# Enzymatic Degradation of Cystine Decreases Nephrolithiasis in a Mouse Model of Cystinuria

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# Cystinuria: One of the First Identified Inborn Errors of Metabolism[1]

## Cystinuria: an Autosomal Recessive Disorder

- Amino acid transporter (Slc3a1/Slc7a9) defect
- Failure to re-absorb cystine from the glomerular filtrate
- Marked and persistent increase in urinary cystine concentration
- Poorly soluble cystine precipitates and forms stones (lithiasis in kidney, bladder and ureter)

## Key Disease Manifestations

- Severe, recurrent flank/abdominal pain and hematuria
- Frequent requirement for multiple procedural interventions often under general anesthesia
- Increased risk of chronic renal failure

## Standard of Care

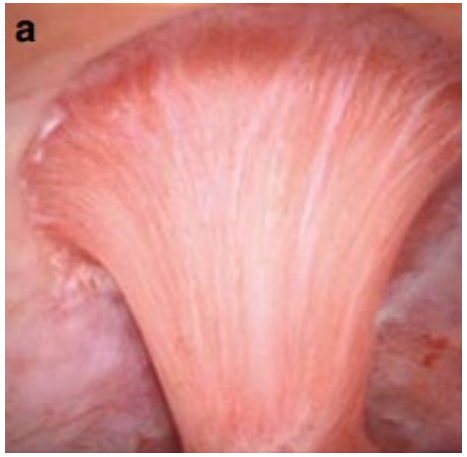
1) Hyperhydration → 3L per day
2) Urine alkalization
3) Cystine-binding thiol drugs (CBTDs) or cystine-reducing agents (i.e. penicillamine, tiopronin)

***Untreated cystine kidney stones***

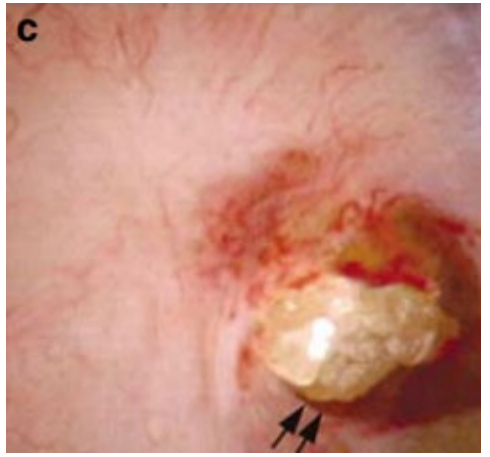



*Bazin et al. J. Appl. Cryst. 2014; 47, 719–725*

***Normal renal papilla***




***Renal papilla from a patient with cystinuria***




*Evan et al. Kidney International 2006; 69, 2227-2235*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[1]*Garrod et al, Lancet 1908;172:1-7*

