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

250 Million in the US

Early stages of CKD (20 Million?)

SCr ≤ 1.4 mg/dL

SCr ≥ 1.5 mg/dL

SCr ≥ 1.7 mg/dL

SCr ≥ 2.0 mg/dL

Renal Replacement Therapy

Parfrey, Sarnak and Levey, from USRDS and NCHS, AJKD 1998
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

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

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 INEFFECTIVE 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 PROGRESSION

• 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

<table>
<thead>
<tr>
<th>Solute</th>
<th>Group</th>
<th>Endogenous Metabolism</th>
<th>Microbiotic Metabolism</th>
<th>Exogenous Intake</th>
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<tbody>
<tr>
<td>ADMA</td>
<td>Dimethylarginines</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em>-Cresyl sulfate</td>
<td>Phenols</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Phenyl acetic acid</td>
<td>Phenols</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Indoxyl sulfate</td>
<td>Indoles</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Indole 3-acetic acid</td>
<td>Indoles</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>AGEs</td>
<td>N/A</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>N/A</td>
<td>X</td>
<td></td>
<td>X^a</td>
</tr>
<tr>
<td>Oxalate</td>
<td>N/A</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

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

Link Between Oxidative Stress and Inflammation

- Antioxidant Depletion
- ↑ ROS Production
- Oxidative Stress
- NFκB Activation
- Cytokines / Chemokines, Adhesion Molecules
- Leukocyte / Macrophage Activation (Inflammation)

Reactive Oxygen Species Formation *in vitro*

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.
Effect of Indoxyl Sulfate on Serum Creatinine in Uremic Rats

(5/6 nephrectomized rats)

A Closer Look at Indoxyl Sulfate

Uremic toxins are absorbed into circulation, leading to deterioration of renal function.

Indoxyl is metabolized in the liver to indoxyl sulfate.

Absorbed into bloodstream.

Excreted with feces.
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 Promotes SA β-gal Expression Marker of Senescence

Indoxyl Sulfate Up Regulates α-SMA Expression Marker of Fibrosis

Indoxyl Sulfate Functions in Proximal Tubular Cells

Indoxyl Sulfate → ROS → p53

- α-SMA
  - ↑ Fibrosis
  - ↓ Cell Proliferation
- SA β-gal
  - ↑ Senescence

Progression of CRF

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

**Klotho mRNA in Rats**

<table>
<thead>
<tr>
<th></th>
<th>DN (n=8)</th>
<th>DN+IS (n=8)</th>
<th>DH (n=8)</th>
<th>DH+IS (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klotho/GAPDH (Fold Increase)</td>
<td>1.00</td>
<td>0.50</td>
<td>0.75</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* *p<0.05 vs DN; **p<0.05 vs DH.

**Klotho Protein in HK-2 Cells**

<table>
<thead>
<tr>
<th></th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klotho/α-tubulin (Fold Increase)</td>
<td>1.25</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* *p<0.05 vs untreated cells.

INDOXYL SULFATE AND CKD

• TGF $\beta$-1
• METALLOPROTEINASE
• PAI-1
• ABNORMALITIES IN TRYPTOPHAN METABOLISM
• CARDIOVASCULAR EFFECTS?
Mechanisms of Indoxyl Sulfate Induced Nephrotoxicity

Luminal Membrane

Basolateral Membrane

Free Radicals

OAT1

OAT3

Indoxyl Sulfate

Tubular Cell Injury

ICAM-1
MCP-1
Osteopontin

ET-1

TGF-β1

Myofibroblast

TIMP-1

Collagen

Interstitial Fibrosis

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.

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

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Protein Metabolite Theory\(^1\)

- Loss of Functioning Nephrons
- Overload of Protein Metabolites on Remnant Nephrons
  (Indoxyl Sulfate, \(p\)-cresylsulfate, etc.)

Collagen ↑
TIMP-1 ↑
TGF-\(\beta\)1 ↑

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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
AST-120 vs Activated Charcoal
Physical Appearance
Excretion Process of Indole by AST-120

Liver → Indoxyl Sulfate → Kidney

G.I. tract

Tryptophan → Indole → AST-120

Excreted with Feces

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-β1, 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-β1, transforming growth factor beta-1; TIMP-1, tissue inhibitor of metalloproteinase-1.

