New and improved medication for cystinosis: Cysteamine prodrug

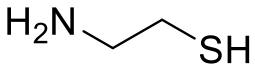
Overview

- Cysteamine and prodrug therapeutics
- CF10 – Rationale, design and pre-clinical data
- CF10 - Future plans
- Additional cysteamine prodrugs for cystinosis

Cysteamine

Advantages

- Removes excess cystine and delays disease progression
- Simple and cheap oral medicine
- Cysteamine forms a mixed disulphide cysteine which utilises the lysosomal lysine (PQLC2) transporter to remove cysteine, and hence reduce cystine levels



Cysteamine free base

Disadvantages

Cysteamine:

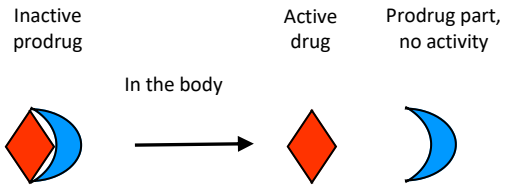
- Bad taste and smell which can cause nausea and vomiting
- Gastrointestinal irritant and can induce ulceration
- Undergoes rapid clearance and hence large doses and frequent dosing are needed for adequate cystine depletion
- Metabolised to volatile noxious compounds which cause halitosis and an unpleasant body odour

These disadvantages can lead to poor adherence

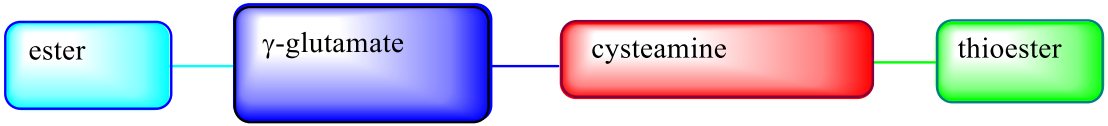
Prodrugs as Therapeutics

Rationale: Prodrugs are inactive forms of a drug that are converted to release the active agent once inside the body. Prodrugs are already used to deliver many medicines:

- Approximately 10% of all drugs in clinical use are prodrugs
- Well-known examples in many therapeutic areas: Lansoprazole, Clopidogrel, Olmesartan medoxomil, Latanoprost, Sulfasalazine, *etc*
- Prodrugs may offer a solution to problems of cysteamine



CF10 Design: An Orally Active Cysteamine Prodrug



- **Thioester** covers up the sulphur in cysteamine to protect the stomach and gut
- **Ester** improves the absorption of the drug through the gut wall and into the blood stream
- **Glutamate** targets **cysteamine** for release at the cell surface of tissues (e.g. kidney) leading to:
  - Increased potency – less frequent and lower dosing
  - Lower circulating cysteamine levels – reduced conversion to volatile compounds with a bad smell and so no/fewer bad breath and body odour problems