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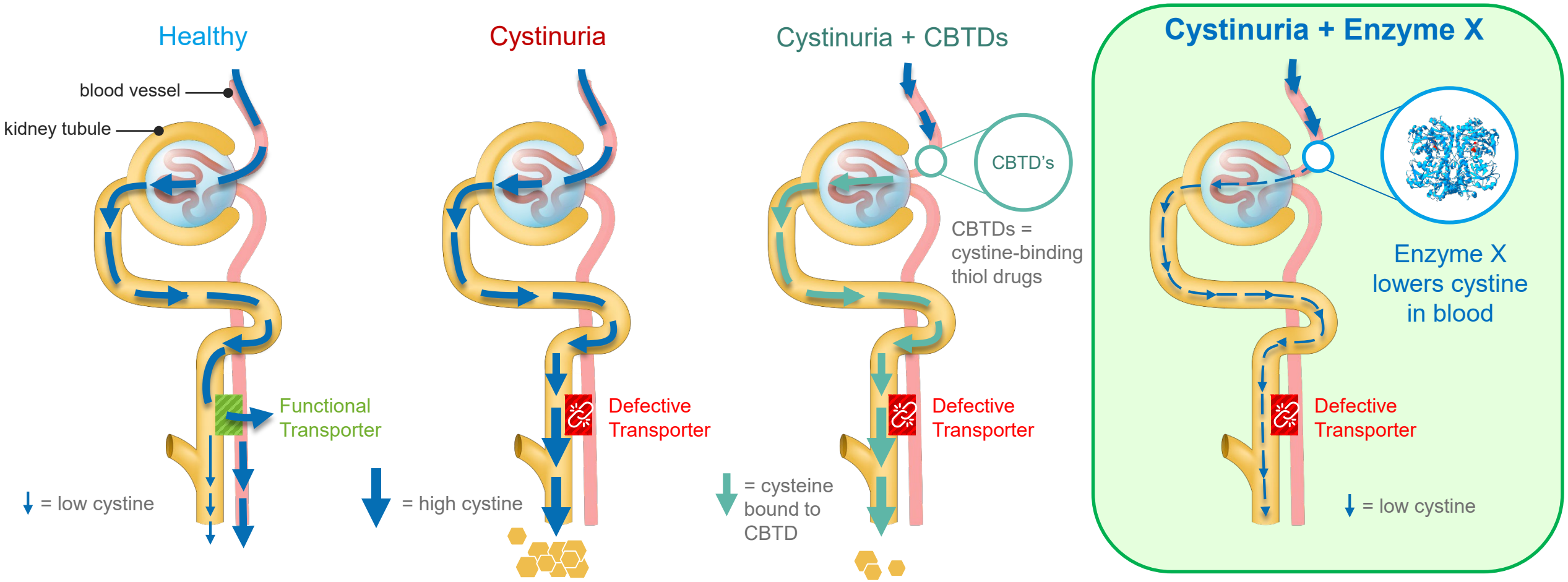
# Enzymatic Reduction of Cystine as a Novel Therapeutic Approach for Cystinuria




| Healthy | Cystinuria | Cystinuria + CBTDs |
|---|---|---|
| Low [cystine] in urine | High [cystine] in urine→ cystine precipitates → stone formation | Increase in soluble [cystine] in urine |
| Healthy | Severe pain[1], surgical interventions[1], kidney failure[2] | Safety concerns and poor therapeutic compliance |

**Hypothesis:**
**Does enzymatic reduction of plasma cystine lower urine cystine and reduce cystine stone formation?**

= kidney stones



[1]*Biyani and Cartledge EAU-EBU Update Series 4 (2006) 175-183*
[2]*Leslie and Nazzal Renal Calculi (Cystinuria, Cystine Stones) (2018)*

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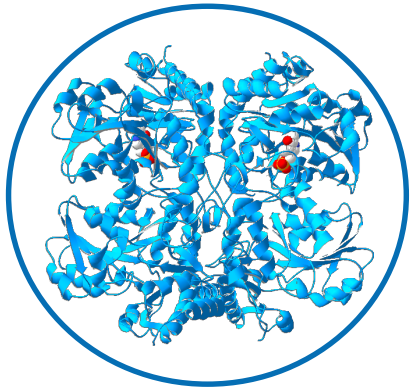
# Design of a Novel Enzyme with Medical Utility for Cystinuria

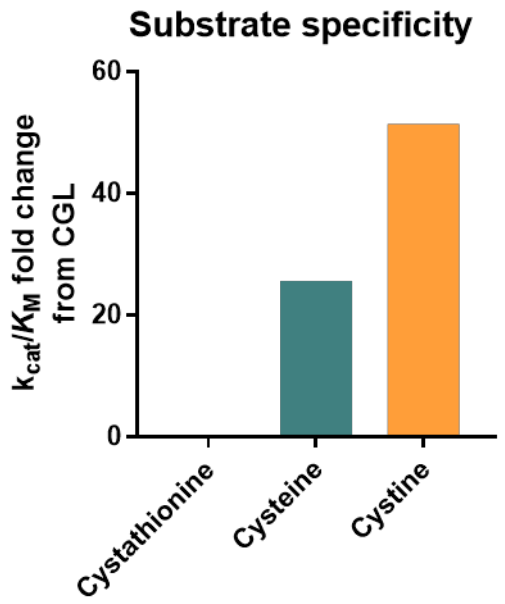
- There are no naturally occurring human enzymes that efficiently degrade cystine
- Human Cystathionine-γ-Lyase (CGL) was engineered to change its native substrate specificity

