

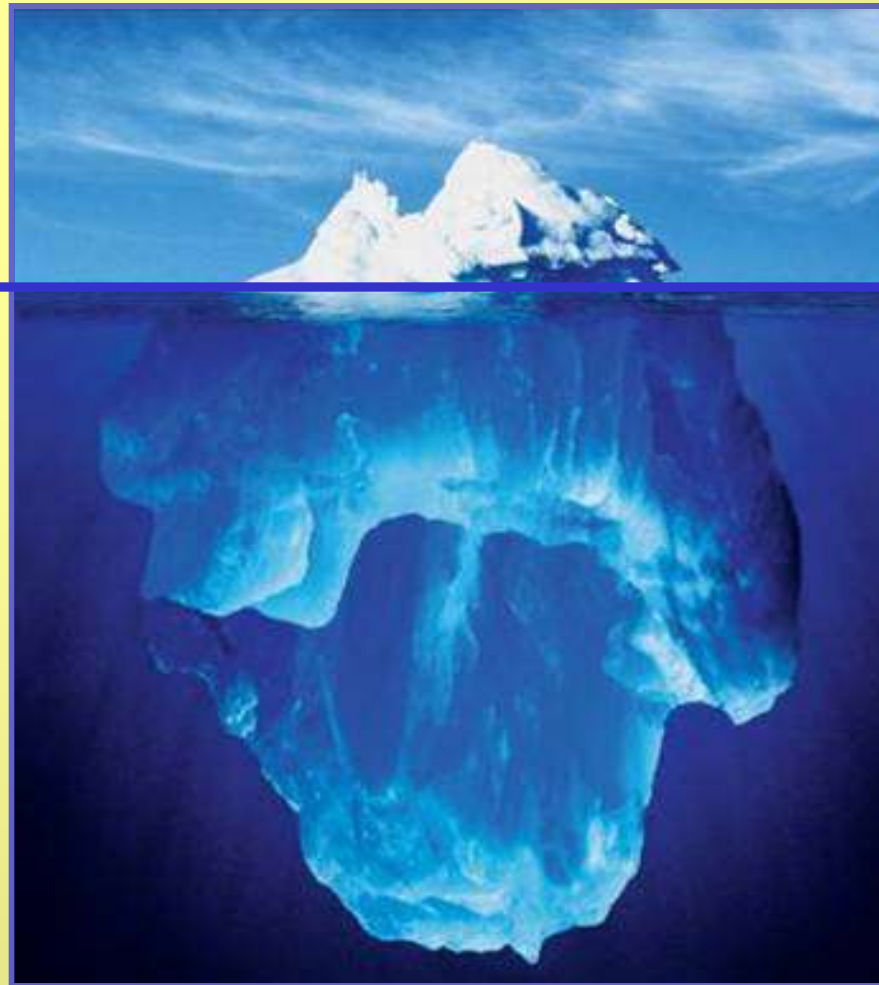
AST-120: A NOVEL DRUG TO PREVENT PROGRESSION OF CKD AND CVD

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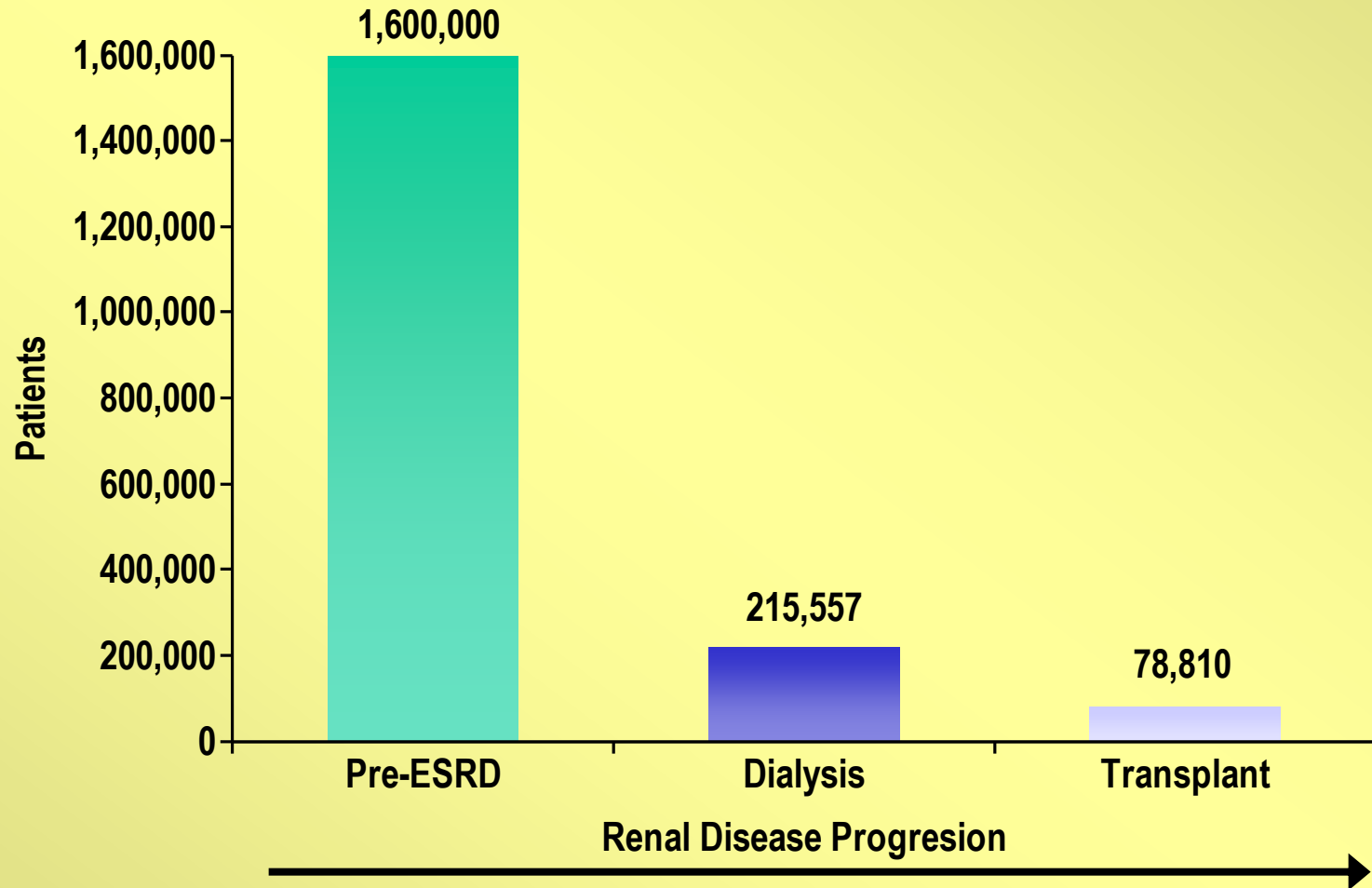
The Renal Disease Iceberg



**DIALYSIS
ESRD**

CHRONIC KIDNEY DISEASE

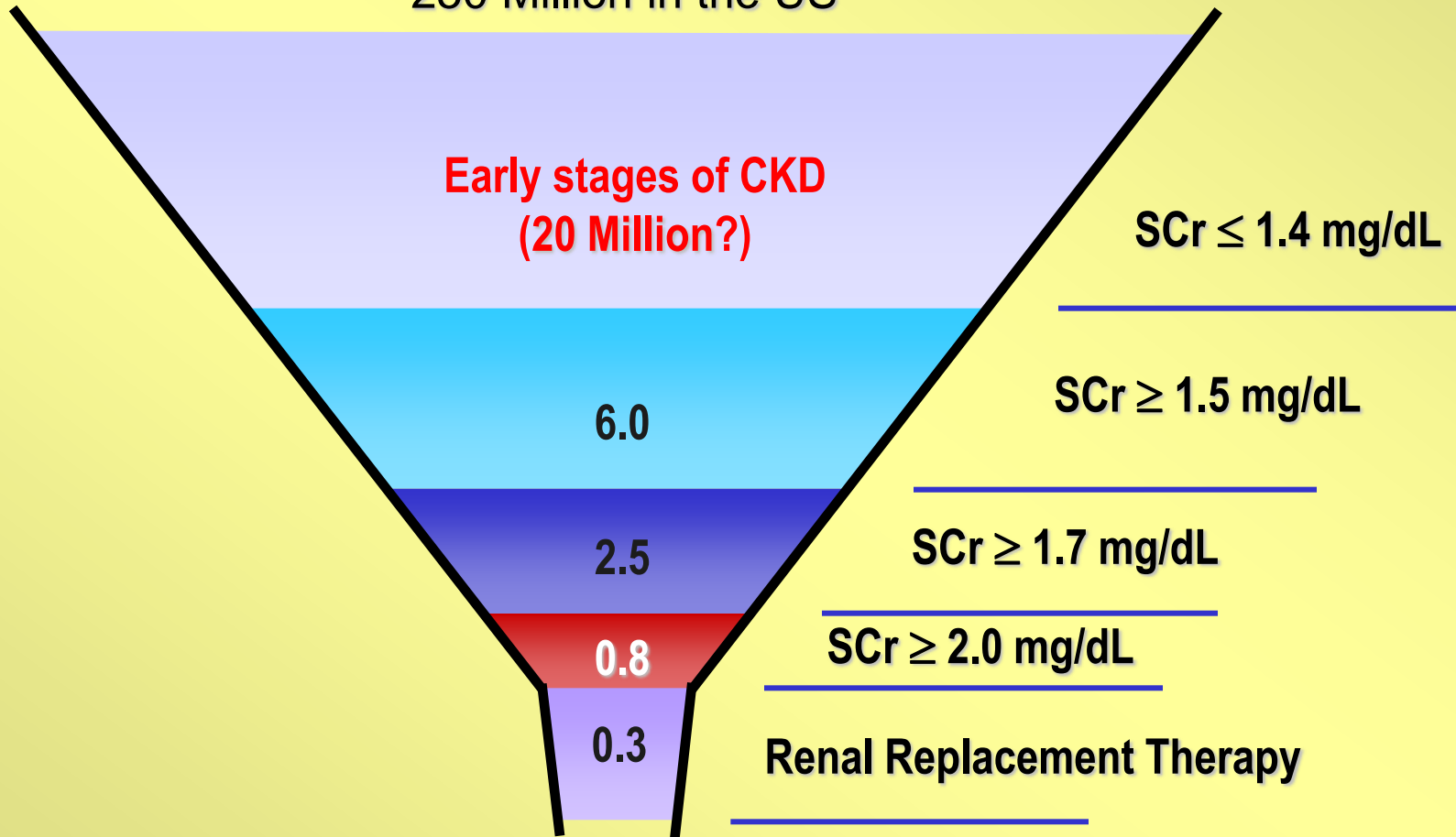
Pre-ESRD Patients Far Outnumber ESRD Patients



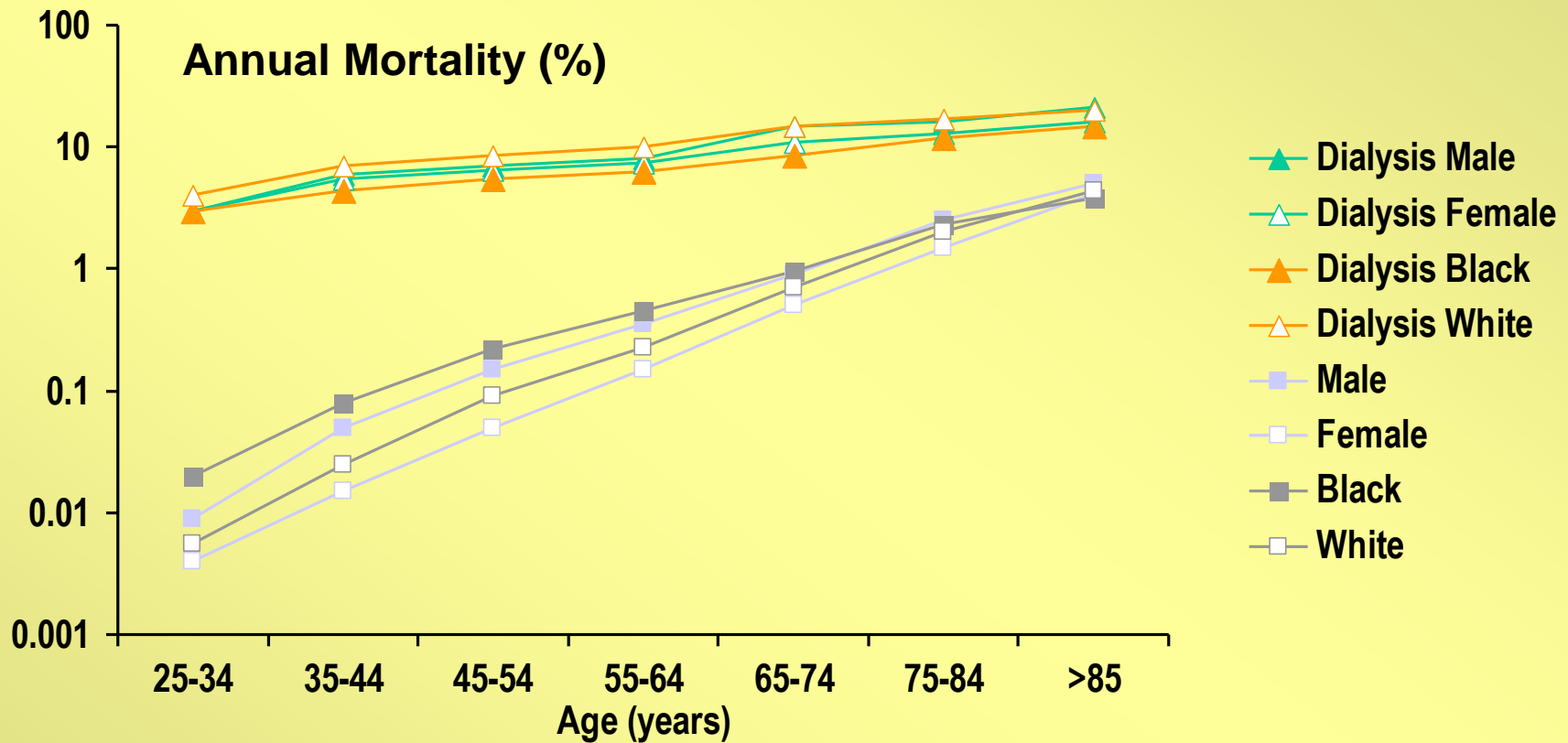
Estimates, Medicare and non-Medicare dialysis patients and Medicare transplant patients from USRDS 1998 ADR, Estimates of pre-ESRD patients from McKinsey and Co.

Estimates of CKD within the USA Based on Creatinine (NHANES III Data)

250 Million in the US



Cardiovascular Mortality in ESRD



MANAGEMENT OF CKD

- **THE NUMBER OF PATIENTS WHO REQUIRE TREATMENT FOR ESRD IS STEADILY INCREASING.**
- **DEVELOPMENT OF NEW THERAPIES TO SLOW DOWN THE PROGRESSION OF KIDNEY DISEASE AND PREVENT CVD IS A CHALLENGE THAT CONFRONTS US.**

UREMIC TOXINS

- **NUMEROUS SUBSTANCES HAVE BEEN PROPOSED TO BE UREMIC TOXINS; SOME PRODUCE UREMIC SYMPTOMS. HOWEVER, SOME OF THESE SUBSTANCES MAY CAUSE PROGRESSIVE LOSS OF RENAL FUNCTION.**
- **DETERIORATION OF RENAL FUNCTION CAUSES AN INCREASE IN CIRCULATING UREMIC TOXINS, WHICH, IN TURN, PROMOTE THE PROGRESSION OF CKD AND THIS FURTHER INCREASES THE BUILD-UP OF UREMIC TOXINS IN THE CIRCULATION. THIS VICIOUS CYCLE EVENTUALLY CULMINATES IN RENAL FAILURE.**

PROGRESSION OF CKD

- ADVANCED CKD IS ASSOCIATED WITH INFLAMMATION, WHICH IS BOTH A CAUSE AND A CONSEQUENCE OF OXIDATIVE STRESS
- INFLAMMATION & OXIDATIVE STRESS CONTRIBUTE TO PROGRESSION OF CKD AND MANY OF THE CKD-ASSOCIATED COMPLICATIONS, INCLUDING: ANEMIA, ATHEROSCLEROSIS, CVD, IMMUNE DEFICIENCY, WASTING AND MALNUTRITION SYNDROME AMONG OTHERS

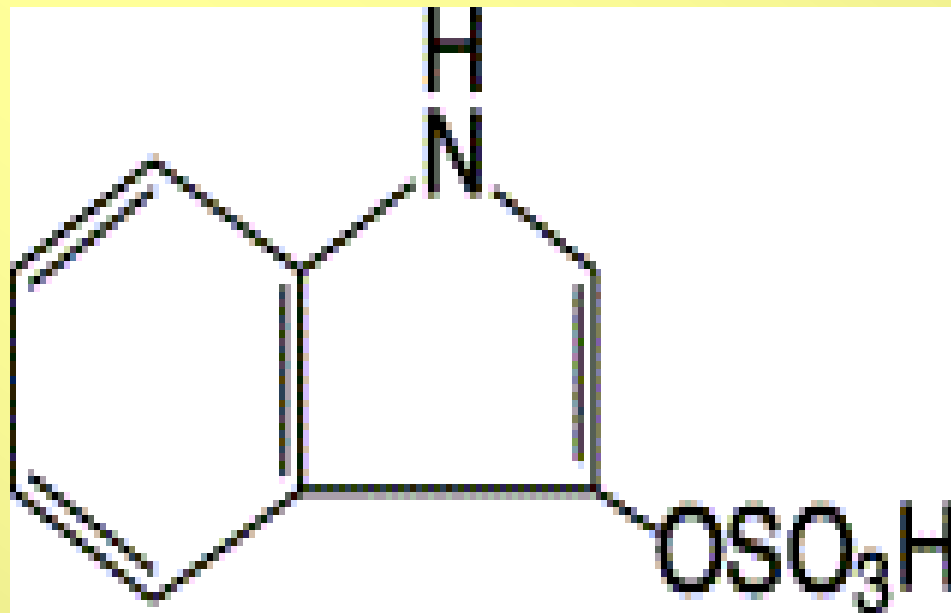
PROPOSED UREMIC TOXINS

Table 1. Main known uremic retention solutes

Small water soluble solutes	Protein-bound solutes	Middle molecules
Asymmetric dimethylarginine	3-Deoxyglucosone	Adrenomedullin
Benzylalcohol	CMPF	Atrial natriuretic peptide
β -Guanidinopropionic acid	Fructoselysine	β_2 -Microglobulin
β -Lipotropin	Glyoxal	β -Endorphin
Creatinine	Hippuric acid	Cholecystokinin
Cytidine	Homocysteine	Clara cell protein
Guanidine	Hydroquinone	Complement factor D
Guanidinoacetic acid	Indole-3-acetic acid	Cystatin C
Guanidinosuccinic acid	Indoxyl sulfate	Degranulation inhibiting protein I
Hypoxanthine	Kinurenine	Delta-sleep-inducing peptide
Malondialdehyde	Kynurenic acid	Endothelin
Methylguanidine	Methylglyoxal	Hyaluronic acid
Myoinositol	N-carboxymethyllysine	Interleukin 1 β
Orotic acid	P-cresol	Interleukin 6
Orotidine	Pentosidine	Kappa-Ig light chain
Oxalate	Phenol	Lambda-Ig light chain
Pseudouridine	P-OHhippuric acid	Leptin
Symmetric dimethylarginine	Quinolinic acid	Methionine-enkephalin
Urea	Spermidine	Neuropeptide Y
Uric acid	Spermine	Parathyroid hormone
Xanthine		Retinol binding protein
		Tumor necrosis factor alpha

CMPF is carboxy-methyl-propyl-furanpropionic acid.