CF10 Metabolism and Pharmacokinetics

In Vivo

University of Sunderland

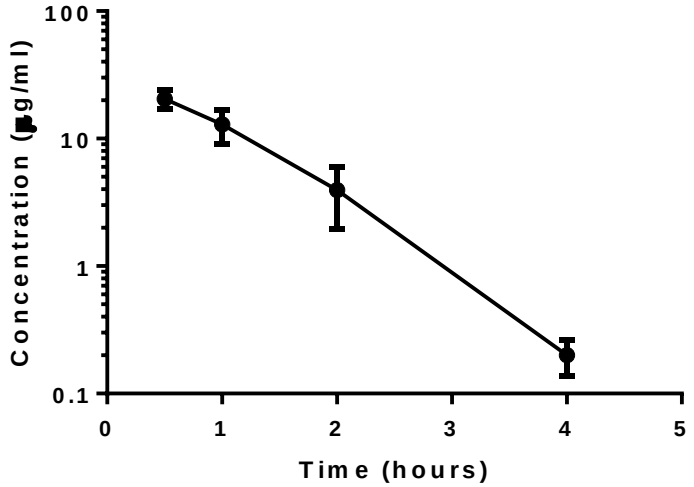
MRC Medical Research Council

CYSTINOSIS FOUNDATION UK

Pharmacokinetic Studies in Rats

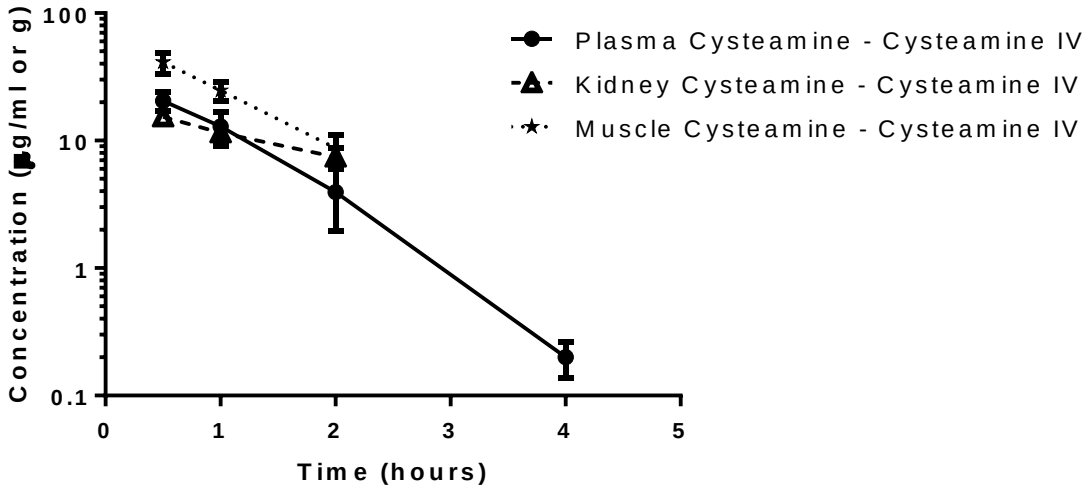
- **IV Administration**
  - Cysteamine – 81 mg/kg, 486 mg/m^2
  - CF10 – 225 mg/kg, 1350 mg/m^2
  - Plasma, kidney, muscle and brain analysed 0.5, 1, 2, 4 and 8 hours after administration – LC/MS assay
- **Oral Administration**
  - CF10 – 304 mg/kg, 1825 mg/m^2
  - Plasma, kidney, muscle and brain analysed 0.5, 1, 2, 4 and 8 hours after administration – LC/MS assay

Cysteamine IV - Plasma cysteamine



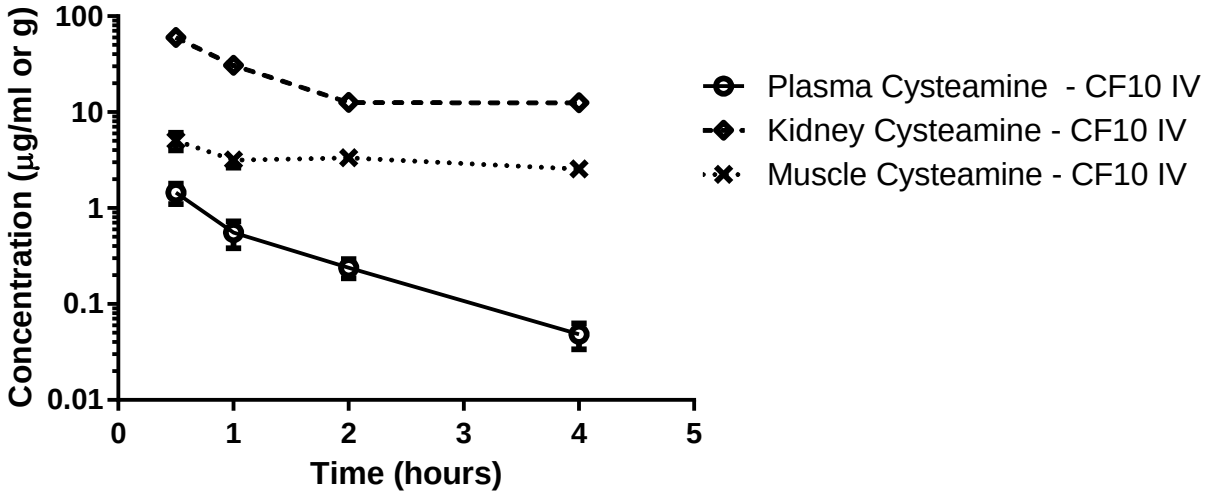
Rapid cysteamine clearance consistent with clinical 6 hour dosing interval