**Scaffold selection** → **Lead Generation** → **Lead Optimization**

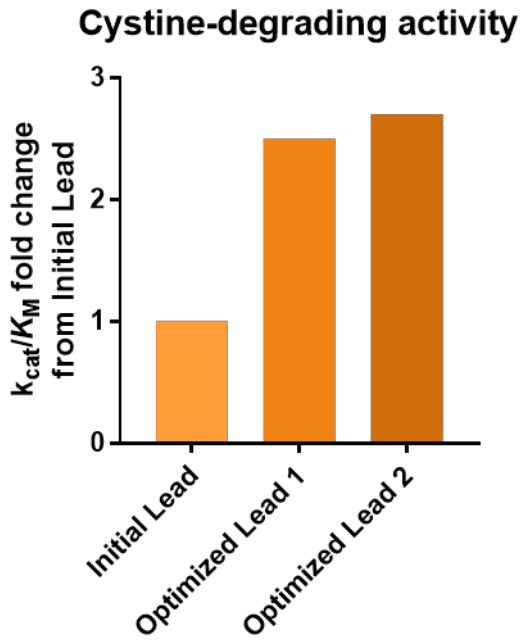


**Potential advantages of the CGL scaffold**

| | |
|---|---|
| **Low immunogenicity** | Human scaffold |
| **Safety** | Generates natural metabolites |
| **Versatility** | One scaffold for multiple targets[1] |
| **Manufacturing** | Scaffold knowledge transfer |
| **High serum stability** | Stable cofactor |
| **Long half-life** | PEGylation |




➢ Introduce new specificity[2]: AEB5100 lead molecule degrades cystine and cysteine




➢ Increase catalytic activity
➢ Increase serum stability
➢ Improve manufacturability

[1]*Daige CL et al, ASHG 2018, Poster 980F*
[2]*Cramer SL et al, 2017 Nature Medicine 23:120-7*



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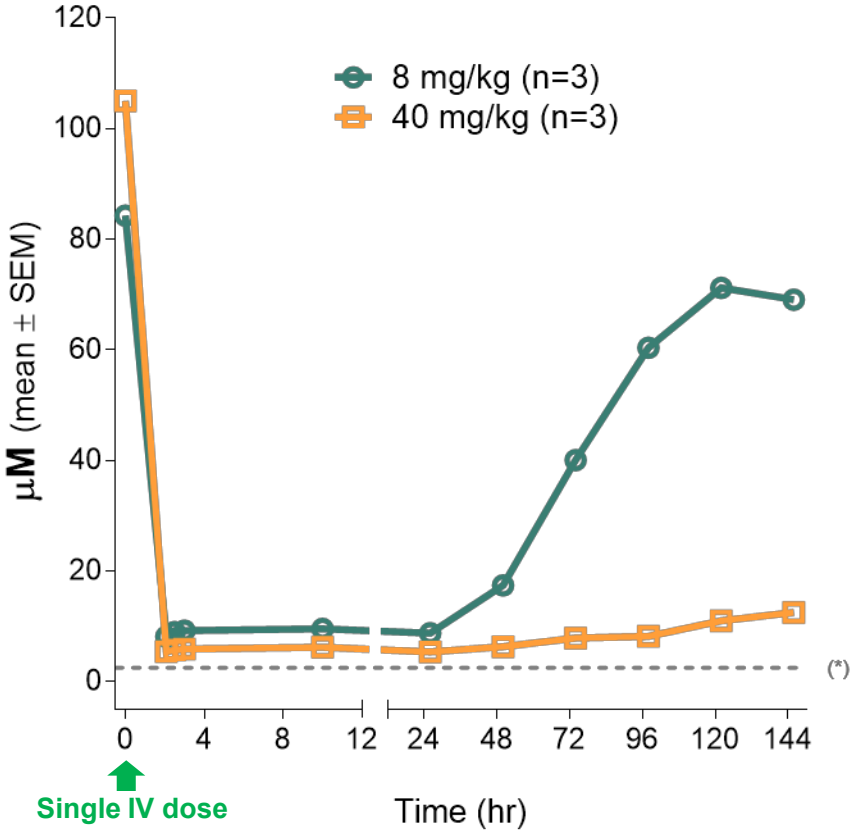
# Reduction of plasma cystine with AEB5100 lowers cystine in urine

