Renal Toxicologic Pathology -
At the interface of science and technology for biomarkers

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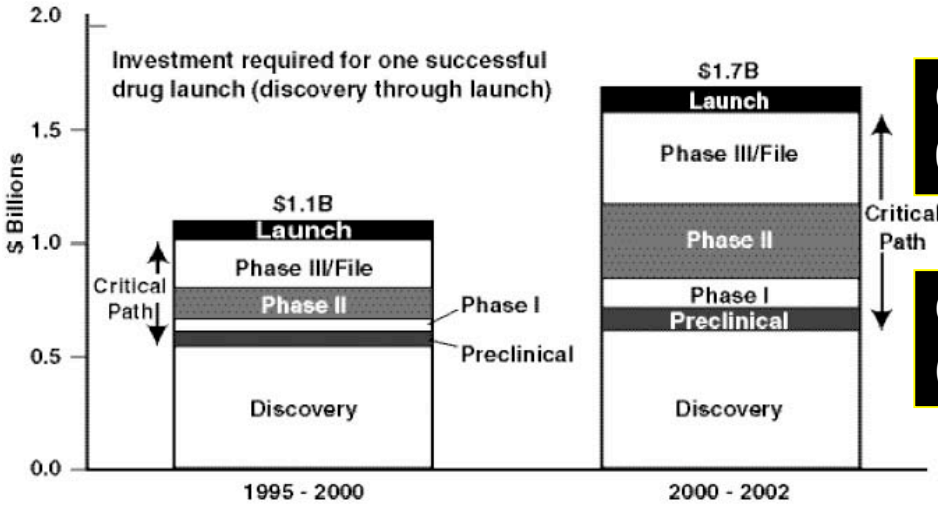
Biomarkers

The Physician: Gerrit DOU Leiden (1613-1675)

- **We need better biomarkers to detect AKI:**
  - Predictive toxicology in drug development
  - Early diagnosis/prognosis of AKI
  - Facilitate clinical trials
- **We need better technologies to quantitate biomarkers**
  - High throughput detection
  - Sensitivity and specificity
  - Reproducible
  - Multiplexing capabilities
  - Requiring low volumes of reagents and samples
  - Economic

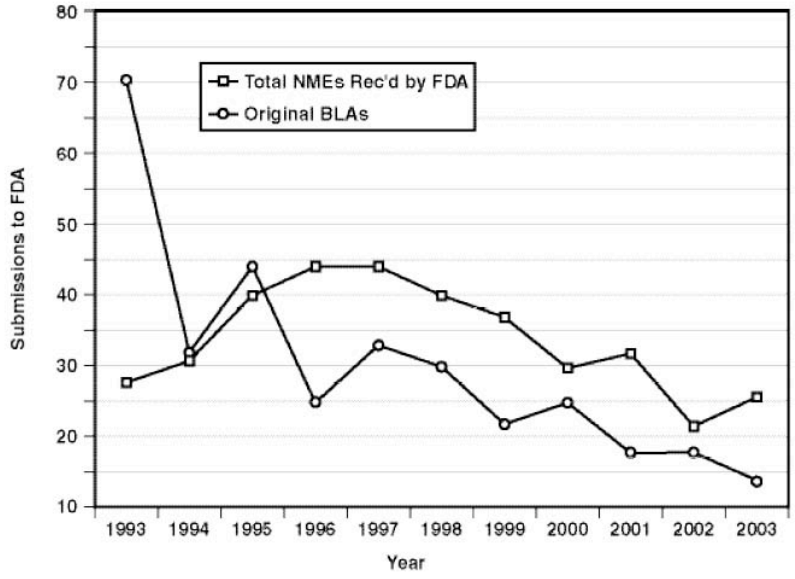
Need for Biomarkers: Facts and Figures



Cost of bringing New drug to Market: $ 0.8 to 1.7 B

Cost of biomedical research funding (2003): $ 94 B

- **8.5 years/drug for FDA approval**
- **20 new drugs approved in 2005 as opposed to 36 in 2004**
- **Slowdown in new drug and biologic submissions to regulatory agencies worldwide.**



Challenge and opportunity on the critical path to new medical products. In: *US FDA* *www.fda.gov/oc/initiatives/criticalpath/whitepaper.html*, 2004.

Need for Biomarkers: From the Horse's Mouth

"Right now, researchers are trying to use 21st century medical innovations to market using 20th century tools to evaluate them"

- Dr. Andrew C. von Eschenbach, Acting FDA commissioner

"A new generation of predictive biomarkers would

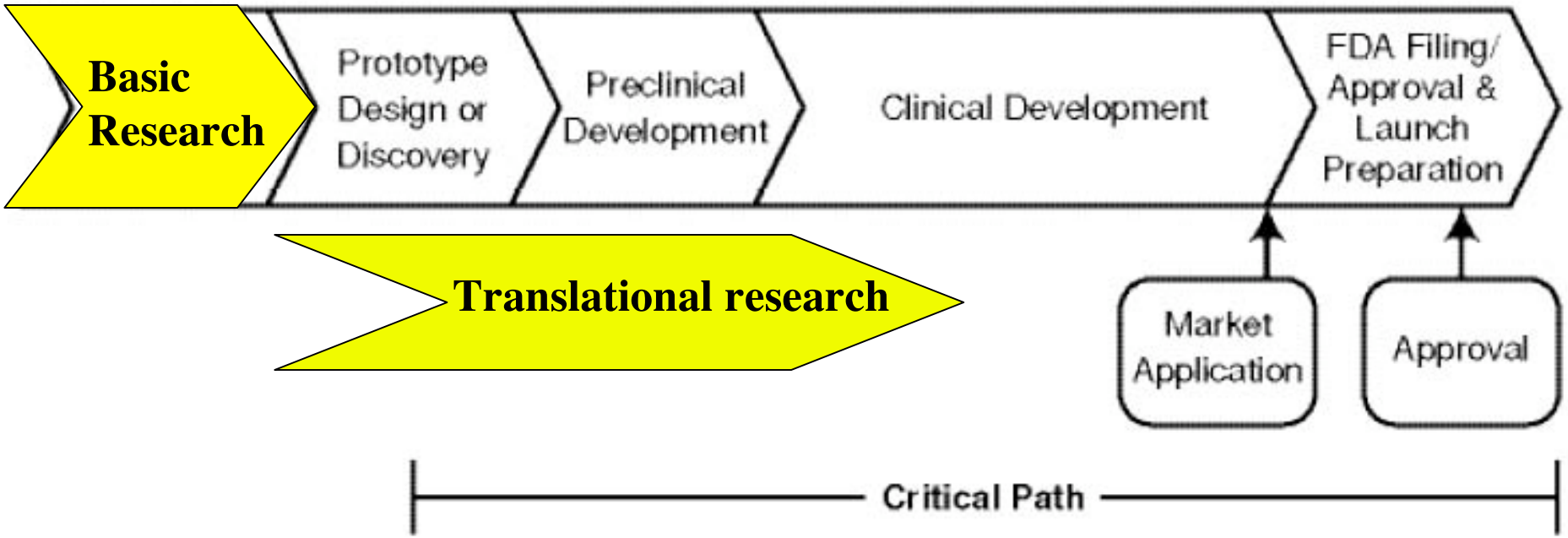
a. dramatically improve the efficiency of product development

b. help identify safety problems before a product is on the market

c. facilitate the development of new types of clinical trials that will produce better data faster"

- Dr. Janet Woodcock, Deputy commissioner, FDA

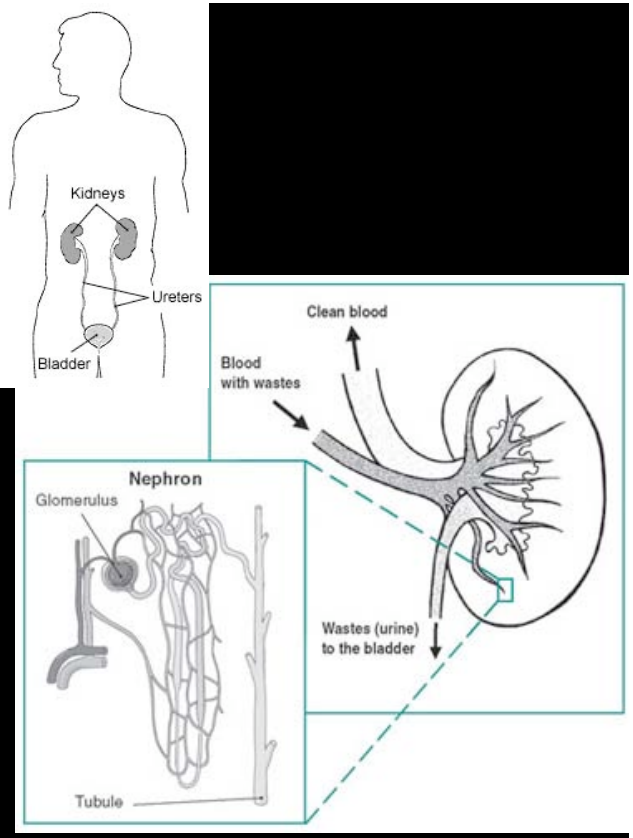
Critical Path for Medical Product Development (FDA and NIH)

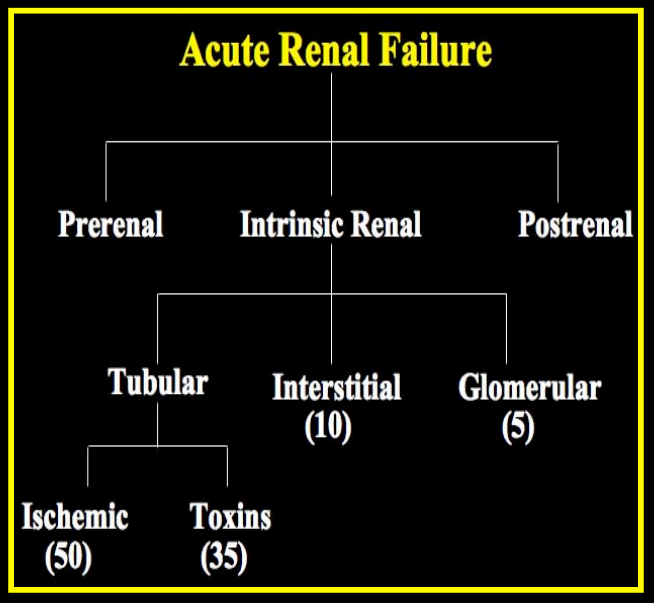


- **Need for Better Biomarkers:**
  - **Preclinical studies**
    - **Eliminate potential toxic drugs early in the drug development process (a 10-percent improvement in predicting failures before clinical trials could save $100 million in development costs per drug)**
    - **Safety to warrant human studies**
  - **Clinical Studies**
    - **Safety in humans**
    - **Diagnosis/Prognosis of Disease**
    - **Early Therapeutic Intervention**

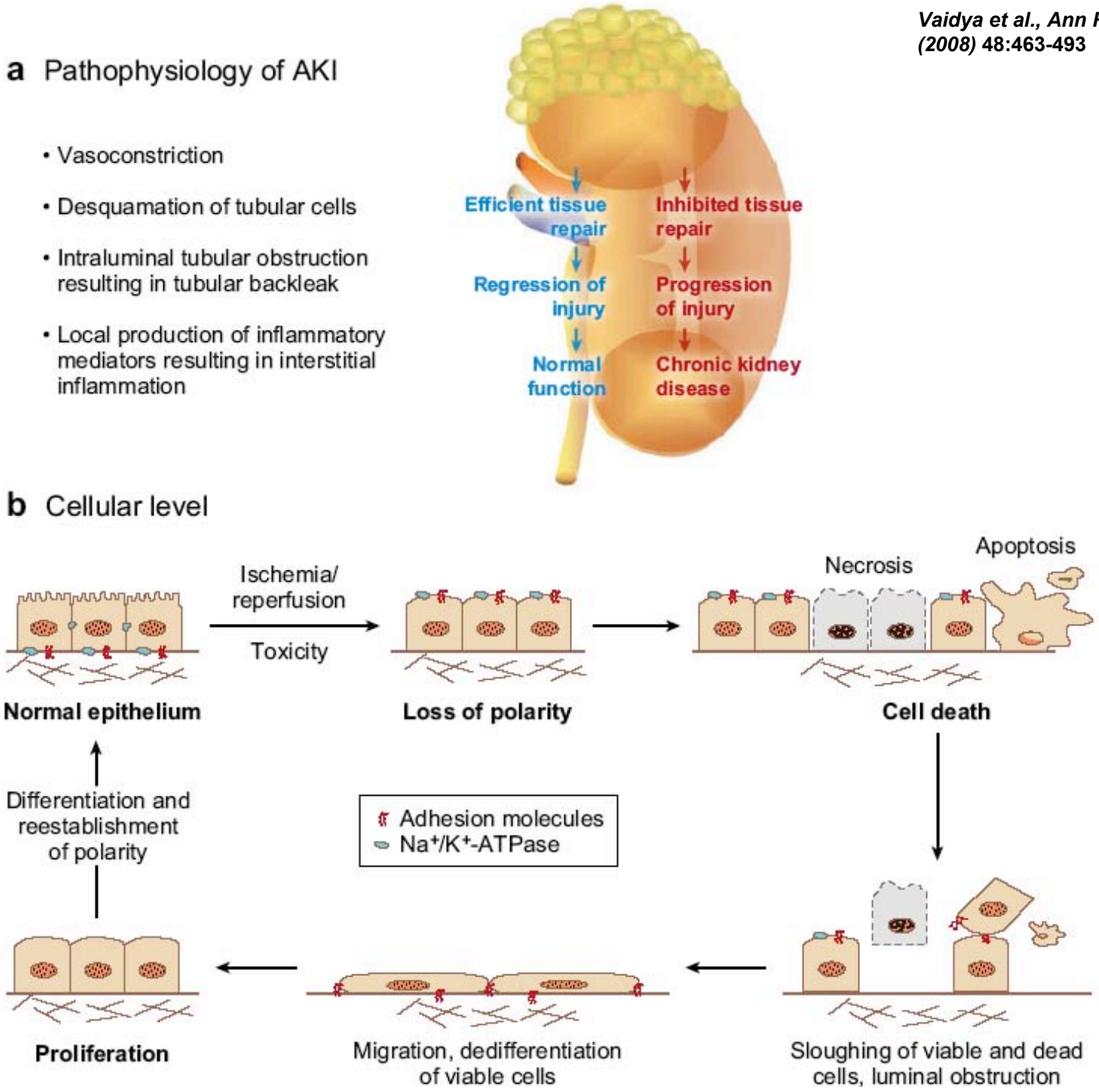
Acute Kidney Injury (AKI)

- Loss of kidney function, measured by decline in glomerular filtration rate (GFR)
- Postoperative renal failure ranges from 24-100%, and 50-70% among patients in intensive care units who require dialysis
- 5 % to 10 % of AKI recognized in hospitalized patients is caused, at least in part, by drugs
- Antibiotics (e.g. aminoglycosides) has been reported to be up to 36 %
- Other nephrotoxic drugs include: cisplatin, cyclosporine, tacrolimus, NSAID's, etc.





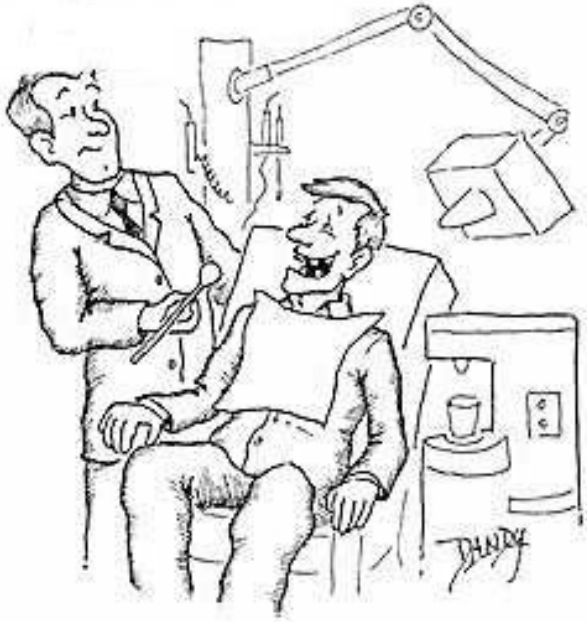
Vaidya et al., Ann Rev Pharm Tox., (2008) **48:463-493**



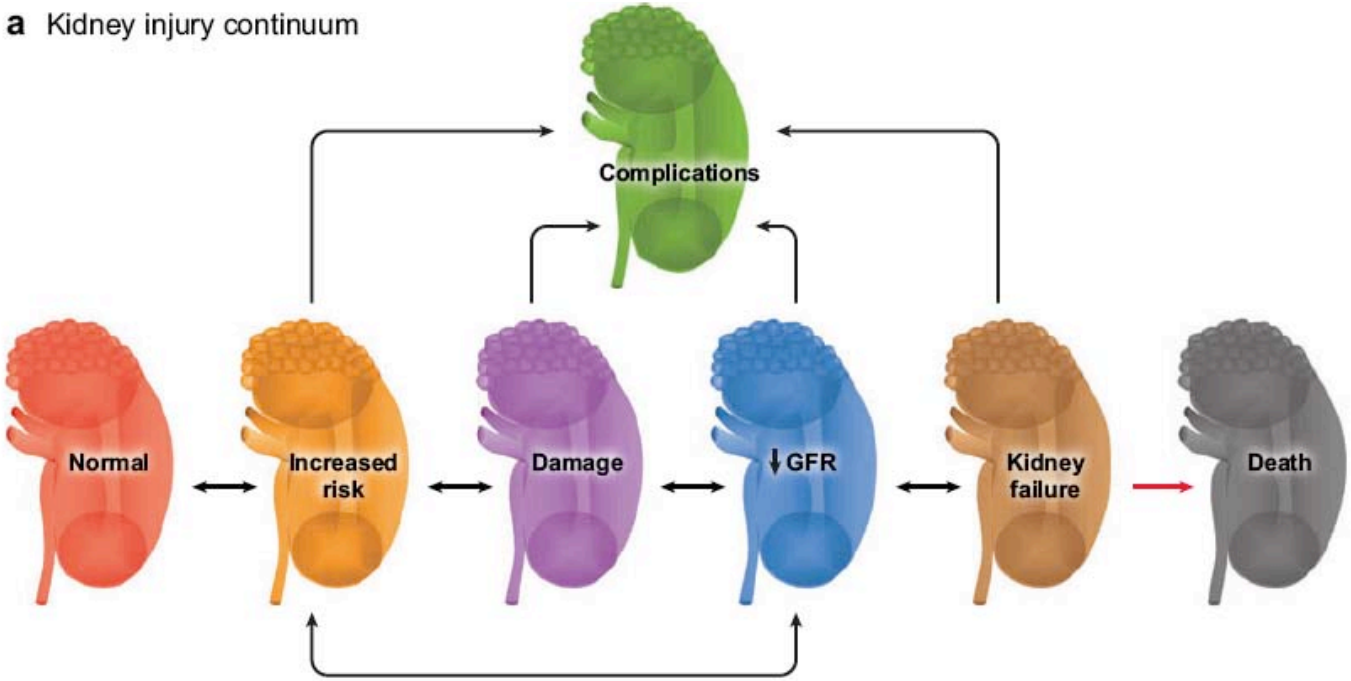
Biomarkers for AKI

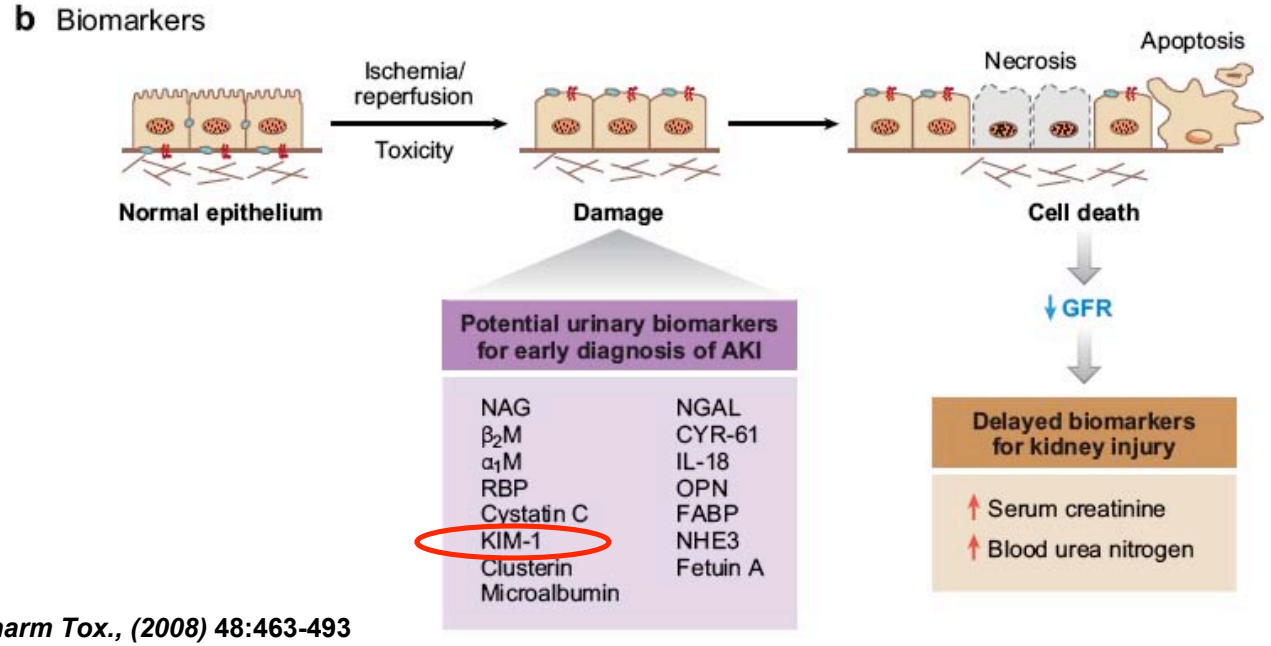
- Serum creatinine, blood urea nitrogen (BUN)
- Urinalysis: urine volume, pH, specific gravity, fluid/electrolyte balance, glycosuria, proteinuria
- Kidney weight (wet weight to dry weight ratio) and histopathology

Too little, Too late!



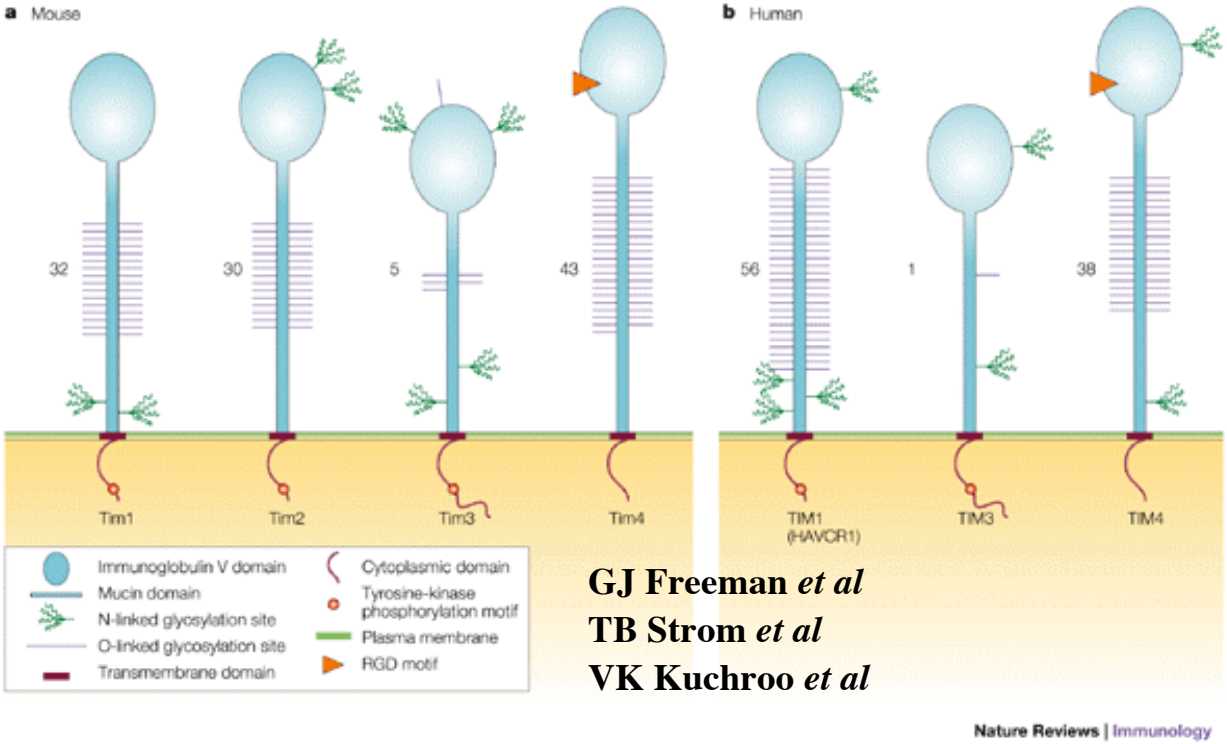
"Do you think drinking milk will help my teeth?"