IS, AST-120 AND ACE-I

Paired **U-test**: \( p = 0.0001 \)

- **Mean s-Cr**: 3.2 (2.0 ~ 4.0)
- **Slope of 1/s-Cr**:
  - Before Kremezin: \(-497 \times 10^{-5}\) dL/mg/week
  - After Kremezin: \(-96 \times 10^{-5}\) dL/mg/week

**No. of patients**: 416

(from Phase IV data)
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.

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

- **2- to 4-Week Screening Phase**
  - Screen Labs
  - Baseline Labs

- **Month (12 Weeks) Double-Blind Treatment Phase**
  - AST-120 2.7 grams/day
  - AST-120 6.3 grams/day
  - AST-120 9.0 grams/day
  - PLACEBO

- **Weeks 4, 8, 12**
  - Laboratory Assessments

- **2-Week Follow-up Phase**

- **Follow-Up Safety**

- **Laboratory Assessments**
<table>
<thead>
<tr>
<th>Parameter</th>
<th>9.0 g Krem.</th>
<th>6.3 g Krem.</th>
<th>2.7 g Krem.</th>
<th>Placebo</th>
<th>Total #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients randomized but never received study drug</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Safety population</td>
<td>39</td>
<td>40</td>
<td>39</td>
<td>39</td>
<td>157</td>
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<tr>
<td><strong>Intent to treat population</strong></td>
<td>38</td>
<td>39</td>
<td>39</td>
<td>38</td>
<td>154</td>
</tr>
<tr>
<td>Per protocol population</td>
<td>31</td>
<td>33</td>
<td>33</td>
<td>35</td>
<td>132</td>
</tr>
<tr>
<td>Completed treatment</td>
<td>33</td>
<td>32</td>
<td>33</td>
<td>34</td>
<td>132</td>
</tr>
<tr>
<td>Not completed treatment</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>25</td>
</tr>
</tbody>
</table>
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

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

Geometric mean values at end of each 7-day treatment period
Figure 1. Carbonaceous Oral Adsorbent’s Effects on Progression of Chronic Kidney Disease (CAP-KD) participant flow chart.
Primary Endpoint: Doubling Serum Creatinine, Creatinine $\geq 6$ mg/dl, Death, Dialysis, Transplant

Akizawa T et al, AJKD: 54, 2009
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 al\textsuperscript{18}) over time, by treatment group. Vertical lines indicate 95\% confidence intervals.

Akizawa T et al, AJKD: 54, 2009
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
Clinical Outcomes Progression of CKD

Cinacalcet HCl

PTH
Calcium
Phosphorus
Indoxyl Sulfate

Effects on the Kidneys

AST-120
Lowers Serum Indoxyl Sulfate

Current Data Do Not Answer The Real Question

Progression of CKD

FDA APPROVAL REQUIRES PROOF

Why EPPIC?

Indoxyl Sulfate Effects on the Kidneys

Lowers

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.

- Primary endpoints: ~291
- Subjects screened: ~1600 pts
- Randomized: ~800 pts (400/arm)
- Duration: 1.5 yrs (FPI) and ~3.5 yrs (LPI)

Diagram shows:
- Screening Phase
  - Labs to include: Chem Profile, CBC, HbA1c, Urinary total protein, Creatinine ratio, Urinalysis, Pregnancy test, ECG

- Baseline Labs
  - Including 24-hour urine collection

- Randomize patients

- Treatment phases:
  - AST-120 9g/day
  - PLACEBO

- Study visits:
  - Week 2 (Visit 3)
  - Week 6 (Visit 4)
  - Week 12 (Visit 5)
  - Week 24 (Visit 6)
  - Week 36 (Visit 7)
  - Week 48 (Visit 8)

- Double-Blind Treatment

- Subsequent Study Visits
# EPPIC Trial Milestones

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Date</th>
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<tbody>
<tr>
<td>Final Protocol</td>
<td>May 2007</td>
</tr>
<tr>
<td>Sites Selected</td>
<td>February – June 2007</td>
</tr>
<tr>
<td>Investigator Meetings</td>
<td><strong>June 2007</strong> (Chicago), <strong>October 2007</strong> (Budapest), and <strong>November 2007</strong> (Milan)</td>
</tr>
<tr>
<td></td>
<td><strong>January 2008</strong> (Buenos Aires), <strong>September 2008</strong> (Kiev), and <strong>October 2008</strong> (Dallas)</td>
</tr>
<tr>
<td>Sites Initiated</td>
<td>June 2007 – November 2009</td>
</tr>
<tr>
<td>First Patient Randomized</td>
<td>August 2007</td>
</tr>
<tr>
<td>Last Patient Randomized</td>
<td>January 2010</td>
</tr>
<tr>
<td>Completed Half of Endpoints</td>
<td>March 2010</td>
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</table>
Global Status