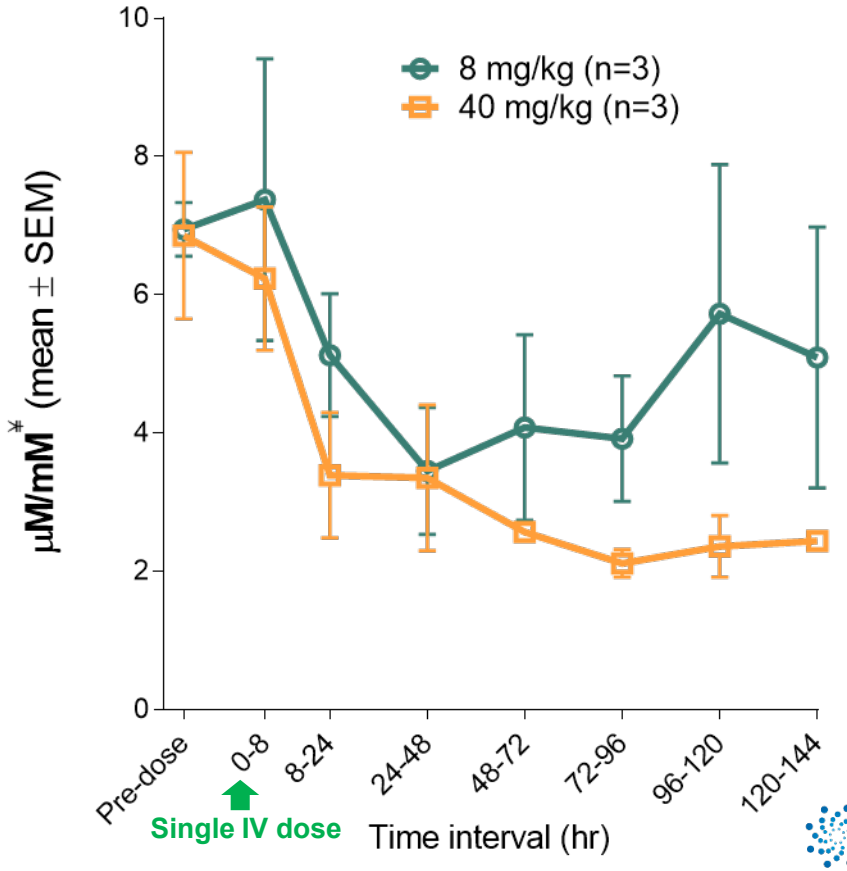
Pre-clinical study demonstrates that a single dose of AEB5100 results in:

- rapid and sustained reduction of cystine and cysteine in plasma
  - Cystine, which is the oxidized form of cysteine, is the predominant form of cysteine in blood
  - For analytical analysis cystine is reduced to cysteine to enable measurement of total cysteine concentrations
- with accompanied by reduction of cystine and cysteine in urine

## Plasma cystine and cysteine




## Urine cystine and cysteine




(*) Lowest cystine and cysteine level quantified in cyno *in vivo*

¥total cysteine (µM) normalized by creatinine (mM)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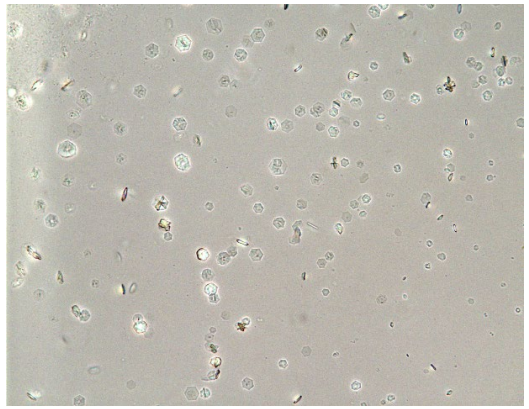
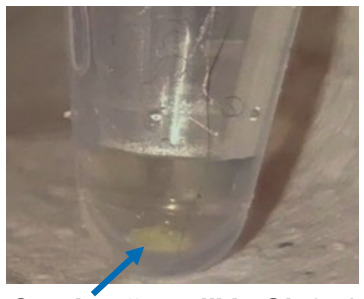
# Cystinuria mouse model