Cysteamine IV - Plasma and tissue cysteamine



Delivery of cysteamine to kidney and muscle, target tissues for cystinosis pathology

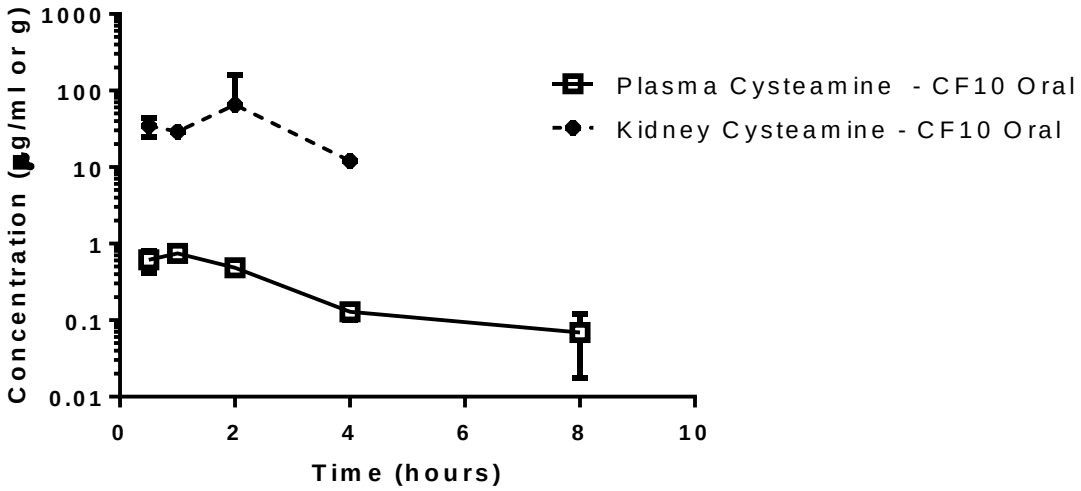
CF10 IV - Plasma and tissue cysteamine



Delivery of cysteamine to kidney and muscle, target tissues for cystinosis pathology

- Tissue:plasma concentration ratios are ~10 for muscle and ~100 for kidney, compared to ~1 following cysteamine
- In comparison to an equimolar dose of parent cysteamine, absolute kidney cysteamine concentrations are higher and drug levels in muscle are more sustained

Oral CF10 - Plasma and kidney cysteamine



Delivery of cysteamine to the kidney, a key target tissue for cystinosis pathology

- Tissue:plasma concentration ratios are ~50 for kidney, indicating effective delivery
- Kidney cysteamine concentrations following oral CF10 administration are the same as following IV CF10 dosing

CF10 – Markedly improved renal cysteamine delivery in rats

Treatment	Kidney:Plasma Concentration Ratio (mean ± SD, 0.5-2 hours, n = 12)
Intravenous Cysteamine (81 mg/kg)	1.28 ± 0.74
Intravenous CF10 (225 mg/kg)	54 ± 21
Oral CF10 (304 mg/kg)	48 ± 26