Vaidya et al., Ann Rev Pharm Tox., (2008) **48:463-493**

Immunologist's interest



TIM-1 is expressed by Th1 and Th2-type cells

TIM-1 is the T cell costimulatory molecule that can be used as an effective adjuvant to enhance T cell immunity.

In humans certain polymorphic variants of TIM-1 are associated with protection against atopy (asthma)

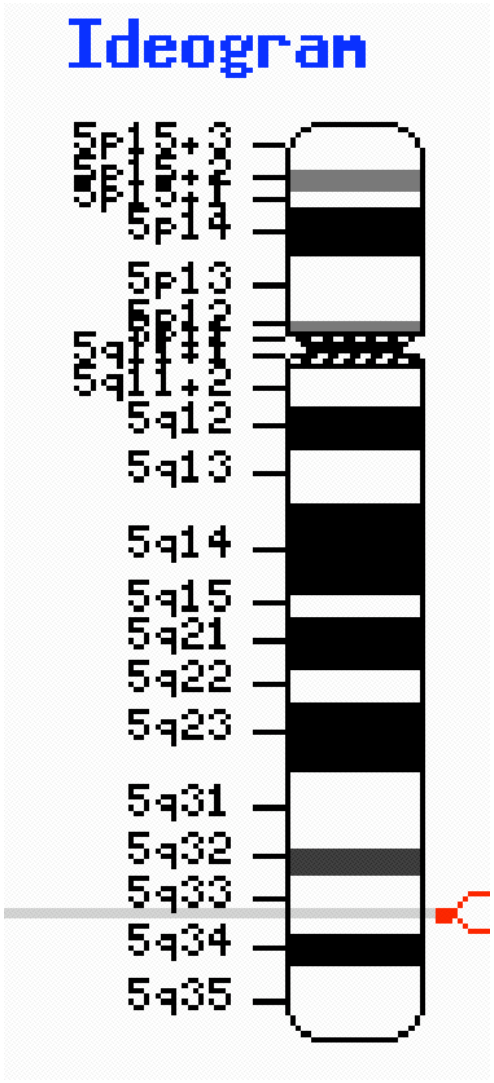
Species	Mouse	Rat	Human
Location	11 B1.1	10q21	5q33
Structure	Ig domain; THR rich Mucin; Transembrane domain and cytoplasmic tail with tyr PO_4 motif		
# of Kim's	8	6	3

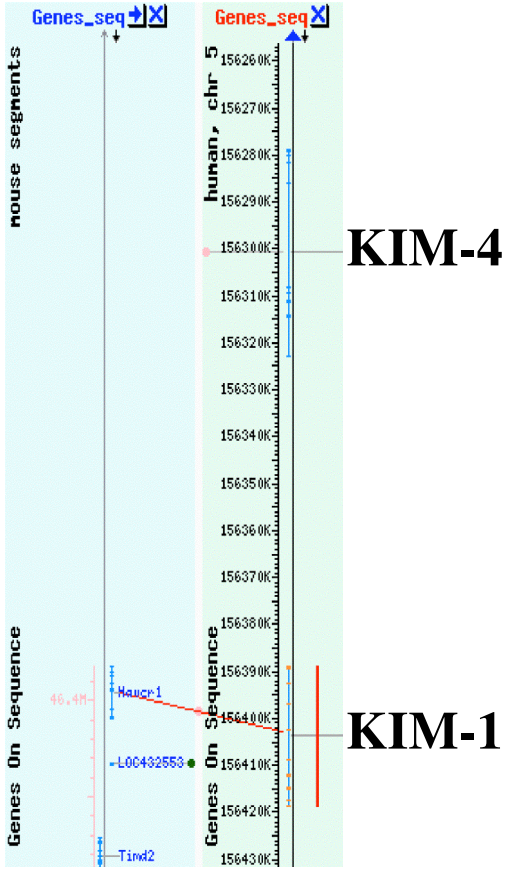
Human: Chr: 5q33

Region: 156,340 K-165,537 K bp

Total Genes: 1190

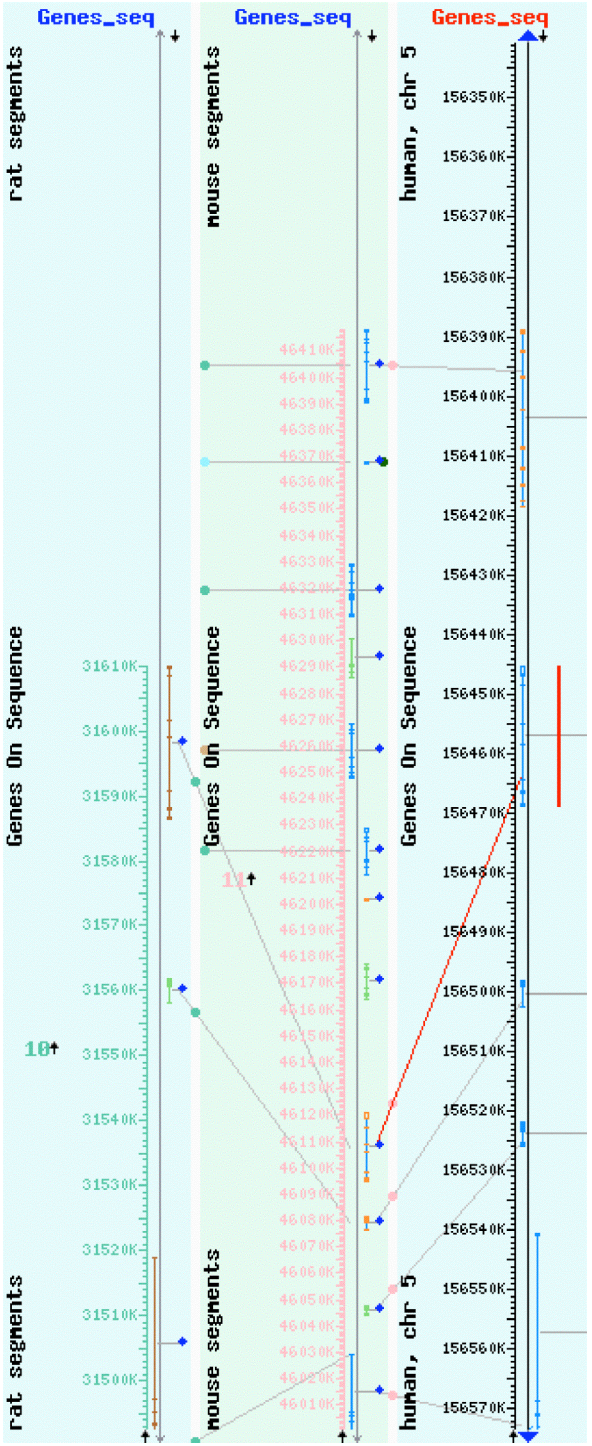
Total Genes in Region: 5





KIM-4

KIM-1



KIM-1

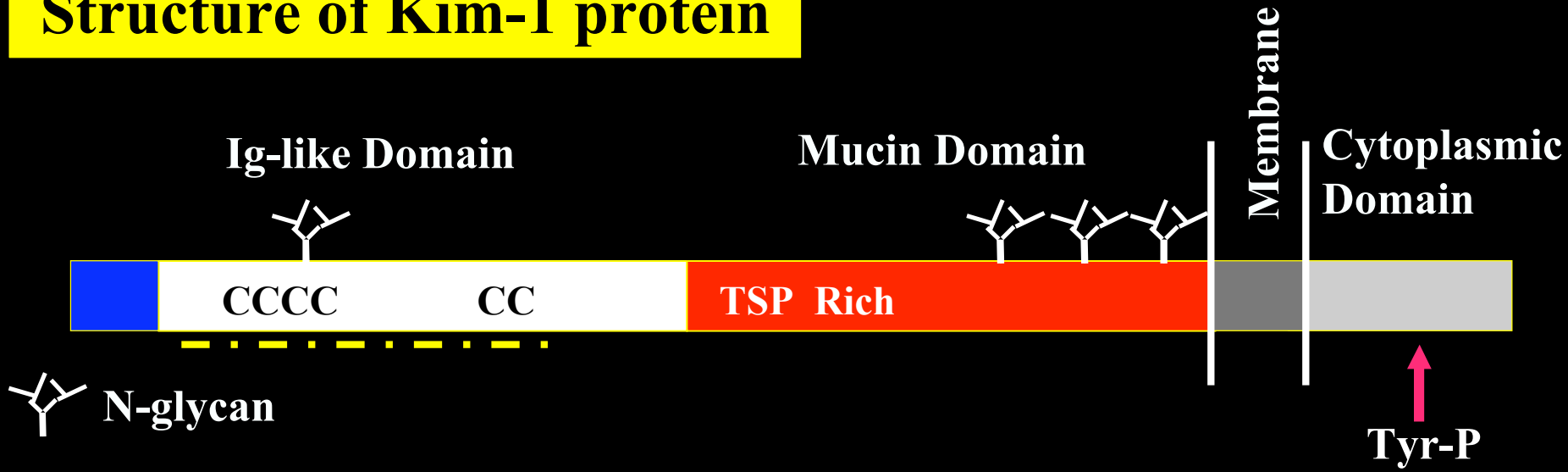
HAVCR2/ KIM-3

CRSP9

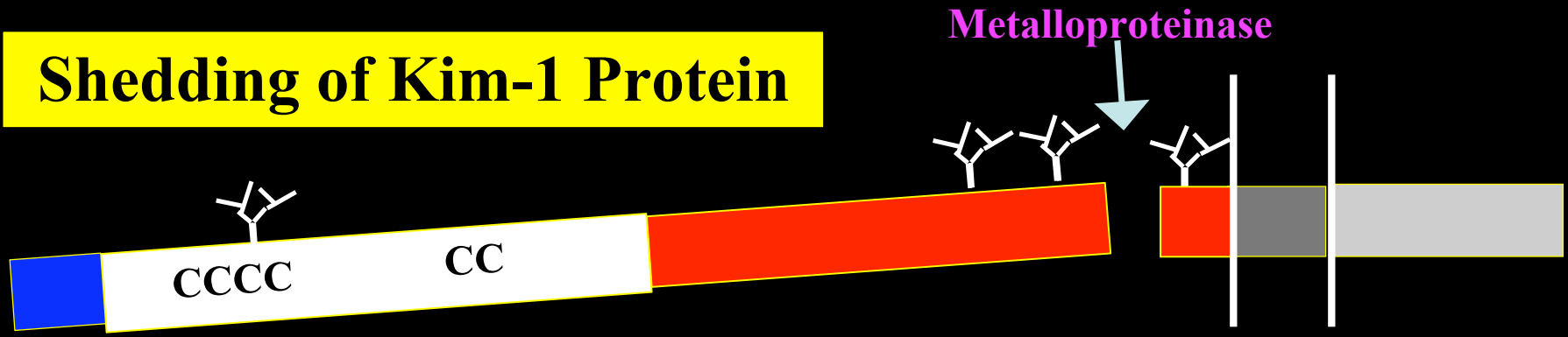
(cofactor required for Sp1 transciptional actvation)

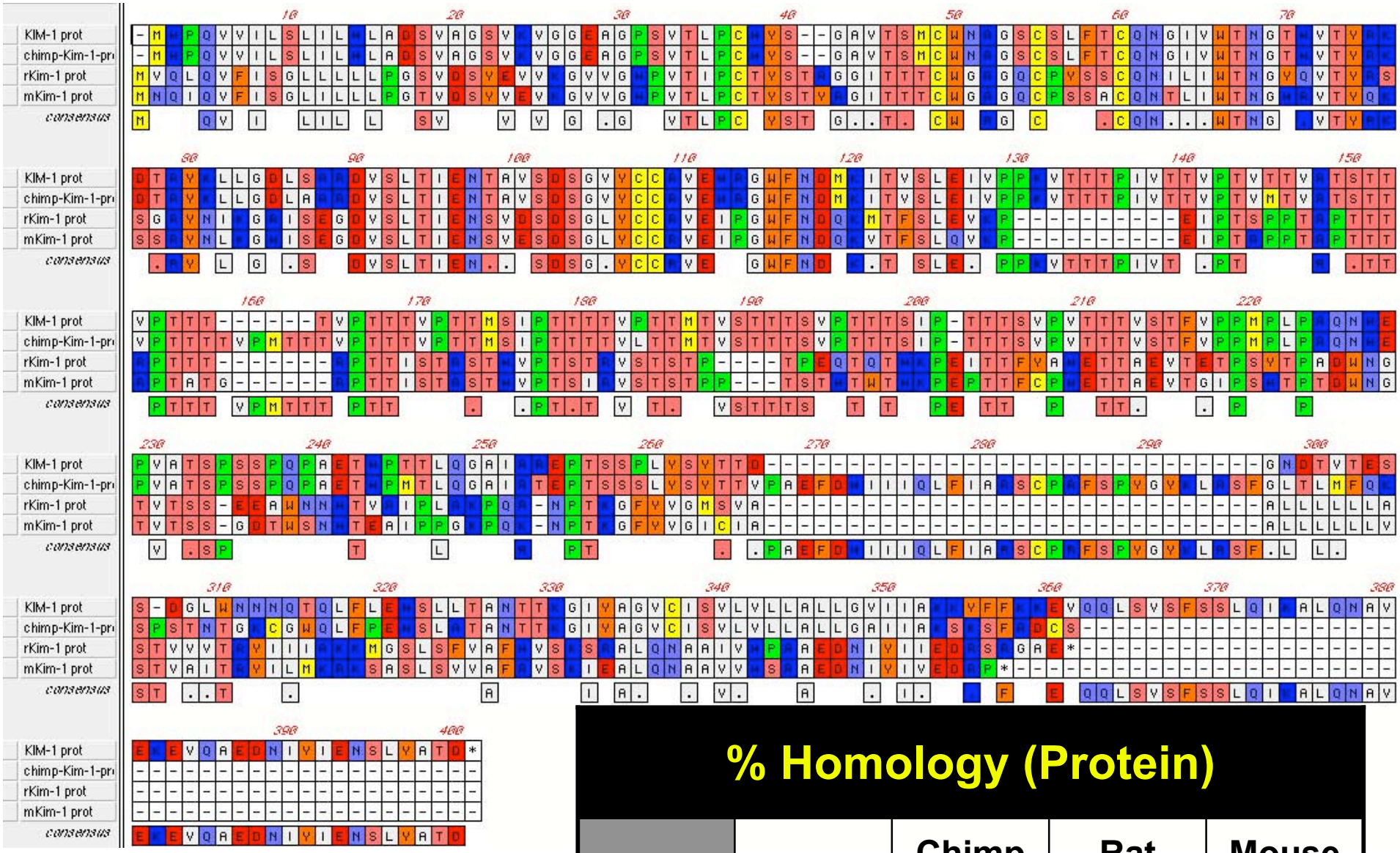
ITK

Structure of Kim-1 protein



Shedding of Kim-1 Protein

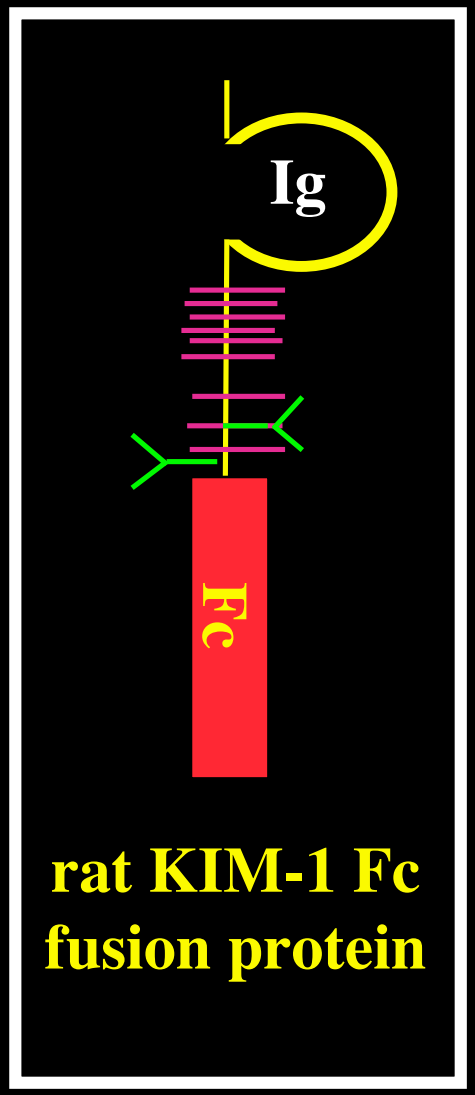




% Homology (Protein)

	KIM-1	Chimp KIm-1	Rat Kim-1	Mouse Kim-1
KIM-1	100 %	79 %	39 %	37 %

Rat Kim-1 Fc fusion protein



- A Construct of Extracellular domain of rat Kim-1 (residues 1 to 234) was attached to Fc (231 aa) portion of human IgG
- Cloned into pEAG347 containing a tandem promoter and dihydrofolate reductase gene for methotrexate selection
- Stably transfected into CHO cell line

❖ Growing rat-Kim-1-Fc CHO cells

- Initially in 10 % MEM minus
- Passed to serum free hybridoma media and grown in a cell factory for large production

Cell Factory

❖ Purification of conditioned media

- Protein A Sepharose columns

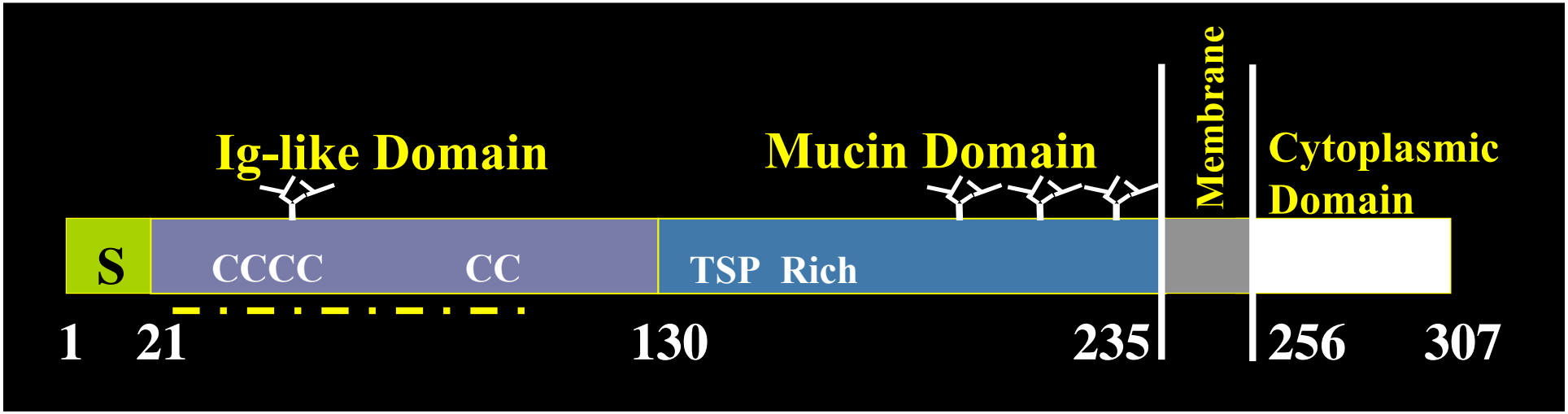
Protein A Column

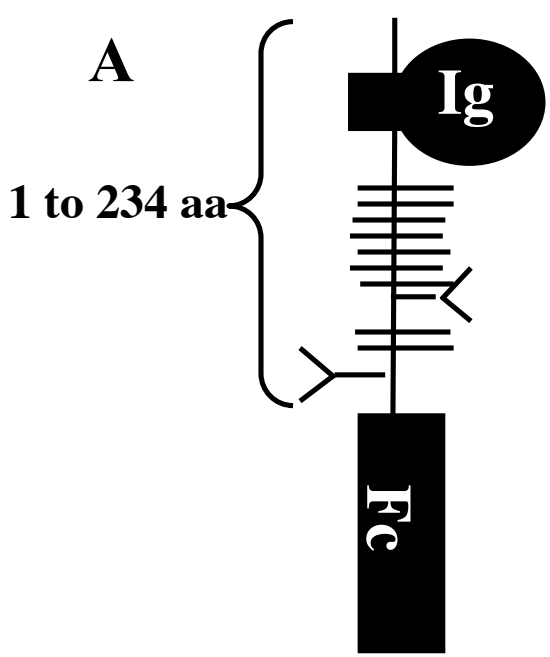
❖ Dialysis and concentration

- Dialysis tubing
- Amicon Centriplus centrifugal filter devices

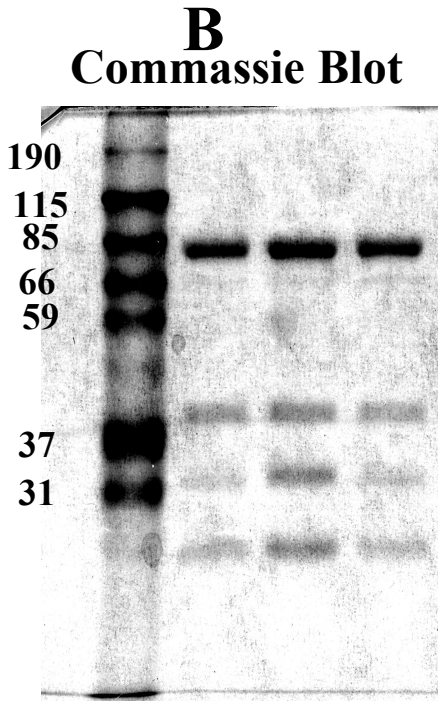
❖ Quantitation

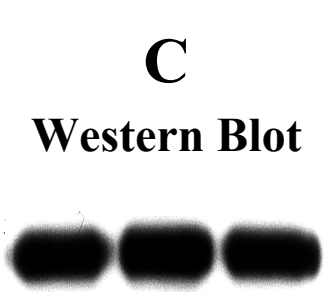
- Elisa using goat anti human Fc as trapping Ab and HRP conjugated antihuman Fc as detecting Ab





rat KIM-1 Fc
fusion protein

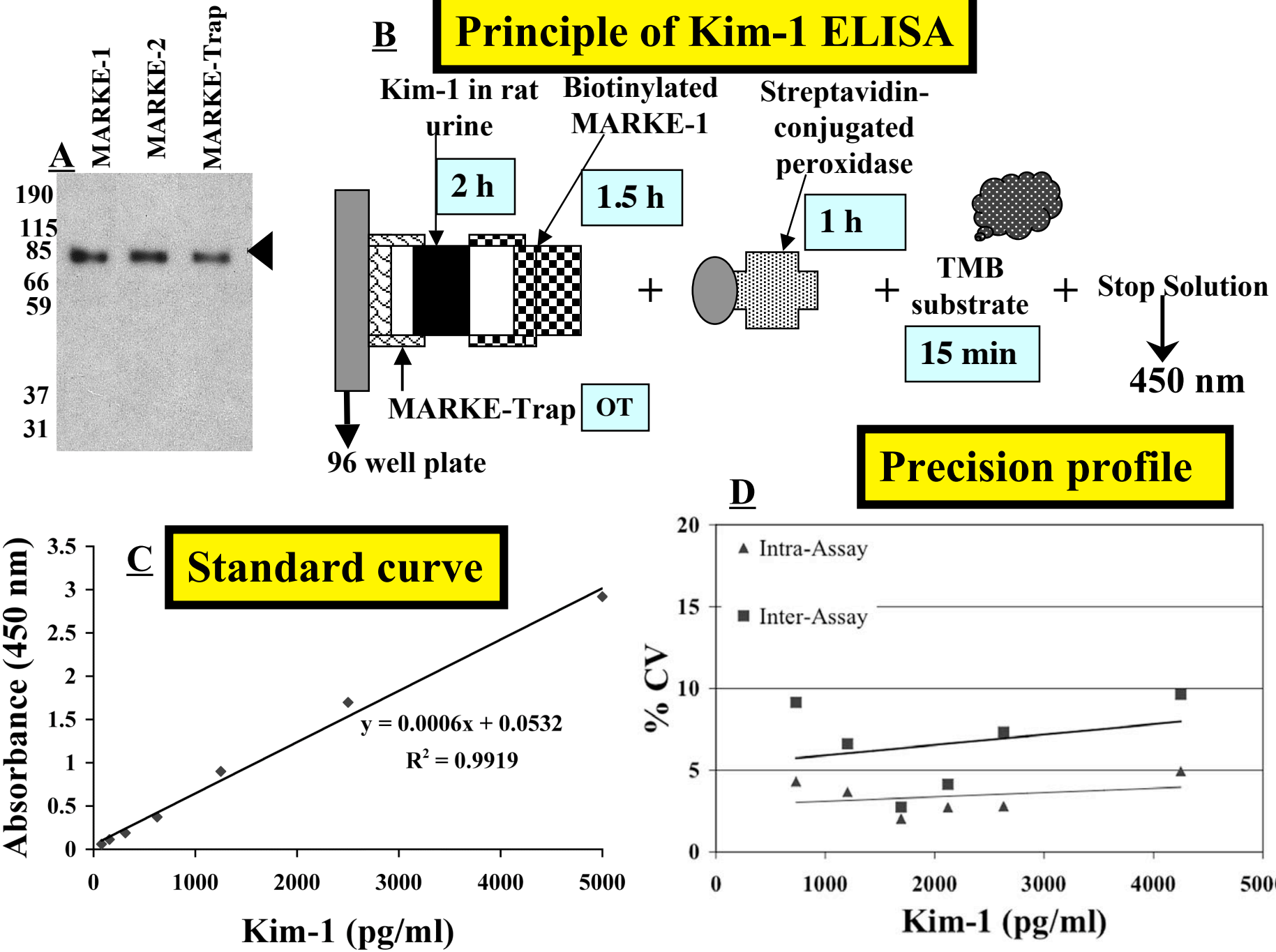




Construction and purification of rat Kim-1 ectodomain fusion protein

Monoclonal antibodies

- 2 clones Monoclonal Anti Rat Kim-1 Ectodomain (MARKE)-1 & 2 were grown in serum free media, protein G purified, dialyzed against PBS, concentrated to yield 2.2 mg/ml. (total ~ 30 mg ea).
- **ELISA failed:**
  - Probably recognize same/similar epitope
  - Recognize rKim-1 ecto individually
  - Both were biotinylated to act as detecting antibodies
- **Hunt for Trapping Antibody (MARKE-Trap):**
  - Hybridoma supernatents from 46 clones that were positive for rat Kim-1 and negative for hIgG-Fc were tested and 16 were selected, 3 were grown and one WORKED!