INDOXYL SULFATE



History of Indoxyl Sulfate

- 1950S
 - FIRST PUBLISHED REFERENCES TO INDOXYL SULFATE IN KIDNEY DISEASE
- 1980S
 - NIWA ET AL STUDIED THE ROLE OF PROTEIN-BOUND UREMIC TOXINS IN COMPLICATIONS OF DIALYSIS PATIENTS DUE TO INEFFICIENT REMOVAL BY HEMODIALYSIS
 - MOST IMPORTANT PROTEIN-BOUND UREMIC TOXINS (BY SERUM LEVELS): INDOXYL SULFATE (IS), P-CRESOL AND CMPF
- 1990S
 - HYPOTHESIS: INTESTINAL ADSORBENTS MIGHT BE ABLE TO REMOVE IS, AS ITS PRECURSOR INDOLE IS SYNTHESIZED IN THE GUT
 - DEMONSTRATED THAT AN INTESTINAL ADSORBENT REDUCES SERUM LEVELS OF IS IN CRF RATS (1991)
 - DEMONSTRATED THAT ADMINISTRATION OF IS OR INDOLE TO CRF RATS REDUCED RENAL FUNCTION, AND STIMULATED PROGRESSION OF CRF (1994)
 - P-CRESOL WAS ADMINISTERED TO RATS, AND NO STIMULATORY EFFECT ON CRF PROGRESSION WAS SEEN
 - CMPF NOT DETECTED IN SERUM OF RATS
 - SERUM LEVELS OF OTHER INDOLIC COMPOUNDS ARE LOW AS COMPARED WITH INDOXYL SULFATE
- 2000S – PRESENT
 - CONTINUED RESEARCH INTO EFFECTS OF IS ON OXIDATIVE STRESS AND IMPACT ON CARDIOVASCULAR DISEASE

ENTERIC FLORA AND PROGRSSION

- THERE ARE MANY CAUSES OF CKD-INDUCED OXIDATIVE STRESS AND INFLAMMATION
- ONE CAUSE IS UREMIC BYPRODUCTS OF THE MICROBIAL FLORA
 - PROTEIN-BOUND UREMIC TOXINS SUCH AS INDOXYL SULFATE AND *P*-CRESYL SULFATE
- BOTH INDOXYL SULFATE AND *P*-CRESYL SULFATE HAVE BEEN SHOWN TO BE ASSOCIATED WITH PROGRESSION OF KIDNEY DISEASE AND CARDIOVASCULAR MORTALITY
- THERE IS A NEED TO REDUCE UREMIC TOXINS OF GI ORIGIN IN ATTEMPTS TO SLOW THE PROGRESSION OF CHRONIC KIDNEY DISEASE

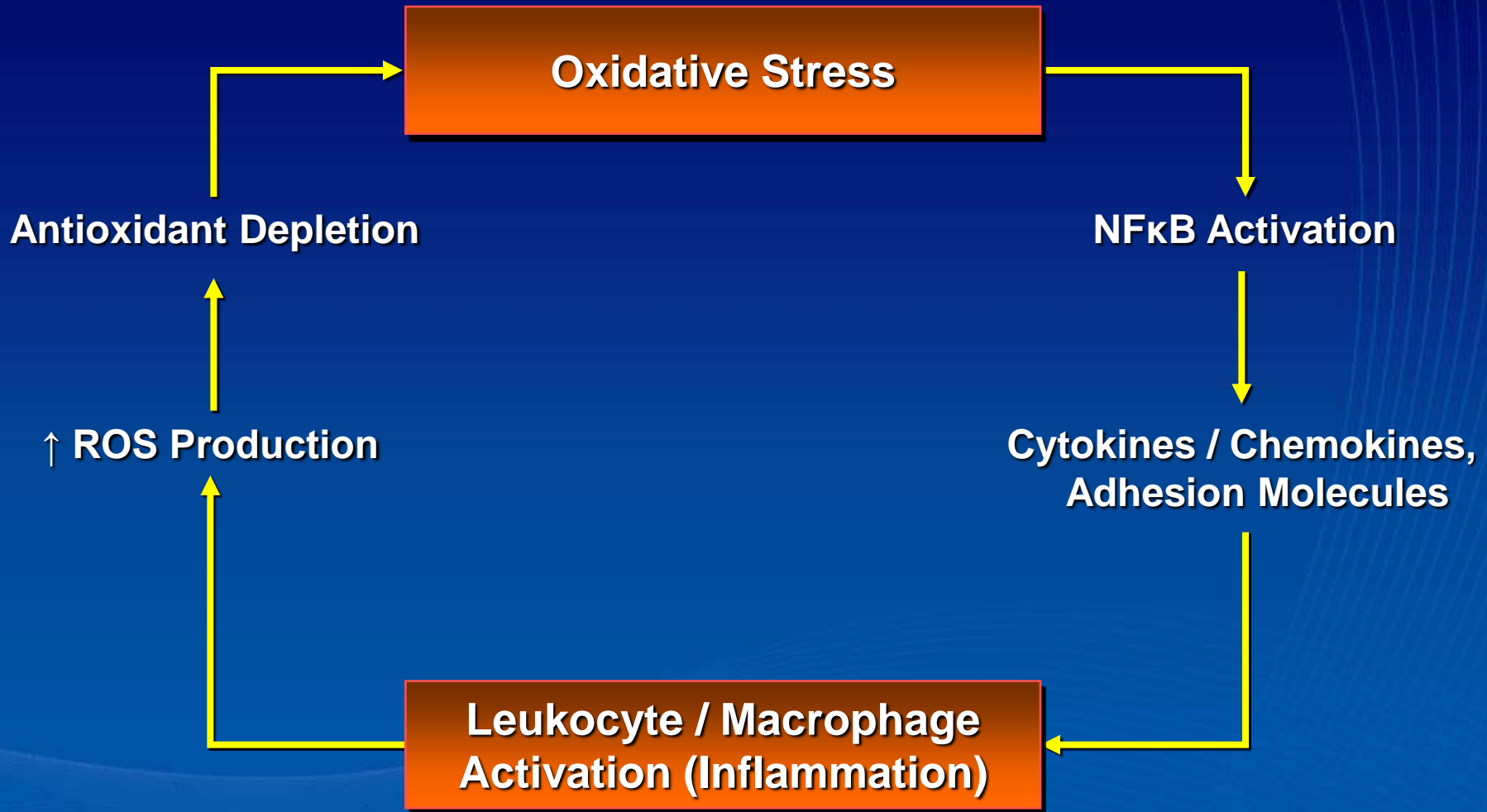
The Origin of Uremic Solutes Associated with Cardiovascular Disease

Solute	Group	Endogenous Metabolism	Microbiotic Metabolism	Exogenous Intake
ADMA	Dimethylarginines	X		
p-Cresyl sulfate	Phenols		X	
Phenyl acetic acid	Phenols		X	
Indoxyl sulfate	Indoles		X	
Indole 3-acetic acid	Indoles		X	
AGEs	N/A	X		X
Homocysteine	N/A	X	X ^a	
Oxalate	N/A	X		X

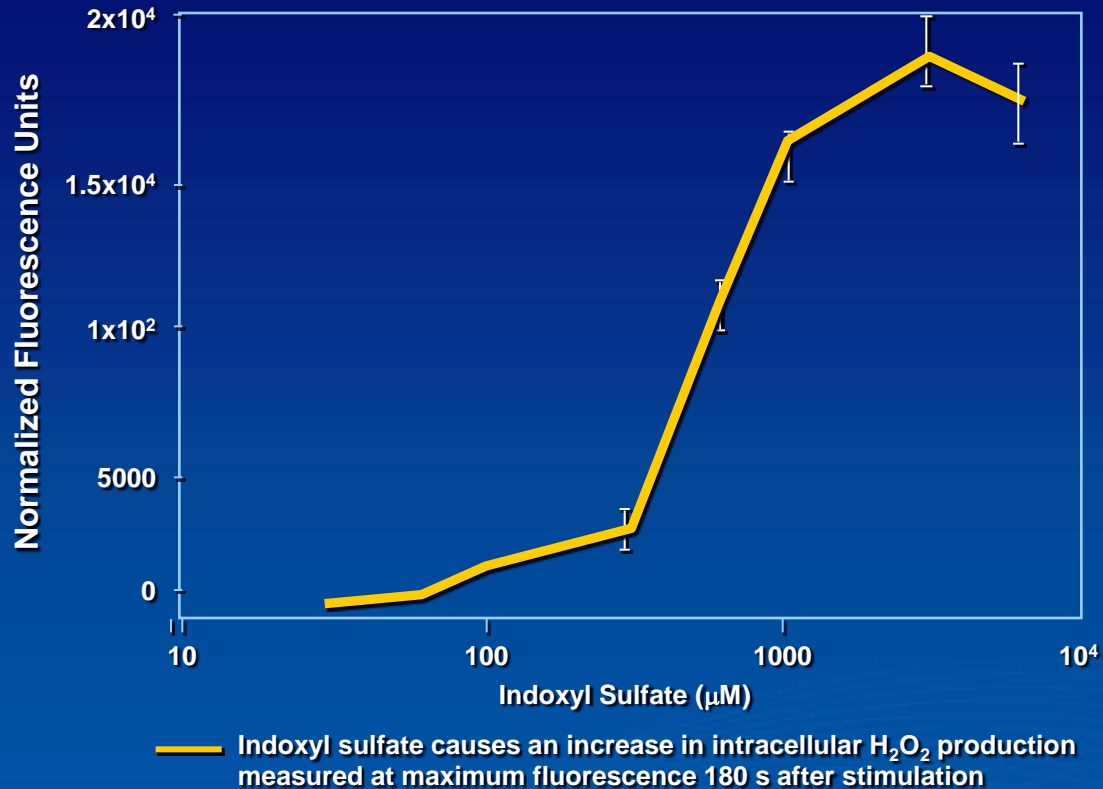
ADMA, asymetrical dimethylarginine; AGEs, advanced glycation end products; N/A, not applicable.

^aThe colonic microbiota produce substantial amounts of folic acid, which in turn lower homocysteine concentrations. Evenepoel P et al. *Kidney International*. 2009;76(Suppl 114):S12–S19.

Link Between Oxidative Stress and Inflammation

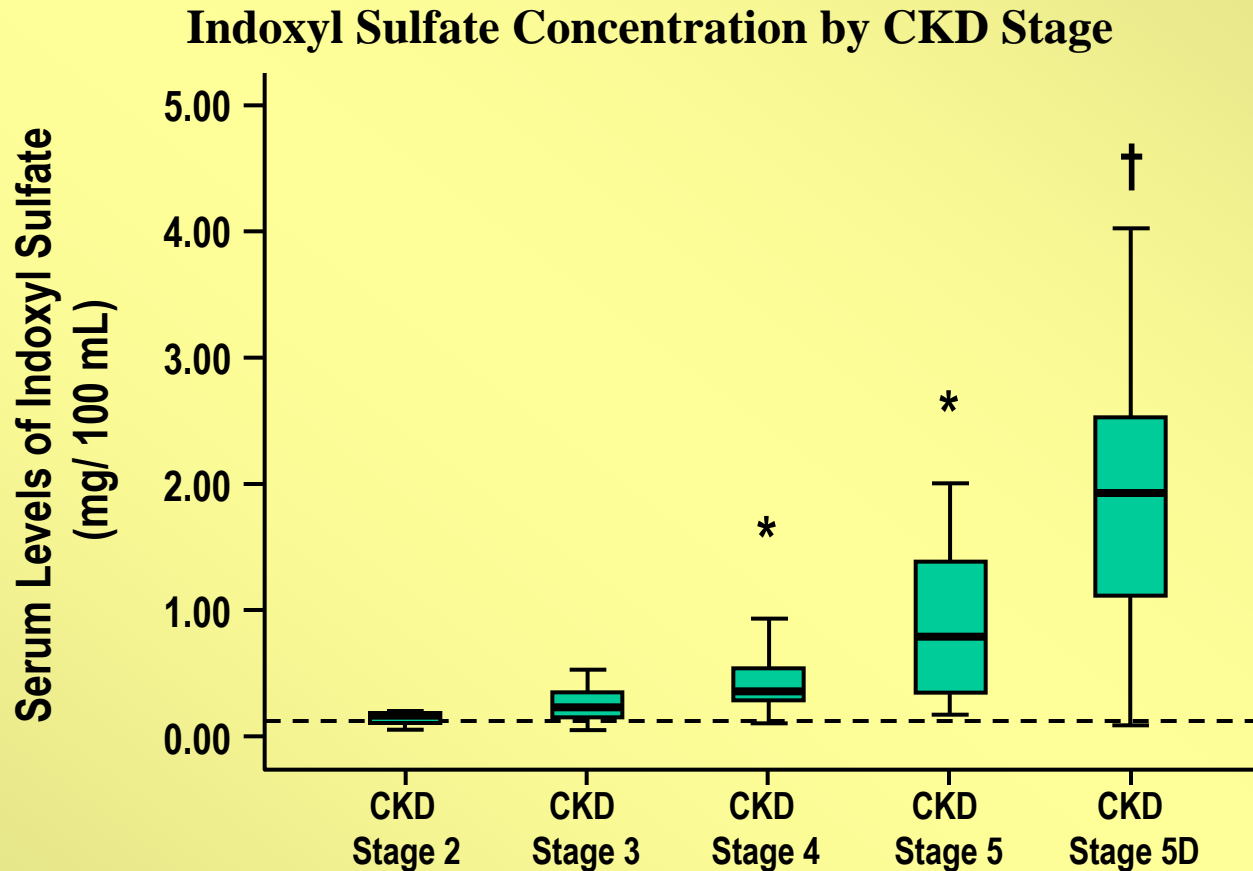


Reactive Oxygen Species Formation *in vitro*



Primary rat renal mesangial cells obtained from cortical sections of kidneys from young male Sprague-Dawley rats. Cells treated with increasing indoxyl sulfate concentrations.
Gelasco AK et al. *Am J Physiol Cell Physiol.* 2006;290:F1551-1558.

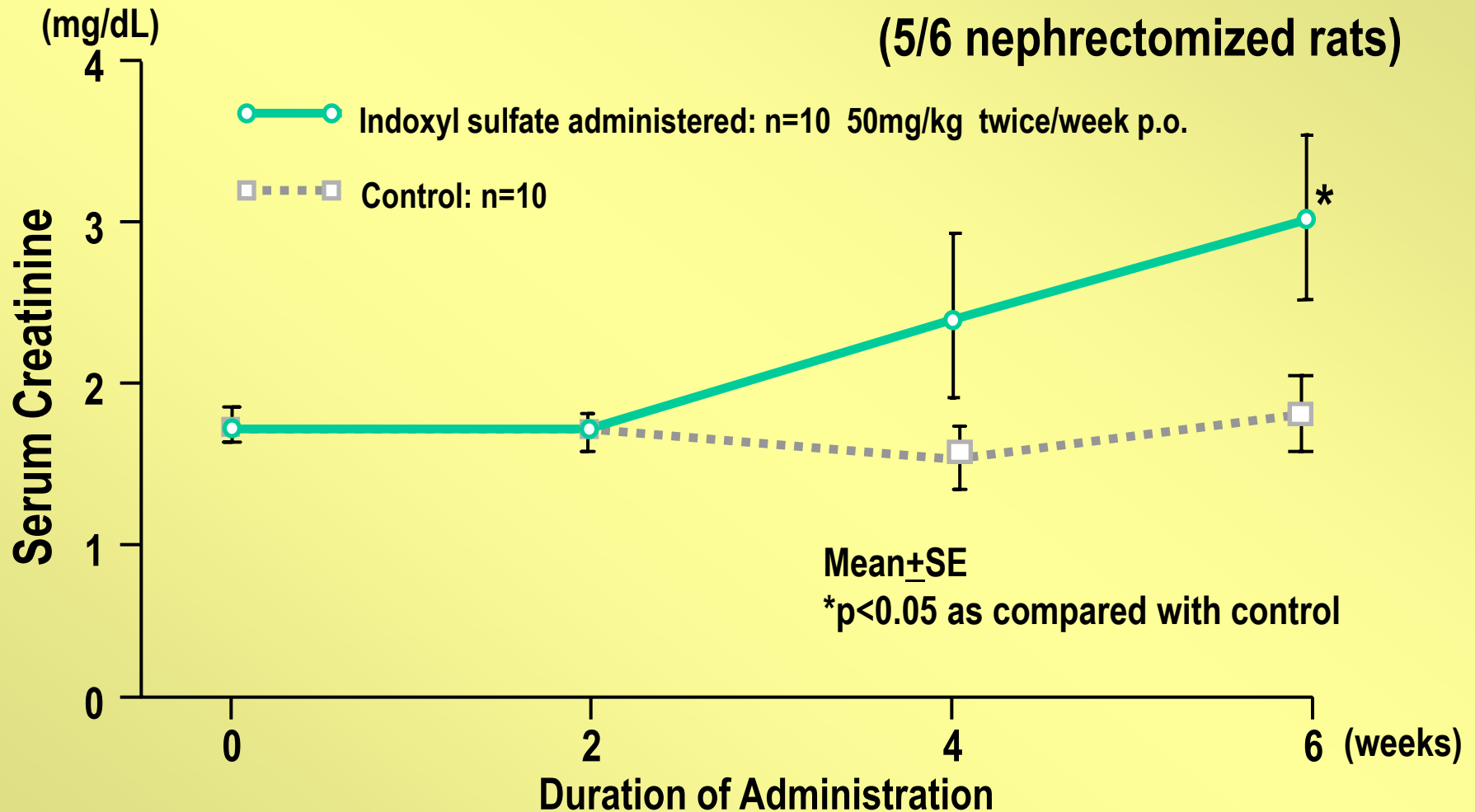
Serum Levels of IS as a Function of CKD Stages



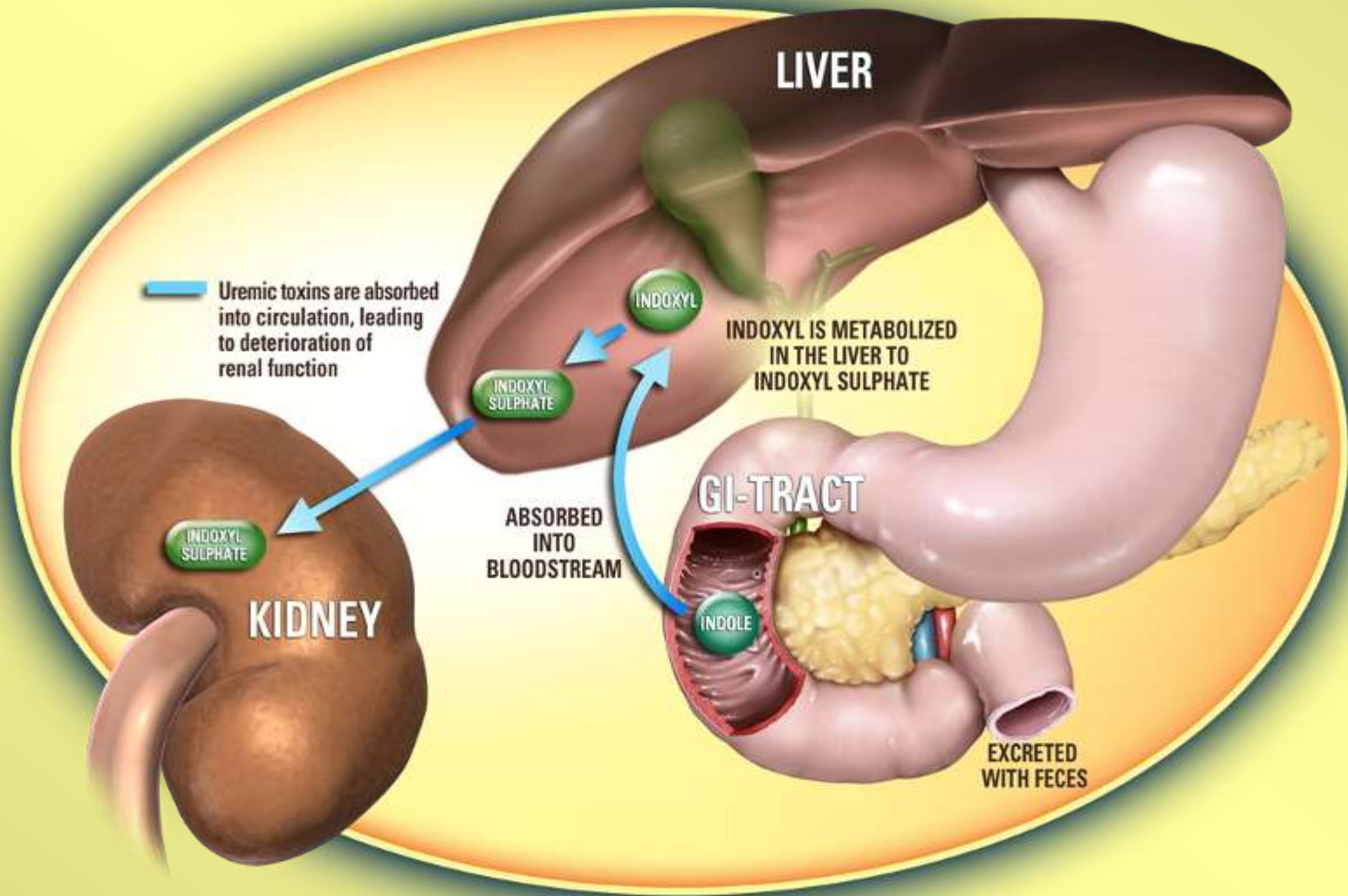
* $P < 0.05$ versus CKD stages 2, 3 or 5D; † $P < 0.05$ versus CKD stages 2, 3, 4, or 5.

F.C. Barreto et al. *Clin J Am Soc Nephrol*.2009; 4: 1551-1558.