The Slc3a1 -/- mouse model mimics human cystinuria stone formation[1], however:

- The number and volume of stones accrue progressively faster than in humans
- The propensity of stone formation occurs in the bladder as opposed to the kidneys

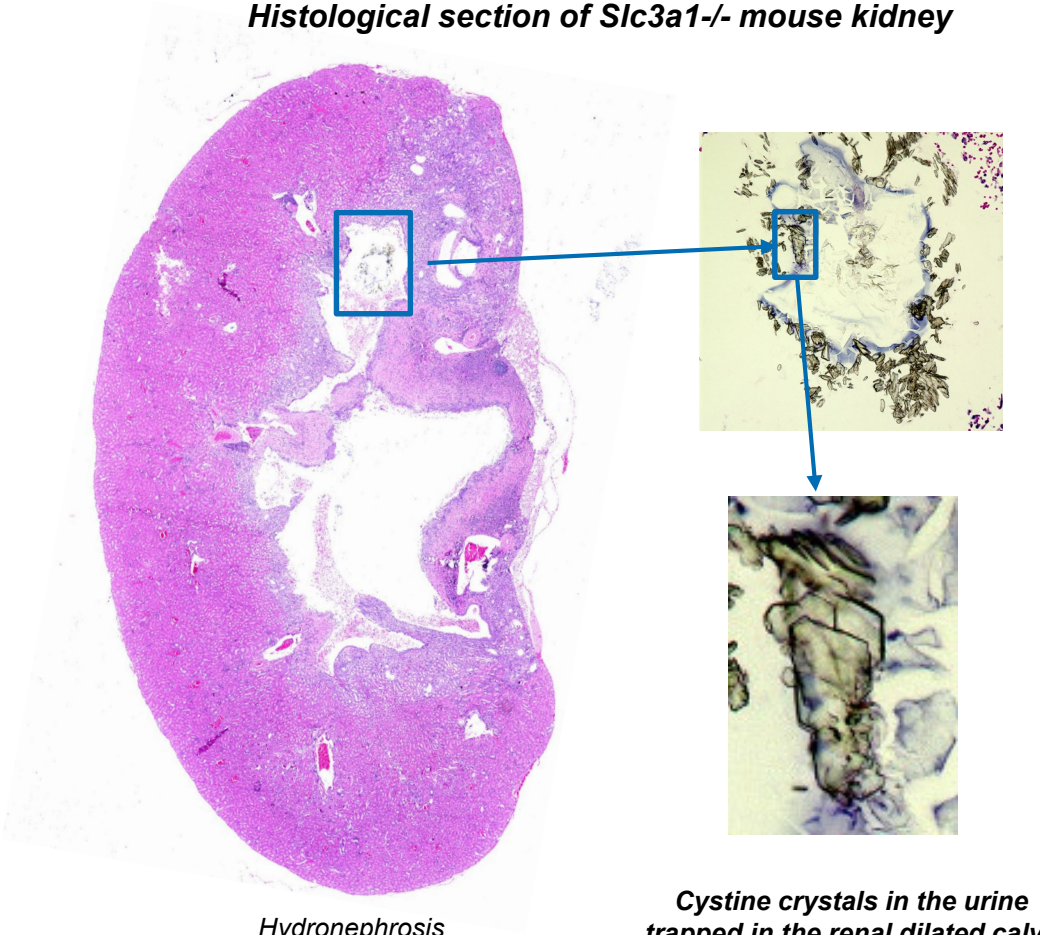
***Slc3a1-/- mouse urine containing cystine crystals***