A
MARKE-1
MARKE-2
MARKE-Trap
190
115
85
66
59
37
31
B
Principle of Kim-1 ELISA
Kim-1 in rat urine
Biotinylated MARKE-1
Streptavidin-conjugated peroxidase
2 h
1.5 h
1 h
TMB substrate
15 min
Stop Solution
450 nm
MARKE-Trap
OT
96 well plate
C
Standard curve
Absorbance (450 nm)
Kim-1 (pg/ml)
y = 0.0006x + 0.0532
R² = 0.9919
D
Precision profile
% CV
Intra-Assay
Inter-Assay
Kim-1 (pg/ml)

Evaluation

Sensitivity: < 39 pg/ml

Assay range: 0-5000 pg/ml

Intra-assay variability: < 5 %

Inter-assay variability: < 10 %

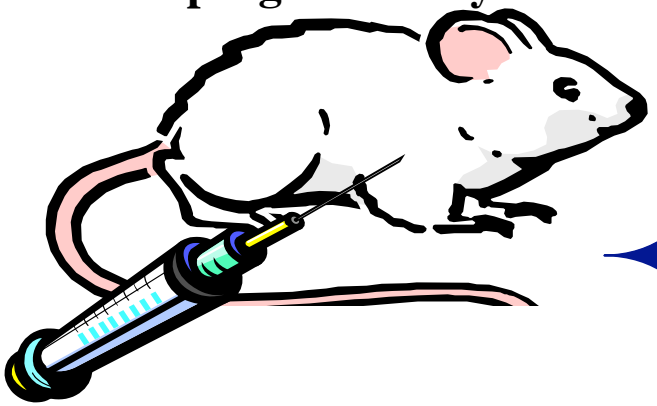
Recovery: 90 to 110 %

Interference: None

Dilution linearity (r=0.95-0.99) with 1:5, 1:10 and 1:20 dilutions

Experimental Protocol

Male Sprague Dawley rats



Cisplatin 2.5, 5, or 7.5 mg/kg, ip

Days of sacrifice

0 1 2 3 4 5

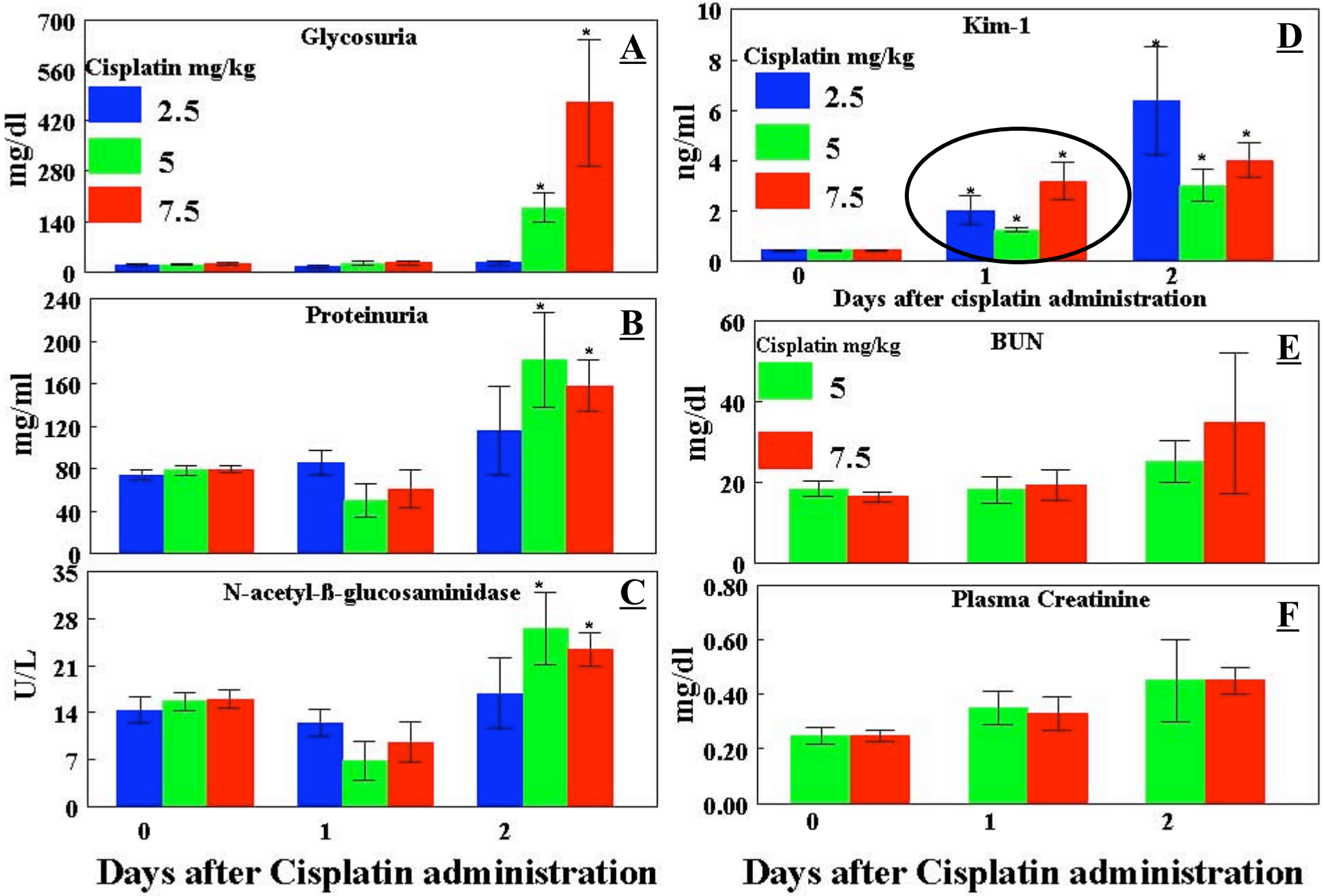
Blood: Blood urea nitrogen, Creatinine

Kidney: Histology, Immunocytochemistry

Days of Urine collection

0 1 2 3 4 5

Urinalysis: Glucose, Protein, N-acetyl-β-glucosaminidase, Creatinine, Kim-1


Glycosuria
A
Cisplatin mg/kg
2.5
5
7.5
mg/dl
700
560
420
280
140
0
Proteinuria
B
mg/ml
240
200
160
120
80
40
0
N-acetyl-ß-glucosaminidase
C
U/L
35
28
21
14
7
0
0
1
2
Days after Cisplatin administration
Kim-1
D
Cisplatin mg/kg
2.5
5
7.5
ng/ml
10
8
6
4
2
0
0
1
2
Days after cisplatin administration
BUN
E
Cisplatin mg/kg
5
7.5
mg/dl
60
40
20
0
Plasma Creatinine
F
mg/dl
0.80
0.60
0.40
0.20
0.00
0
1
2
Days after Cisplatin administration

Renal Ischemic Injury Model

Days of sacrifice/Urine collection

0 **1**

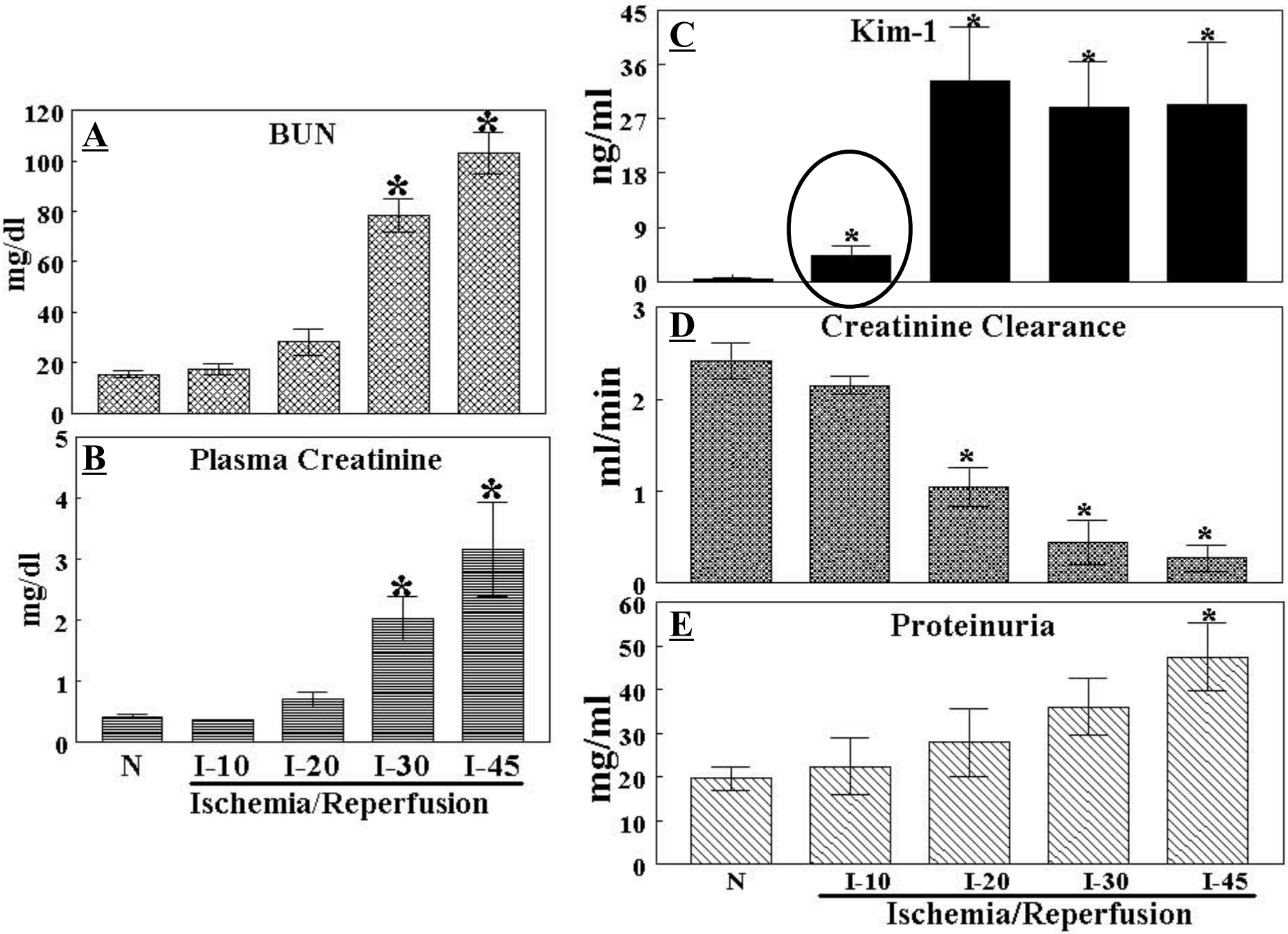
Male Sprague Dawley rats

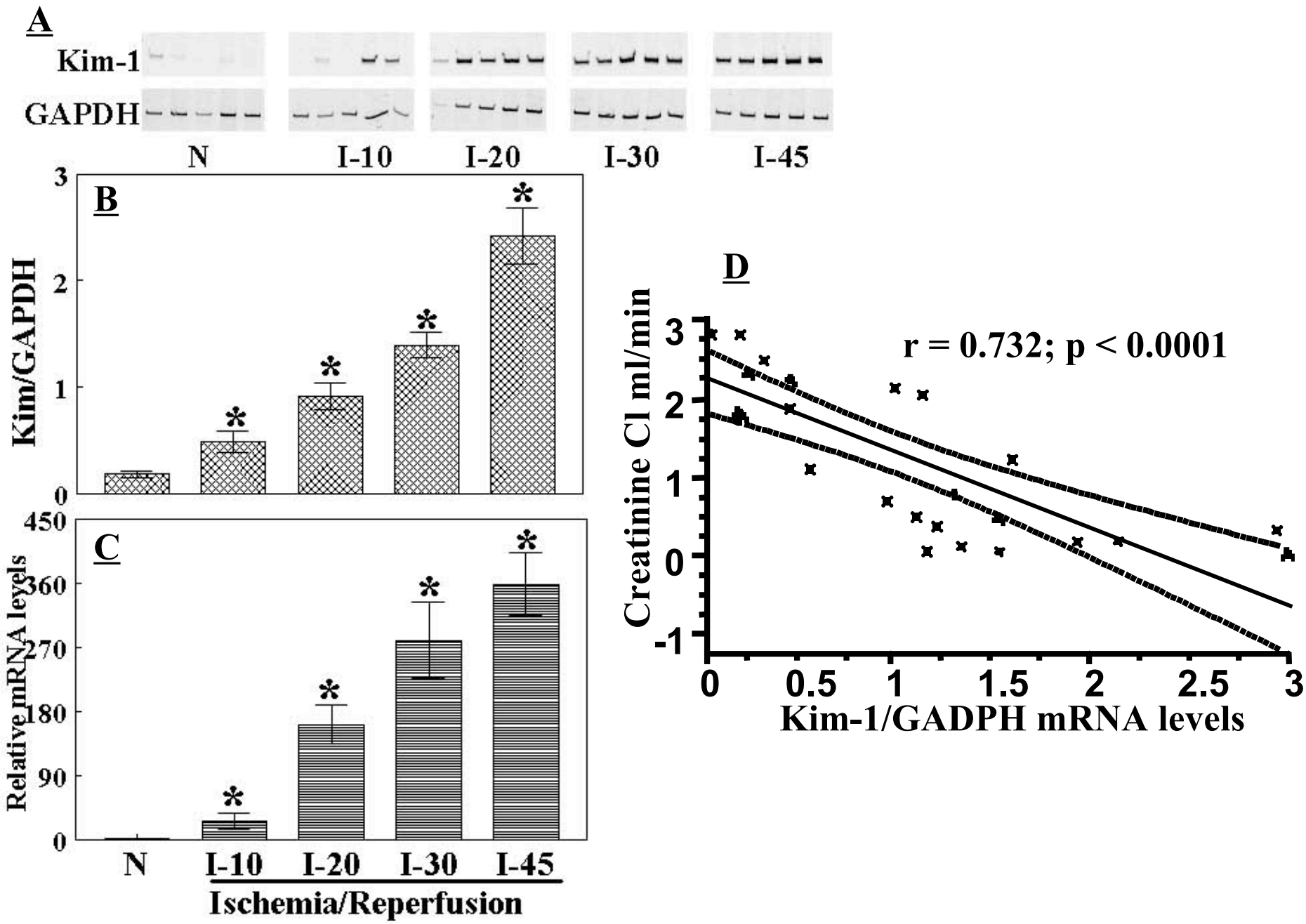
Sham/Bilateral Ischemia: 10, 20, 30, and 45 min

Blood: Blood urea nitrogen, Creatinine

Kidney: Histology, Immunocytochemistry

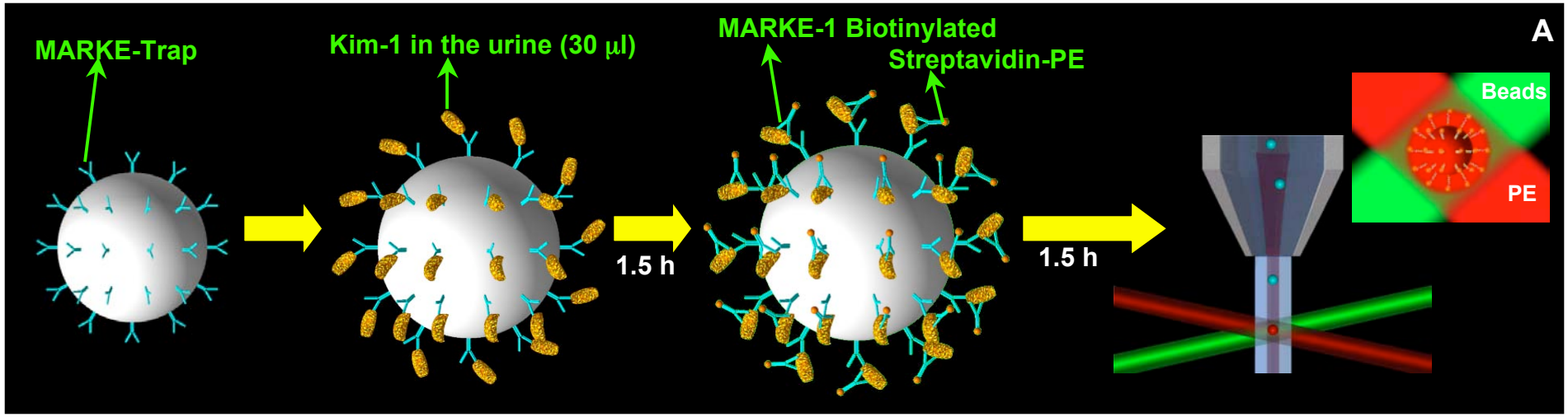
Urinalysis: Protein, Creatinine, Kim-1

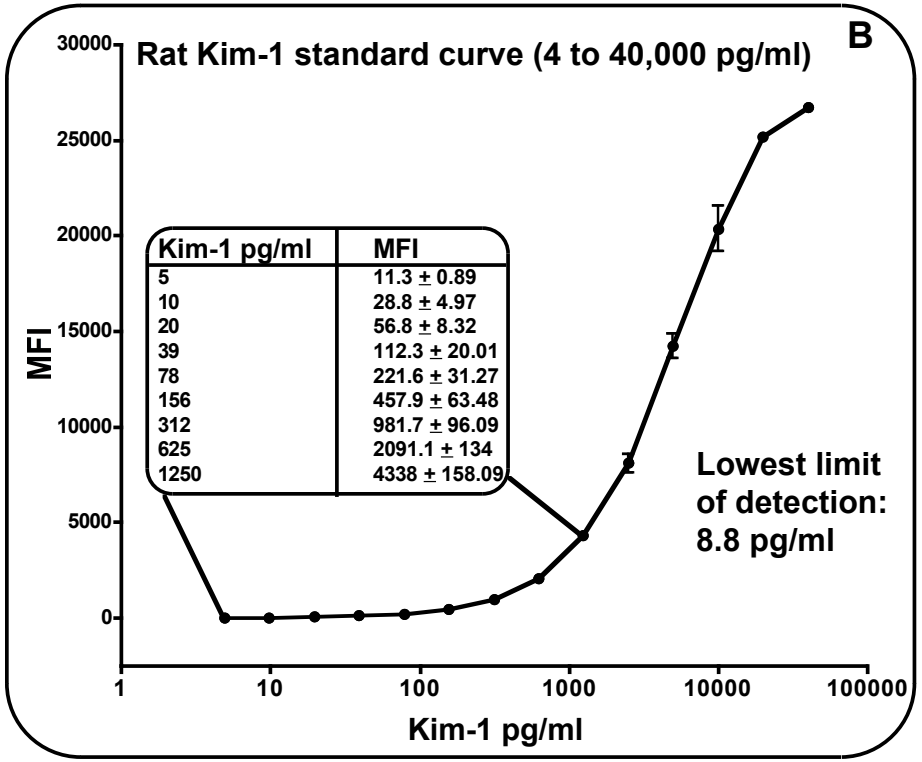

A
BUN
mg/dl
120
100
80
60
40
20
0
B
Plasma Creatinine
mg/dl
5
4
3
2
1
0
N
I-10
I-20
I-30
I-45
Ischemia/Reperfusion
C
Kim-1
ng/ml
45
36
27
18
9
0
D
Creatinine Clearance
ml/min
3
2
1
0
E
Proteinuria
mg/ml
60
50
40
30
20
10
0
N
I-10
I-20
I-30
I-45
Ischemia/Reperfusion

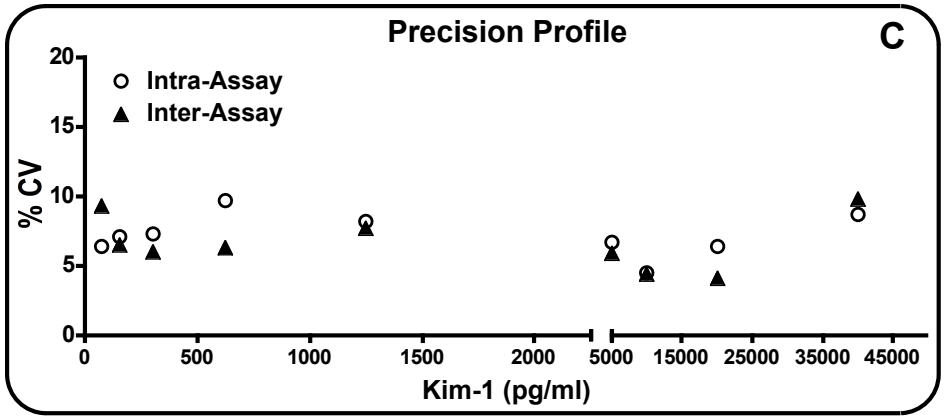


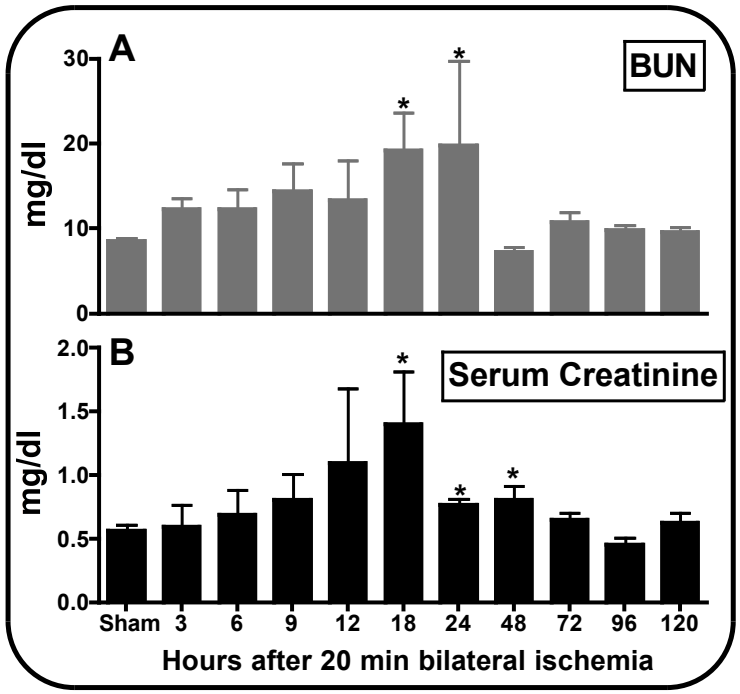
Vaidya, *et al*., Am J. Phys. Renal Phys., 2006; 290:F517-529