Effect of Indoxyl Sulfate on Serum Creatinine in Uremic Rats

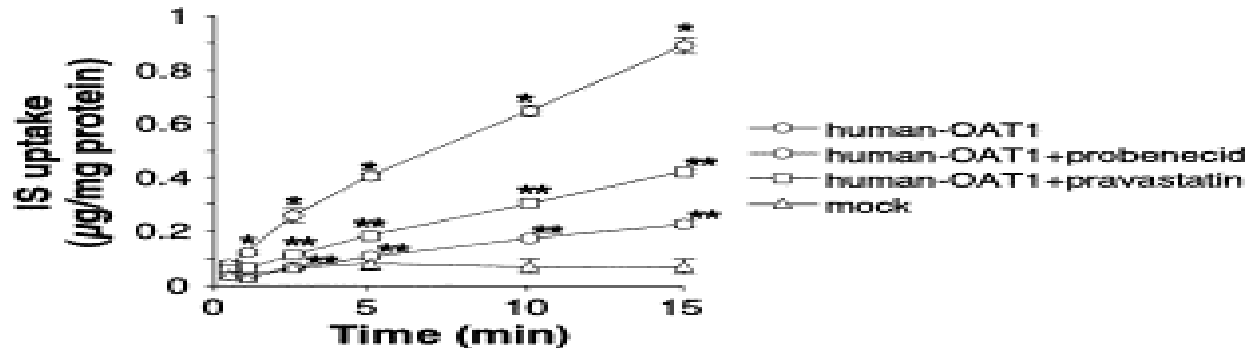


A Closer Look at Indoxyl Sulfate

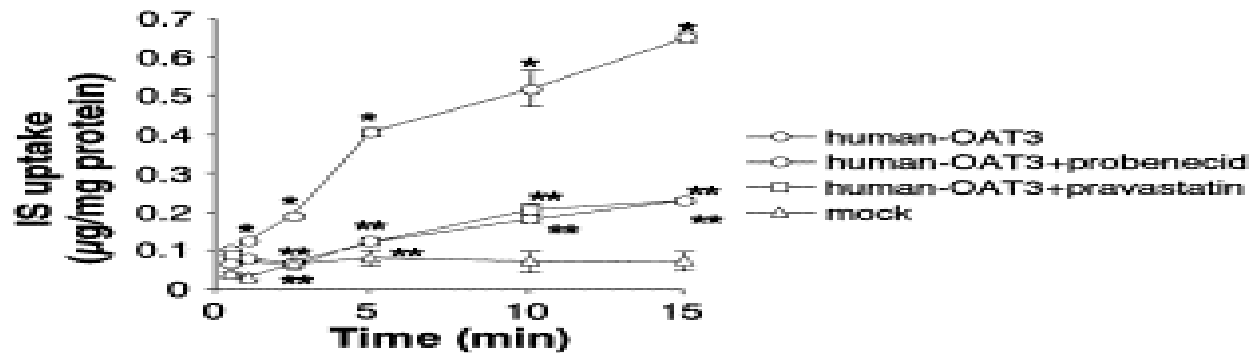


INDOXYL SULFATE UPTAKE

A Human-OAT1



B Human-OAT3



C Human-OAT4

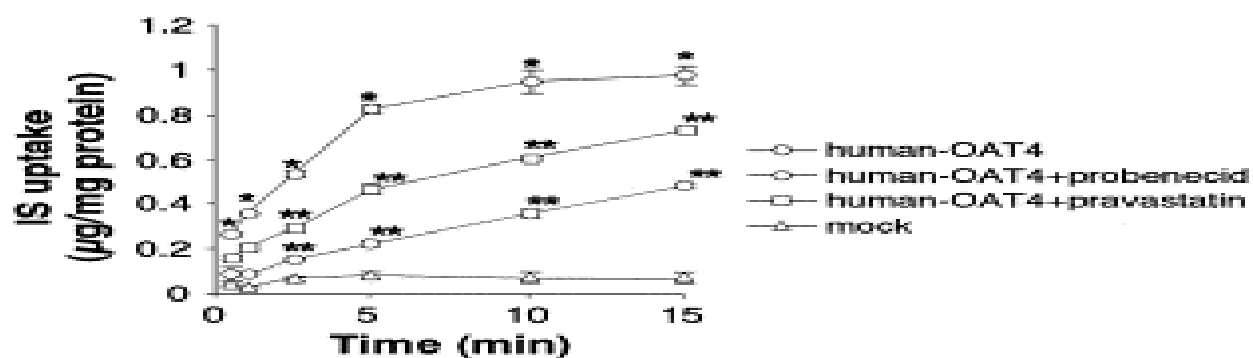
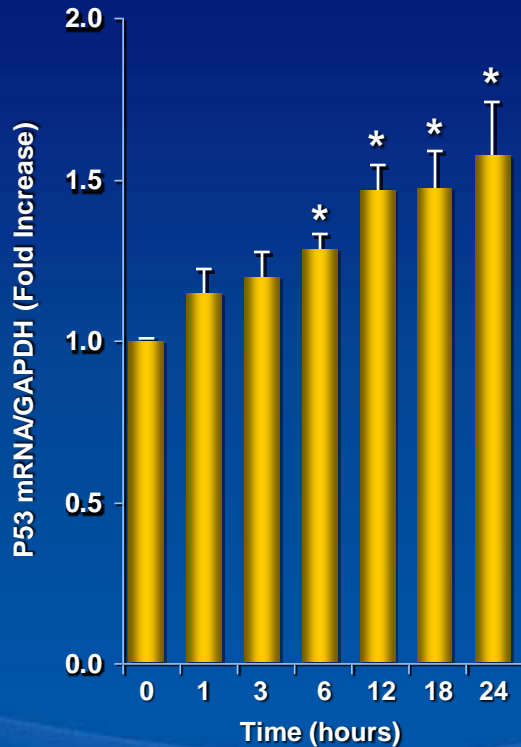


Fig. 4. Indoxyl sulfate (IS) uptake by human-OATs. S2 human-OAT1, S2 human-OAT3, S2 human-OAT4 and mock were incubated in solution containing at 37 °C for 0.5, 1, 2.5, 5, 10 or 15 min. Each value represents the mean ± S.E. of six monolayers from two separate experiments. * $P < 0.01$ vs. mock and ** $P < 0.01$ between human-OATs and human-OATs in the presence of probenecid or pravastatin.

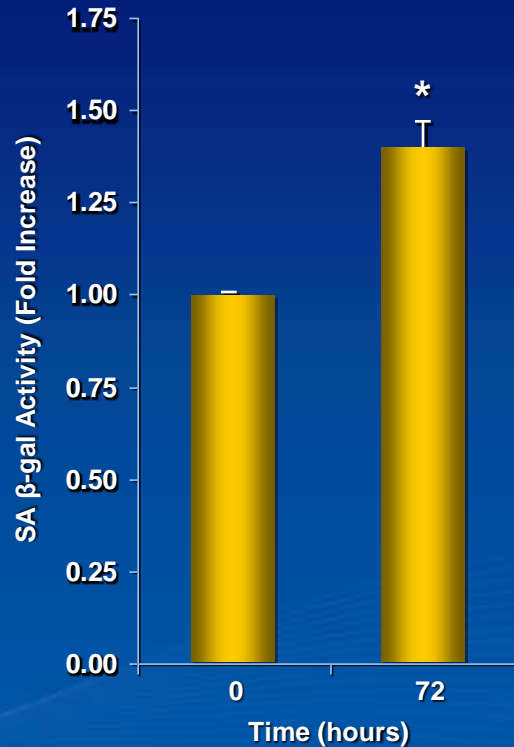
Senescence and Dysfunction of Proximal Tubular Cells associated with Activated p53 Expression by Indoxyl Sulfate

Indoxyl Sulfate Induces Activated p53 Expression Suppresses Cell Proliferation



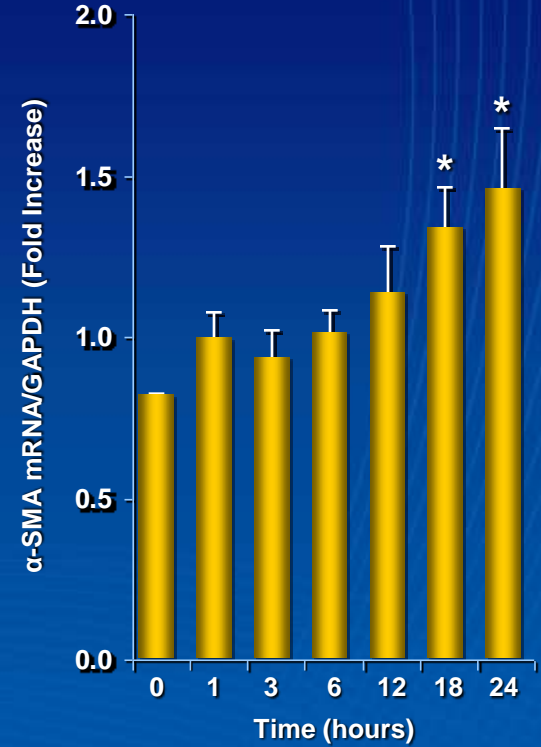
Indoxyl Sulfate (250 μM)

Indoxyl Sulfate Promotes SA β-gal Expression Marker of Senescence



Indoxyl Sulfate (250 μM)

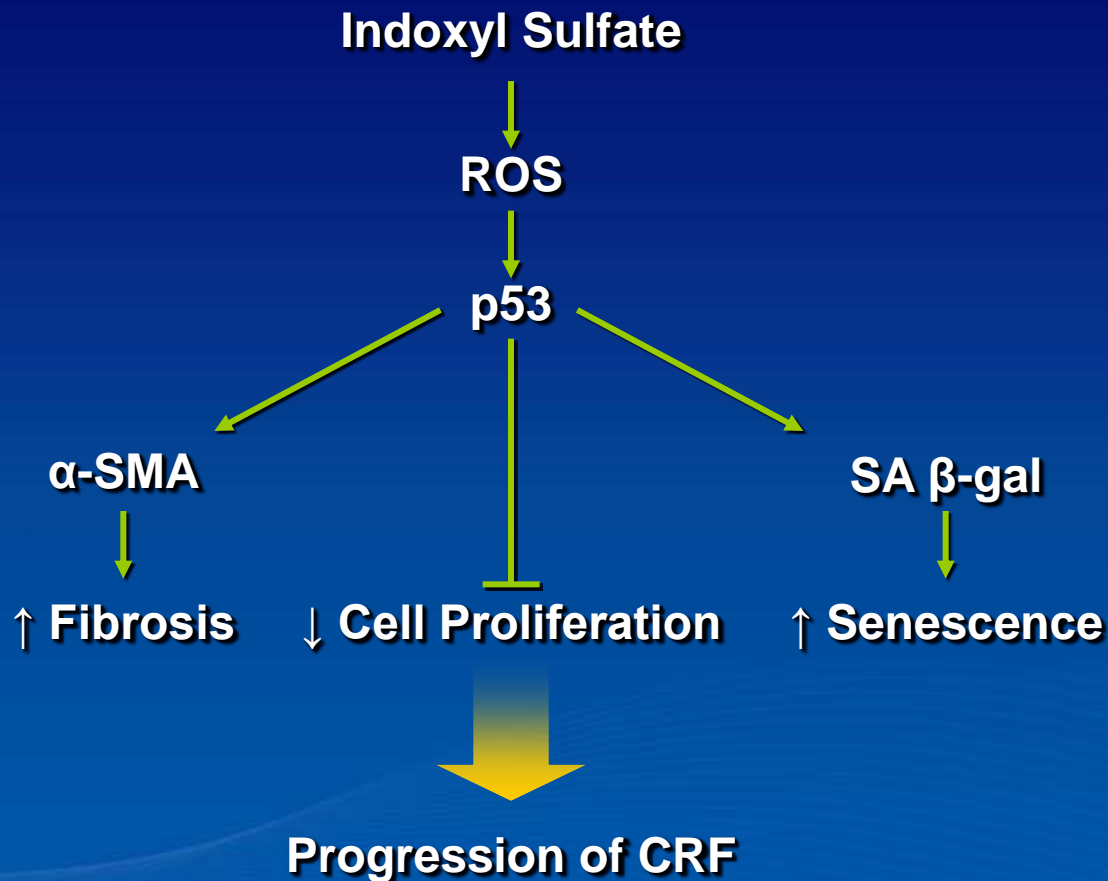
Indoxyl Sulfate Up Regulates α-SMA Expression Marker of Fibrosis



Indoxyl Sulfate (250 μM)

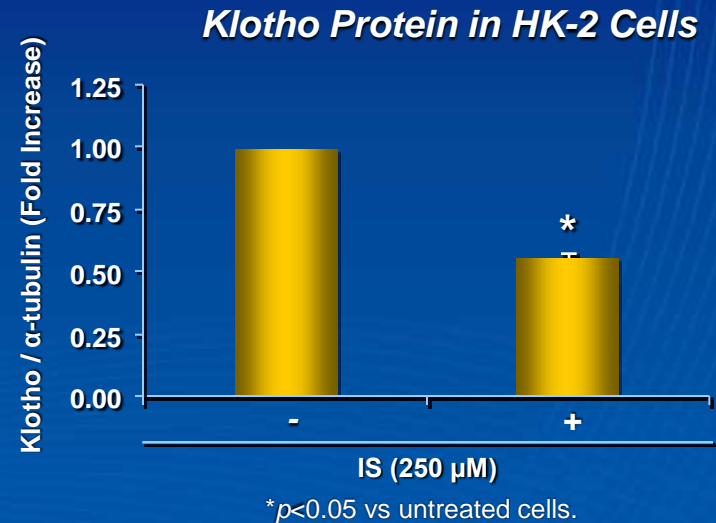
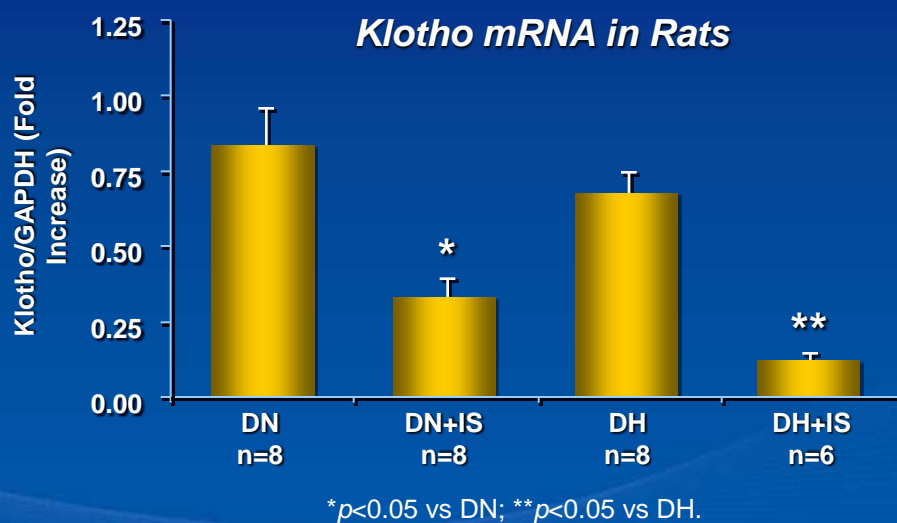
Serum-starved HK-2 cells incubated with indoxyl sulfate. * p < 0.05 vs untreated cells. Shimizu H et al. *Am J Physiol Cell Physiol.* 2010;299:C1110-C1117.

Indoxyl Sulfate Functions in Proximal Tubular Cells



Indoxyl Sulfate Down-Regulates *Klotho* Expression in Dahl Rat Kidney

- *Klotho*, an anti-aging gene, is expressed in the kidneys, and its renal expression is decreased in CKD
- Indoxyl sulfate administration to DH rats reduced renal expression of *klotho* and promoted cell senescence which was accompanied by renal fibrosis

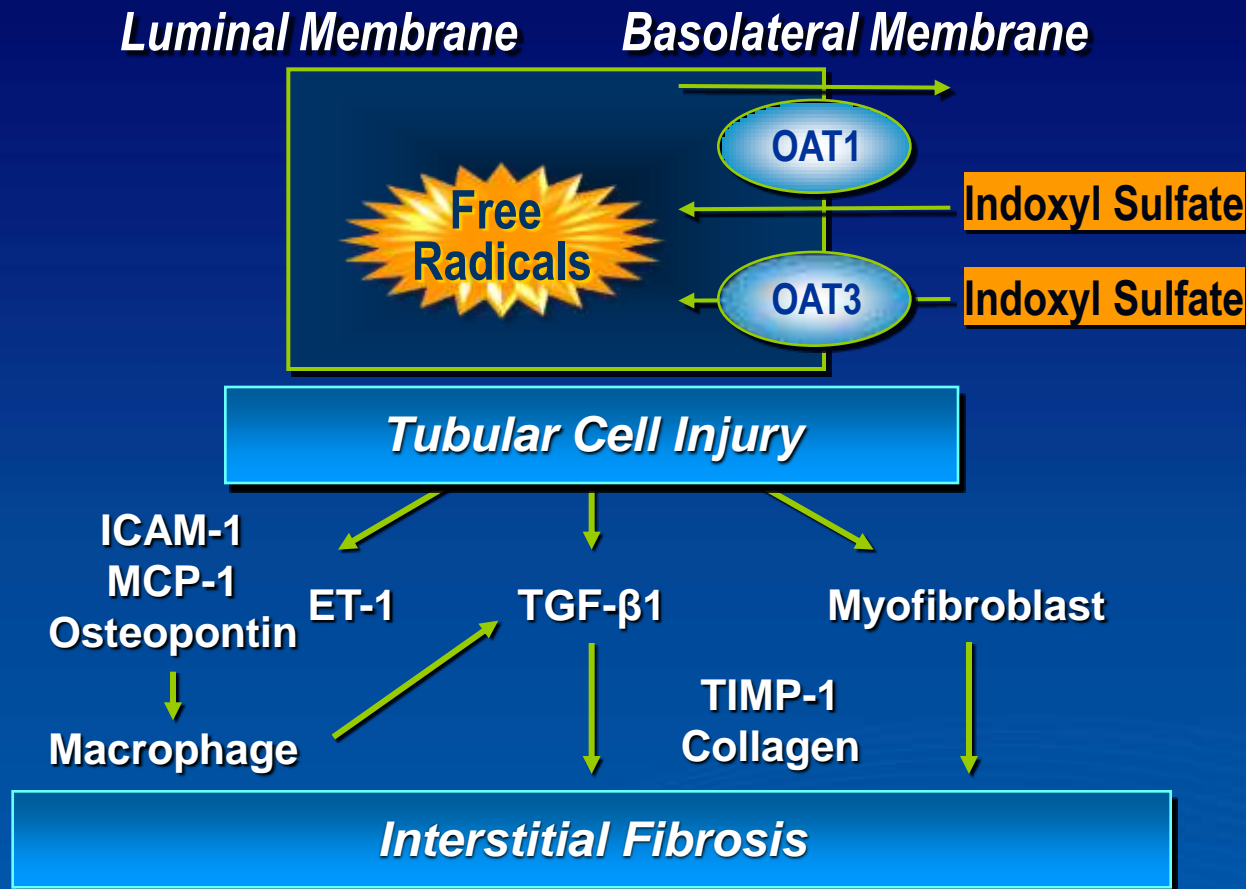


DN, Dahl salt-resistant normotensive rats; DN+IS, Dahl salt-resistant normotensive indoxyl sulfate-administered rats; DH, Dahl salt-sensitive hypertensive rats; DH+IS, Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats
Adijiang A et al. *J Ren Nutr.* 2011;21:105-109. Shimizu H et al. *A J Neph.* 2011;33:319-324.

INDOXYL SULFATE AND CKD

- TGF β -1
- METALLOPROTEINASE
- PAI-1
- ABNORMALITIES IN TRYPTOPHAN METABOLISM
- CARDIOVASCULAR EFFECTS?

Mechanisms of Indoxyl Sulfate Induced Nephrotoxicity

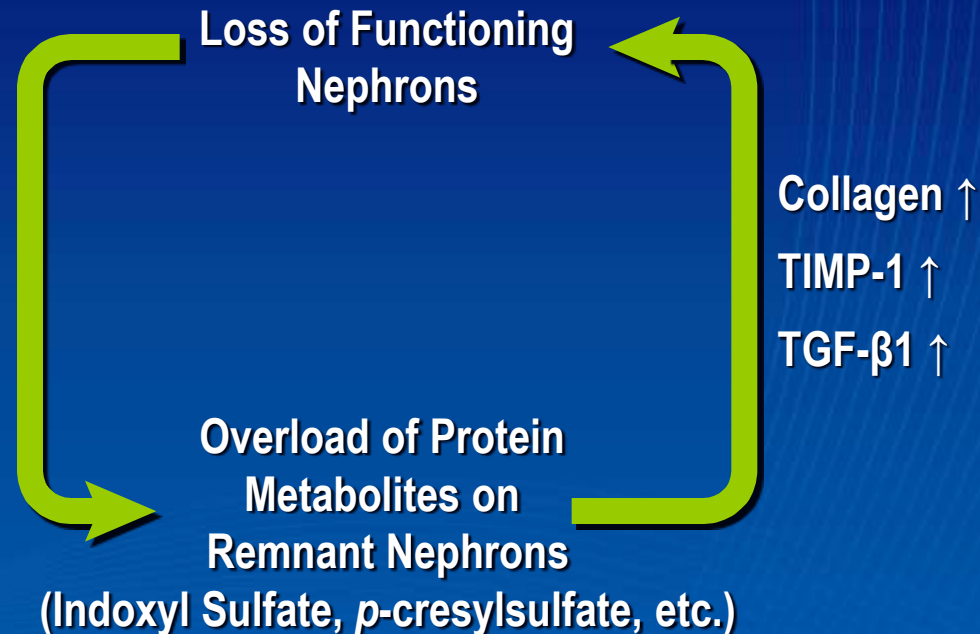


ET-1, endothelin-1; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemotactic protein-1; OAT, organic anion transporters; TGF-β1, transforming growth factor beta-1; TIMP-1, tissue inhibitor of metalloproteinase-1.
 Niwa T. *J Renal Nutr.* 2010;20:S2–S6.