***Cystine "sand" in Slc3a1-/- mouse urine***

***Histological section of Slc3a1-/- mouse kidney***



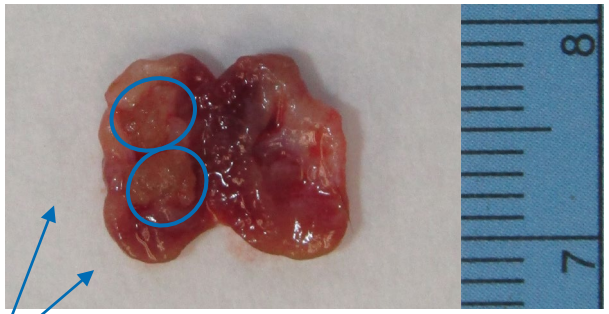
*Hydronephrosis*
*Calyceal dilatation*
*Intraluminal cystine crystals*



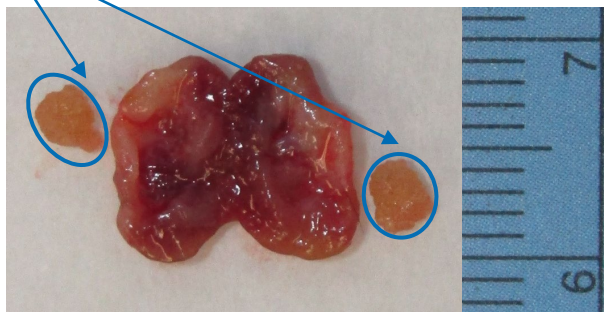


***Cystine crystals in the urine trapped in the renal dilated calyx***

***Slc3a1-/- mouse kidney containing cystine stones***



Stones





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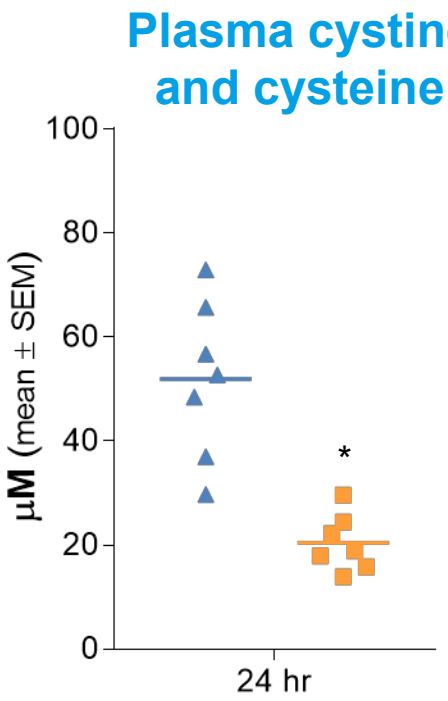
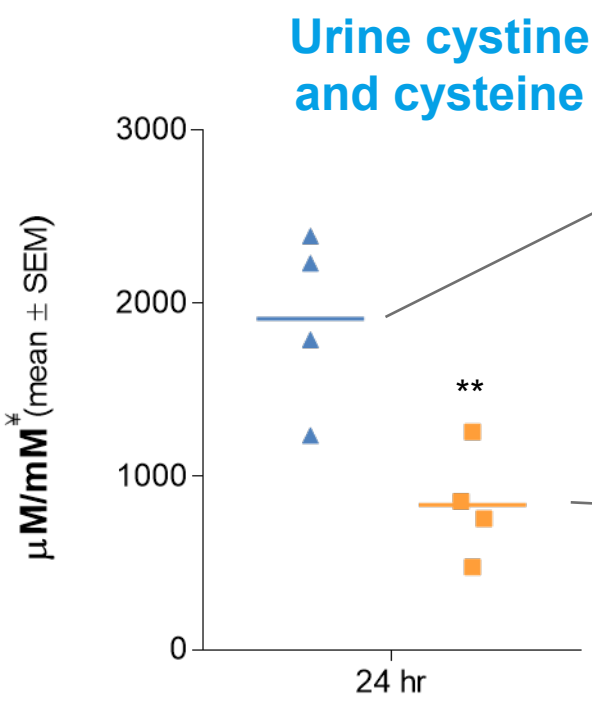
[1]Ercolani et al., Int Urol Nephrol. 2010 Mar;42(1):57-63

# Depletion of plasma cystine with AEB5100 reduces cystine crystals in urine

- Single IP dose of AEB5100 lead molecule in Slc3a1-/- mice reduces:
  - Plasma cystine and cysteine levels
  - Urine cystine and cysteine levels
  - Urine cystine crystals