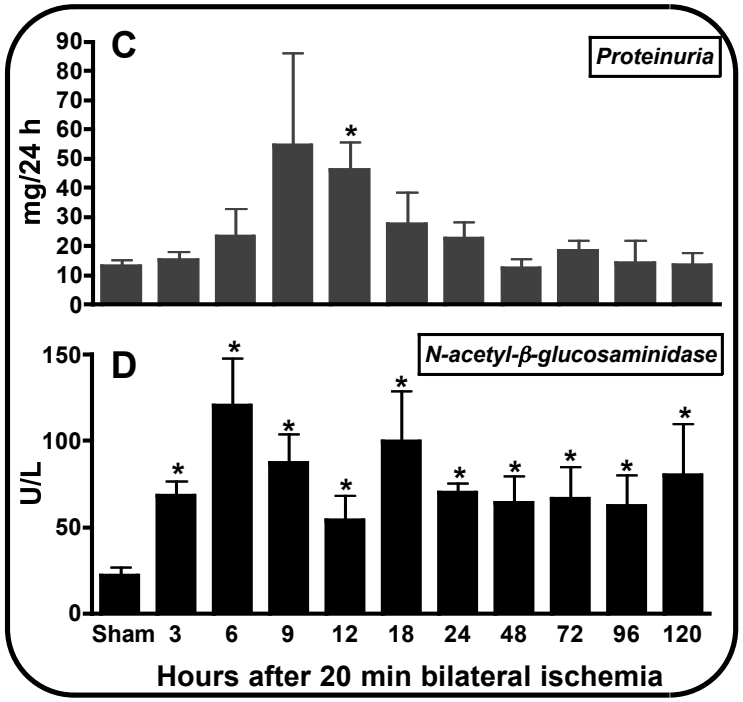
- Disadvantages of ELISA:
  - Dynamic range: 78 pg/ml to 5000 pg/ml
  - Duration: 6 hours

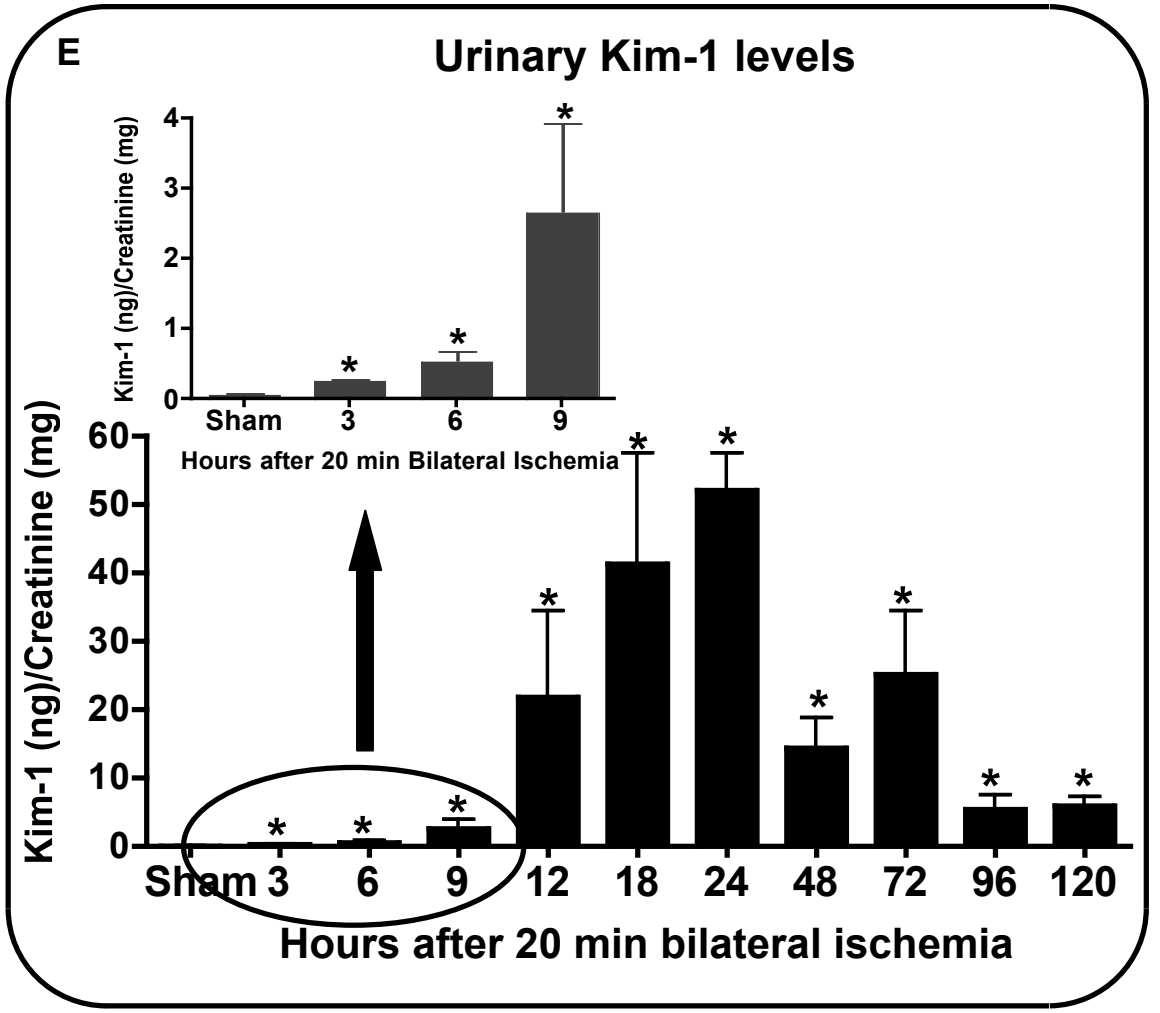

A
MARKE-Trap
Kim-1 in the urine (30 µl)
MARKE-1 Biotinylated
Streptavidin-PE
1.5 h
1.5 h
Beads
PE


B
Rat Kim-1 standard curve (4 to 40,000 pg/ml)
MFI
Kim-1 pg/ml
Lowest limit of detection: 8.8 pg/ml
Kim-1 pg/ml | MFI
5 | 11.3 ± 0.89
10 | 28.8 ± 4.97
20 | 56.8 ± 8.32
39 | 112.3 ± 20.01
78 | 221.6 ± 31.27
156 | 457.9 ± 63.48
312 | 981.7 ± 96.09
625 | 2091.1 ± 134
1250 | 4338 ± 158.09

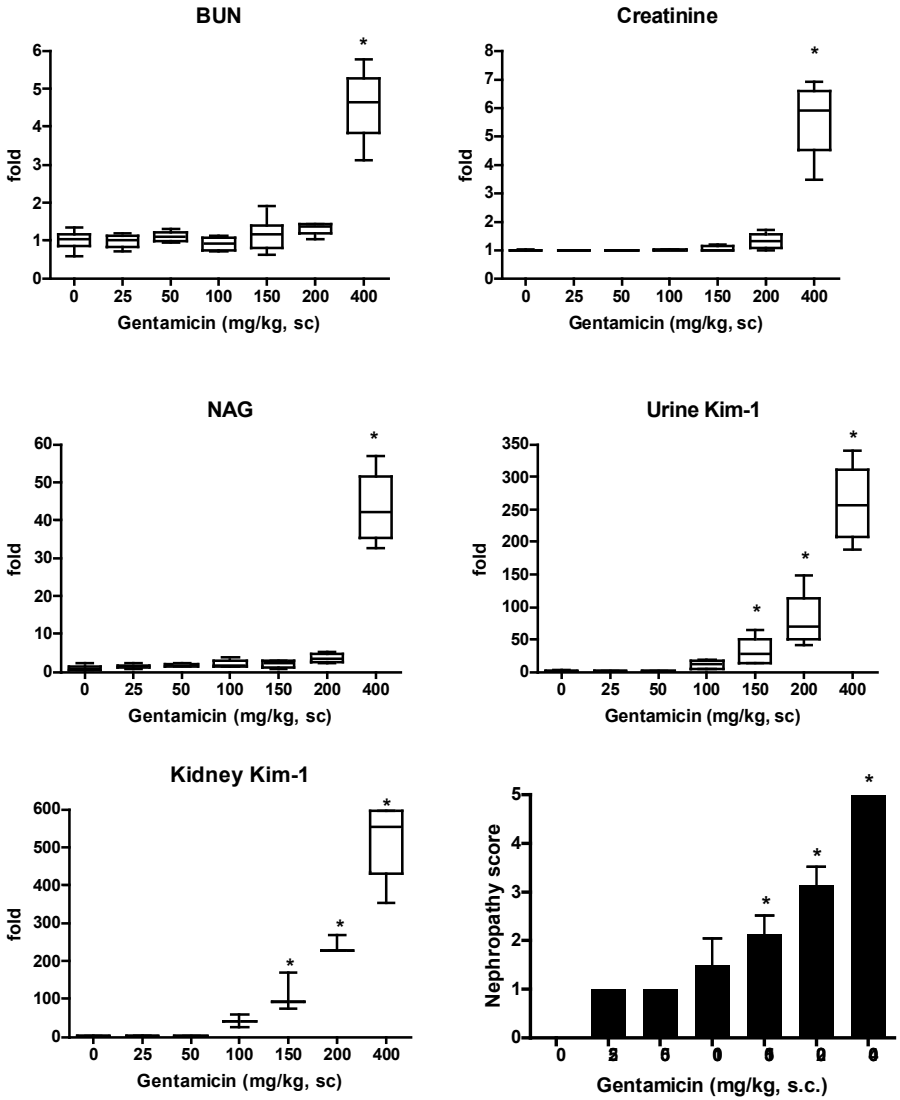

C
Precision Profile
Intra-Assay
Inter-Assay
% CV
Kim-1 (pg/ml)

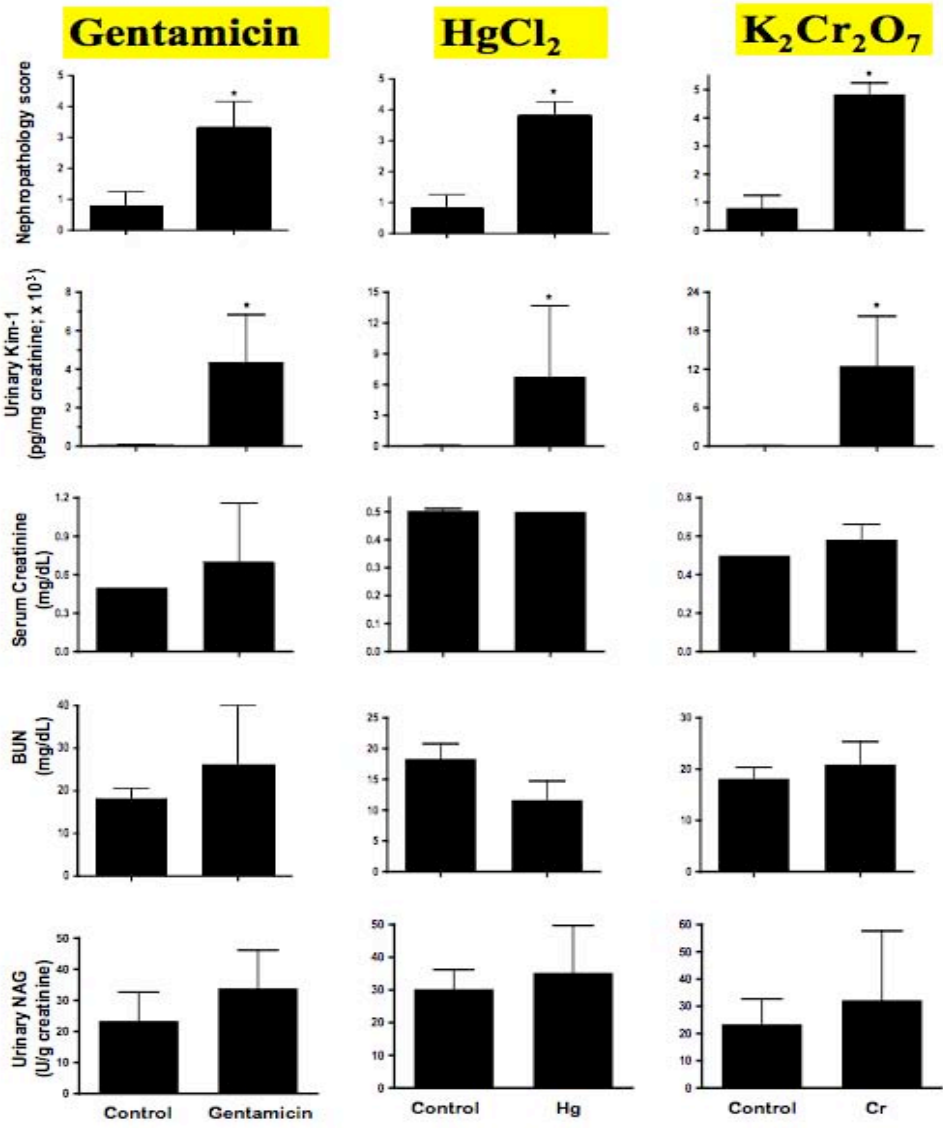

A
BUN
mg/dl
30
20
10
0
B
Serum Creatinine
mg/dl
2.0
1.5
1.0
0.5
0.0
Sham 3 6 9 12 18 24 48 72 96 120
Hours after 20 min bilateral ischemia


C
Proteinuria
mg/24 h
90
80
70
60
50
40
30
20
10
0
D
N-acetyl-β-glucosaminidase
U/L
150
100
50
0
Sham 3 6 9 12 18 24 48 72 96 120
Hours after 20 min bilateral ischemia


E
Urinary Kim-1 levels
Kim-1 (ng)/Creatinine (mg)
4
3
2
1
0
Sham 3 6 9
Hours after 20 min Bilateral Ischemia
Kim-1 (ng)/Creatinine (mg)
60
50
40
30
20
10
0
Sham 3 6 9 12 18 24 48 72 96 120
Hours after 20 min bilateral ischemia

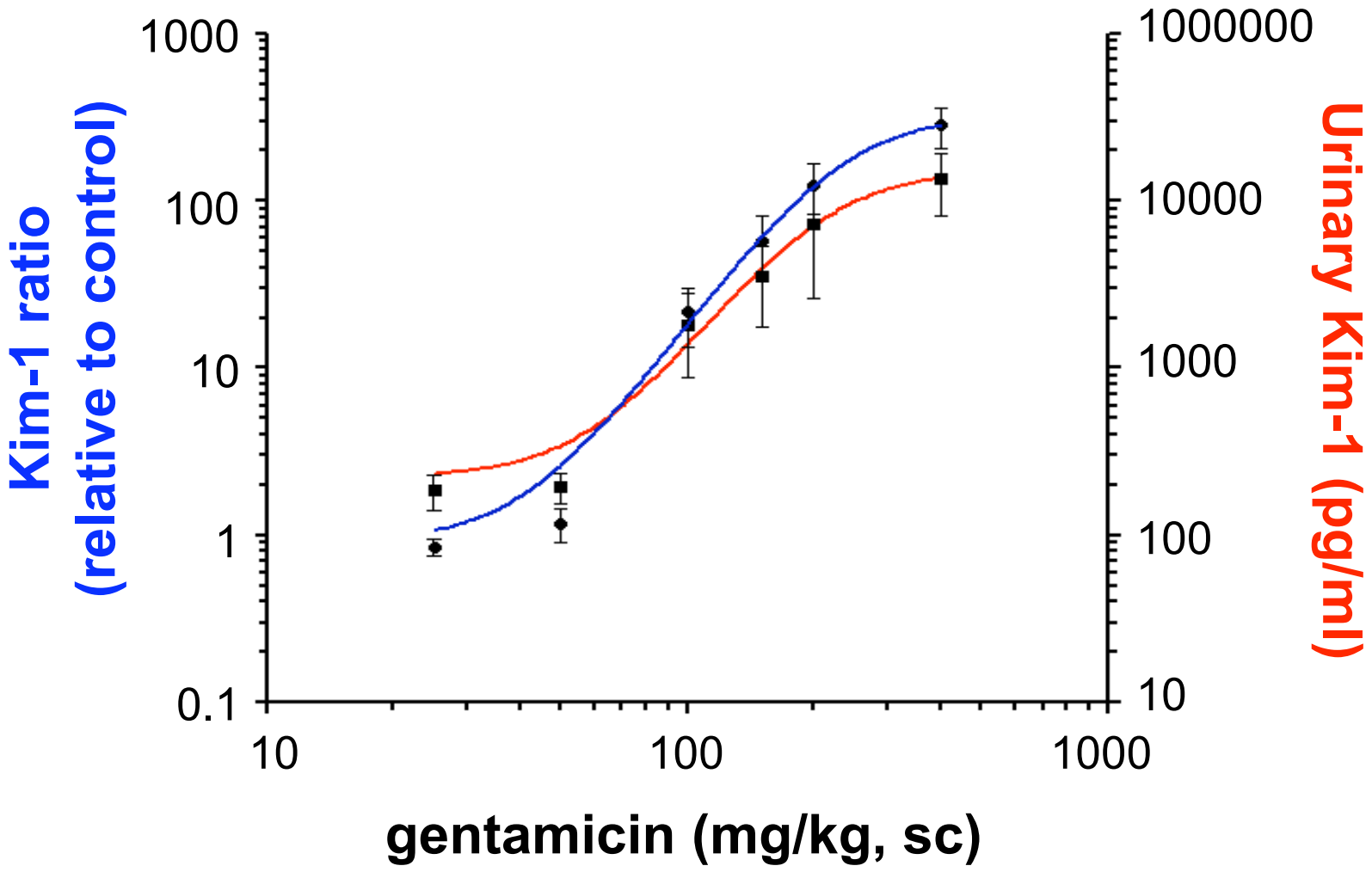
Kim-1 in preclinical model of gentamicin, mercuric cloride and chromium



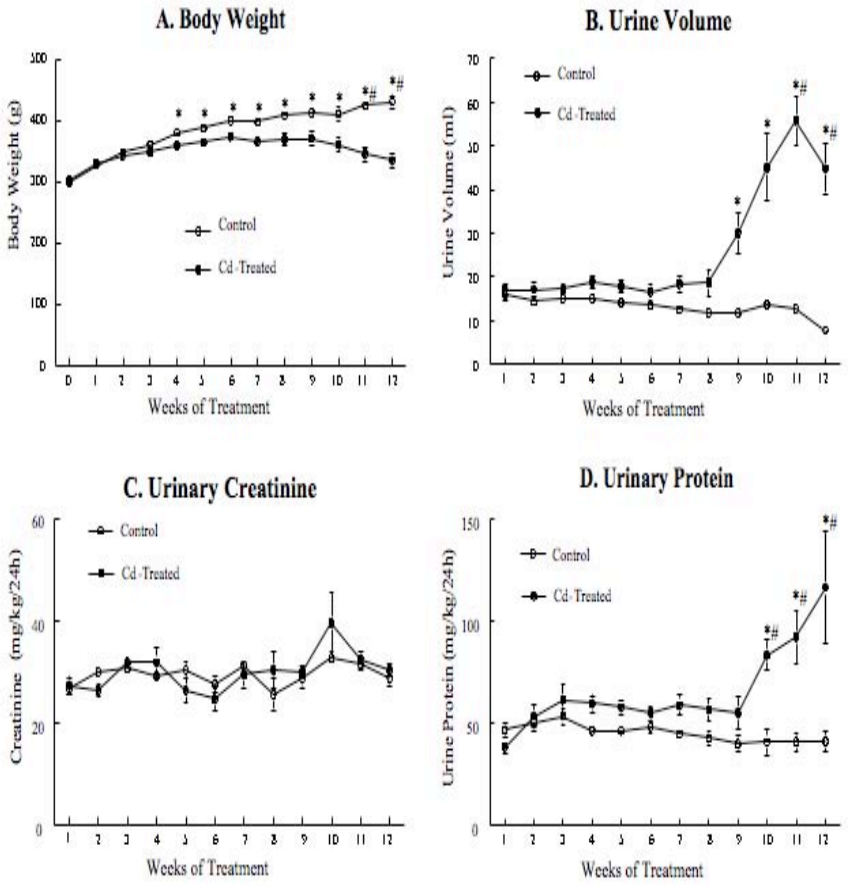


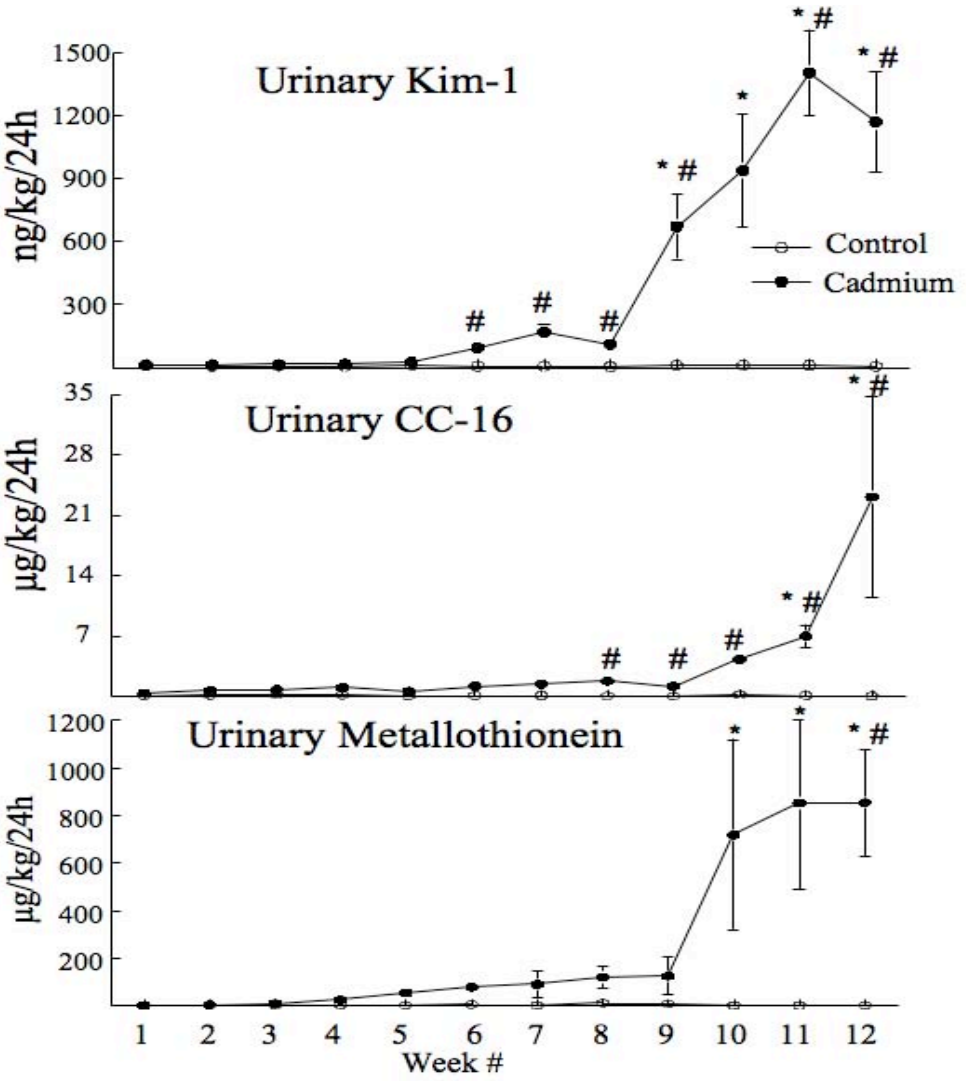
Peter Goering et al., FDA

Kim-1 – kidney mRNA vs urine protein



Kim-1 in preclinical model of Cadmium





Walter Prozialeck et al., Midwestern univ, IL

MERCK STUDY

Collaboration with Josef Ozer and Frank Sistare; In submission

4 Nephrotoxicants

Compound	Tubule	Glom.	Coll. D.	Mode of Toxicity
Gentamicin	x	(x)		Lysosomal phospholipidosis
Cisplatin	x	(x)	(x)	Direct DNA alkylation of DNA, Ox. stress
Cyclosporine	x	(x)		Complex (vasoconstrict., calcification...)
Thioacetamide	x			Oxidative stress (free radicals)

2 Hepatotoxicants

Carbon tetrachloride	Free radicals
Bromotrichloromethane	Free radicals

1 Cardiotoxicant

Isoproterenol	Oxidative stress

NOVARTIS STUDY

Collaboration with Frank Dieterle; In submission

8 Nephrotoxicants

Compound	Tubul.	Glom.	Coll. D.	Mode of Toxicity
Gentamycin	x	(x)		Lysosomal phospholipidosis
Puromycin	x (2nd)	x		Damage to podocytes
Vancomycin	x			Oxidative stress (free radicals)
Doxorubicin	x (2nd)	x		Oxidative stress to glom. filtr. membrane
Furosemide	x			Mineralization
Lithium carbonate	x	(x)	x	Influences formation of intracellular cyclic adenosine monophosphate
Cisplatin	x	(x)	(x)	Direct DNA alkylation of DNA, Ox. stress
Tacrolimus	x	(x)		Complex (vasoconstrict., calcification...)