THE ROLE OF PROTEIN METABOLITES IN CKD PROGRESSION

- Strategies that alleviate the overload of uremic toxins may interrupt the chain of events that lead to further renal damage^{1,2}
- These strategies may include:^{1,2}
 - Dietary protein restriction
 - Limiting absorption of toxic protein-derived byproducts in the gut

Protein Metabolite Theory¹



1. Niwa T. *J Renal Nutr.* 2010;20:S2–S6.

2. Aoyama I et al. *Miner Electrolyte Metab.* 1999;25:365–372.

**A NEXUS OF PROGRESSION OF
CHRONIC KIDNEY DISEASE,
TRYPTOPHAN, INDOXYL
SULFATE AND PROFIBROTIC
CYTOKINES AND CHARCOAL**

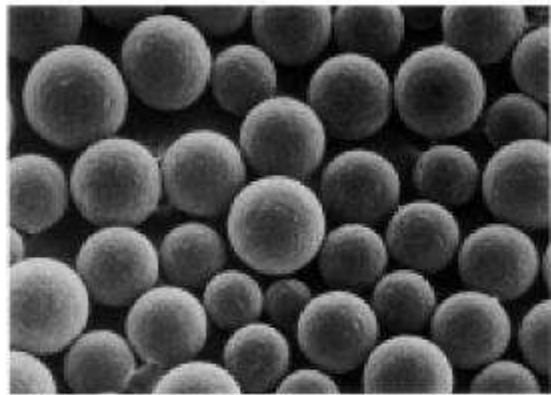
AST-120

- **AST-120 is an orally administered adsorbent that was approved in Japan in 1991 for prolonging the time to initiation of hemodialysis and improving uremic symptoms in patients with chronic kidney disease (CKD).**

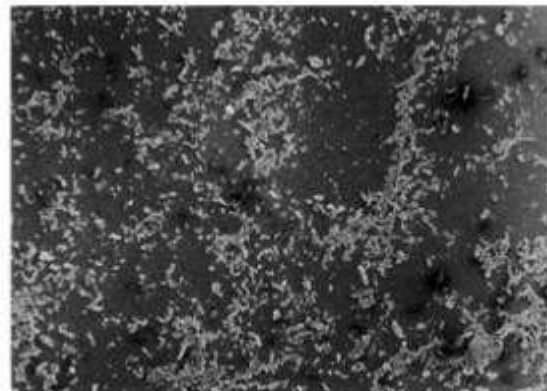
AST-120

- **AST-120 consists of black spherical particles ca. 0.2 to 0.4 mm in diameter. Composed mainly of carbon (approximately 96%), AST-120 exhibits similar or superior adsorption-ability to activated charcoal for certain acidic and basic organic compounds that are known to be increased in renal failure patients. The clinical utility of AST-120, therefore, is believed to reside in its ability to adsorb uremic toxins in the gastrointestinal (GI) tract, thereby reducing systemic absorption of uremic toxins and related contributions to the CKD disease process**

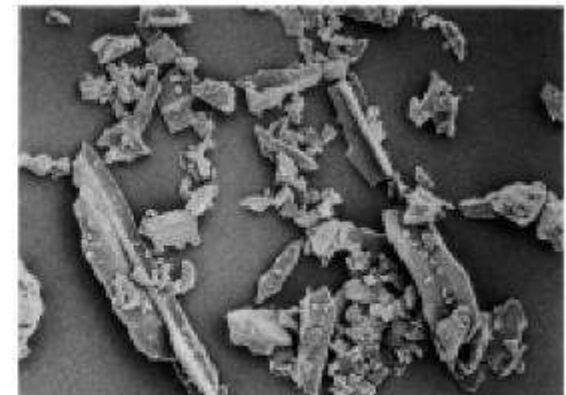
Difference between AST-120 and Activated Charcoal



0.5mm



0.5mm



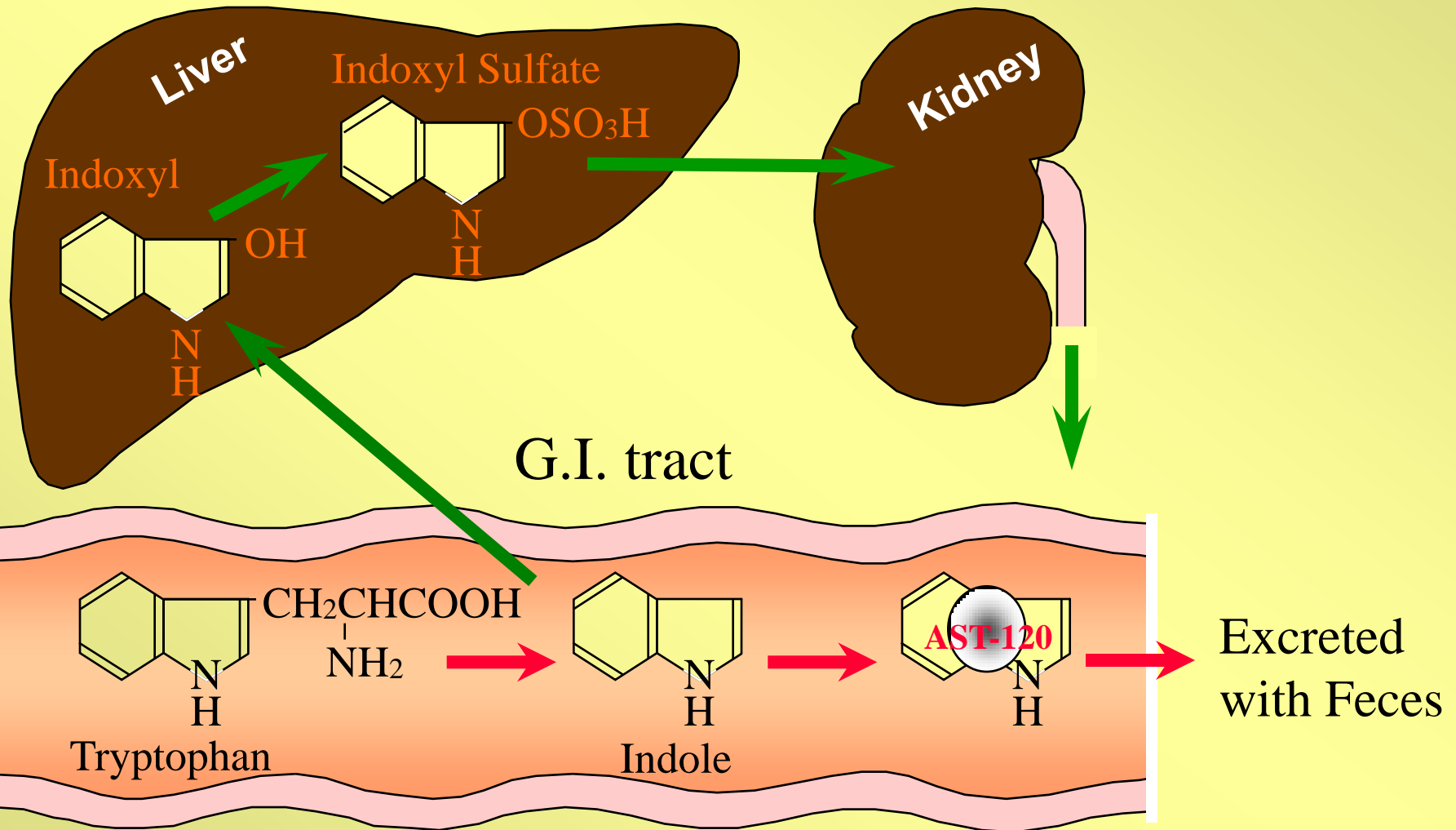
0.05mm

AST-120 vs Activated Charcoal

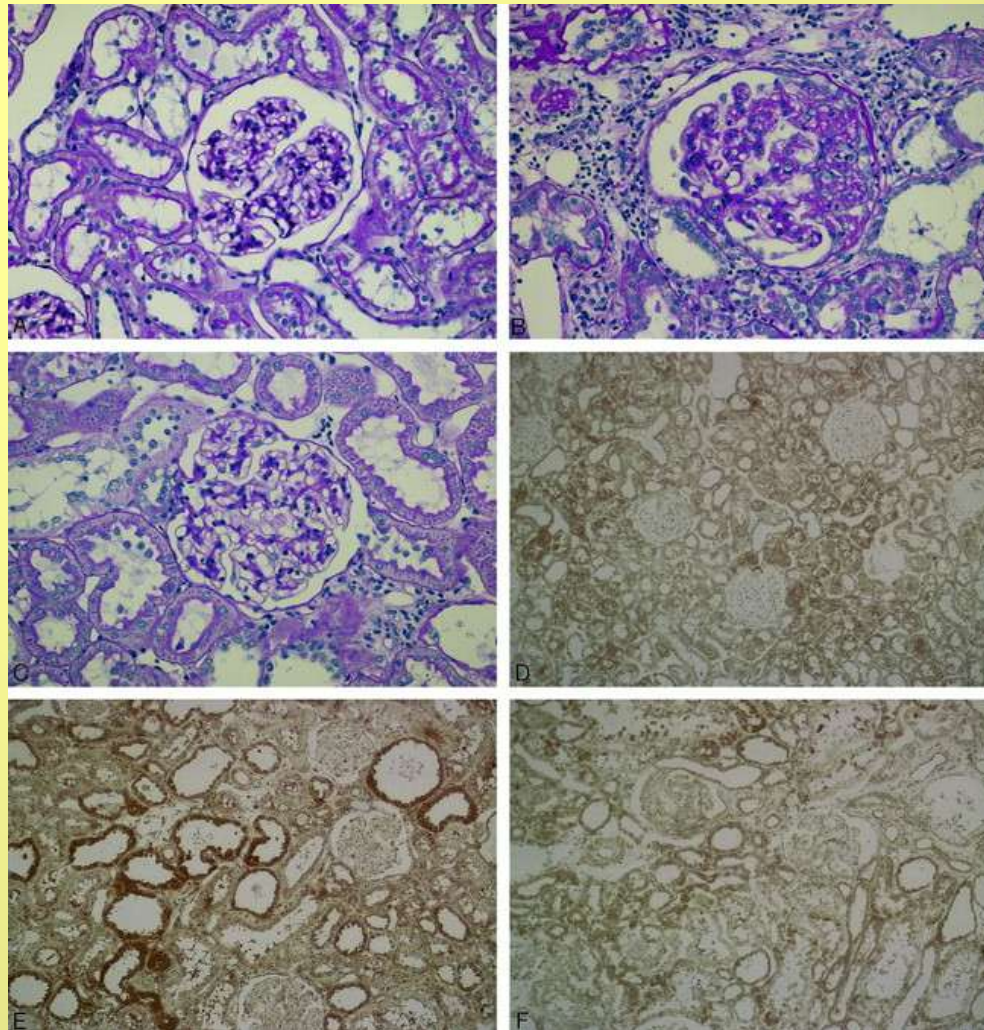
Physical Appearance



Excretion Process of Indole by AST-120

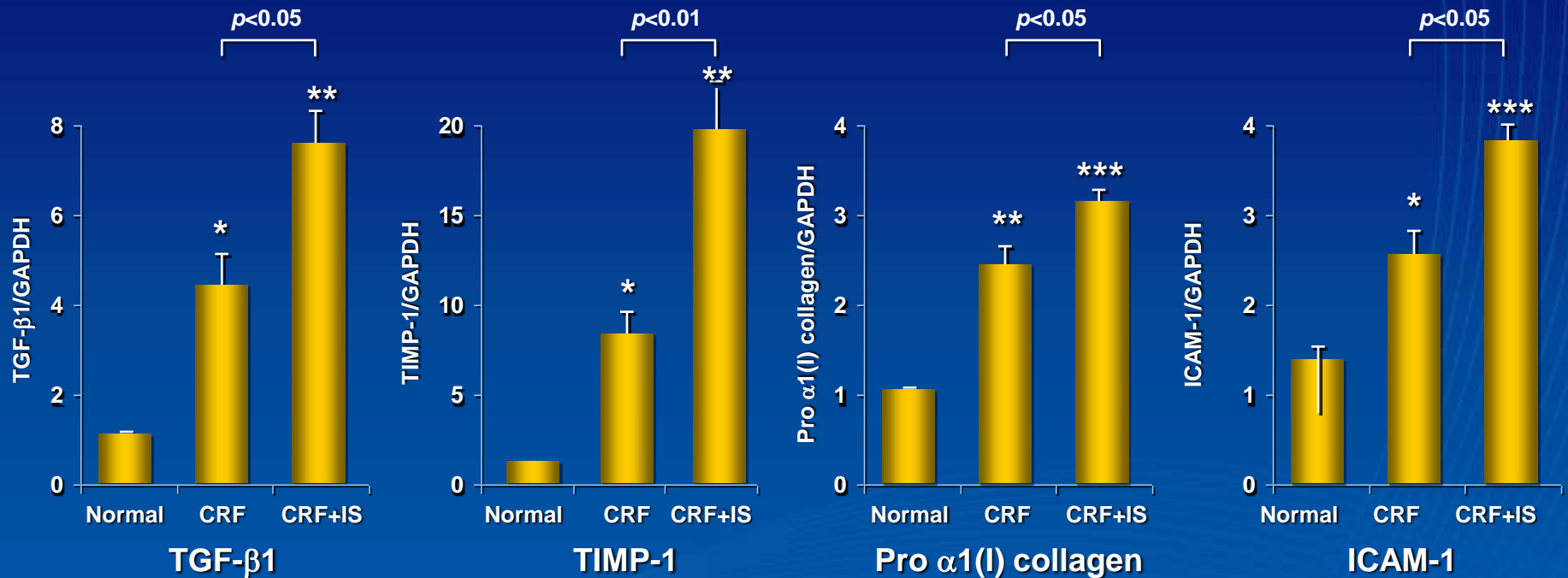


PAS staining of the renal cortices in a normal rat (A), a control uraemic rat (B), and a uraemic rat treated with AST-120 (C) (x200), and immunostaining of indoxyl sulphate in the renal cortices of a normal rat (D), a control uraemic rat (E), and a uraemic rat treated with AST-120 (F) using a monoclonal anti-indoxyl sulphate antibody (x100)



Indoxyl Sulfate Increases Expression of Genes Related to Tubulointerstitial Fibrosis in Animals with CKD

- Indoxyl sulfate administration to uremic rats resulted in significant increases in TGF- β 1, TIMP-1, pro- α (I) collagen, and ICAM-1

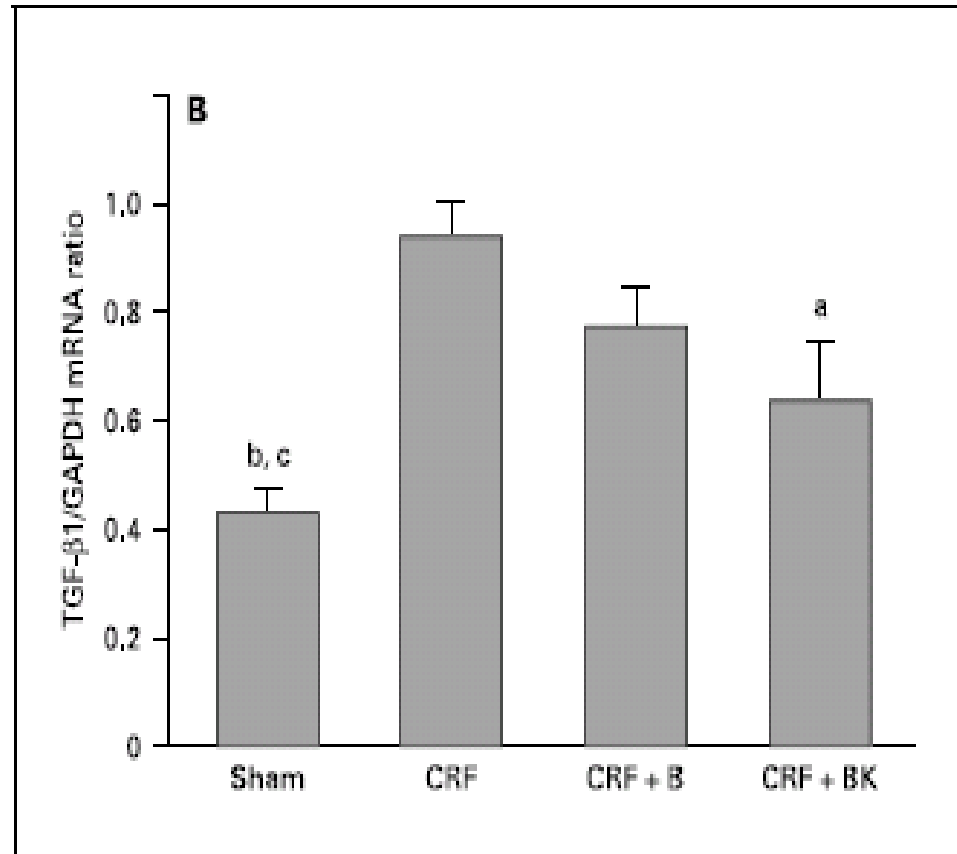
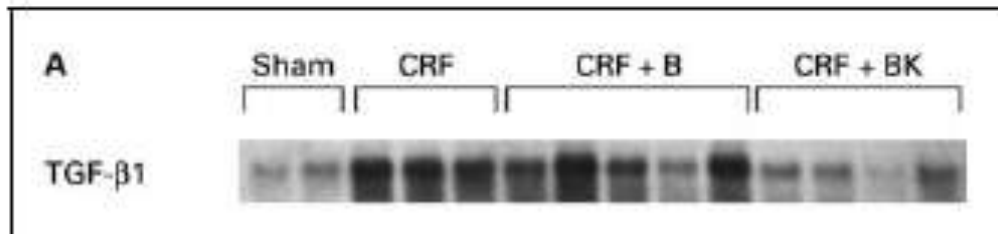


5/6 nephrectomized uremic rats

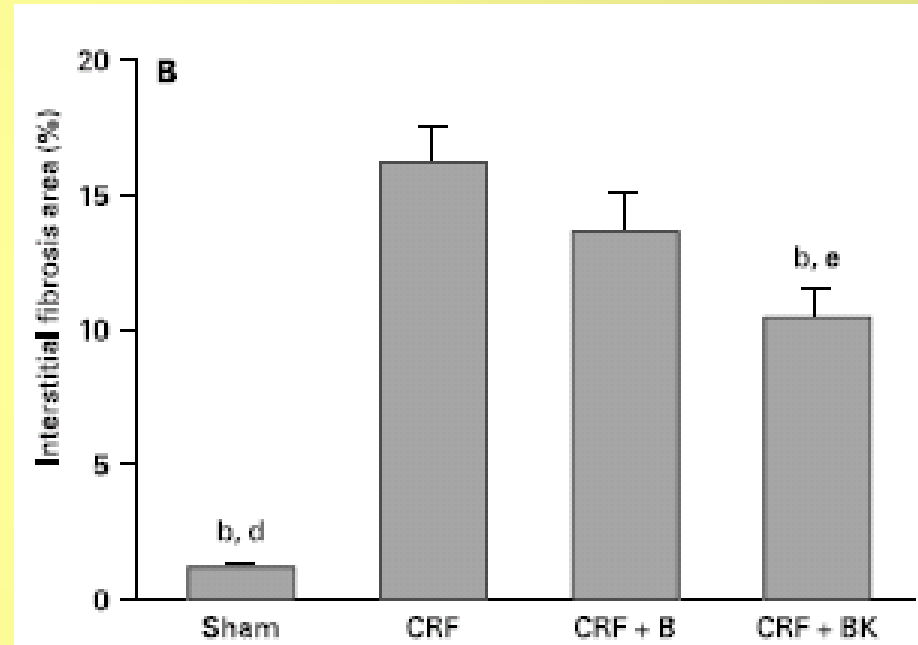
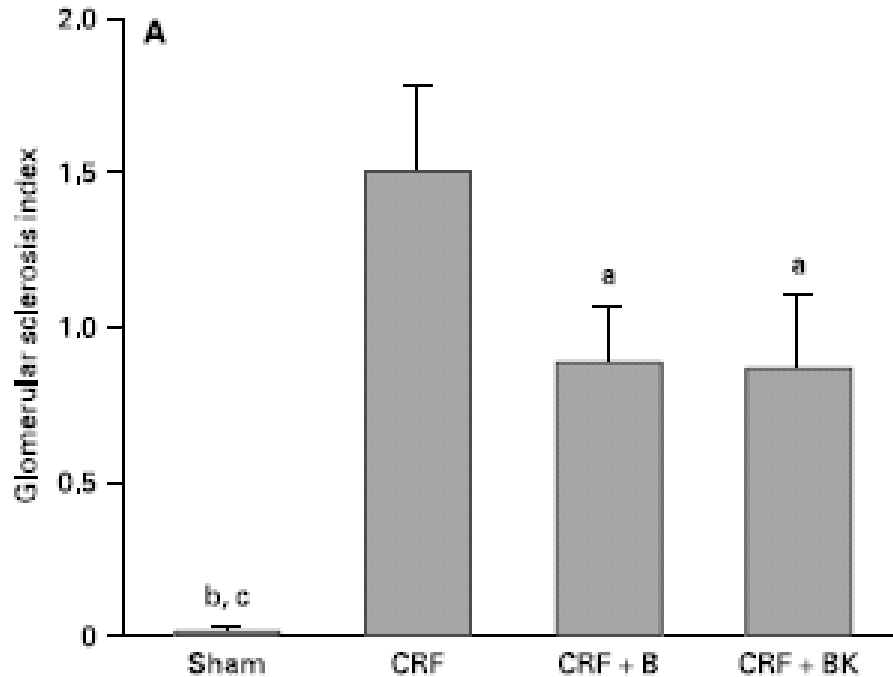
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared with normal rats

ICAM, intracellular adhesion molecule-1; TGF- β 1, transforming growth factor beta-1; TIMP-1, tissue inhibitor of metalloproteinase-1.

Miyazaki T et al. *Kidney International*.1997;63:S211-S214.



