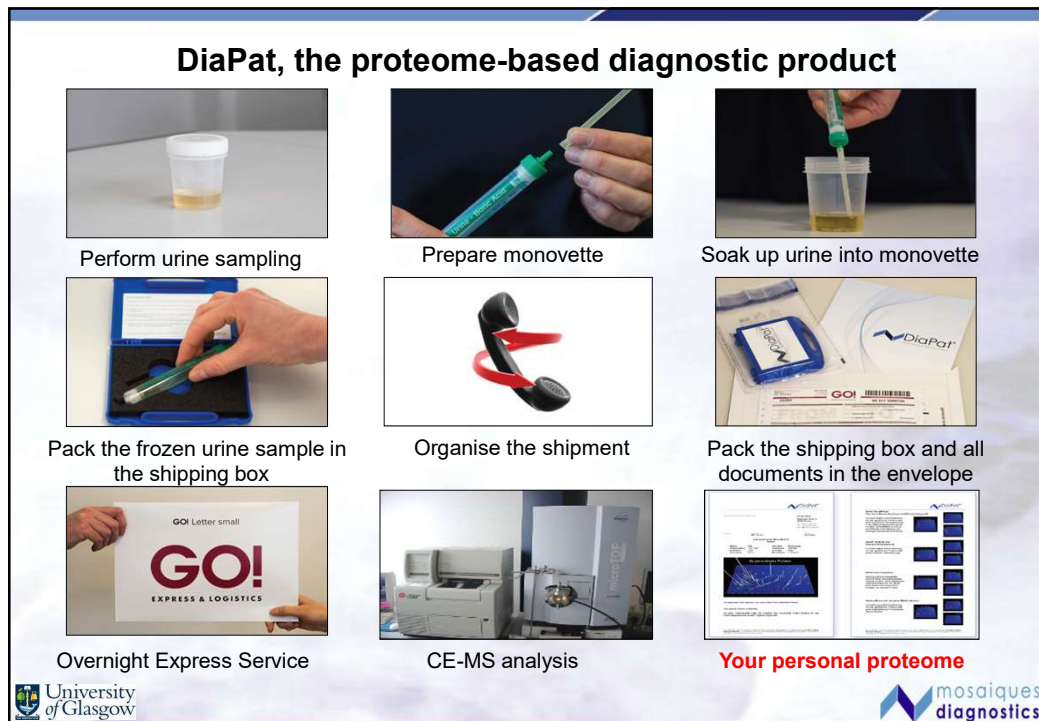
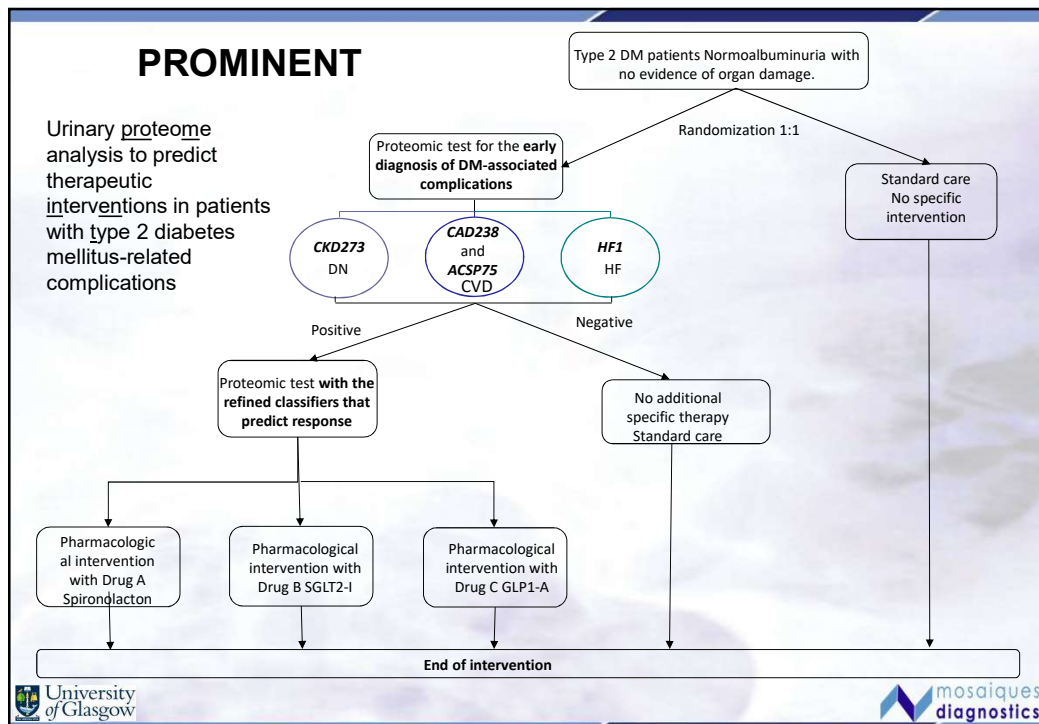
Kaplan Meier survival curve depicting the cumulative percentage with an ACS event based on an ACSP75 score above (red solid line) and below (black spotted line) the threshold of 0.041

Htun et al. Prediction of acute coronary syndromes by urinary proteome analysis. *PLOS ONE* 2017

## The next step: one dataset, multiple pathologies assessed







## Conclusions

- ➔ CE-MS-based proteomic panels enable (early) detection, prognosis, and assessment of therapy of a variety of diseases, with 80 - 98% sensitivity and specificity.
- ➔ Biomarkers and biomarker panels were validated in independent multi-centric blinded studies.
- ➔ Variability in single biomarkers is counteracted by well defined, e.g. SVM-based classifiers that tolerate instability and inconsistency of individual polypeptides/biomarkers
- ➔ Proteomic biomarkers can be employed to guide early intervention, and significantly reduce end organ damage
- ➔ In concert with physiology, histopathology, and additional omics data urinary proteome analysis enables assessment of disease on a molecular level, definition of new, more appropriate therapeutic targets and drugs.
- ➔ **High number of subjects (best n>1000) is key to success**

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