2 Hepatotoxicants

α-Naphthyisothiocyanate (ANIT)	Cholangitis
Methapyrilene	Hepatocarcinogen (chronic treatment)

FDA News

FOR IMMEDIATE RELEASE
June 12, 2008

Media Inquiries:
Christopher DiFrancesco, 301-827-6242
Consumer Inquiries:
888-INFO-FDA

FDA, European Medicines Agency to Consider Additional Test Results When Assessing New Drug Safety

Collaborative effort by FDA and EMEA expected to yield additional safety data

In the first use of a framework allowing submission of a single application to the two agencies, the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) worked together to allow drug companies to submit the results of seven new tests that evaluate kidney damage during animal studies of new drugs. The tests measure the levels of seven key proteins or "biomarkers" found in urine that can provide additional information about drug-induced damage to kidney cells, also known as renal toxicity.

The new biomarkers are KIM-1, Albumin, Total Protein, β2-microglobulin, Cystatin C, Clusterin, and Trefoil Factor-3. For decades, both FDA and EMEA have required drug companies to submit the results of two blood tests, called blood urea nitrogen (BUN) and serum creatinine, to evaluate renal toxicity. In addition to those tests, the FDA and EMEA will now consider results from the seven new tests as part of their respective drug review processes. Although a decision by the sponsor to collect information using the new tests is voluntary, if collected, it must be submitted to FDA.

"The development of these and other biomarkers can result in important tools for better understanding the safety profile of new drugs," said Janet Woodcock, M.D., director of FDA's Center for Drug Evaluation and Research. "We hope these biomarkers will lead to human tests that detect drug-induced kidney injury in people earlier than is now possible, and help health care professionals better manage potential kidney damage from drugs."

Woodcock added that such human tests could one day open the door to the approval of more powerful drugs, especially for diseases where renal toxicity currently prevents promising experimental drugs from being approved. With more sensitive tests for renal toxicity, FDA could approve such drugs because health care professionals could closely monitor patients and halt the drug if early signs of renal toxicity appear.

Development of the new biomarkers was led by the Predictive Safety Testing Consortium (PSTC), whose members include scientists from 16 pharmaceutical companies. The PSTC was organized and led by the Critical Path Institute, a nonprofit organization that works to support FDA research collaborations that improve the development of medical products.

Researchers from Merck & Co., Whitehouse Station, N.J., and Novartis AG, Basel, Switzerland, identified the new biomarkers, tested them to prove their accuracy and usefulness, and then shared their findings with the other consortium members for further study. The consortium then submitted applications for use of the biomarkers to FDA and EMEA.

The project is the first in which a group of drug companies has worked together to propose and qualify new safety tests and then present them jointly to the FDA and EMEA for consideration. The FDA and EMEA laid the groundwork for these specific joint-agency biomarker reviews in 2004 when they developed a framework called the Voluntary Exploratory Data Submission review process.

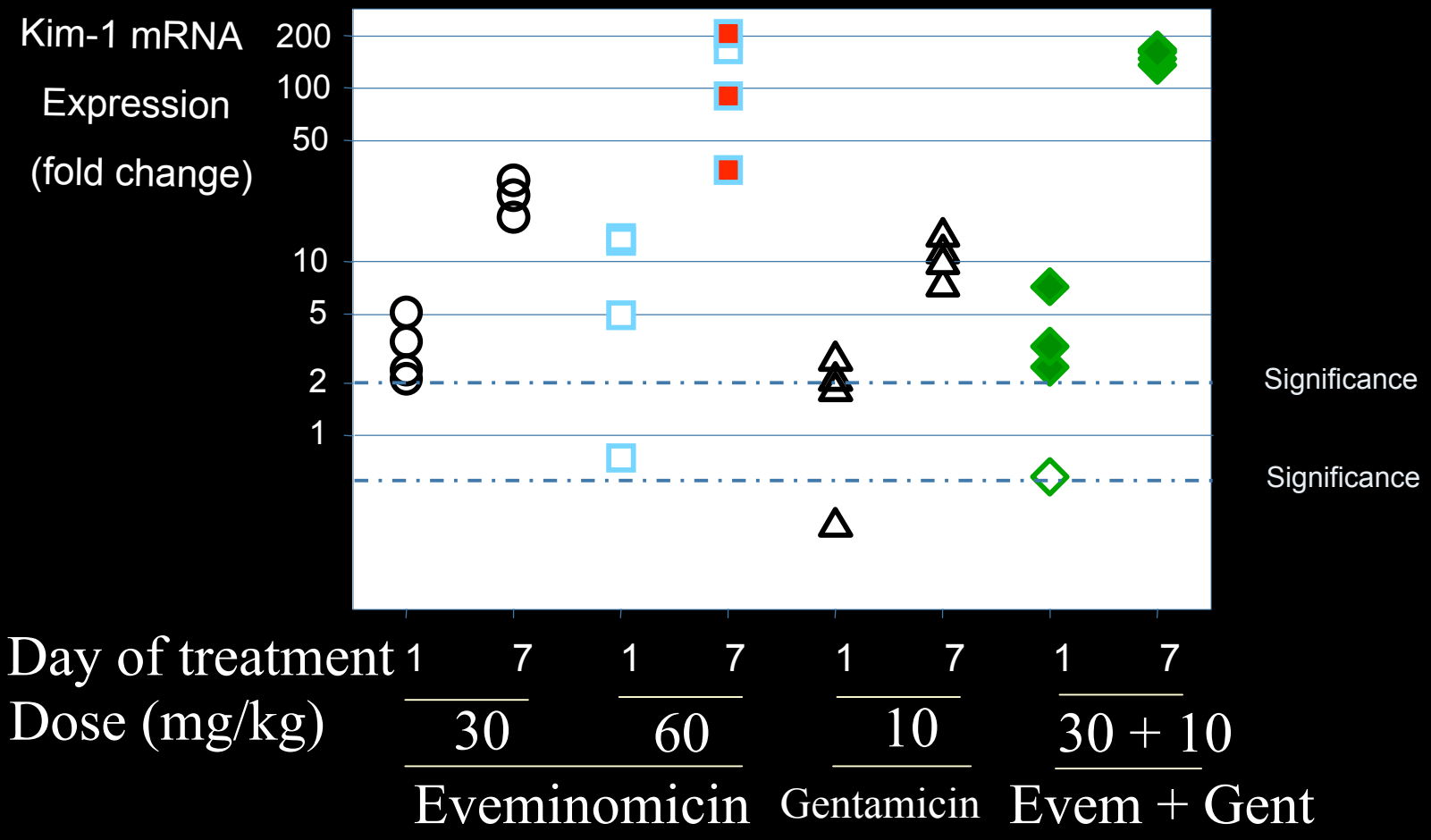
The new process allowed the PSTC to submit a single biomarker data application to both regulatory agencies, and then to meet jointly with scientists from both agencies to discuss it in detail and to address additional scientific questions posed by the regulators. Each regulatory agency then reviewed the application separately and made independent decisions on use of the new biomarkers.

FDA scientists believe that the seven new tests may provide important advantages over the BUN and creatinine tests. For example, in experiments using rats, the two traditional tests can only detect kidney damage a week after it has begun to occur. The new tests, however, are more sensitive and can detect cellular damage within hours. And while BUN and serum creatinine show that damage has occurred somewhere in the kidneys, the new tests can pinpoint which parts of the kidney have been affected.

The seven new tests were developed and will be carried out initially in rats. These tests were selected because other studies have shown that identical biomarkers are produced in human kidney cells. While the FDA and EMEA will consider these biomarkers in rat studies initially, the PSTC has begun work to further qualify the biomarkers for use in human studies. If successful, the PSTC will present a new biomarker data application to the two agencies to seek acceptance of the human biomarkers.

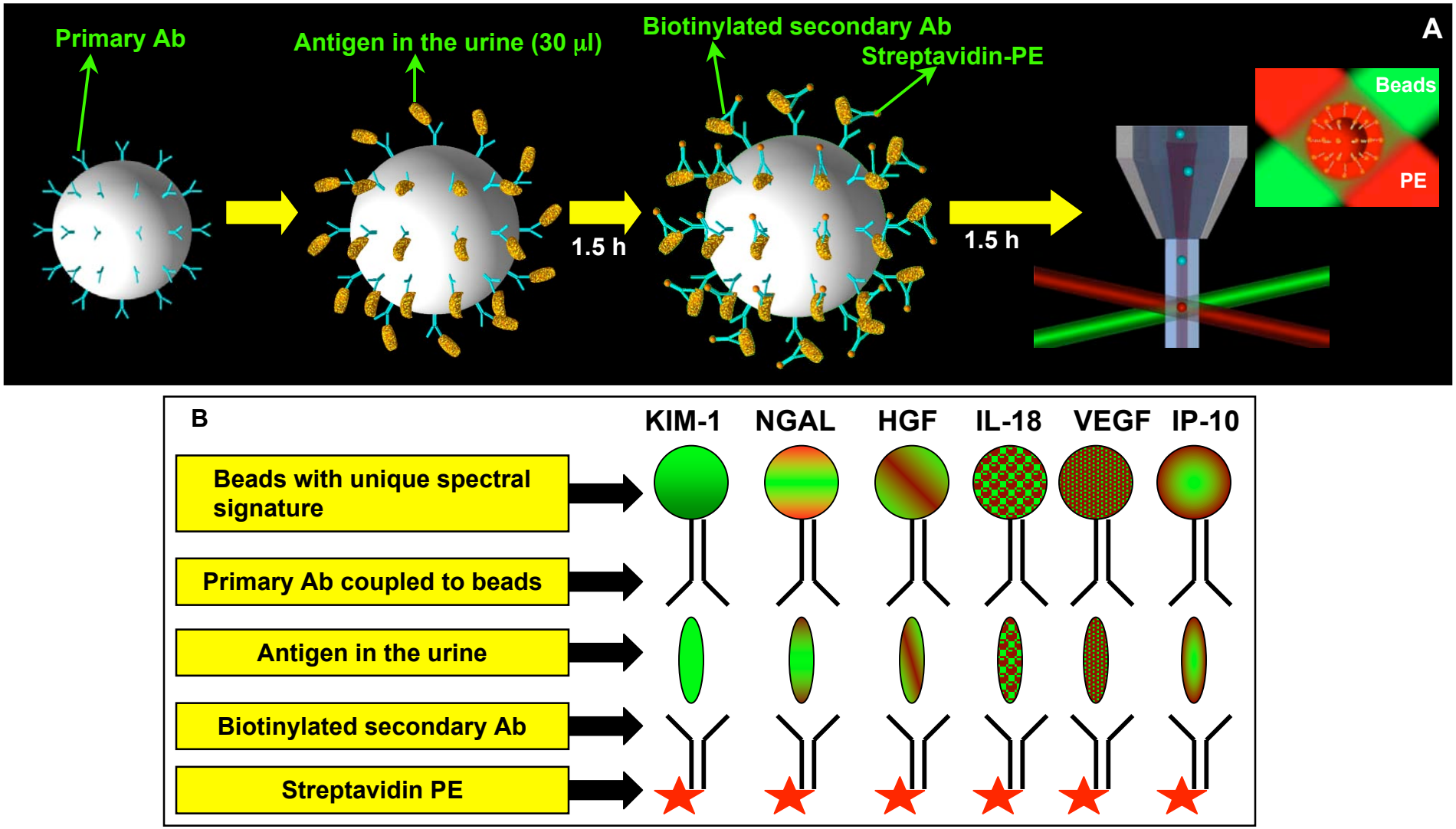
Link to the Predictive Safety Testing Consortium:
http://c-path.org/PredictiveSafetyTestingConsortium/tabid/219/Default.aspx

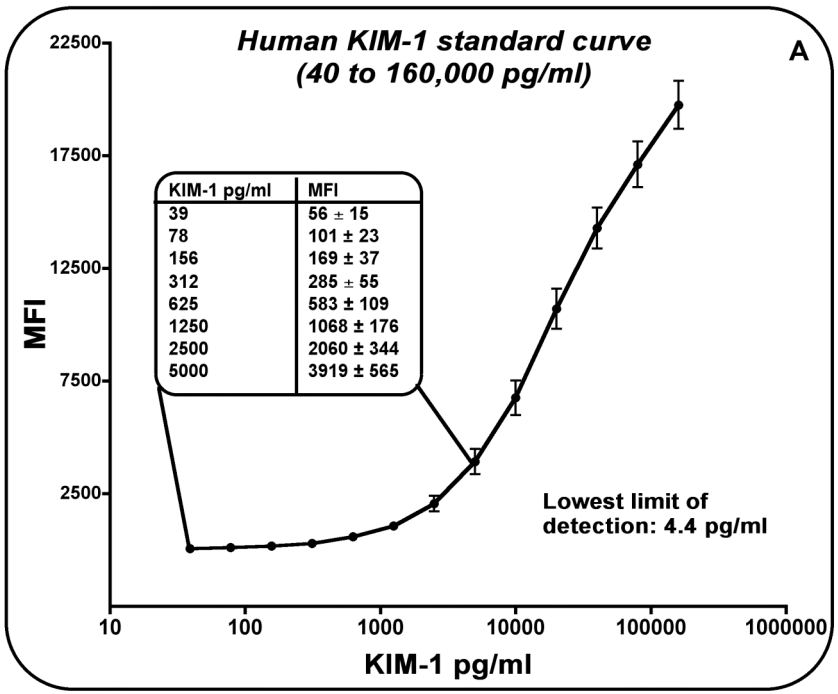
Induction of Kim-1 mRNA in Monkey Kidneys

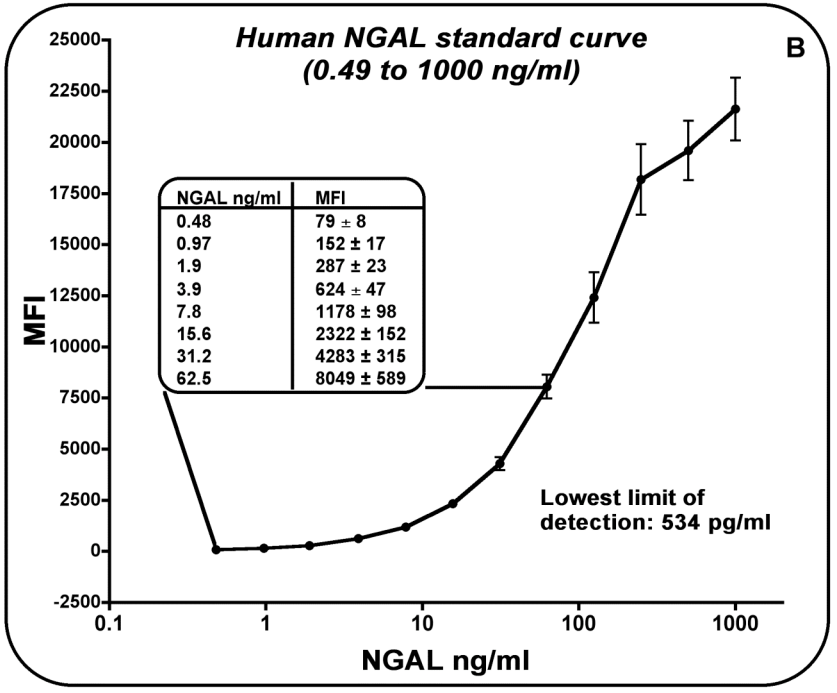


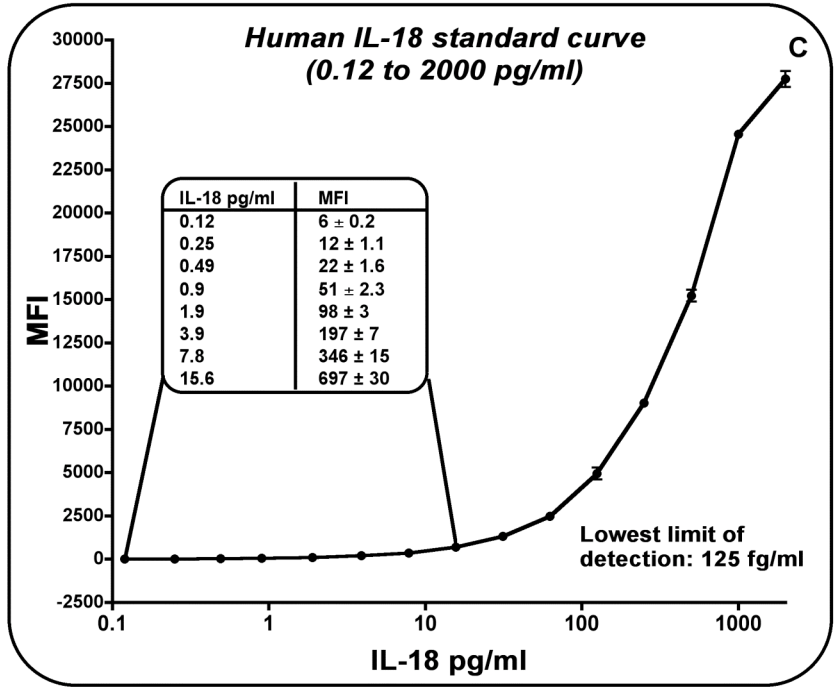
Filled symbols indicate animals with histopathological (renal) lesions

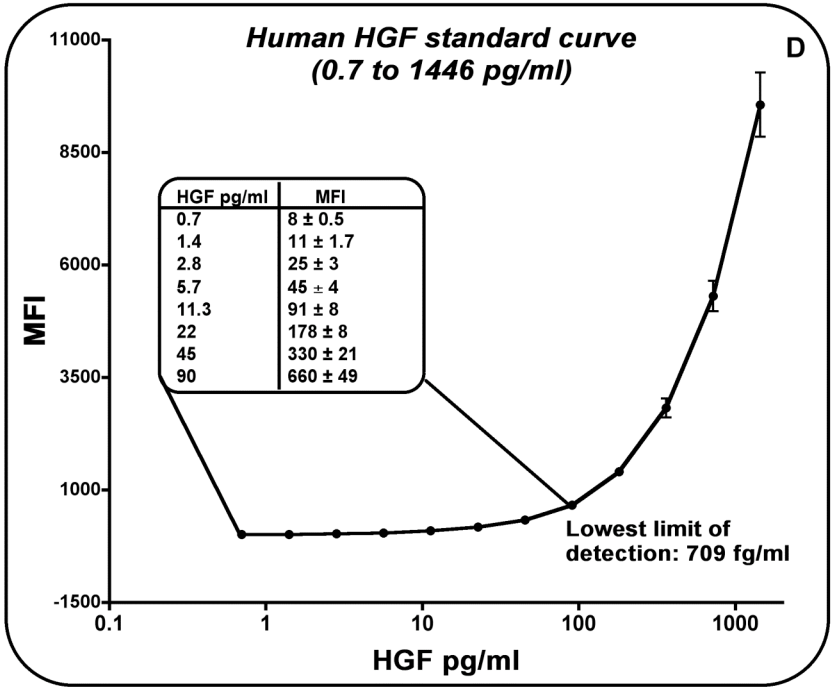
JW Davis II, FM Goodsaid, et al.
Molecular Toxicology Group
Schering-Plough Research Institute

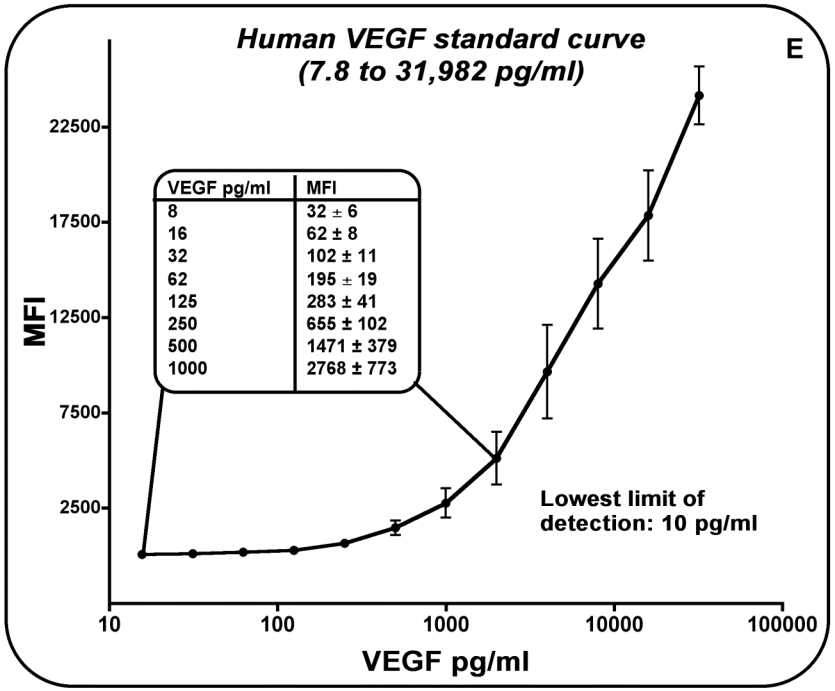

A
Primary Ab
Antigen in the urine (30 µl)
Biotinylated secondary Ab
Streptavidin-PE
1.5 h
1.5 h
Beads
PE
B
KIM-1
NGAL
HGF
IL-18
VEGF
IP-10
Beads with unique spectral signature
Primary Ab coupled to beads
Antigen in the urine
Biotinylated secondary Ab
Streptavidin PE