IS, AST-120 AND ACE-I



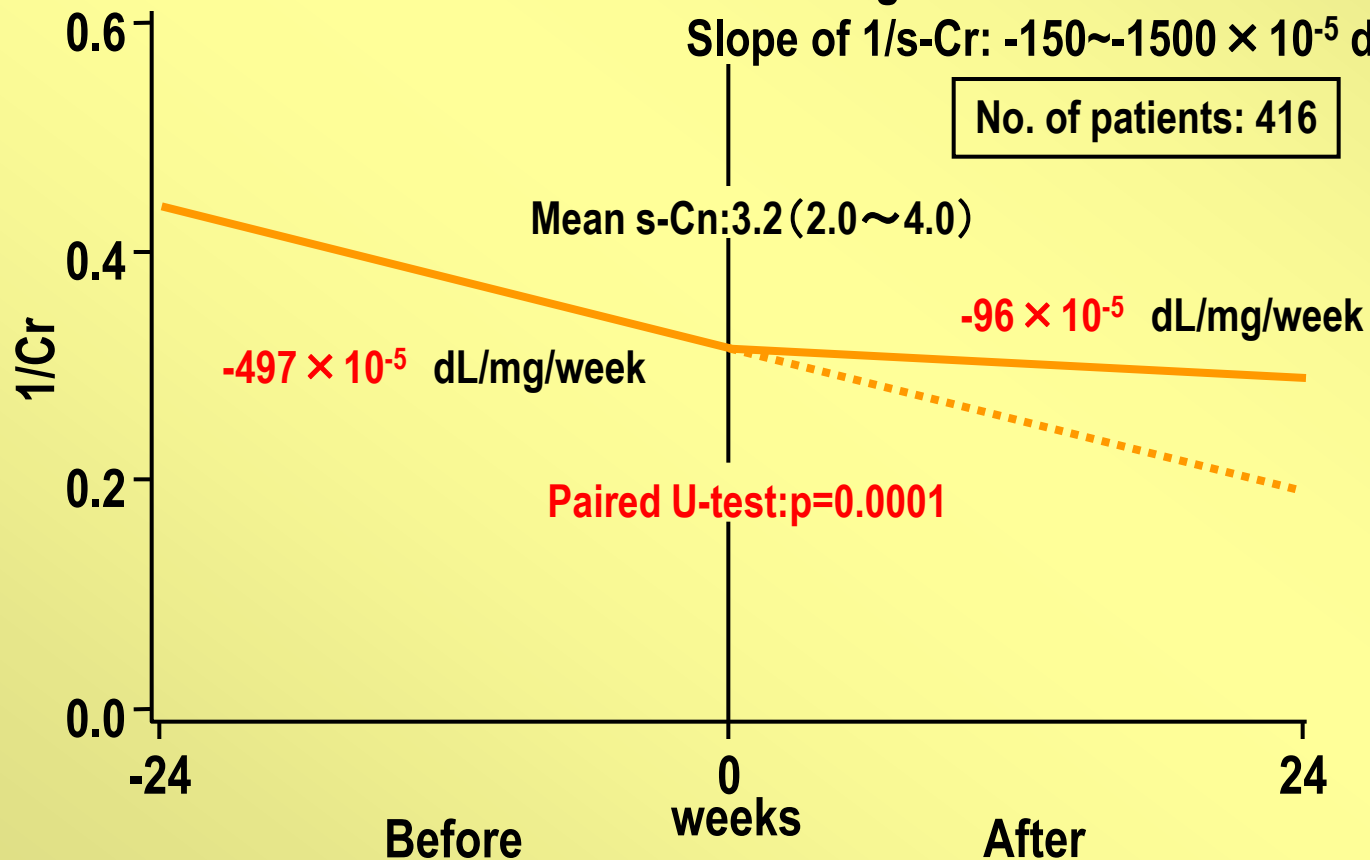
1/s-Cr slope Before and After the Initiation of Kremezin Administration

(from Phase IV data)

Cr=2~4 mg/dL

Slope of 1/s-Cr: $-150 \sim -1500 \times 10^{-5}$ dL/mg/week

No. of patients: 416



AST-120 and Progression of CKD Clinical Experience in Japan (6 g/day)

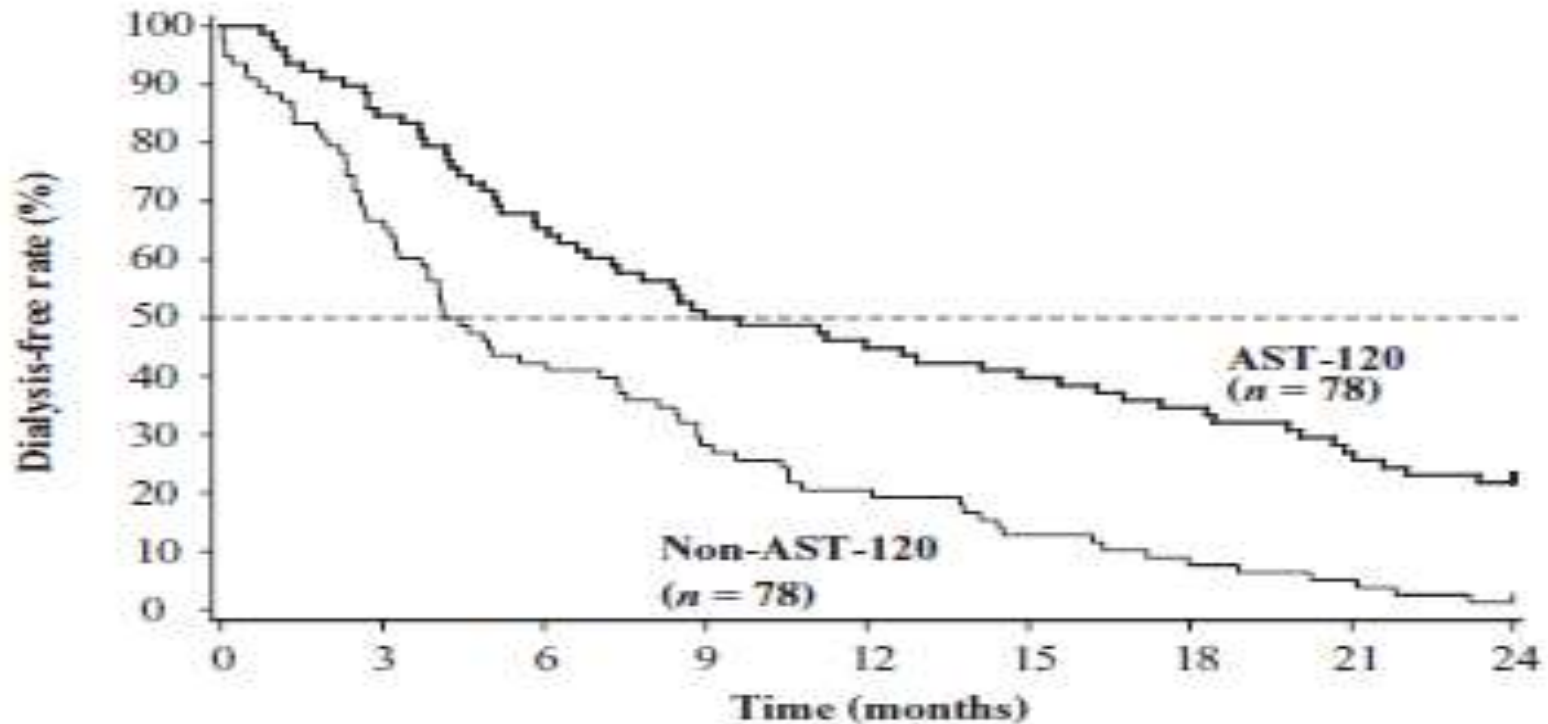


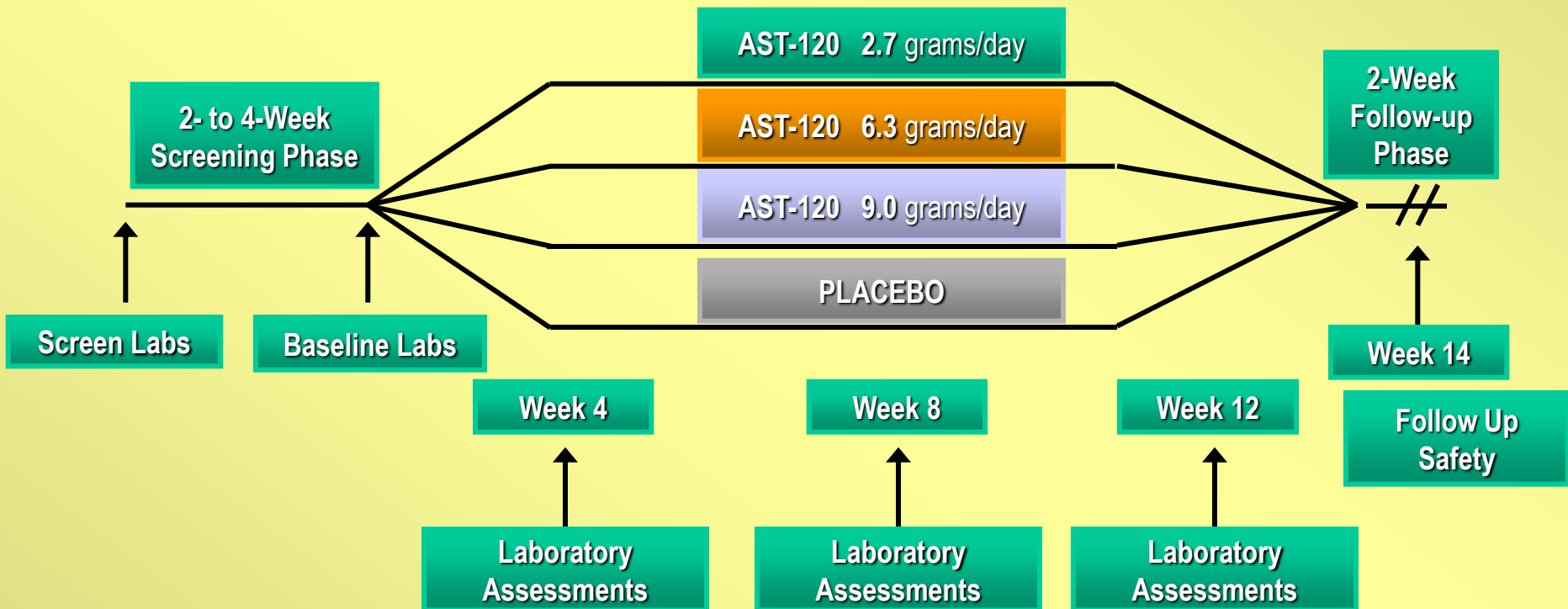
FIG. 1. Effect of AST-120 on dialysis initiation in all pair-matched patients. Cumulative percentages of non-dialyzed patients who were given (AST-120) or not given (Non-AST-120) the adsorbent for 24 months.

US PHASE II AST-120 STUDY: PRIMARY OBJECTIVE

- THE PRIMARY OBJECTIVE OF THIS STUDY WAS TO EVALUATE THE POTENTIAL NEPHROPROTECTIVE EFFECT(S) OF 3 DOSES OF AST-120 COMPARED WITH PLACEBO AS PRIMARILY ASSESSED BY CHANGE FROM BASELINE IN SERUM-INDOXYL SULFATE (S-IS) LEVELS, A PUTATIVE UREMIC TOXIN WITH POTENTIAL TO ACCELERATE PROGRESSION OF CHRONIC KIDNEY DISEASE.

Study Schematic

3-Month (12 Weeks) Double-Blind Treatment Phase



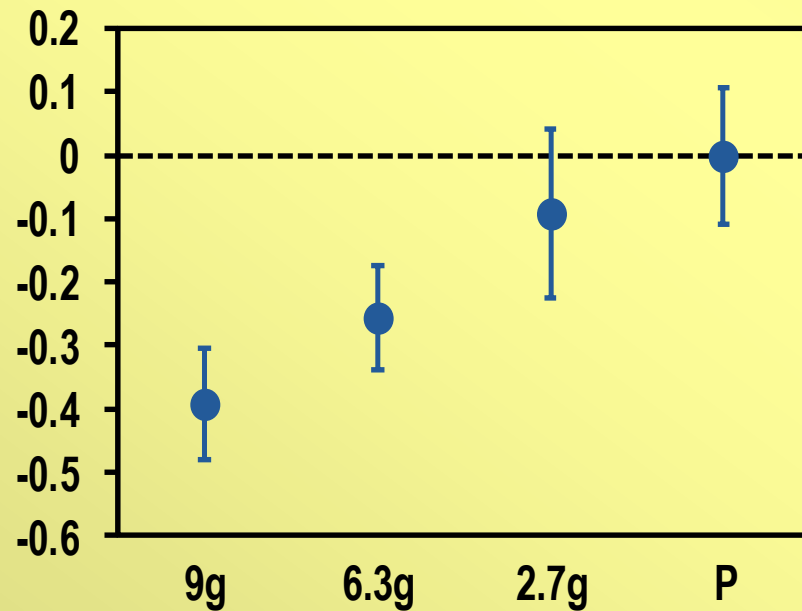
PATIENT DISPOSITION (number)

Parameter	9.0 g Krem.	6.3 g Krem.	2.7 g Krem.	Placebo	Total #
Patients randomized but never received study drug	2	1	1	3	7
Safety population	39	40	39	39	157
Intent to treat population	38	39	39	38	154
Per protocol population	31	33	33	35	132
Completed treatment	33	32	33	34	132
Not completed treatment	6	8	6	5	25

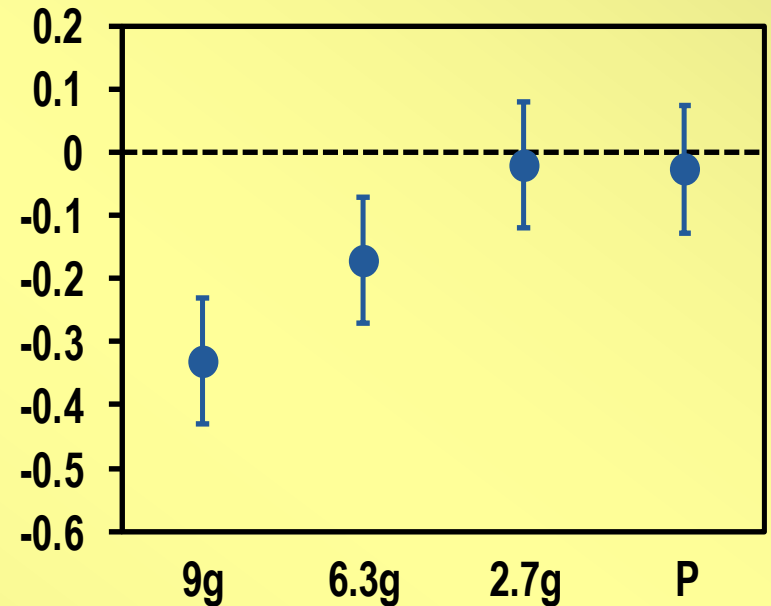
MEAN CHANGE IN SERUM IS FROM BASELINE TO WEEK 8 AND WEEK 12

(Mean + 95% Confidence Interval)

Week 8



Week 12



URINARY CREATININE EXCRETION STUDY (KRM-102): RESULTS

- Randomized, double-blind, cross-over study
 - 2 7-day treatment periods: AST-120 (3 g, 3 x daily) and placebo
 - 20 patients with mild CKD (mean baseline sCr: ~2 mg/dL)
 - In controlled Phase I unit during each treatment period

	AST-120 9.0 g/day	Placebo	Geometric Mean Ratio (90% CI)
Urinary Creatinine Excretion (mg/dL)	1264.7	1286.1	0.98 (0.91 – 1.07)
Creatinine Clearance (mL/min)	46.1	45.4	1.02 (0.92 – 1.12)
Serum Creatinine (mg/dL)	1.73	1.79	0.97 (0.91 – 1.02)

Geometric mean values at end of each 7-day treatment period

JSN AST-120 STUDY

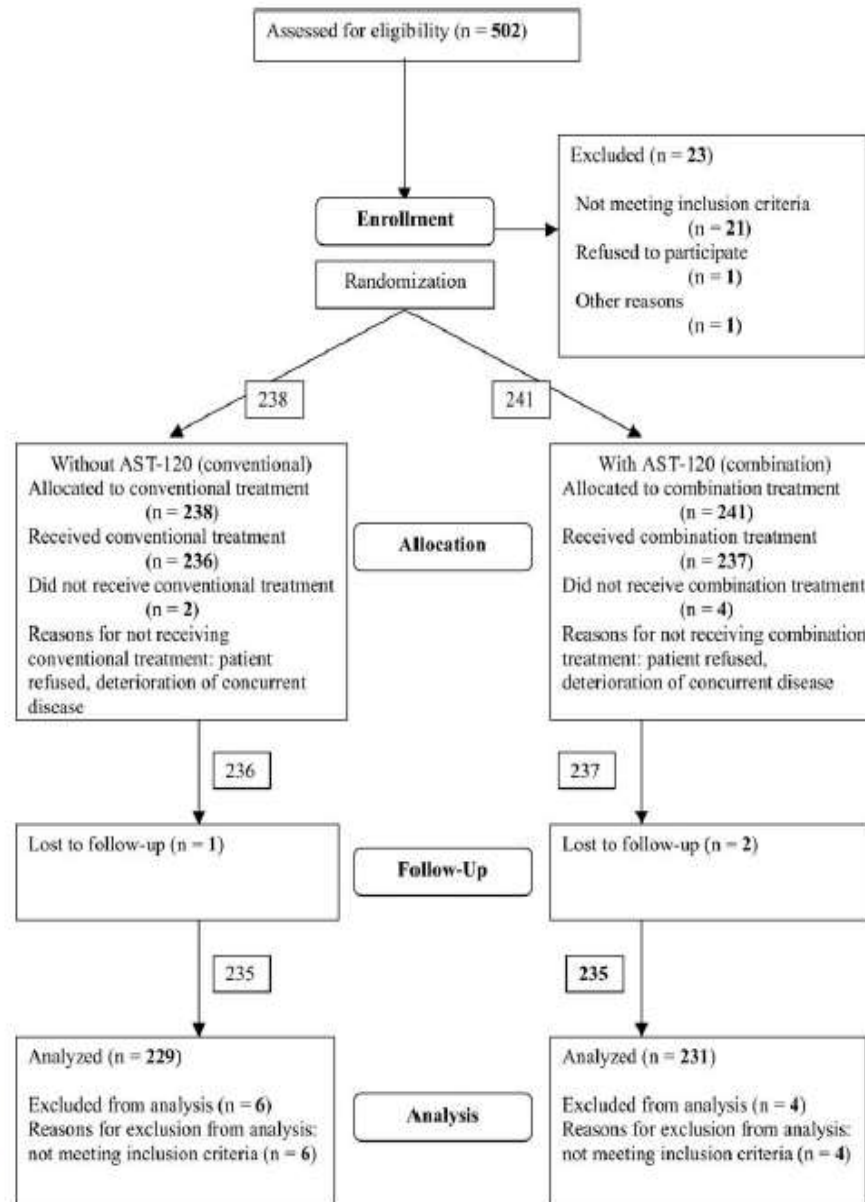
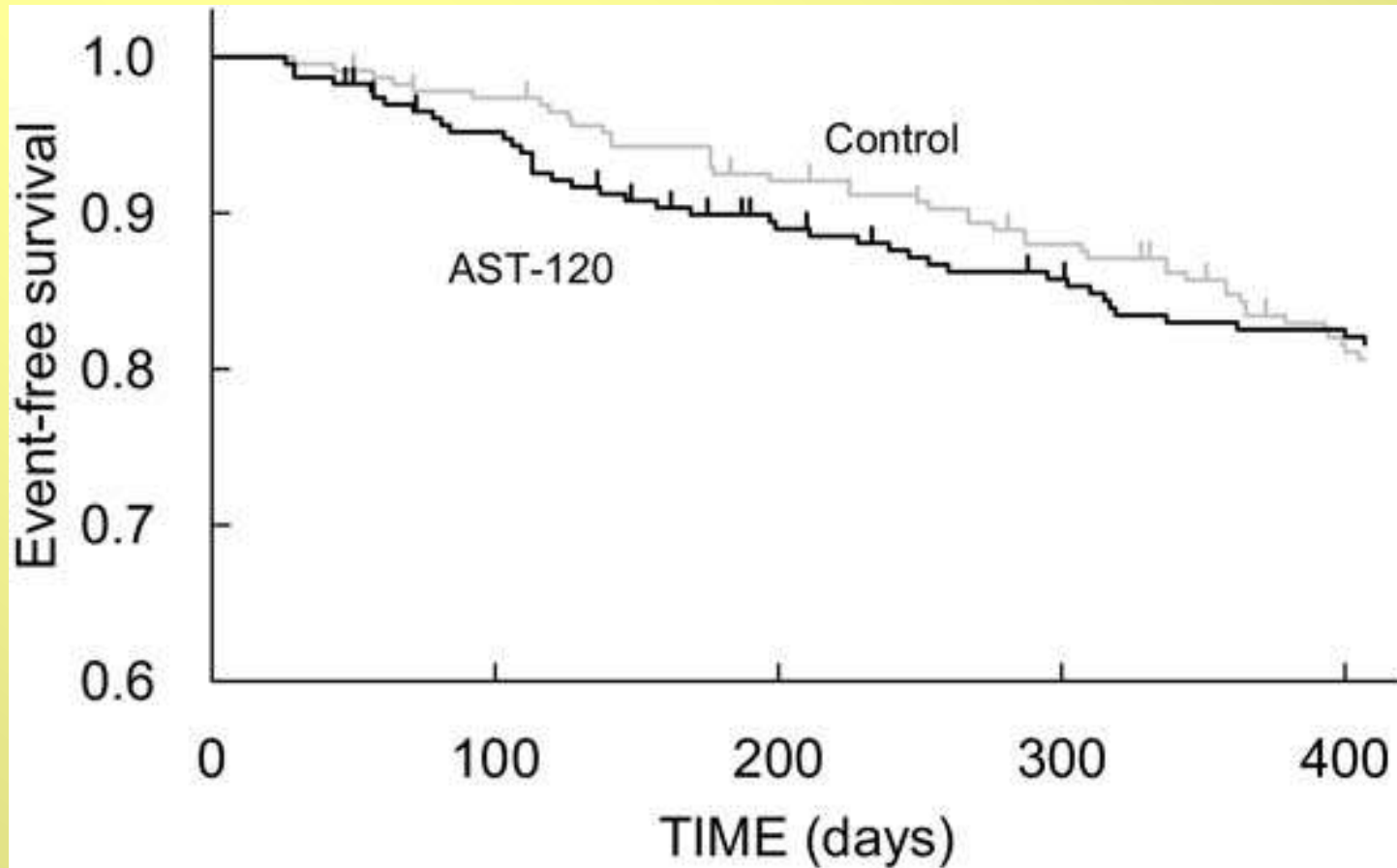


Figure 1. Carbonaceous Oral Adsorbent's Effects on Progression of Chronic Kidney Disease (CAP-KD) participant flow chart.