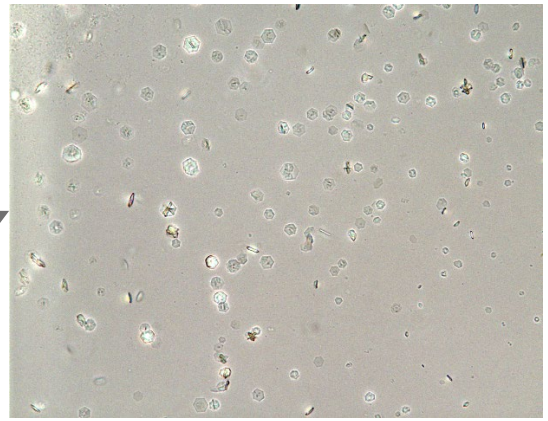



* p<0.005

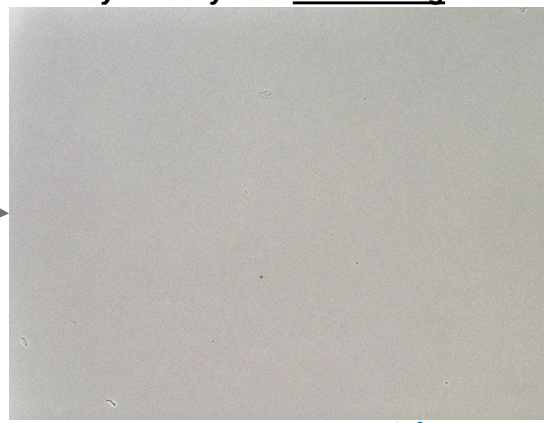



** p<0.05

*Slc3a1-/- mouse* ***urine cystine crystals before dosing***



*Slc3a1-/- mouse* ***urine cystine crystals after dosing***



▲ Slc3a1-/- Vehicle

■ Slc3a1-/- AEB5100 lead molecule

*total cysteine (µM) normalized by creatinine (mM)



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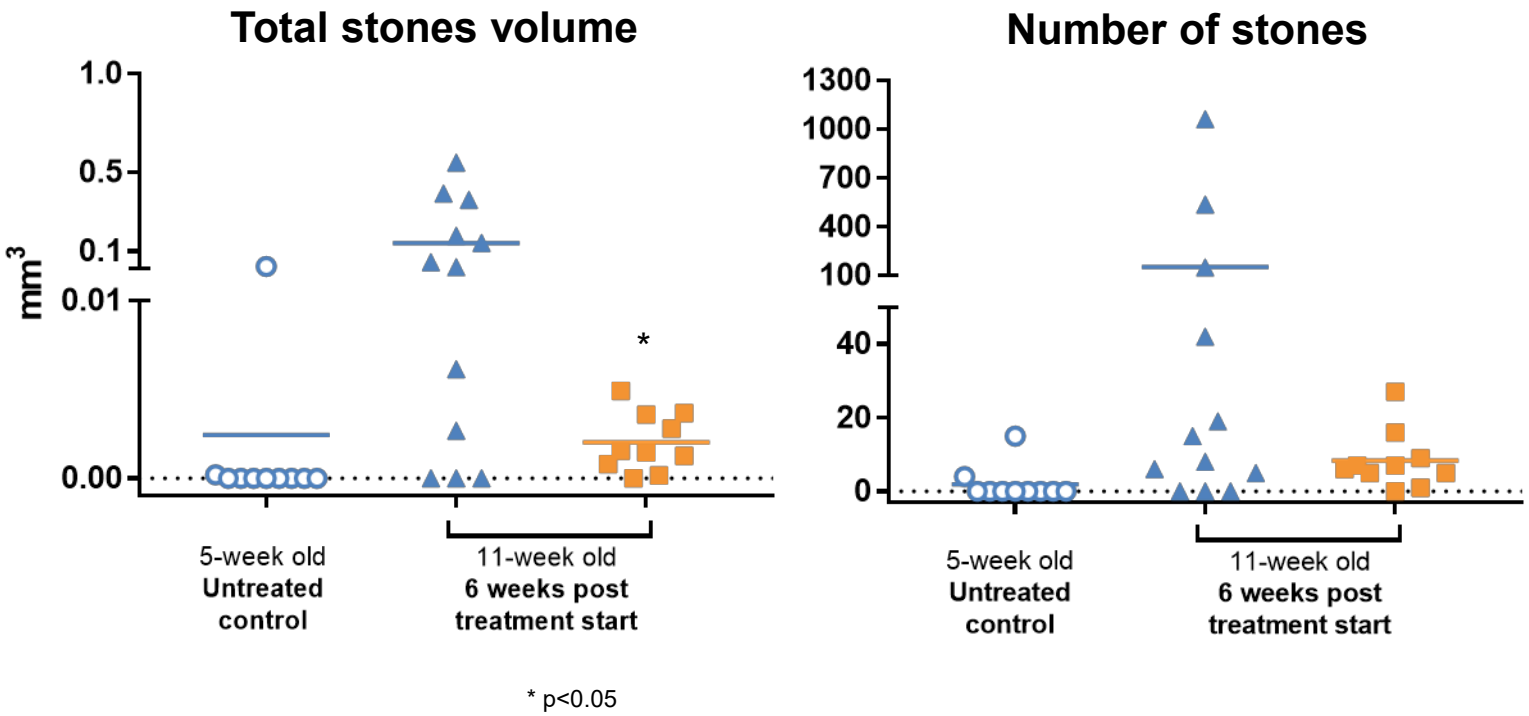
# AEB5100 effectively reduces stones in a cystinuria mouse model