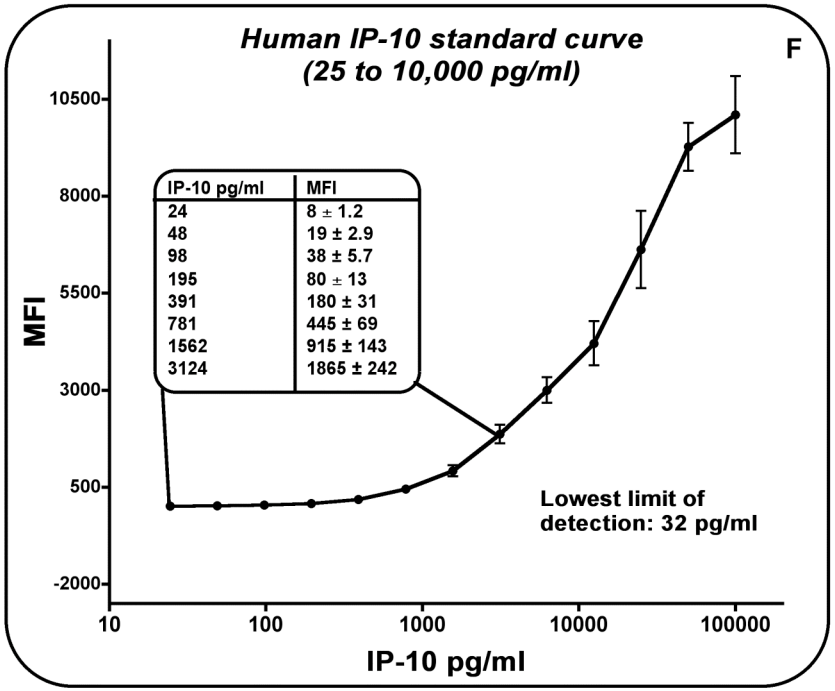

Human KIM-1 standard curve
(40 to 160,000 pg/ml)
A
KIM-1 pg/ml | MFI
39 | 56 ± 15
78 | 101 ± 23
156 | 169 ± 37
312 | 285 ± 55
625 | 583 ± 109
1250 | 1068 ± 176
2500 | 2060 ± 344
5000 | 3919 ± 565
Lowest limit of detection: 4.4 pg/ml
MFI
KIM-1 pg/ml


Human NGAL standard curve
(0.49 to 1000 ng/ml)
B
NGAL ng/ml | MFI
0.48 | 79 ± 8
0.97 | 152 ± 17
1.9 | 287 ± 23
3.9 | 624 ± 47
7.8 | 1178 ± 98
15.6 | 2322 ± 152
31.2 | 4283 ± 315
62.5 | 8049 ± 589
Lowest limit of detection: 534 pg/ml
MFI
NGAL ng/ml

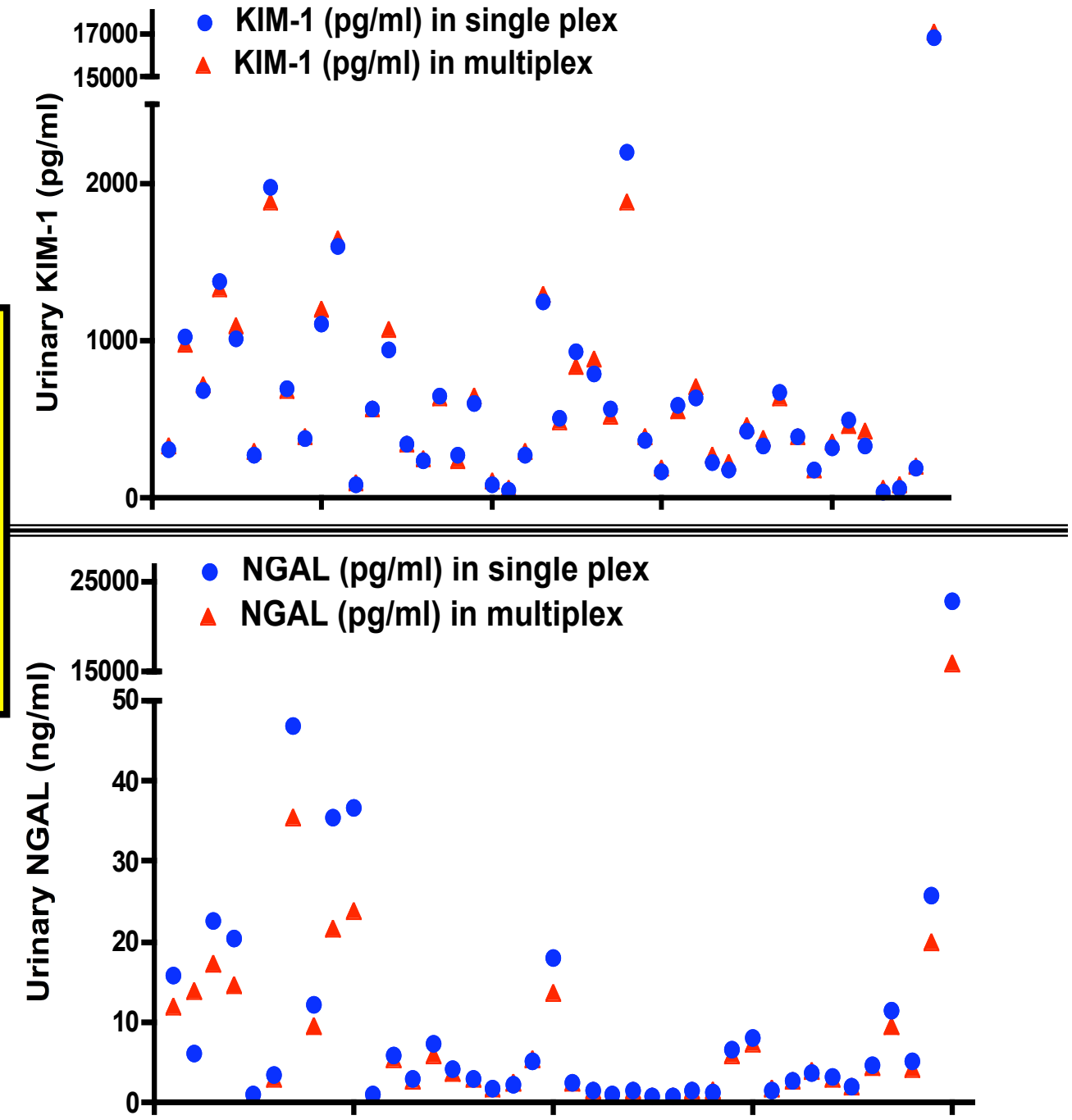

Human IL-18 standard curve
(0.12 to 2000 pg/ml)
C
IL-18 pg/ml | MFI
0.12 | 6 ± 0.2
0.25 | 12 ± 1.1
0.49 | 22 ± 1.6
0.9 | 51 ± 2.3
1.9 | 98 ± 3
3.9 | 197 ± 7
7.8 | 346 ± 15
15.6 | 697 ± 30
Lowest limit of detection: 125 fg/ml
MFI
IL-18 pg/ml

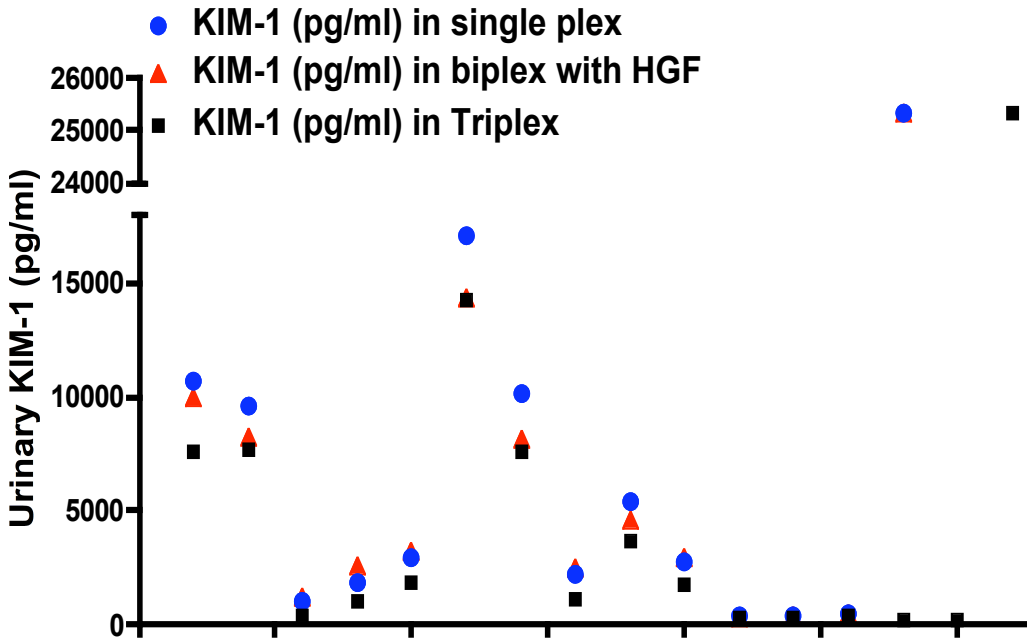

Human HGF standard curve
(0.7 to 1446 pg/ml)
D
HGF pg/ml | MFI
0.7 | 8 ± 0.5
1.4 | 11 ± 1.7
2.8 | 25 ± 3
5.7 | 45 ± 4
11.3 | 91 ± 8
22 | 178 ± 8
45 | 330 ± 21
90 | 660 ± 49
Lowest limit of detection: 709 fg/ml
MFI
HGF pg/ml

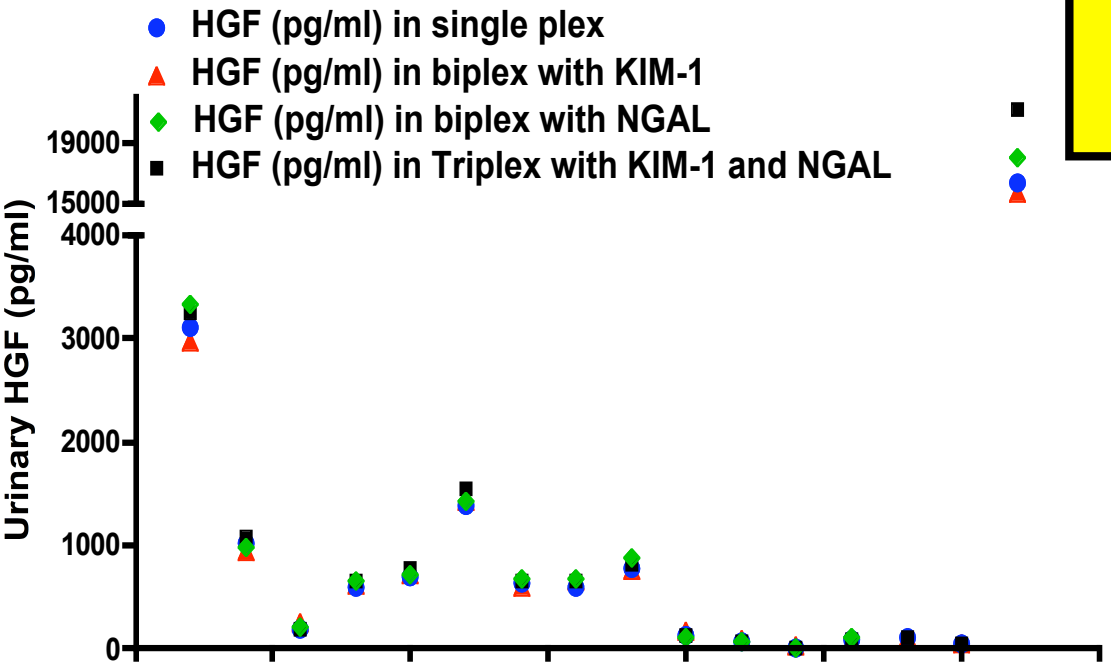

Human VEGF standard curve
(7.8 to 31,982 pg/ml)
E
VEGF pg/ml | MFI
8 | 32 ± 6
16 | 62 ± 8
32 | 102 ± 11
62 | 195 ± 19
125 | 283 ± 41
250 | 655 ± 102
500 | 1471 ± 379
1000 | 2768 ± 773
Lowest limit of detection: 10 pg/ml
MFI
22500
17500
12500
7500
2500
10
100
1000
10000
100000
VEGF pg/ml


Human IP-10 standard curve
(25 to 10,000 pg/ml)
F
IP-10 pg/ml | MFI
24 | 8 ± 1.2
48 | 19 ± 2.9
98 | 38 ± 5.7
195 | 80 ± 13
391 | 180 ± 31
781 | 445 ± 69
1562 | 915 ± 143
3124 | 1865 ± 242
Lowest limit of detection: 32 pg/ml
MFI
10500
8000
5500
3000
500
-2000
10
100
1000
10000
100000
IP-10 pg/ml

NO Interference with biplexing between KIM-1 and NGAL







NO Interference in KIM-1 or HGF values with triplexing as compared to mono or biplexing

Multiplexing compatibilities

Antigens	Bead #	KIM-1	NGAL	HGF	IL-18
KIM-1	25		√	√	√
NGAL	43	√		X	X
HGF	62	√	X		√
IL-18	42	√	X	√	

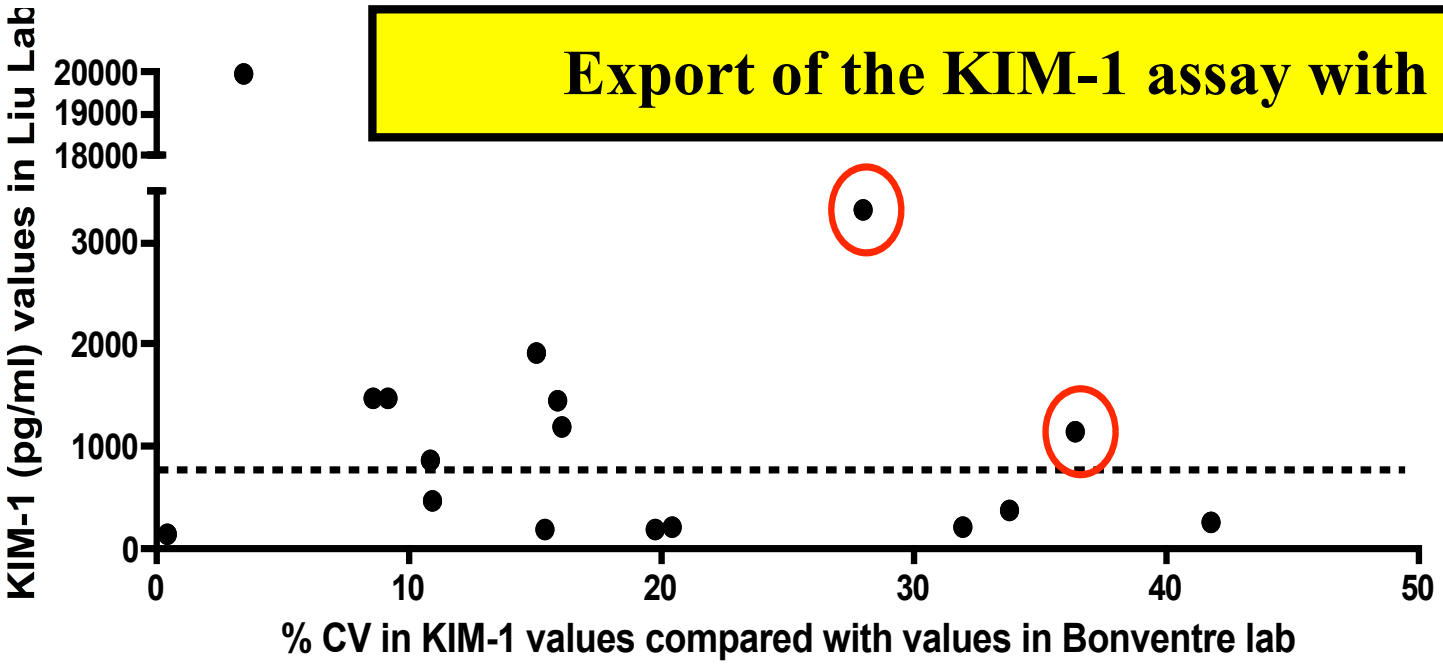
Multiplexing compatibilities

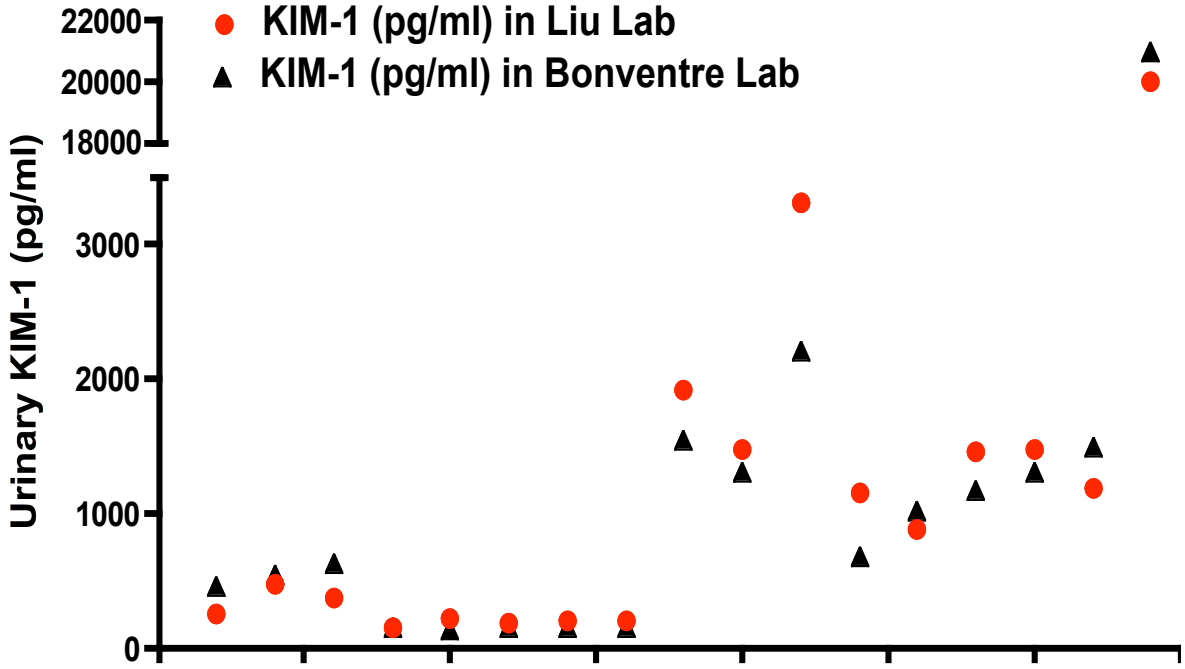
Antigens	Bead #	KIM-1	NGAL	HGF	IL-18
KIM-1	25				
NGAL	43				
HGF	62				
IL-18	42				

Multiplexing compatibilities

Antigens	Bead #	KIM-1	NGAL	HGF	IL-18	VEGF	IP-10	FABP
KIM-1	25							
NGAL	43							
HGF	62							
IL-18	43							
VEGF	42							
IP-10	41							
FABP	42							

Export of the KIM-1 assay with R^2=0.99

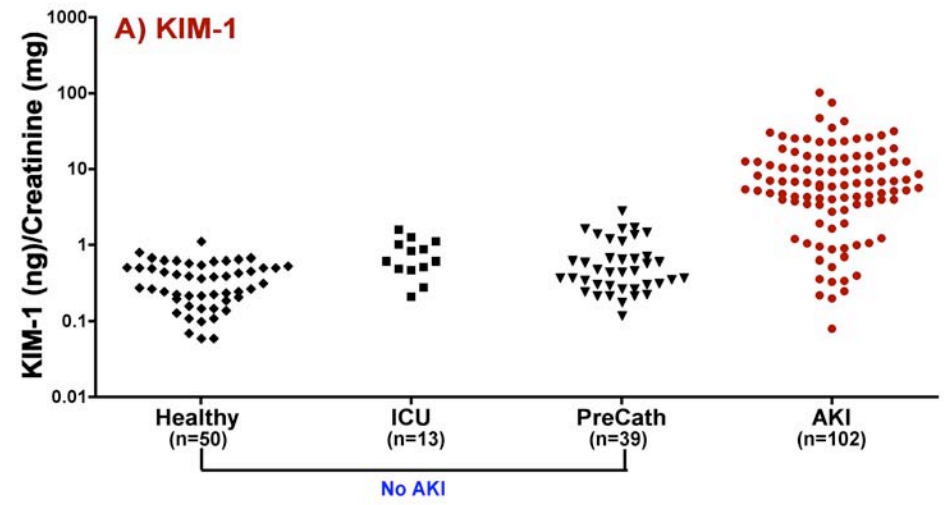


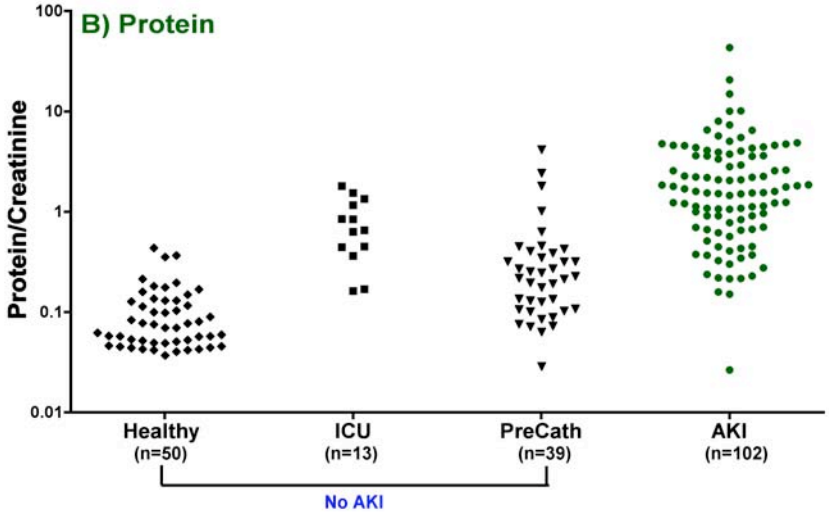


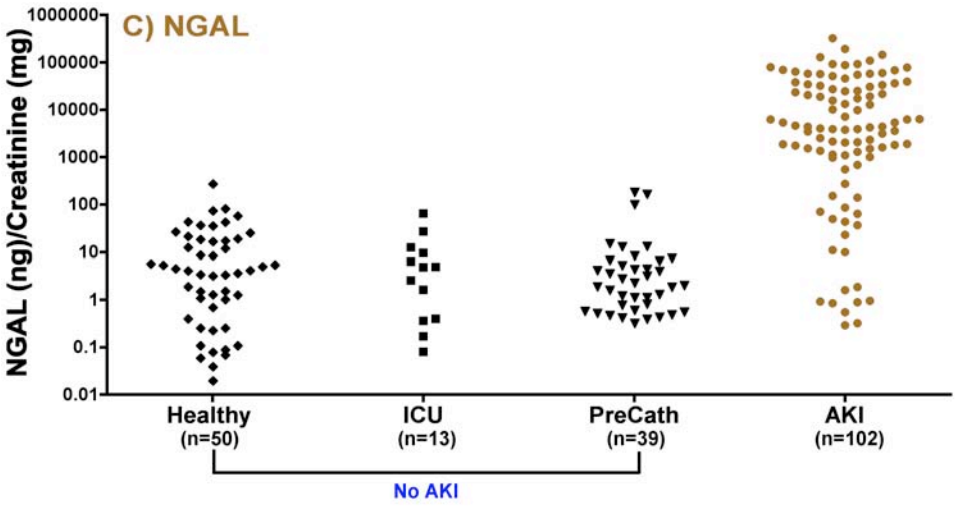
Patient selection in cross sectional study

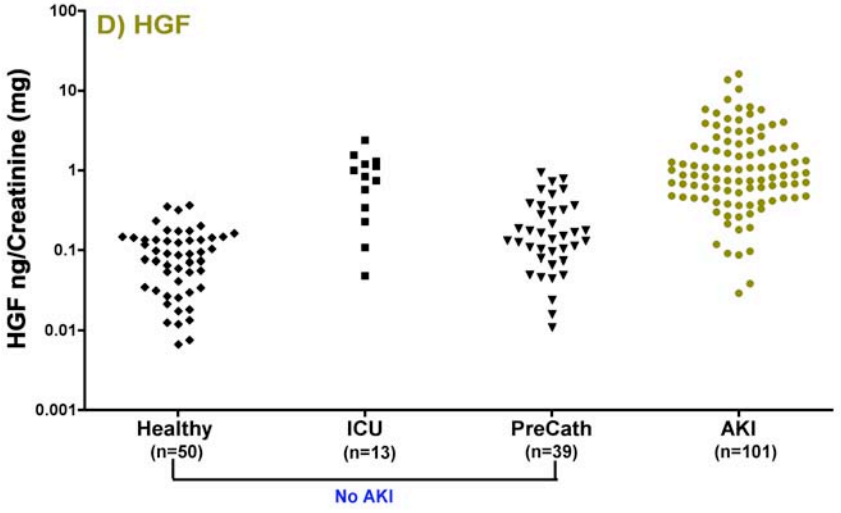
- **AKI (n=102):** ≥ 50% increase in serum creatinine over baseline values caused by ischemia, sepsis, or nephrotoxicants