Akizawa et al. AJKD 54:459-467; 2009

Primary Endpoint: Doubling Serum Creatinine, Creatinine ≥ 6 mg/dl, Death, Dialysis, Transplant



Effect of AST-120 on estimated Glomerular Filtration Rate Over Time in Japan (6 g/day)

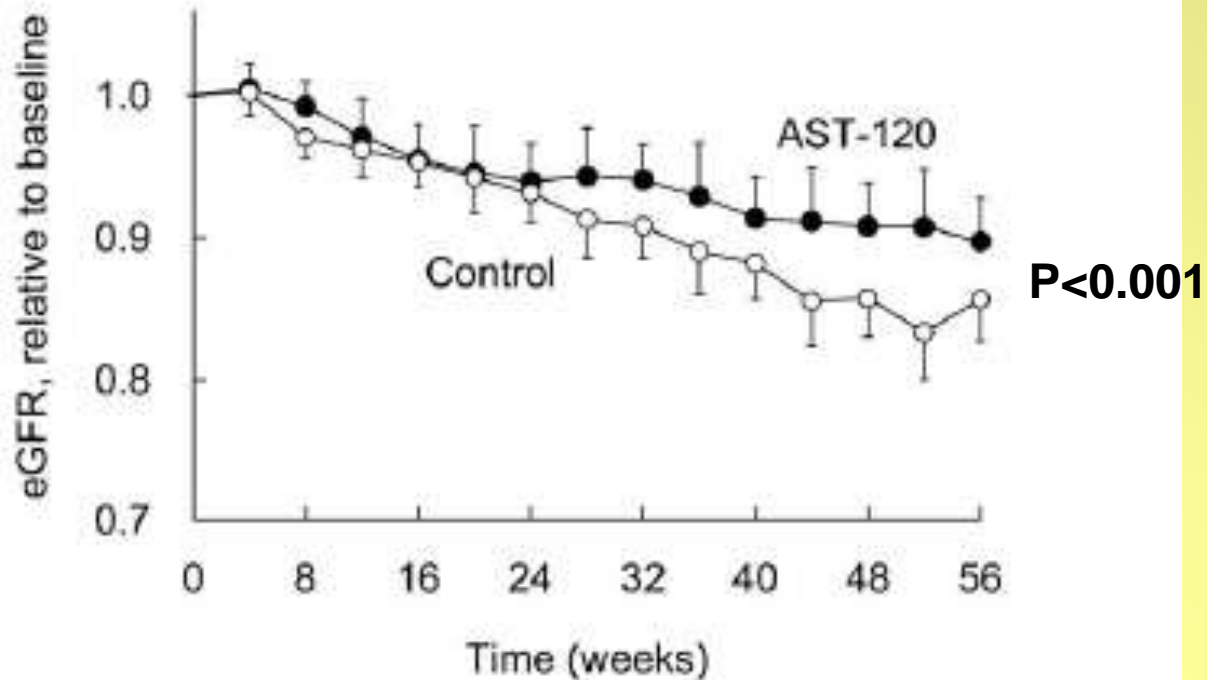


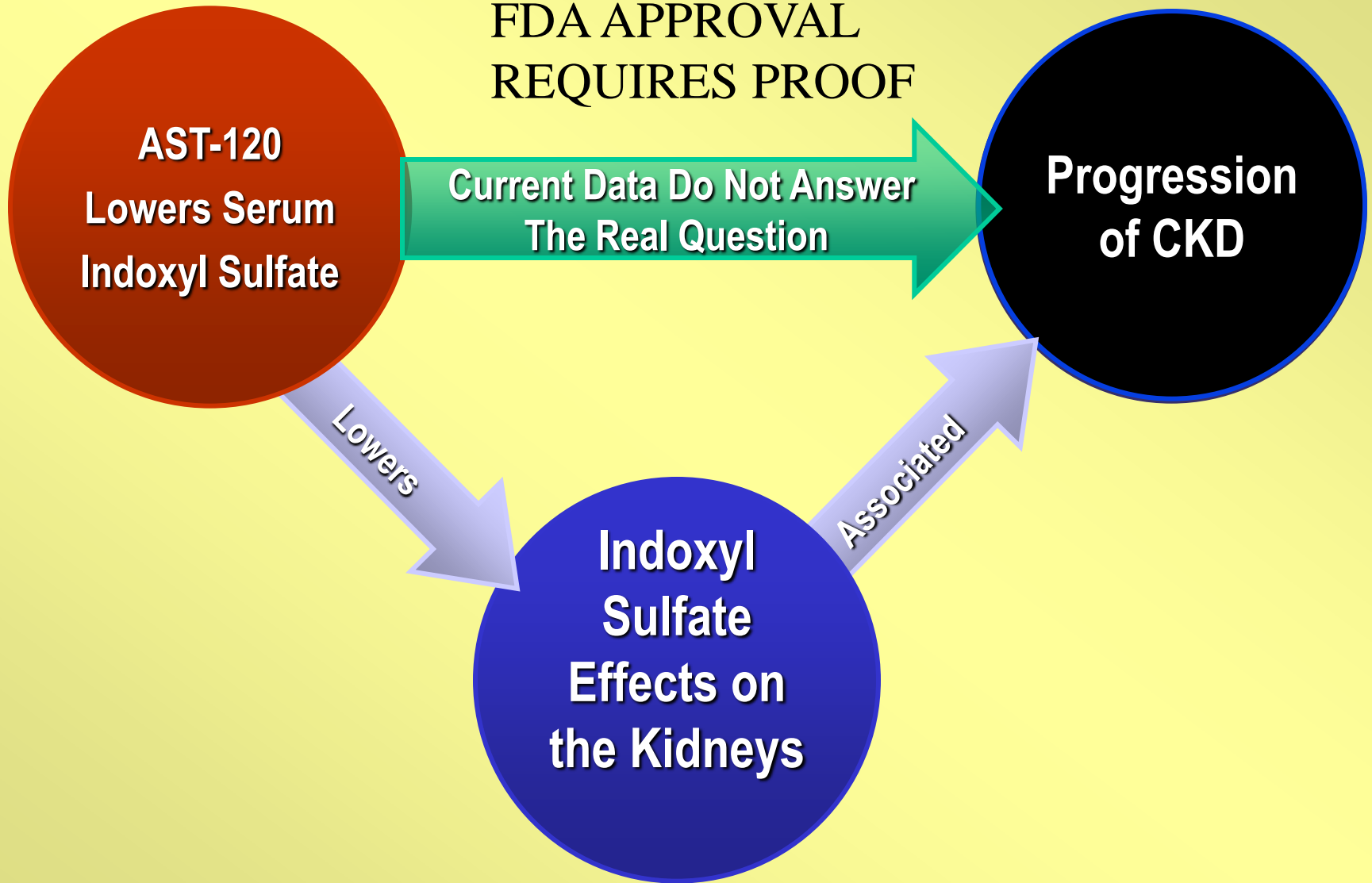
Figure 3. Estimated glomerular filtration rate (eGFR; estimated as described in Matsuo et al¹⁸) over time, by treatment group. Vertical lines indicate 95% confidence intervals.

Limitations of the Study by Akizawa et. al.

- 1 YEAR STUDY/RELATIVELY SMALL STUDY
- FEW DIABETICS
 - ~23% WITH DIABETES
- TOO FEW EVENTS: PRIMARY END POINTS NOT REACHED
 - BASELINE CREATININE; ~2.65 mg/dl (233 mmol/l)
 - THEREFORE, MEAN eGFR WOULD HAVE HAD TO BE < 11 ml/min BEFORE THE INITIATION OF DIALYSIS
- MAJORITY OF SUBJECTS CKD IV AND V
 - BASELINE eGFR: ~22 ml/min

Why EPPIC?

FDA APPROVAL
REQUIRES PROOF



AST-120
Lowers Serum
Indoxyl Sulfate

Current Data Do Not Answer
The Real Question

**Progression
of CKD**

Lowers

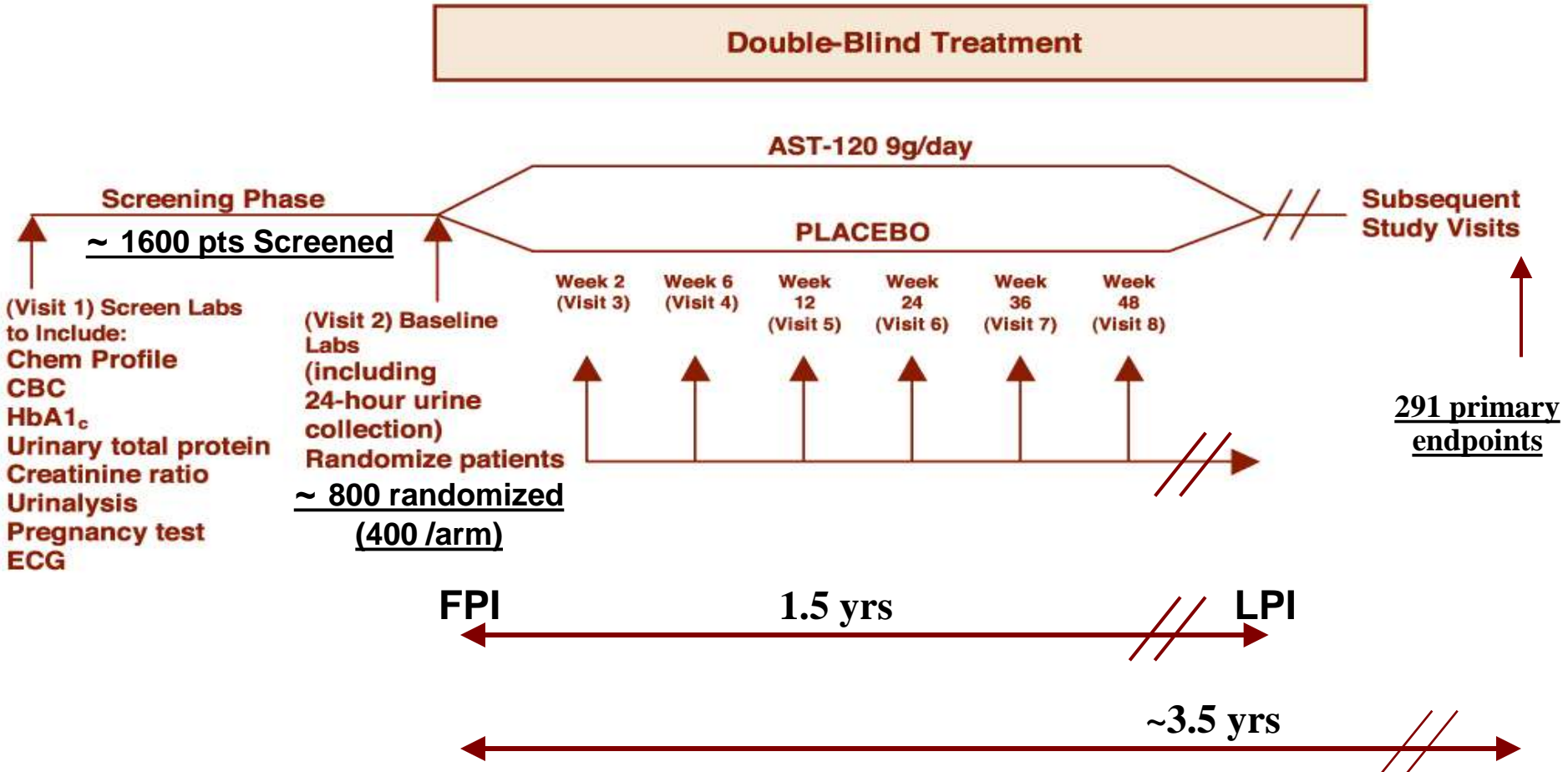
**Indoxyl
Sulfate
Effects on
the Kidneys**

Associated

Primary Objectives

- Demonstrate that AST-120 reduces the risk of developing a component of the triple composite endpoint—“time to”
 - Doubling of sCr
 - Dialysis
 - Transplantation
- Assess long-term safety of AST-120 in CKD patients

Study Design



Two clinical trials will be conducted at a total of approximately 240 study centers worldwide

EPPIC Trial Milestones

Milestone	Date
Final Protocol	May 2007
Sites Selected	February – June 2007
Investigator Meetings	June 2007 (Chicago), October 2007 (Budapest), and November 2007 (Milan)
	January 2008 (Buenos Aires), September 2008 (Kiev), and October 2008 (Dallas)
Sites Initiated	June 2007 – November 2009
First Patient Randomized	August 2007
Last Patient Randomized	January 2010
Completed Half of Endpoints	March 2010

Global Status

Patient Status as of December 30, 2010

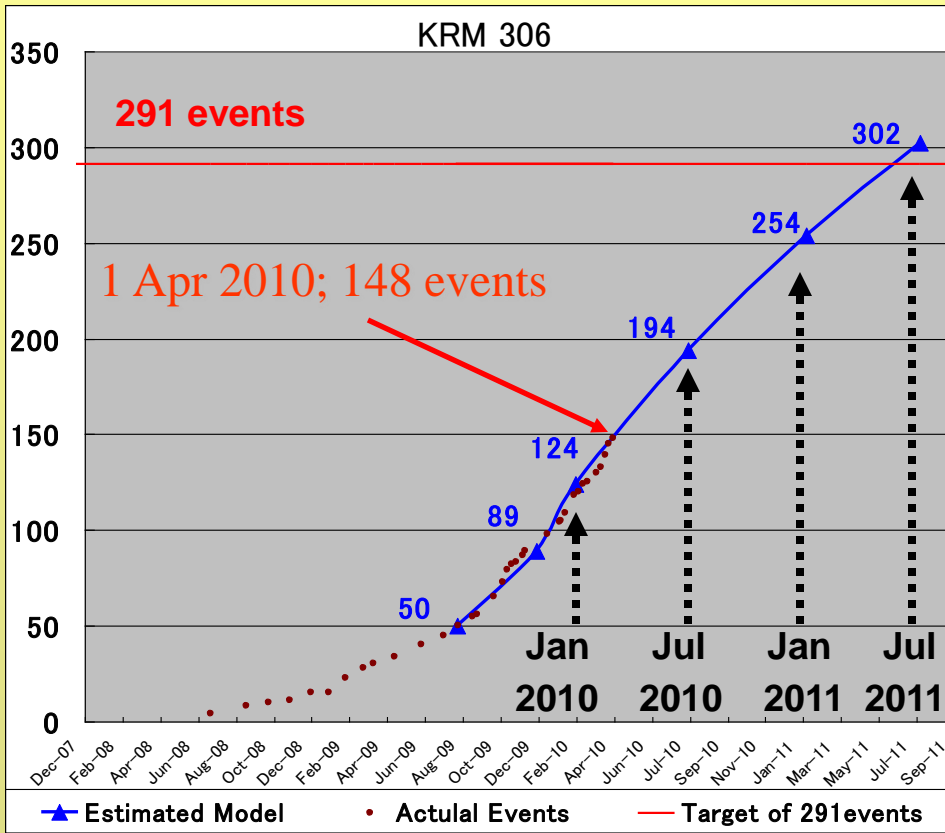
	Total Screenings	Randomized	Early Term	Discontinued in IVRS	Primary Endpoints	Secondary Endpoints
KRM-306	2026	1021	224	245	268	102
KRM-307	2195	1015	207	246	265	115
Total	4221	2036	431	491	533	217

SAE Status as of December 30, 2010

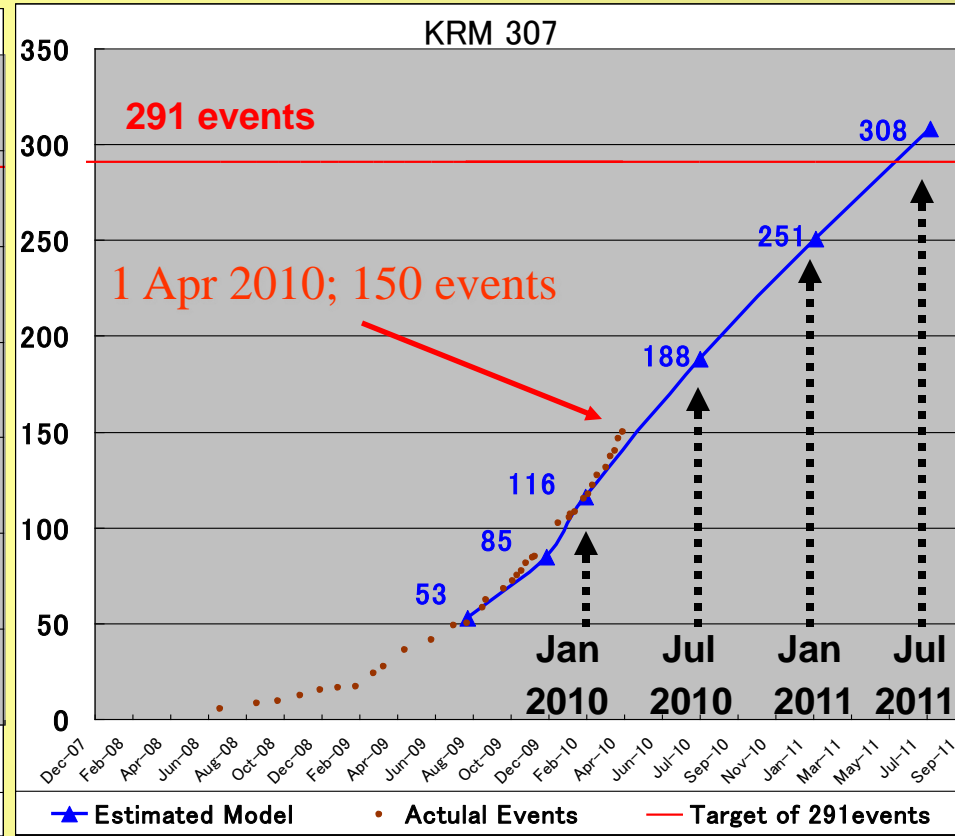
	Initial SAEs reported	Actual SAEs
KRM-306	552	501
KRM-307	465	444
Total	987	945

EVENT PREDICTION

KRM 306

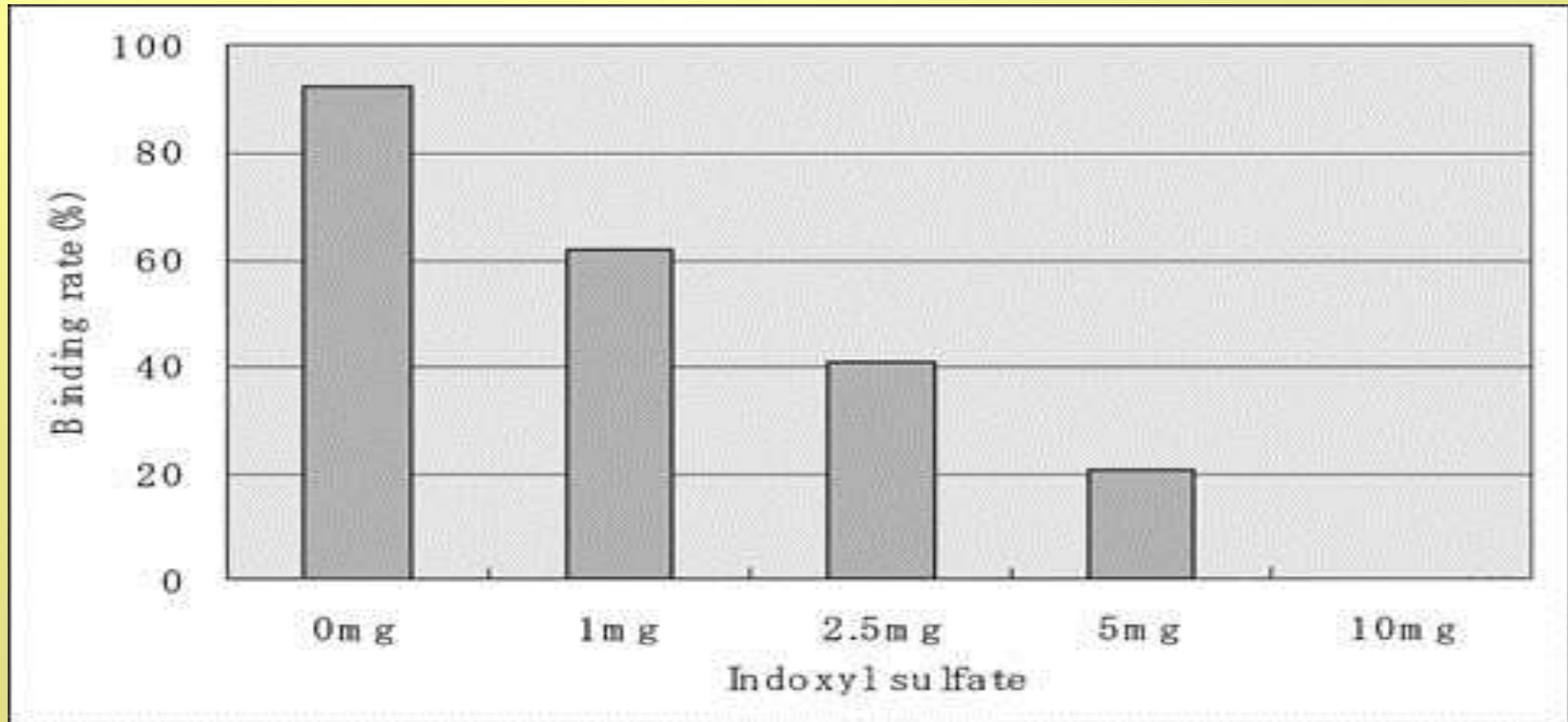


KRM 307



291 Primary Endpoints estimated to be adjudicated by July 2011

BINDING RATE OF TRP WITH BSA IN THE COMPETITION WITH IS IN VITRO STUDY.