CF10 Efficacy

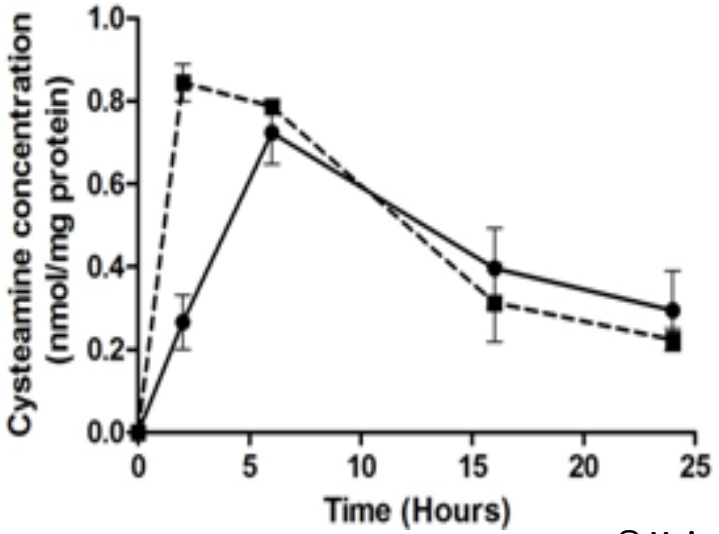
University of Sunderland

MRC Medical Research Council

CYSTINOSIS FOUNDATION UK

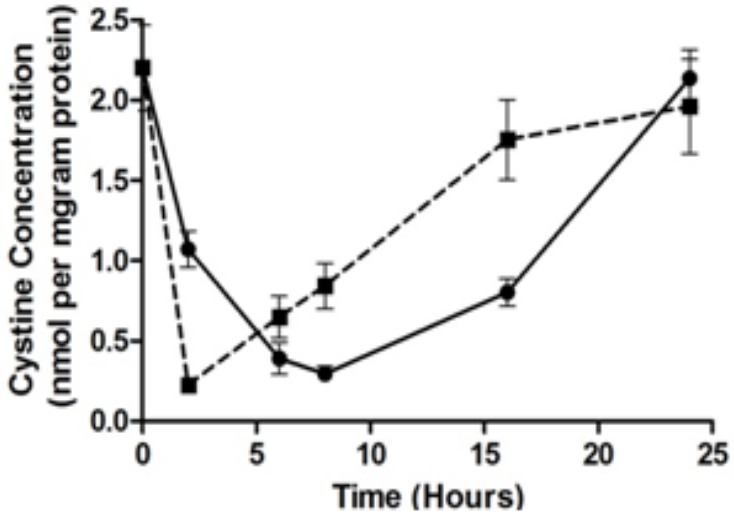
Cysteamine Concentrations in Kidney Cells after Exposure to Cysteamine or CF10

- Concentration of cysteamine in proximal tubule epithelial kidney cells treated with 20 µM CF10 (solid line) or cysteamine (dashed line)