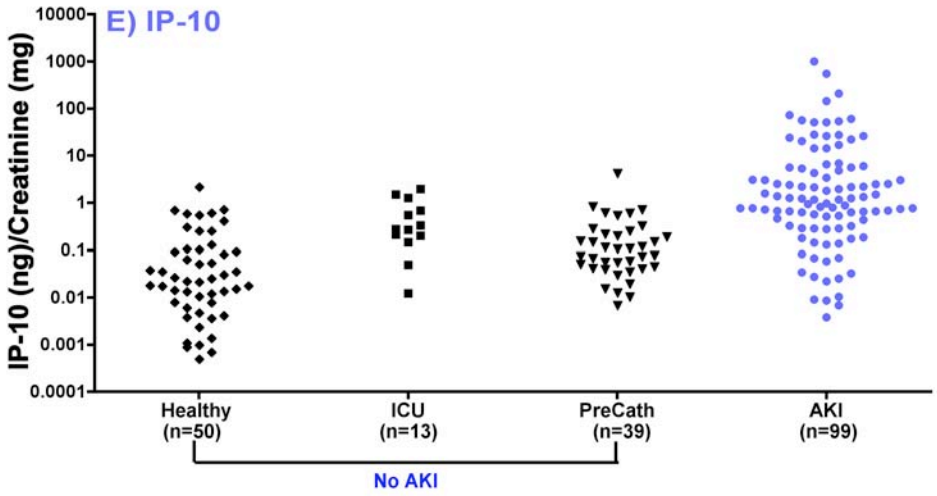
- **NO AKI (n=102)**
  - **Healthy Volunteers (n=50):** no known kidney disease, excluded if they reported a recent hospitalization, diagnosis of CKD, or current treatment with nephrotoxic medications (non-steroidal anti-inflammatory drugs were allowed)
  - **ICU patients (n=13):** Patients admitted in intensive care unit without any clinically diagnosed kidney disease (stable < 1.3 mg/dL and urine output)
  - **Precath (n=39):** Patients undergoing cardiac catheterization with eGFR > 50 ml/min/1.73 m^2 (pre procedure samples)

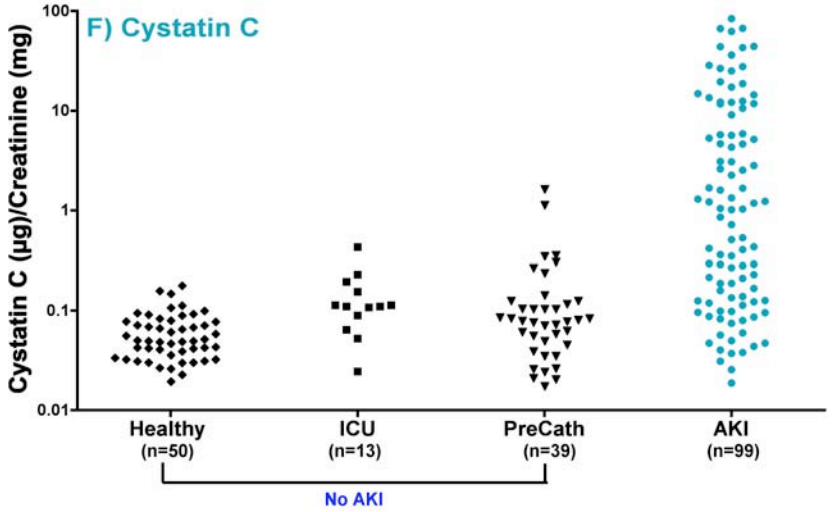

A) KIM-1
KIM-1 (ng)/Creatinine (mg)
1000
100
10
1
0.1
0.01
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=102)
No AKI

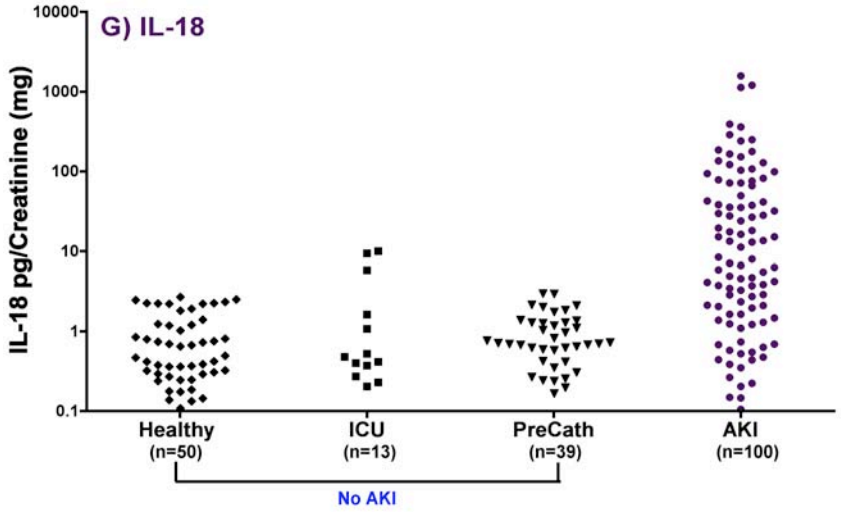

B) Protein
Protein/Creatinine
100
10
1
0.1
0.01
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=102)
No AKI

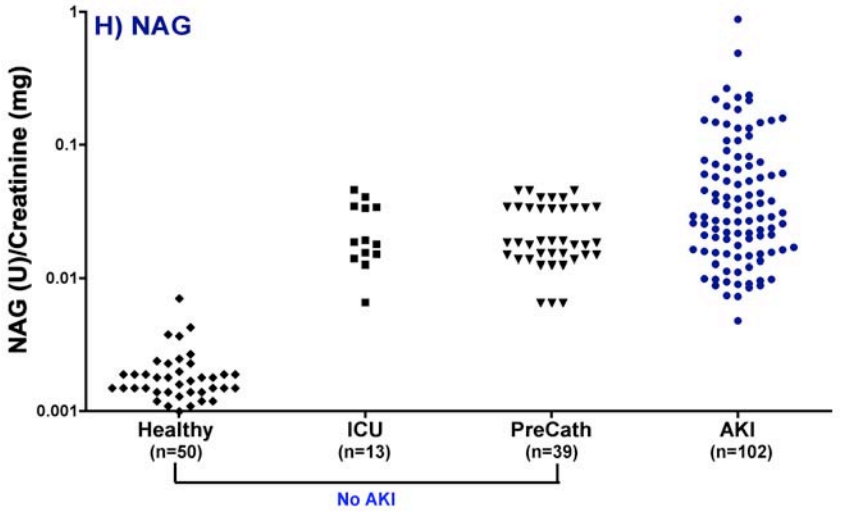

C) NGAL
NGAL (ng)/Creatinine (mg)
1000000
100000
10000
1000
100
10
1
0.1
0.01
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=102)
No AKI

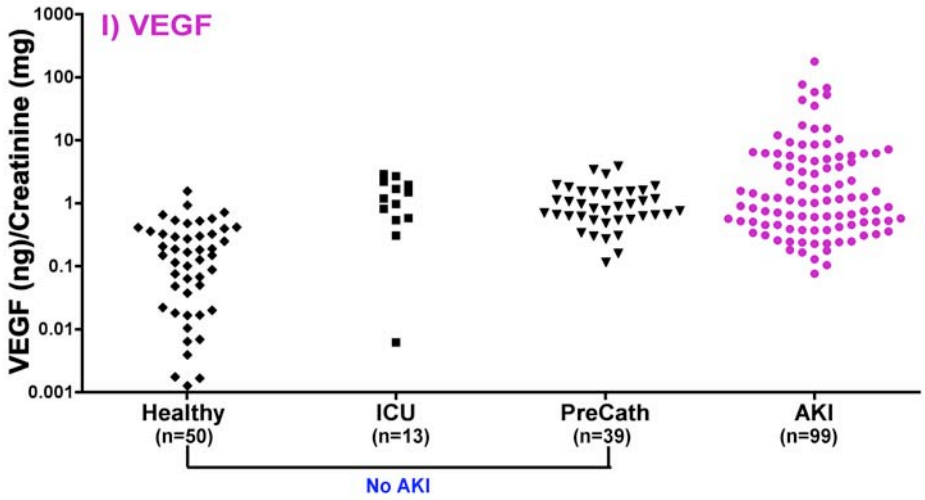

D) HGF
HGF ng/Creatinine (mg)
100
10
1
0.1
0.01
0.001
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=101)
No AKI


E) IP-10
IP-10 (ng)/Creatinine (mg)
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=99)
No AKI


F) Cystatin C
Cystatin C (µg)/Creatinine (mg)
100
10
1
0.1
0.01
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=99)
No AKI


G) IL-18
IL-18 pg/Creatinine (mg)
10000
1000
100
10
1
0.1
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=100)
No AKI


H) NAG
NAG (U)/Creatinine (mg)
1
0.1
0.01
0.001
Healthy (n=50)
ICU (n=13)
PreCath (n=39)
AKI (n=102)
No AKI


I) VEGF
VEGF (ng)/Creatinine (mg)
1000
100
10
1
0.1
0.01
0.001
Healthy
(n=50)
ICU
(n=13)
PreCath
(n=39)
AKI
(n=99)
No AKI

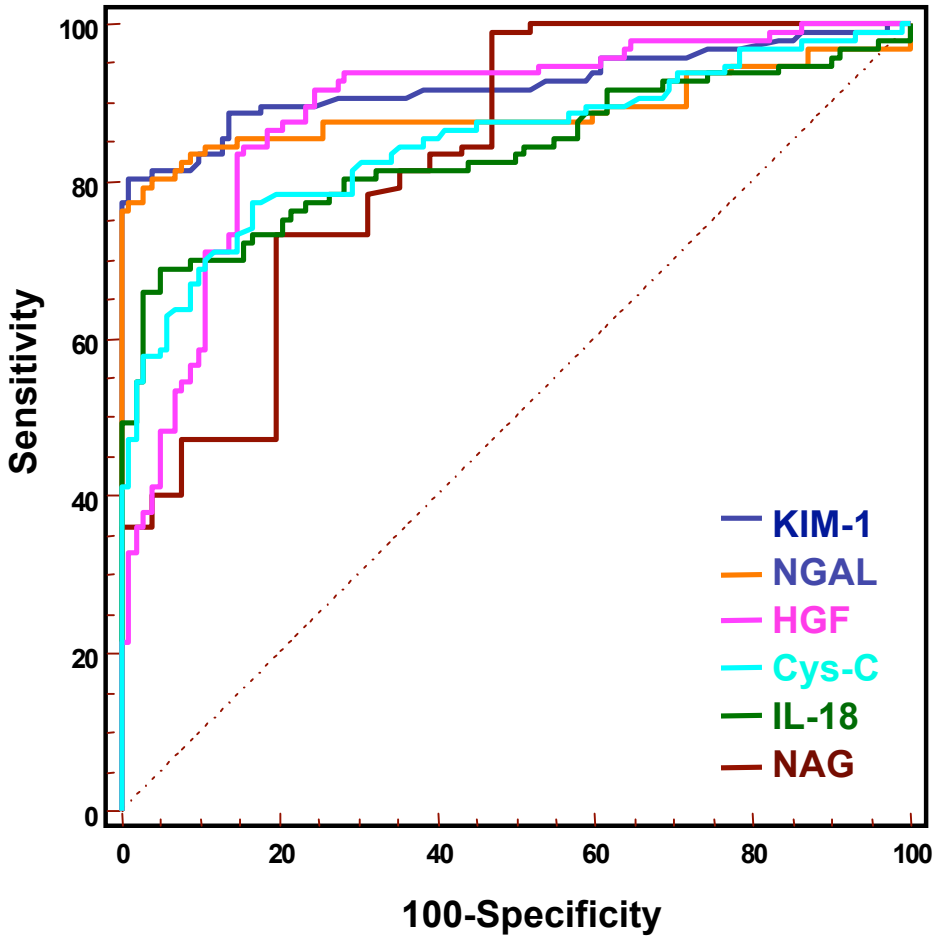
Performance characteristics of 9 urinary biomarkers

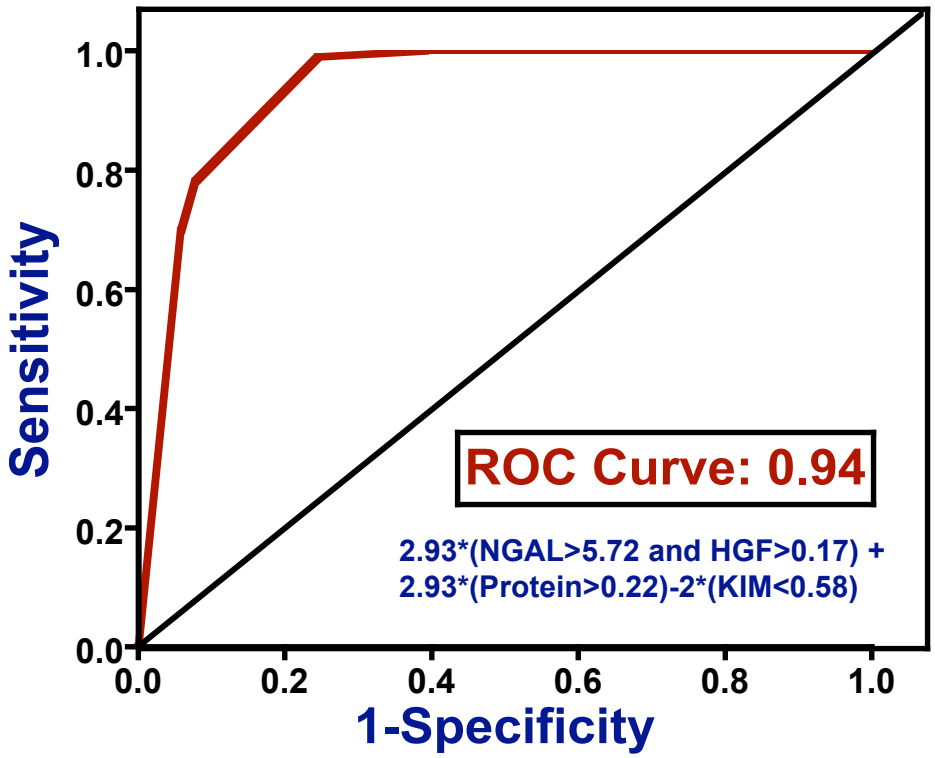
	AKI (N = 102) vs healthy individuals (N = 50)				AKI (N = 102) vs all non-AKI controls (N = 102)			
Biomarker*	**AUC-ROC (95% CI)**	**Cutoff**	**Sensitivity**	**Specificity**	**AUC-ROC (95% CI)**	**Cutoff**	**Sensitivity**	**Specificity**
KIM-1 (ng/mg)	0.95 (0.90 – 0.98)	0.70	90%	96%	0.93 (0.88 – 0.96)	1.73	80%	99%
Protein (mg/mg)	0.98 (0.94 – 1.00)	0.22	96%	94%	0.91 (0.87 – 0.95)	0.46	81%	87%
NGAL (ng/mg)	0.89 (0.83 – 0.94)	83.0	80%	98%	0.89 (0.84 – 0.93)	82.7	80%	96%
HGF (ng/mg)	0.96 (0.92 – 0.99)	0.23	91%	94%	0.89 (0.84 – 0.93)	0.37	84%	84%
IP-10 (ng/mg)	0.89 (0.83 – 0.93)	0.13	85%	80%	0.84 (0.79 – 0.89)	0.62	69%	89%
Cystatin C (ug/mg)	0.90 (0.84 – 0.94)	0.11	78%	94%	0.85 (0.80 – 0.90)	0.12	78%	83%
IL-18 (pg/mg)	0.85 (0.78 – 0.90)	2.30	69%	92%	0.83 (0.77 – 0.88)	2.74	68%	95%
NAG (U/mg)	1.00 (0.98 – 1.00)	0.007	99%	100%	0.83 (0.77 – 0.88)	0.015	80%	65%
VEGF (ng/mg)	0.90 (0.84 – 0.94)	0.43	77%	84%	0.73 (0.66 – 0.79)	0.64	62%	62%
Urine creatinine (mg)	0.78 (0.70 – 0.84)	62	67%	76%	0.72 (0.65 – 0.78)	37	45%	92%

Urinary biomarkers with respect to clinical outcome

	In hospital mortality (36%)			Renal replacement therapy (46%)			Mortality or renal replacement therapy (60%)		
	Died	Survived	P value	Yes	No	P value	Yes	No	P value
Cystatin C (ug/mg)	1.19	0.72	0.63	1.21	0.69	0.87	1.03	0.85	0.60
HGF (ng/mg)	1.23	0.77	0.07	1.13	0.76	0.24	1.15	0.74	0.03
IL-18 (pg/mg)	16.89	6.12	0.27	16.22	5.90	0.29	15.19	4.93	0.29
IP-10 (ng/mg)	1.21	0.97	0.74	1.25	0.92	0.66	1.38	0.85	0.29
KIM-1 (ng/mg)	10.17	5.19	0.008	7.24	5.19	0.37	6.84	4.80	0.10
Protein (mg/mg)	2.20	1.51	0.13	2.21	1.14	0.02	2.20	1.13	0.02
NGAL (ng/mg)	5384.4	3113.2	0.94	12883.3	2063.0	0.14	6389.1	2044.3	0.40
NAG (U/mg)	0.05	0.03	0.02	0.06	0.02	0.003	0.06	0.02	<0.001
VEGF (ng/mg)	1.63	0.91	0.07	1.24	0.95	0.11	1.55	0.75	0.008

Biomarker combination approach using Logic Regression

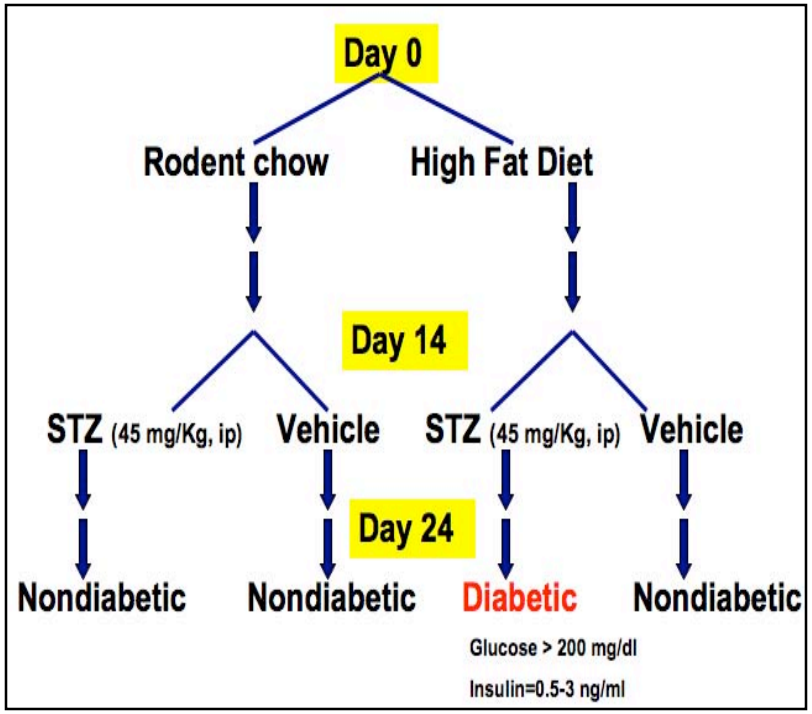




Patient selection in prospective study

Prospective Cohort	Study design	N	Aim of the study
Pts with malignant mesothelioma undergoing lung resection with intracavitary cisplatin	• Dose 225 mg/m2 cisplatin x 1 h in thoracic cavity after resection • Samples collected prior to surgery, 4h post cisplatin, every 24 h for 5 d	30	Compare biomarkers in cisplatin nephrotoxicity

Rat model of type 2 diabetic nephropathy



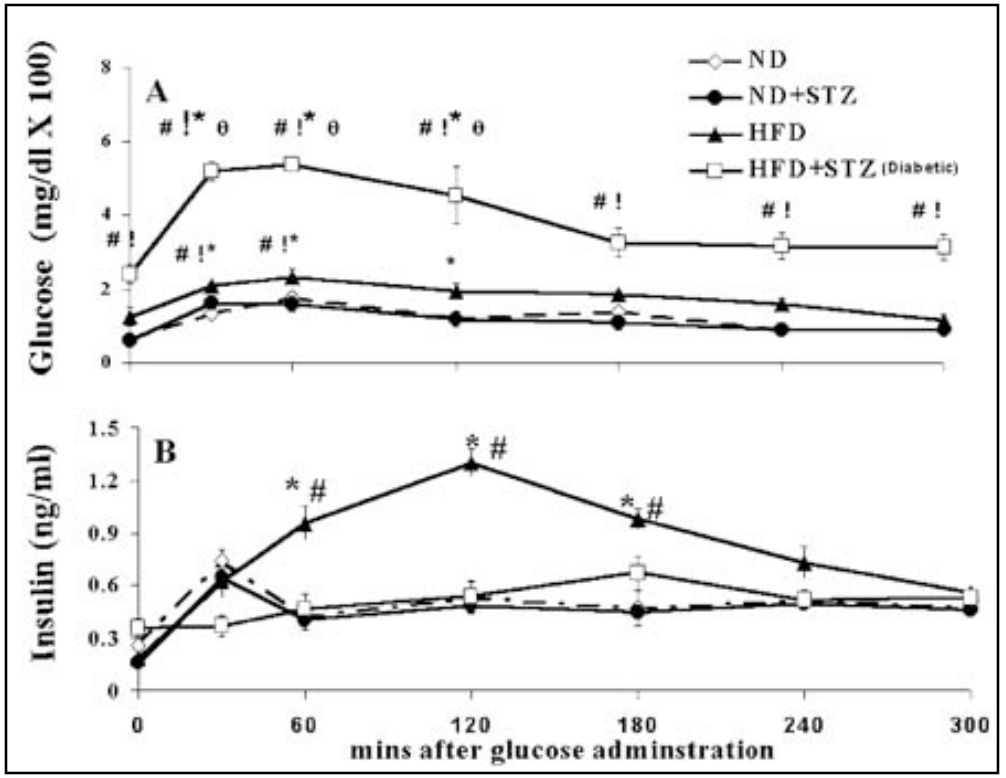
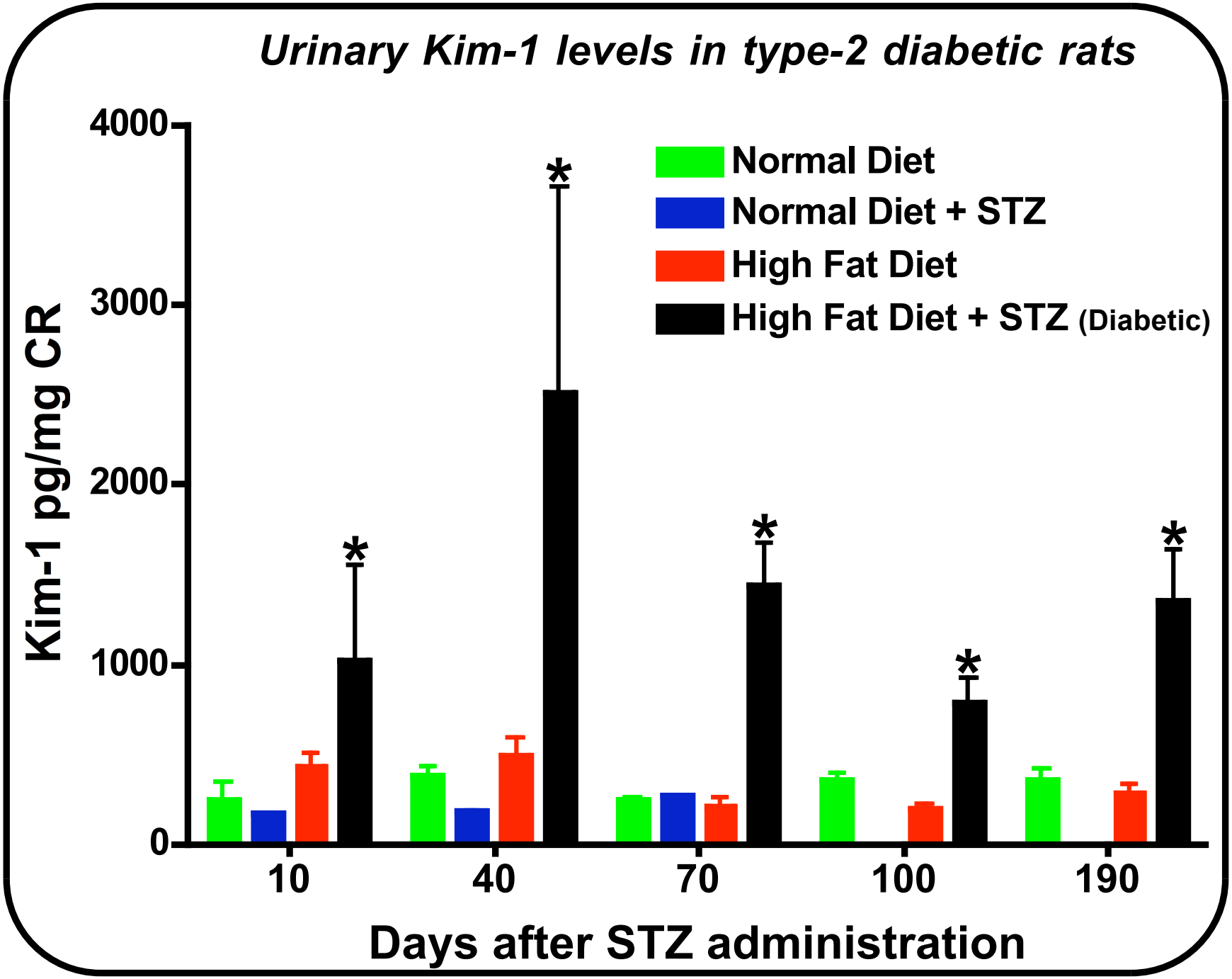