EFFECT OF AST-120 ON TRP AND IS CONCENTRATIONS IN PATIENTS WITH

	TRP			Total IS
	Total	Free	Bound (%)	
Before	0.90 ± 0.08*	0.28 ± 0.06*	68.7 ± 6.8*	1.79 ± 1.01*
After	1.16 ± 0.18* †	0.19 ± 0.03†	83.1 ± 3.8* †	1.15 ± 0.85* †
Healthy	2.45 ± 0.45	0.20 ± 0.06	92.0 ± 1.4	0.06 ± 0.01

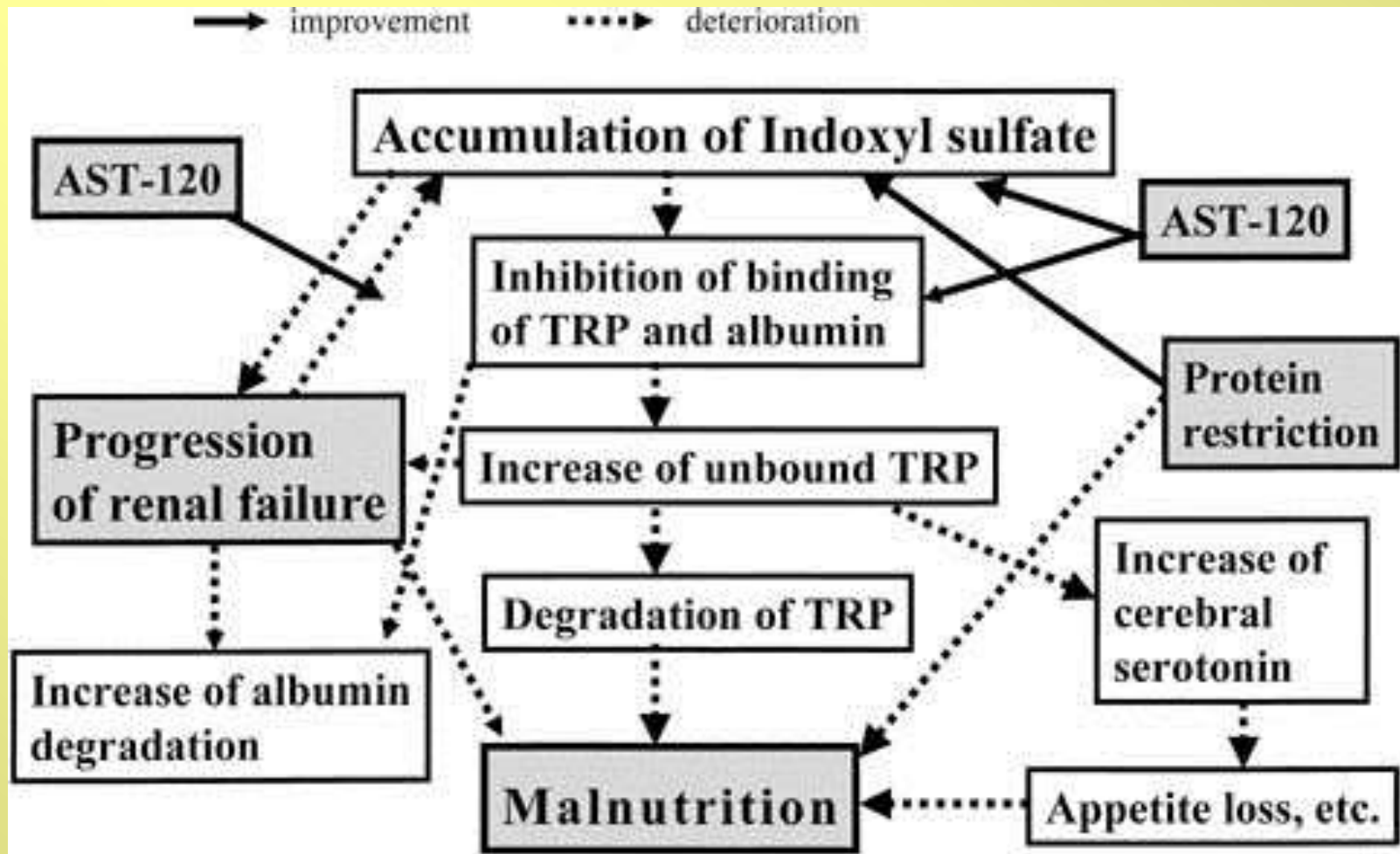
NOTE. Values expressed as mean ± SD. Values are serum levels in five patients with CRF before and after 2 weeks of AST-120 administration, in five healthy volunteers.

Abbreviations: Total, serum total concentration; free, serum protein-unbound concentration; bound, serum protein-bound rate.

* Significant difference ($P < 0.05$) versus healthy subjects.

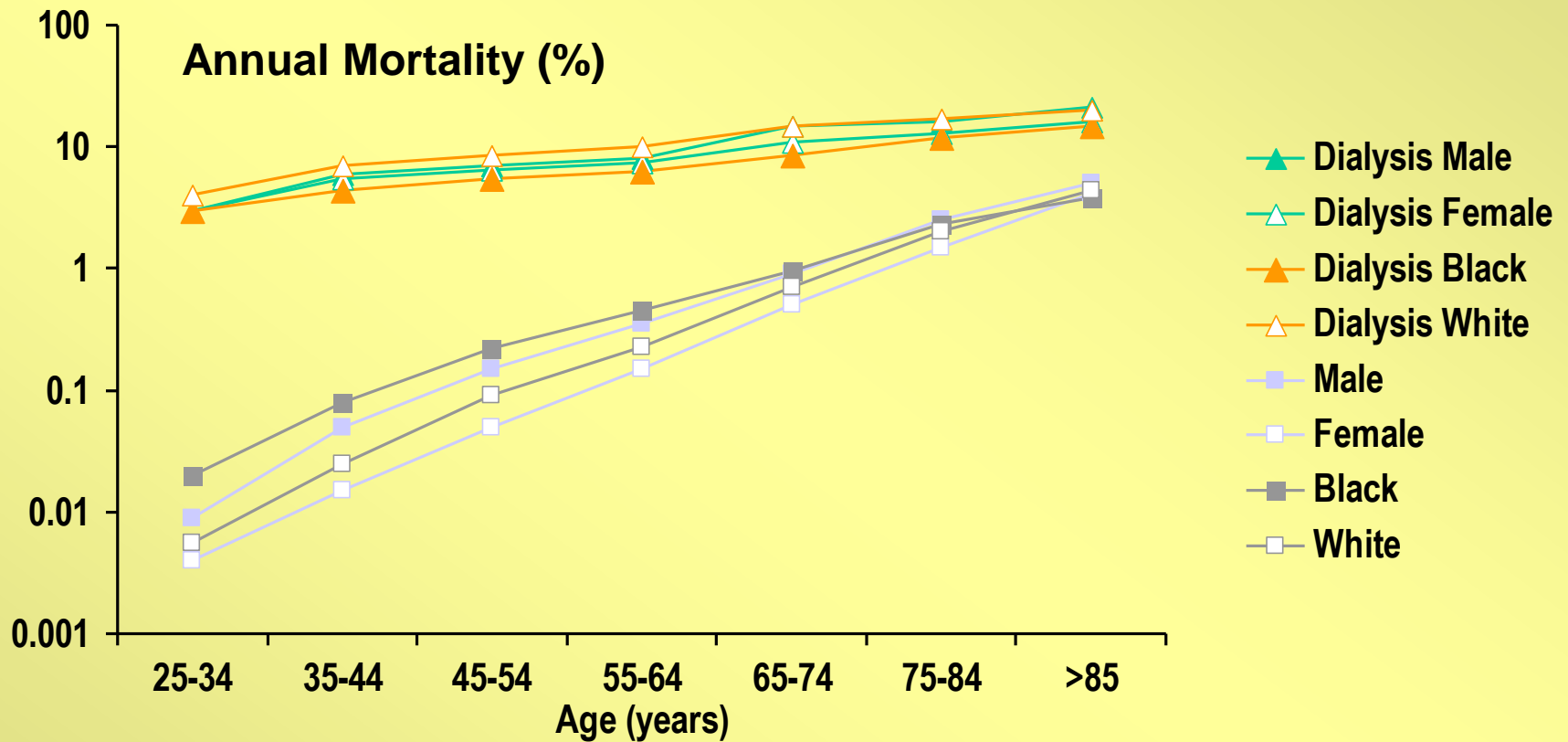
†Significant difference ($P < 0.05$) versus before AST-120 administration.

ABNORMALITIES IN TRYPTOPHAN IN CKD



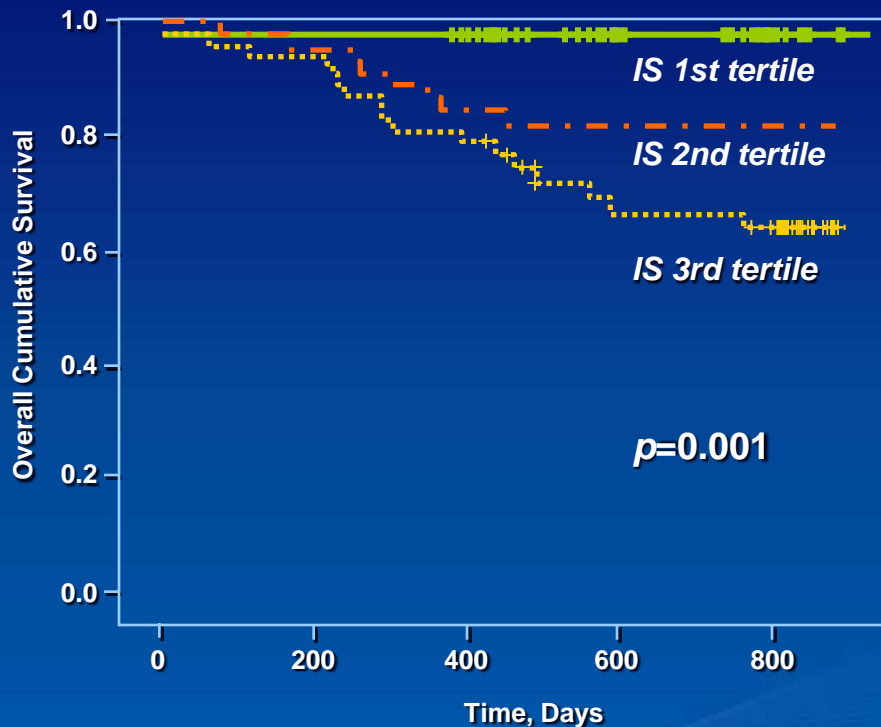
THE ROLE OF UREMIC TOXINS IN CARDIOVASCULAR DISEASE

Cardiovascular Mortality in ESRD

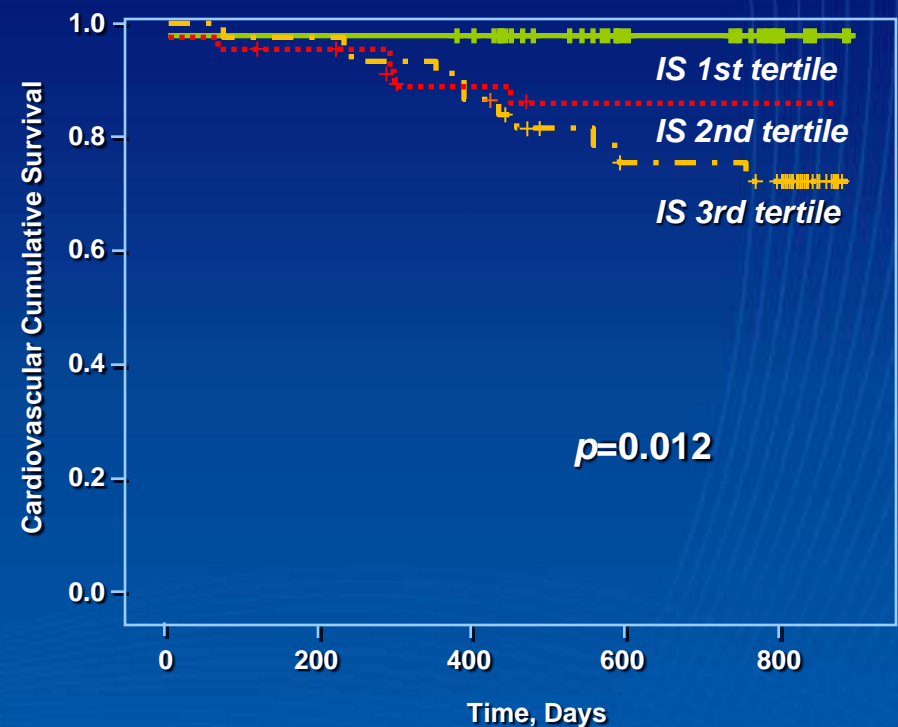


Serum Levels of Indoxyl Sulfate Are Associated with Overall and Cardiovascular Mortality

Overall Cumulative Survival



Cardiovascular Cumulative Survival



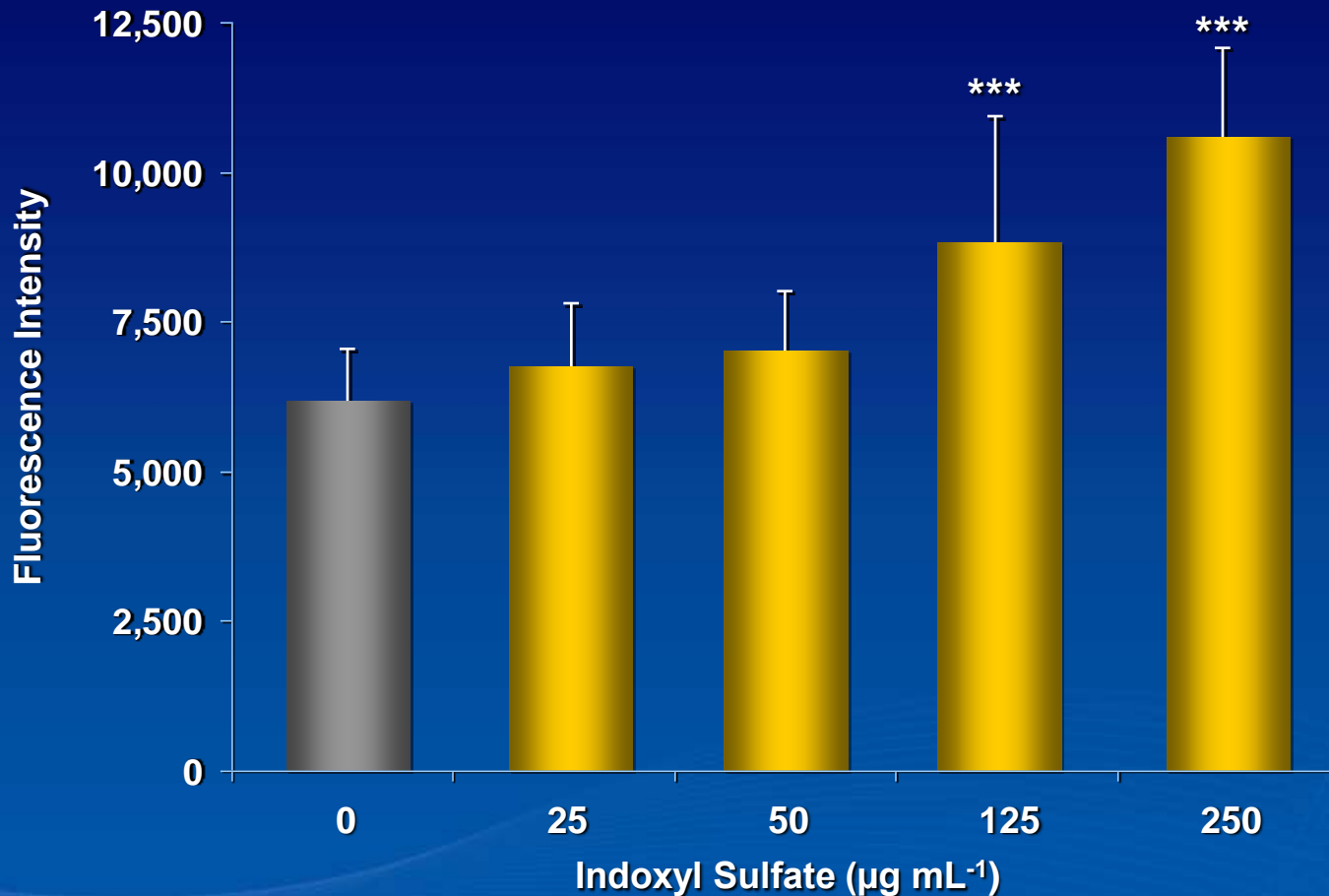
IS, indoxyl sulfate. 139 CKD patients. Serum IS levels were assessed.

Death records were made prospectively by considering all patients at least 1 yr before the study end date (June 30, 2008).

Cardiovascular mortality defined as any death directly related to cardiovascular system dysfunction (stroke, myocardial infarction, congestive heart failure, or sudden death).

Barretto FC et al. *Clin J Am Soc Nephrol*. 2009;4:1551–1558.

Indoxyl Sulfate Induces Oxidative Stress in Endothelial Cells

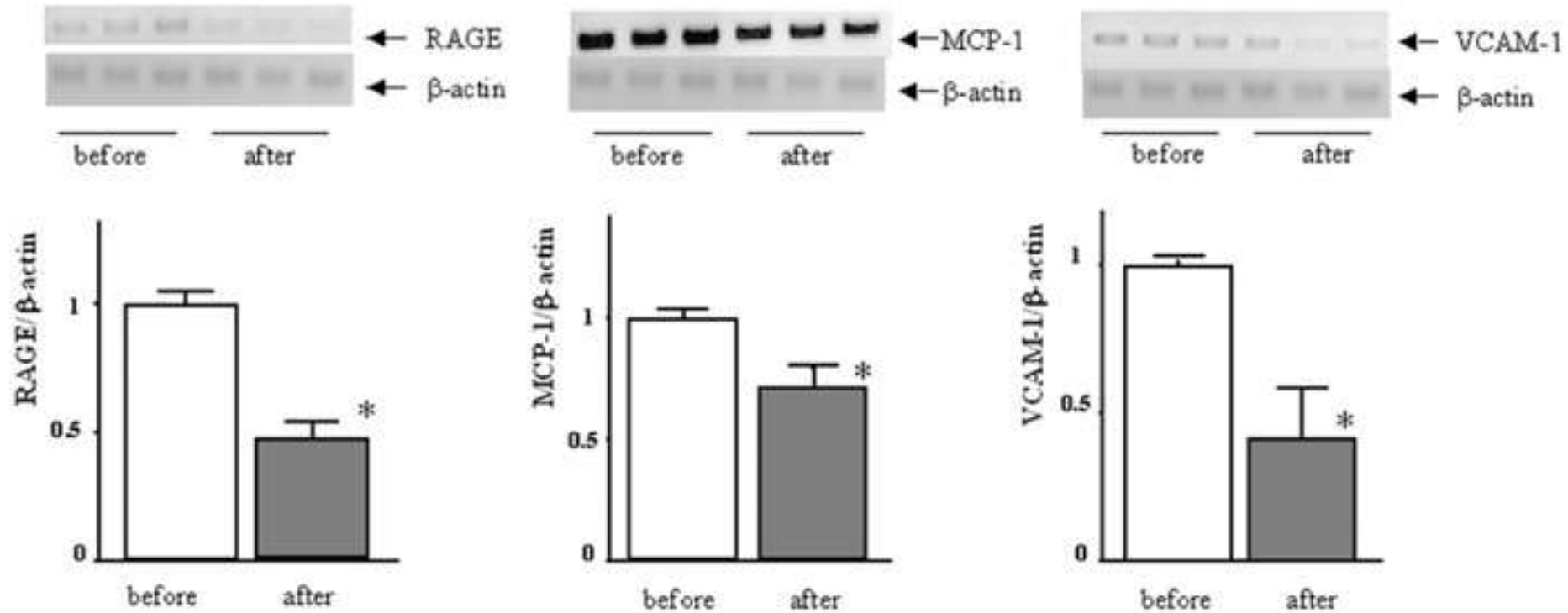


*** $p < 0.001$ vs control

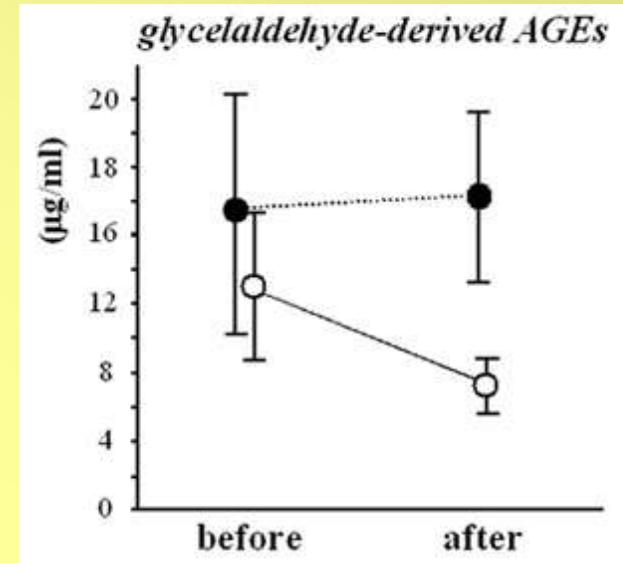
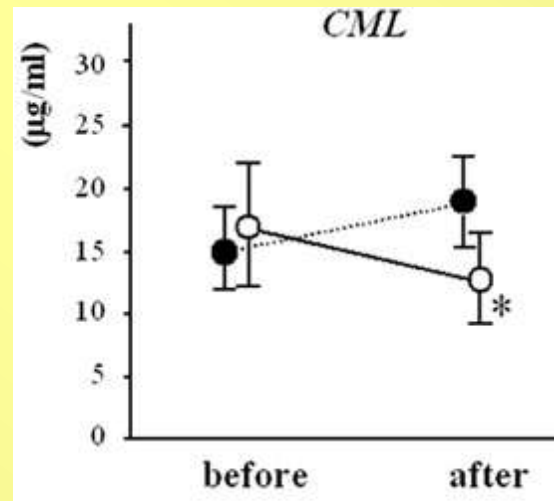
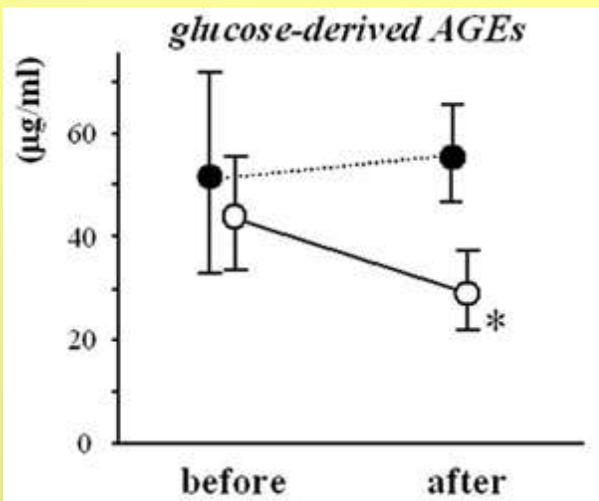
Human umbilical vein endothelial cells (HUVEC) were incubated for 5 h in medium without and with different concentrations of indoxyl sulfate, and then ROS production was measured by cytofluorimetry.

Dou L et al. *J Thrombosis and Haemostasis*. 2007;5:1302-1308.