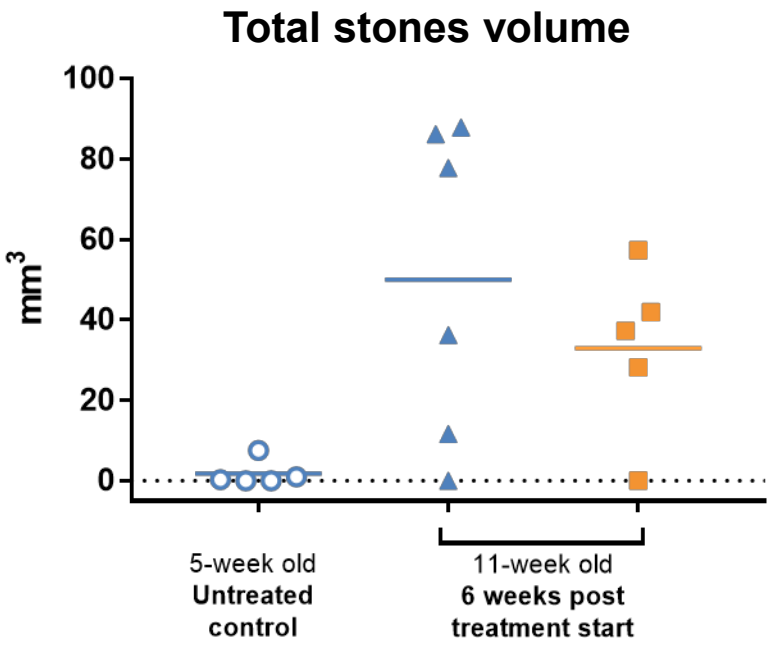
- IP administration of AEB5100 lead molecule for 6 weeks reduces the propensity for kidney stone formation in Slc3a1-/- mice

## Kidneys




* p<0.05

## Bladder




- Slc3a1-/- Untreated
- Slc3a1-/- Vehicle
- Slc3a1-/- AEB5100 lead molecule



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# Summary and Conclusion

- CGL provides a novel therapeutic scaffold that has been engineered to mediate a unique enzymatic reaction: cystine degradation
- AEB5100-mediated lowering of plasma cystine levels in a mouse model of cystinuria reduces:
  1. Urine cystine levels
  2. Urine cystine crystals
  3. Kidney stone formation
- This data support the advancement of the AEB5100 program into IND-enabling studies

**Enzymatic reduction of systemic cystine provides a novel therapeutic approach for the treatment of cystinuria**



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