Patient Status as of December 30, 2010

<table>
<thead>
<tr>
<th></th>
<th>Total Screenings</th>
<th>Randomized</th>
<th>Early Term</th>
<th>Discontinued in IVRS</th>
<th>Primary Endpoints</th>
<th>Secondary Endpoints</th>
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<tr>
<td>KRM-306</td>
<td>2026</td>
<td>1021</td>
<td>224</td>
<td>245</td>
<td>268</td>
<td>102</td>
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<tr>
<td>KRM-307</td>
<td>2195</td>
<td>1015</td>
<td>207</td>
<td>246</td>
<td>265</td>
<td>115</td>
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<tr>
<td>Total</td>
<td>4221</td>
<td>2036</td>
<td>431</td>
<td>491</td>
<td>533</td>
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SAE Status as of December 30, 2010

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<th>Initial SAEs reported</th>
<th>Actual SAEs</th>
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<tr>
<td>KRM-306</td>
<td>552</td>
<td>501</td>
</tr>
<tr>
<td>KRM-307</td>
<td>465</td>
<td>444</td>
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<tr>
<td>Total</td>
<td>987</td>
<td>945</td>
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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 CRF

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

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

- Accumulation of Indoxyl sulfate
  - Inhibition of binding of TRP and albumin
  - Increase of unbound TRP
  - Degradation of TRP
  - Malnutrition
  - Increase of cerebral serotonin
  - Appetite loss, etc.

- Progression of renal failure
  - Increase of albumin degradation

- AST-120
  - Improvement
  - Deterioration
  - Protein restriction

- Increase of albumin degradation
THE ROLE OF UREMIC TOXINS IN CARDIOVASCULAR DISEASE
Cardiovascular Mortality in ESRD

Parfrey, Sarnak and Levey, from USRDS and NCHS, AJKD 1998
Serum Levels of Indoxyl Sulfate Are Associated with Overall and Cardiovascular Mortality


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).
Indoxyl Sulfate Induces Oxidative Stress in Endothelial Cells

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.


**p<0.001 vs control**
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.
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

<table>
<thead>
<tr>
<th>Histological Findings</th>
<th>DN (n=8)</th>
<th>DN + IS (n=8)</th>
<th>DH (n=8)</th>
<th>DH + IS (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcification area (μm²)</strong></td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>2005 ± 1161</td>
<td>13784 ± 4677**</td>
</tr>
<tr>
<td><strong>Wall Thickness (μm)</strong></td>
<td>226 ± 4*</td>
<td>238 ± 8</td>
<td>258 ± 13</td>
<td>318 ± 6***</td>
</tr>
</tbody>
</table>

* 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
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

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)**

![Graph showing relationship between Indoxyl Sulfate and Aortic Calcification Score](image)

**Relationship Between Serum IS and PWV (n=139)**

![Graph showing relationship between Indoxyl Sulfate and PWV](image)

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.

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

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

* Versus healthy controls, p < 0.01. ** Versus before, p < 0.05.
Other Adverse Effects of Indoxyl Sulfate

**Bone formation**¹

- 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 infection**²

- 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

**Anemia**³

- Indoxyl sulfate may promote anemia via an increase in hemolysis

PTH, parathyroid hormone; ROS, reactive oxygen species; MCP-1, Monocyte chemoattractant protein-1.
Overall Toxicity of Indoxyl Sulfate

Free Radicals
Impaired Antioxidative System

Indoxyl Sulfate
OAT3
OAT1

NADPH oxidase

Cellular Toxicity

VSMC
Endothelial cells

Tubular cells
Mesangial cells

Progression of CKD

Cardiovascular Disease

NADPH, nicotinamide adenine dinucleotide phosphate; OAT, organic anion transporters.
The Gut-Kidney Axis
Indoxyl sulfate, $p$-cresyl sulfate and CKD Progression

THANKS FOR YOUR ATTENTION