Effects of patient serum on atherosclerosis-related gene expression in HUVECs. HUVECs were treated with patient serum before or after AST-120 treatment for 12 h, and RAGE, VCAM, and MCP-1 mRNA levels were determined



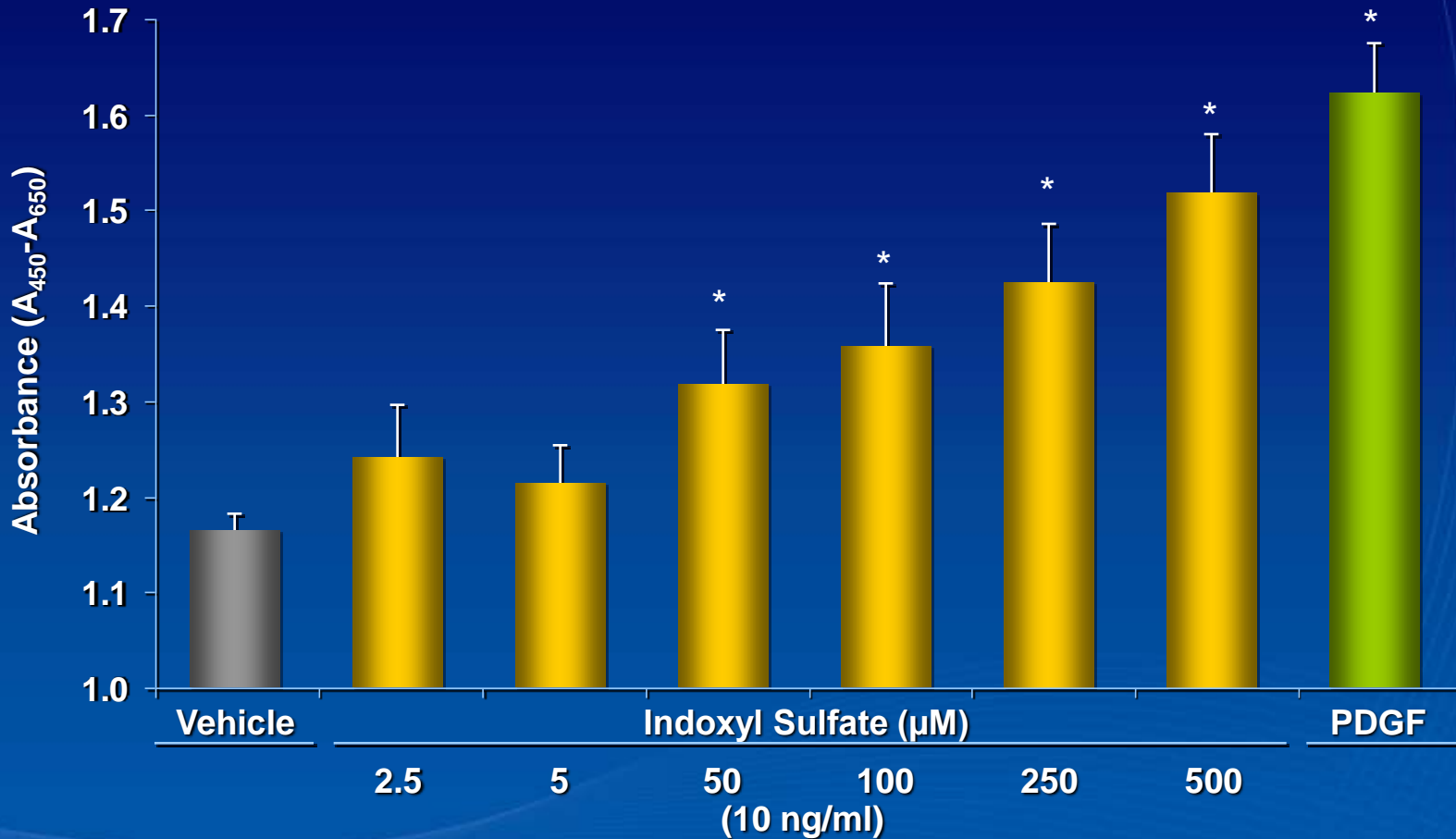
Effects of oral administration of AST-120 on serum levels of AGEs in patients with nondiabetic CR



FAST-treated patients (n = 10); , control patients without AST treatment(n = 6).

*P < 0.05 compared with the value before treatment.

Indoxyl Sulfate Stimulates Proliferation of Vascular Smooth Muscle Cells



* $p < 0.05$ compared with the vehicle.

VSMC, vascular smooth muscle cells; PDGF, platelet derived growth factor.

VSMCs were isolated from the aortas of male Sprague-Dawley rats.

Yamamoto H et al. *Kidney Intl.* 2006;69:1780-1785.

AST-120 and Atherosclerosis

- ApoE Deficient Mice: Uni- and Subtotal Nephrectomy
- AST-120 given immediately or 4 weeks after renal ablation
- Decreased aortic deposition of Indoxyl Sulfate
- Decreased atherosclerotic burden

Increased Aortic Calcification and Wall Thickness with Indoxyl Sulfate in Hypertensive Rats

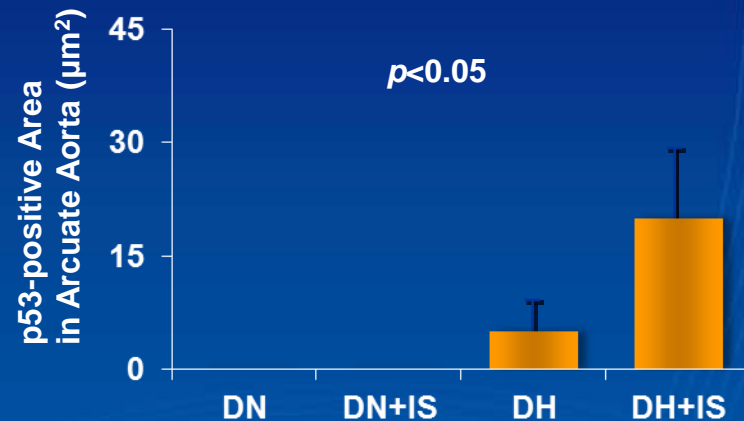
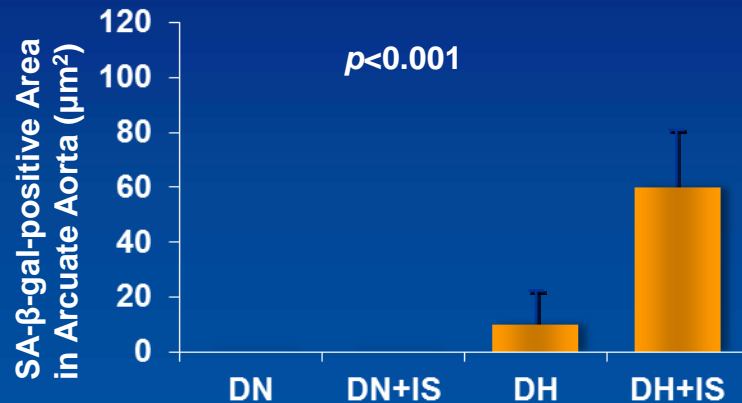
<i>Histological Findings</i>	<i>DN (n=8)</i>	<i>DN + IS (n=8)</i>	<i>DH (n=8)</i>	<i>DH + IS (n=8)</i>
<i>Calcification area (μm^2)</i>	0 ± 0	0 ± 0	2005 ± 1161	$13784 \pm 4677^{**}$
<i>Wall Thickness (μm)</i>	$226 \pm 4^*$	238 ± 8	258 ± 13	$318 \pm 6^{***}$

* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$ by Fisher's LSD test (ANOVA) as compared with DH
DN, Dahl salt-resistant normotensive rats; DN+IS, Dahl salt-resistant normotensive indoxyl sulfate-administered rats;
DH, Dahl salt sensitive hypertensive rats; DH+IS, Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats
Adijiang A et al. *Biochem and Biophys Res Commun.* 2010;399:637-641.

Indoxyl Sulfate Promotes Cell Senescence in Aorta of Hypertensive Rats

*Indoxyl Sulfate Promotes SA β -gal Expression
Marker of Senescence*

*Indoxyl Sulfate Induces Activated p53 Expression
Suppresses Cell Proliferation*

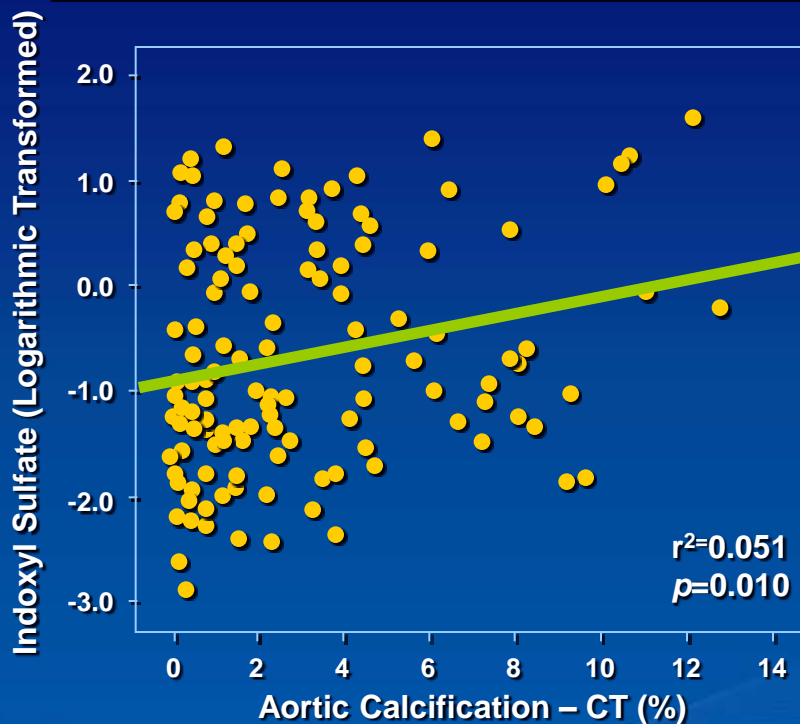


DN, Dahl salt-resistant normotensive rats; DN+IS, Dahl salt-resistant normotensive indoxyl sulfate-administered rats; DH, Dahl salt sensitive hypertensive rats; DH+IS, Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats

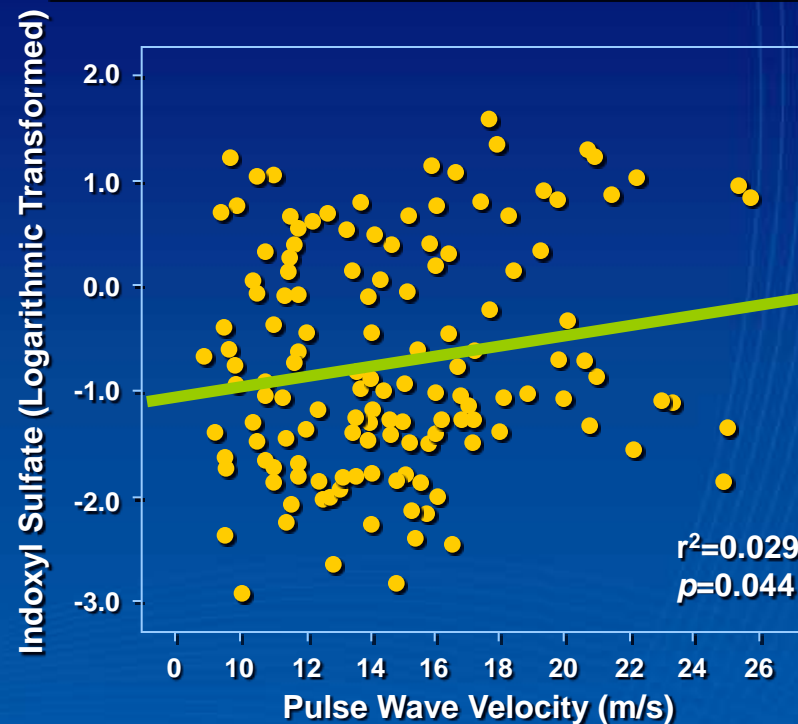
Adijiang A et al. *Biochem and Biophys Res Commun.* 2010;399:637-641.

Positive and Linear Relationship Between Indoxyl Sulfate and Vascular Stiffness and Aortic Calcification Measures

Relationship Between Serum IS and the Aortic Calcification Score as Quantified by MSCT (n=129)



Relationship Between Serum IS and PWV (n=139)



PWV, pulse wave velocity; MSCT, multislice computer tomography
139 CKD patients. Serum IS levels, aortic calcification and vascular stiffness (measured by pulse wave velocity) were assessed.

Barretto FC et al. *Clin J Am Soc Nephrol.* 2009;4:1551–1558.

AST-120 AND CHANGES IN ARTERIAL PULSE WAVE VELOCITY AND CAROTID ARTERY INTIMA-MEDIA THICKNESS

	AST-120	No AST-120	Healthy controls
PWV, cm/s			
Before	1,980 ± 330*	1,940 ± 360*	1,280 ± 240
12 months	1,840 ± 280**	2,020 ± 380	
24 months	1,780 ± 260**	2,140 ± 410**	
IMT, mm			
Before	0.90 ± 0.22*	0.88 ± 0.20*	0.64 ± 0.14
12 months	0.84 ± 0.20	0.90 ± 0.24	
24 months	0.78 ± 0.18**	0.93 ± 0.26	

* Versus healthy controls, $p < 0.01$. ** Versus before, $p < 0.05$.

Other Adverse Effects of Indoxyl Sulfate

Bone formation¹

- Indoxyl sulfate may exert adverse effects on bone formation
 - Promotion of oxidative stress in osteoblasts
 - Downregulation of PTH receptors
- The combination of these effects may induce skeletal resistance to PTH, resulting in low bone turnover and osteodystrophy

Risk of infection²

- Toxic effects of indoxyl sulfate may increase susceptibility to infection via
 - Induced apoptosis of neutrophils by increased production of ROS
 - Decreased expression of MCP-1, a monocyte chemoattractant

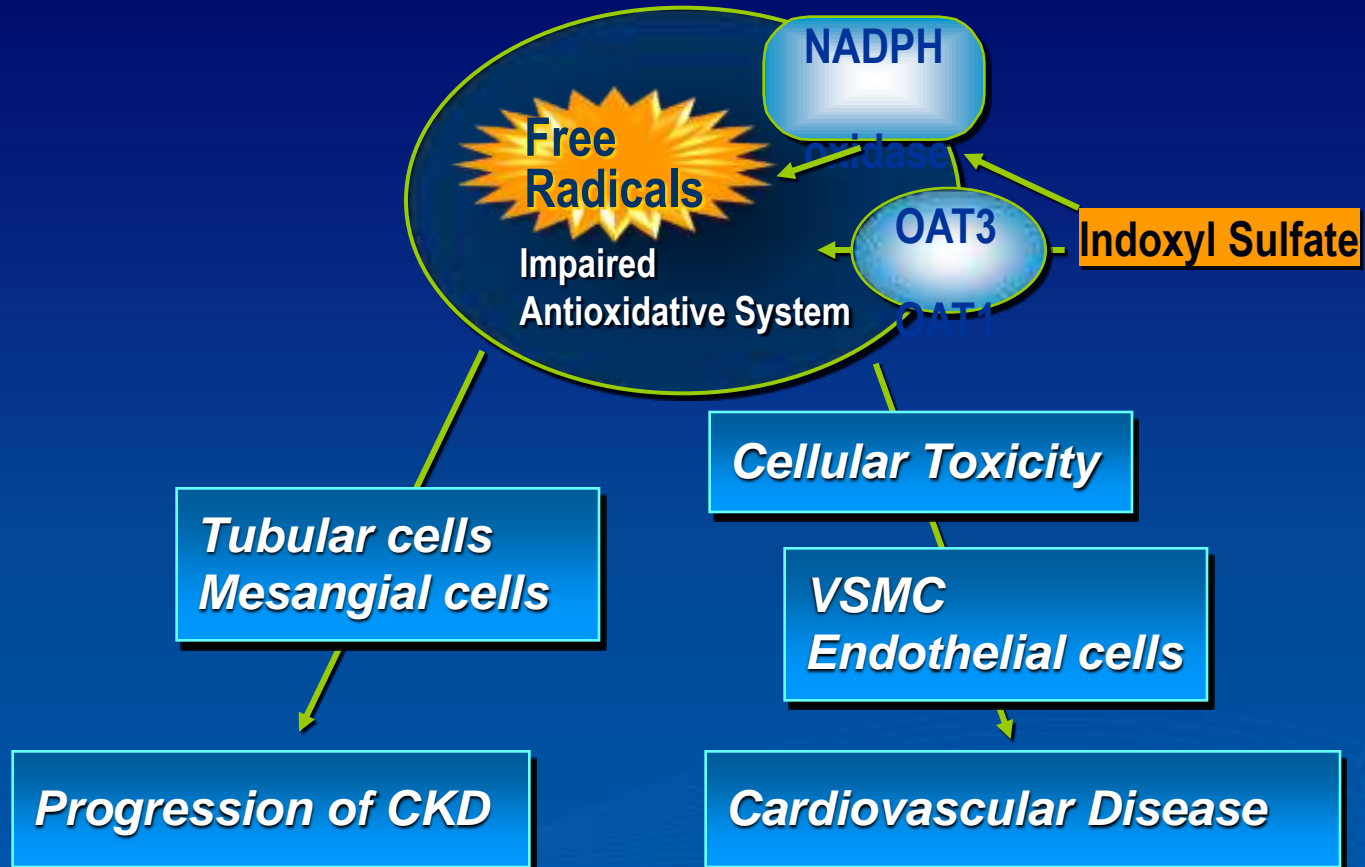
Anemia³

- Indoxyl sulfate may promote anemia via an increase in hemolysis

PTH, parathyroid hormone; ROS, reactive oxygen species; MCP-1, Monocyte chemoattractant protein-1 .

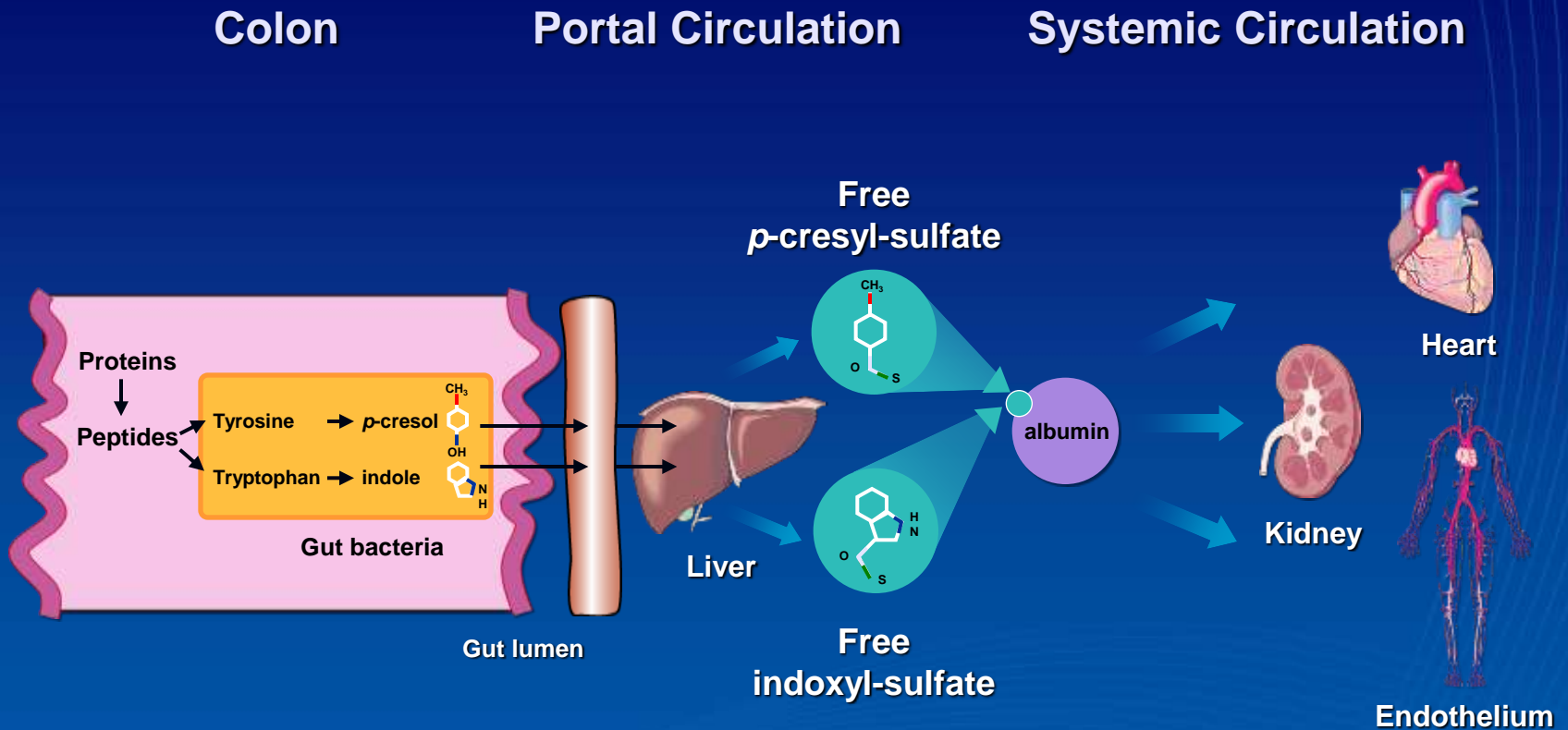
1. Nii-Kono T et al. *Kidney International*. 2007;71:738–743. 2. Cruz EF et al. Abstract presented at: ASN 2010; November 16–21, 2010; Denver, CO. Abstract SA-PO2232. 3. Hirata J et al. Abstract presented at ISURT 2011; May 12-14, 2011; Nagoya, Japan. Abstract P1-09.

Overall Toxicity of Indoxyl Sulfate



The Gut-Kidney Axis

Indoxyl sulfate, *p*-cresyl sulfate and CKD Progression



THANKS FOR YOUR
ATTENTION