TABLE 1
Induction and characterization of type 2 diabetes

Parameters	Normal Diet-Fed Rats				High Fat Diet-Fed Rats			
	ND Nondiabetic		+STZ (ND + STZ) Nondiabetic		HFD Nondiabetic		+STZ (HFD + STZ) Diabetic	
	24 Day	6 Month	24 Day	6 Month	24 Day	6 Month	24 Day	6 Month
Plasma glucose (mg/dl)	123 ± 10	150 ± 20	164 ± 42	140 ± 32	142 ± 18	130 ± 10	450 ± 66[a]	530 ± 40[a]
Plasma insulin (ng/dl)	1.6 ± 0.4	2.6 ± 0.5	1.4 ± 0.3	2.4 ± 0.3	2.3 ± 0.2	2.9 ± 0.2	1.8 ± 0.4	0.7 ± 0.2[a]
Plasma leptin (ng/dl)	1.4 ± 0.1	3.1 ± 0.3	2.8 ± 0.25	2.9 ± 0.4	2.4 ± 0.2	2.5 ± 0.2	0.7 ± 0.05[a]	0.3 ± 0.08[a]
Plasma triglycerides (mg/dl)	68 ± 10	67 ± 12	70 ± 6	72 ± 13	141 ± 15	138 ± 14	1141 ± 20[a]	1267 ± 38[a]
Plasma free fatty acids (mEq/l)	54 ± 4	55 ± 10	52 ± 5	54 ± 12	73 ± 18	71 ± 23	124 ± 14[a]	234 ± 13[a]
Glycated Hb (%)	2 ± 0.02	1.8 ± 0.05	2 ± 0.05	1.9 ± 0.06	2 ± 0.02	1.91 ± .05	4.3 ± 0.23[a]	5.22 ± 0.3[a]
Metformin treatment[b] (plasma glucose, mg/dl)							175 ± 35[c]	
Rosiglitazone treatment[b] (plasma glucose, mg/dl)							135 ± 34[c]	


Urinary Kim-1 levels in type-2 diabetic rats
Kim-1 pg/mg CR
4000
3000
2000
1000
0
Normal Diet
Normal Diet + STZ
High Fat Diet
High Fat Diet + STZ (Diabetic)
*
*
*
*
10
40
70
100
190
Days after STZ administration

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Regression of Microalbuminuria in Type 1 Diabetes

Bruce A. Perkins, M.D., M.P.H., Linda H. Ficociello, M.Sc., Kristen H. Silva, B.A., Dianne M. Finkelstein, Ph.D., James H. Warram, M.D., Sc.D., and Andrzej S. Krolewski, M.D., Ph.D.

Microalbuminuria and the Risk for Early Progressive Renal Function Decline in Type 1 Diabetes

Bruce A. Perkins,*† Linda H. Ficociello,* Betsy E. Ostrander,* Kristen H. Silva,* Janice Weinberg,‡ James H. Warram,*§ and Andrzej S. Krolewski*§‖

Journal of the American Society of Nephrology J Am Soc Nephrol 18: 1353–1361, 2007

High-Normal Serum Uric Acid Is Associated with Impaired Glomerular Filtration Rate in Nonproteinuric Patients with Type 1 Diabetes

Elizabeth T. Rosolowsky,*† Linda H. Ficociello,* Nicholas J. Maselli,* Monika Niewczas,*‡ Amanda L. Binns,* Bijan Roshan,* James H. Warram,* and Andrzej S. Krolewski*‡

*Research Division, Joslin Diabetes Center, †Division of Endocrinology at Children's Hospital Boston, and ‡Department of Medicine at Brigham and Women Hospital, Harvard Medical School, Boston, Massachusetts

STUDY POPULATION

patients with T1DM
from the 2nd Joslin Study of the Natural History of Microalbuminuria

T1DM; Age: 15-64 years;
Duration of diabetes <40 years
Albuminuria status determined on the basis
of multiple ACR measurements over two year period of time
preceding the examination

PHASE 1

Pilot cohort n =156

Low normoalbuminuria
ACR: 0 – 12.7; n=78

High microalbuminuria
ACR: 53-342, n=78

PHASE 2

Entire study n=685

normoalbuminuria
ACR: 10.26±5.05; n=370

microalbuminuria
ACR: 77.86±65.61 n=315

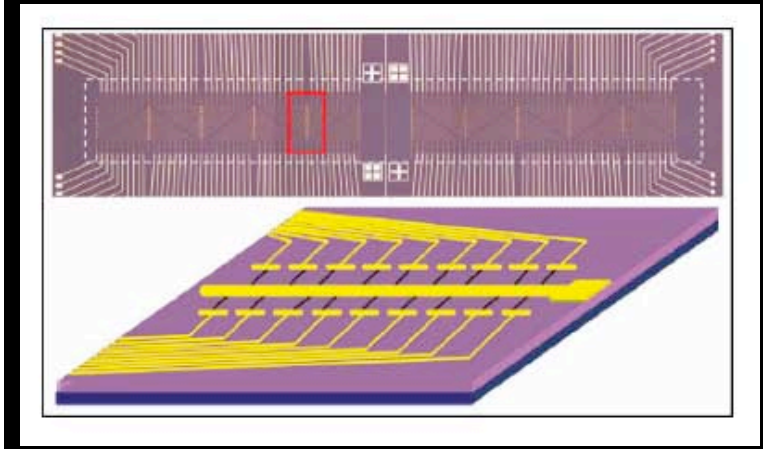
Inclusion/Exclusion Criteria

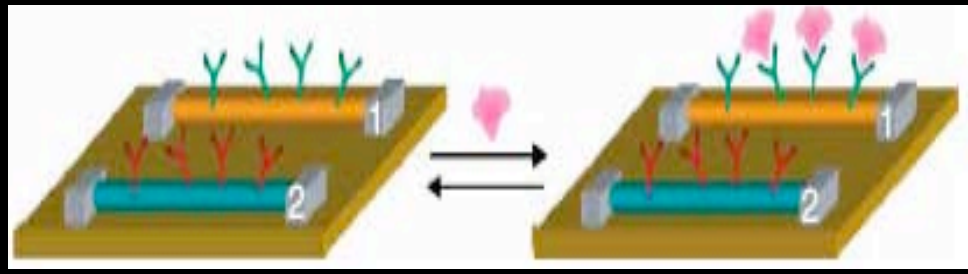
AT STUDY START

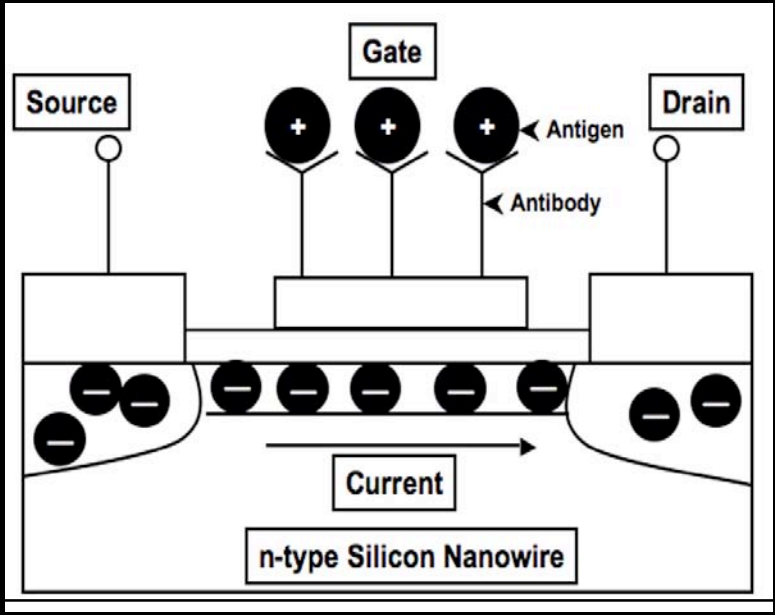
- Aged 15 – 64 years and New England Resident
- Duration of diabetes < 40 years
- At least 2 measurements of HbA1c in past two years
- ACRs over the past two years can be classified as normoalbuminuria, or microalbuminuria
- NO prior kidney transplant, significant non-diabetes related kidney disease, or major health problems
- Defined as having type 1 diabetes:
  - Diagnosis of DM before age 20
  - Not diagnosed after 40
  - If diagnosed between 20 and 40 then must have started insulin within 2 years of diagnosis and be on at least 20 units of insulin.

Nanotechnology

Lieber, CM et al., Nat. Biotech., 2005



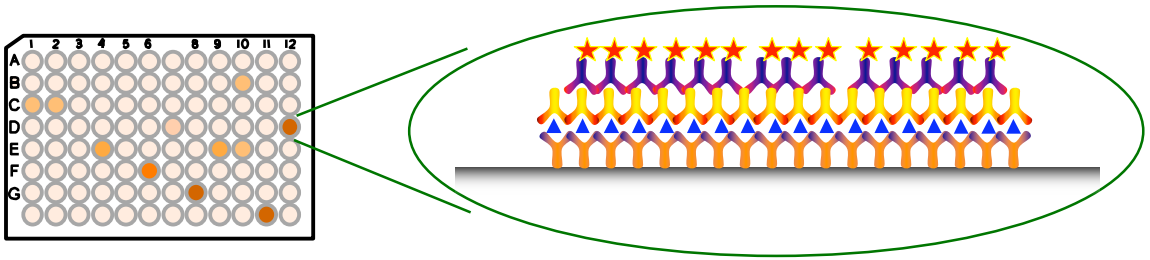




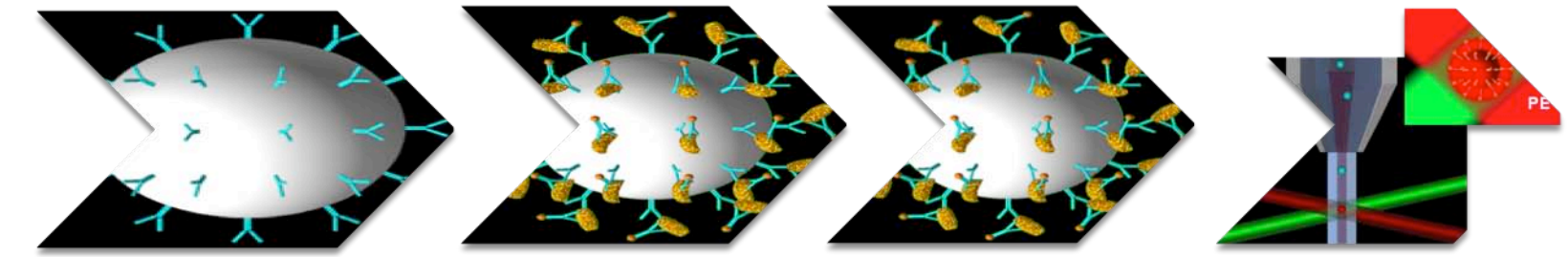
- Field effect transistors (FETs) exhibit a conductivity change in response to variations in electric field or potential at the surface.
- Silicon nanowires (SiNw) are better than CHEMFETs
- First of its kind to show sensitivity, selectivity, speed and multiplexing capability.

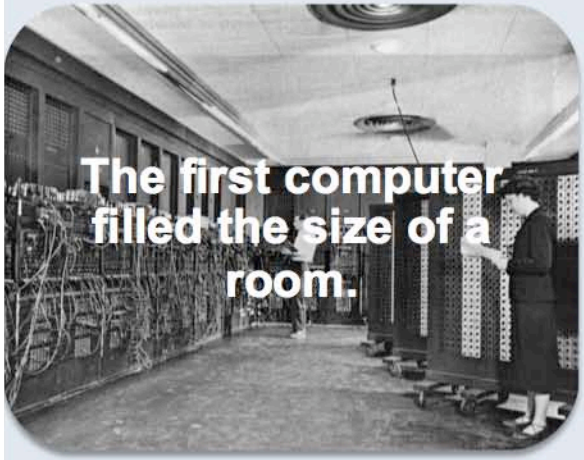
Current methodology for detection of Kim-1

- **ELISA**



- **Luminex microbead assay**

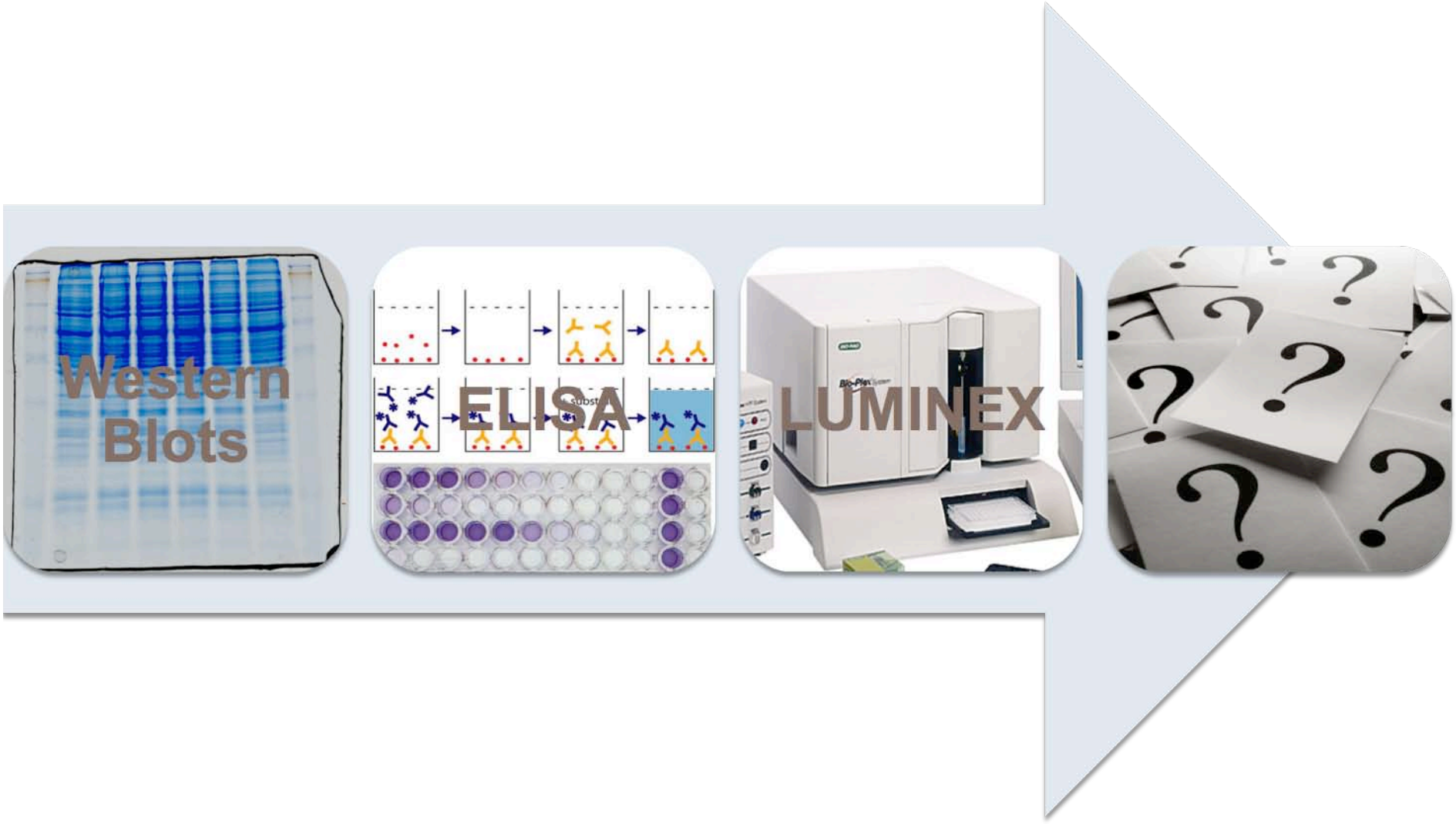



The first computer filled the size of a room.


The first IBM-PC


The Apple iPhone

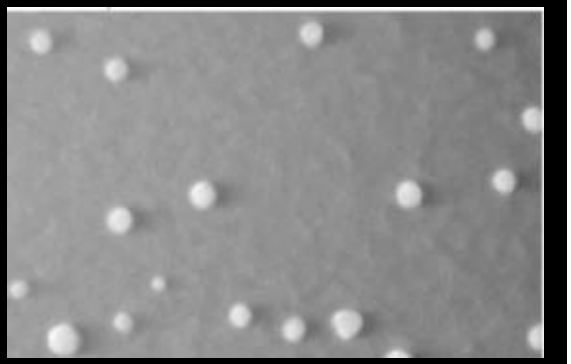
Current methodology for detection of Kim-1



Convenient Nano-Gold Tests for Point-of-Care and Field Surveillance

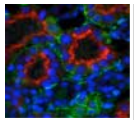
The “dipstick”

- Visual readout (Red color is due to plasmon resonance of gold radicals in stabilized nano-particles)
- High sensitivity (usually in pg/ml range)
- Convenient (requires 70 µl of urine)
- Rapid (results within 30 mins)



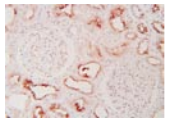
KIM-1

98



Ichimura *et al*:
Kim-1 protein and mRNA is upregulated in 48-hr post ischemic kidney

02



Baily *et al*: Human KIM-1 is cleaved by MMP's and shed

Han *et al*: Shed human KIM-1 can be detected and quantitated in the urine of patients with ATN

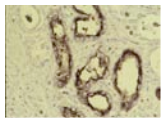
Kuehn *et al*: Kim-1 is expressed in subset of cysts in PKD

04

Ichimura *et al*: Upregulation of rat Kim-1 in the kidney and detection of shed rat Kim-1 in urine of rats post nephrotoxicity

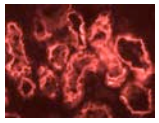
Amin *et al*: Kim-1 as a putative gene based marker following nephrotoxicity

05



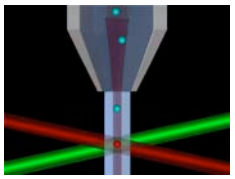
Han *et al*: shed KIM-1 is a tissue and urinary tumor marker of renal cell carcinoma

06

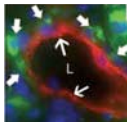


Vaidya *et al*: developed and evaluated an ELISA assay to detect urinary Kim-1 in rodents

07

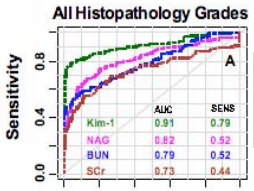


Vaidya *et al*: developed and evaluated a microbead based assay to detect urinary Kim-1 in rodents



Ichimura *et al*: a novel phosphatidyl serine receptor that confers phagocytic phenotype to epithelial cells

08



Vaidya *et al*: PSTC consortium manuscript in submission

Vaidya *et al*: "RenaStick" manuscript in submission

van Timmeren et al. (A)

de Borst et al.

Chen et al.

Prozialeck et al.

van Timmeren et al. (B)

Lesur et al.

Perez-Rojas et al.

Liangos et al.

Esparandi et al.

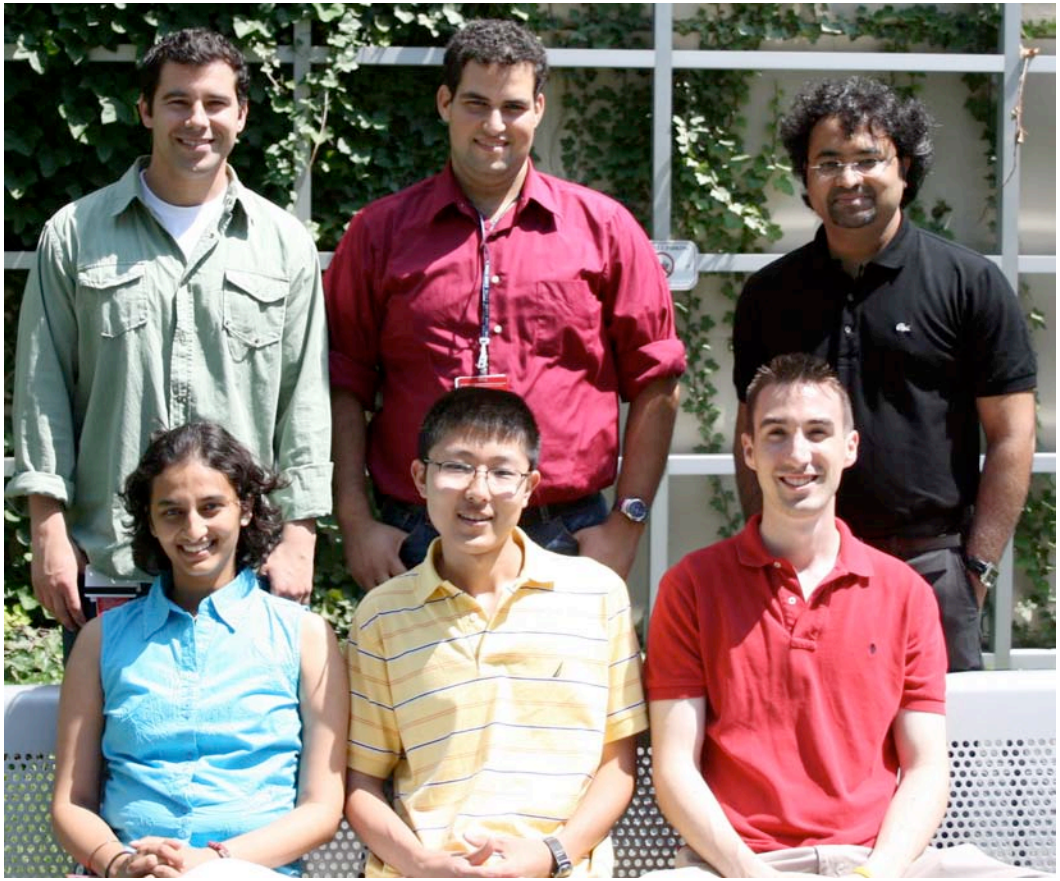
Zhou et al.

Zhang et al.

Vaidya/Waikar et al.

FDA/EMEA submission and announcement

Laboratory of Kidney Toxicology and Regeneration



Matthew Clement, Daniel Engel, Vishal Vaidya,
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BWH

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- Norma Bobadilla, PhD
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- Ronald Brown, PhD
- Parveneh Espandiari, PhD

Midwestern U

- Walter Prozialeck, PhD

Merck

- Josef Ozer, PhD
- Warren Glabb
- Sean Troth
- Frank Sistare, PhD

Novartis

- Frank Dieterle, PhD

Bioassay Works Inc.

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