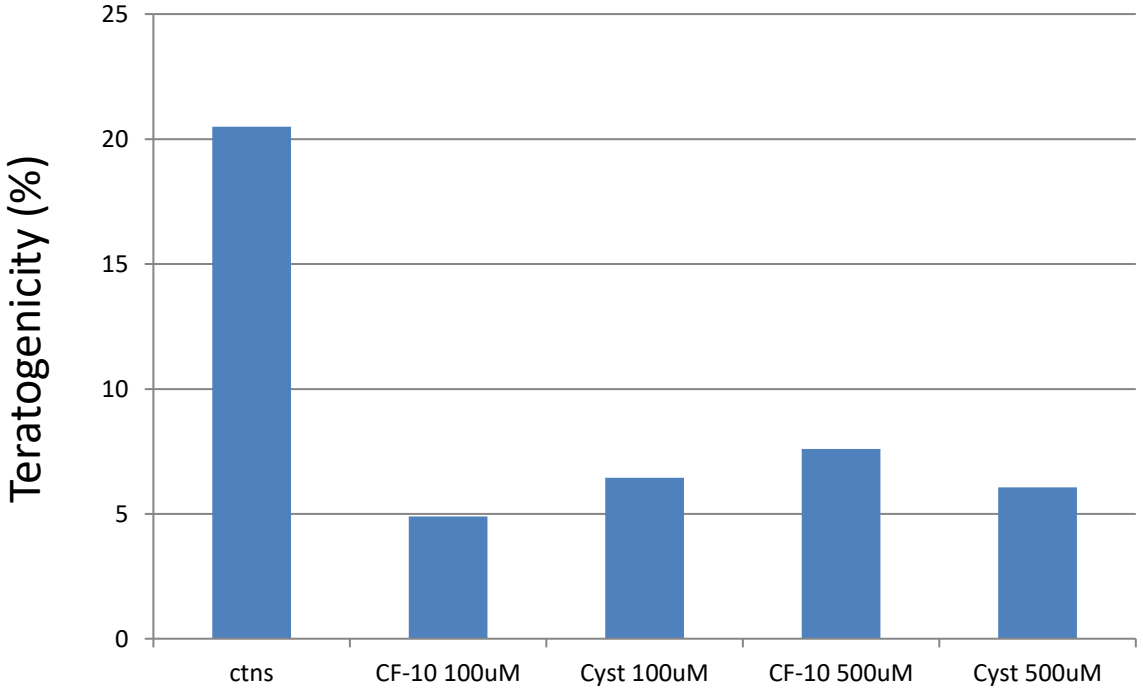
Cystine Depletion in Cystinotic Cells

- Concentration of cystine in cystinotic fibroblasts treated with 20 µM prodrug CF10 (solid line) or cysteamine (dashed line).



CF10 Efficacy and Toxicity Studies in a Zebrafish Model of Cystinosis – Comparison with cysteamine

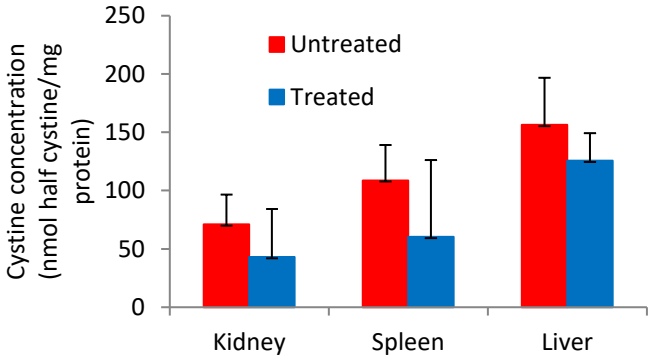
- Deformities in *CTNS*$^{-/-}$ zebrafish larvae treated with CF10 or cysteamine



Model: Elmonem *et al*. Scientific Reports (2017) **7**: 42583

Tissue Cystine Depletion in Cystinotic Mice Following CF10 Treatment

– 28 Day treatment with CF10 at a dose equivalent to 68 mg cysteamine/kg/day administered in 2 divided oral doses as an aqueous solution 12 hours apart



	Kidney	Spleen	Liver
Cystine reduction by CF10 (%)	40%	45%	20%
Cystine reduction by cysteamine (%)*	50%	13%	77%

– Results comparable to 60 days treatment with cysteamine (400 mg/kg/day Cystagon®, equivalent to 101 mg/kg/day cysteamine free base) (*Cherqui *et al*. Mol. Cell. Biol. (2002) **22**:7622–7632)

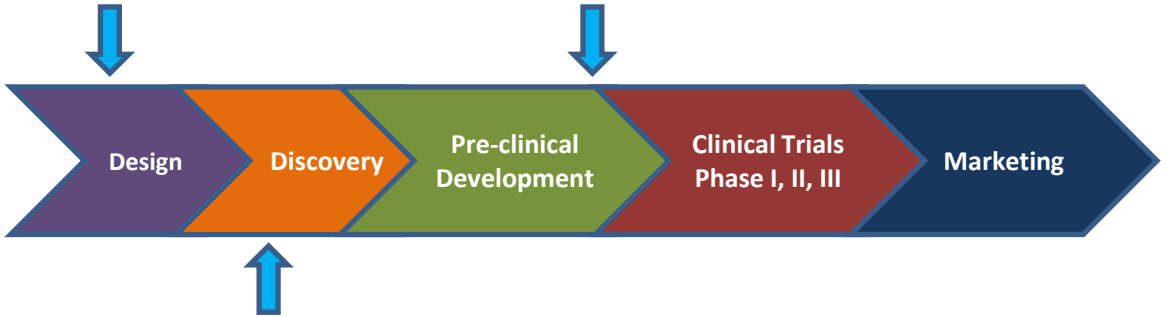
CF10 - Achievements

- Minimal smell and good pharmaceutical properties
- Depletion of cystine in cystinotic cells grown in the laboratory, in the tissues of cystinotic mice, and active without side effects in a Zebrafish model of cystinosis
- Reduced or no damage to the gut in laboratory studies, and no evidence of unwanted side effect in regulatory safety studies
- In comparison to cysteamine, equal or greatly improved delivery of cysteamine to tissues in laboratory studies
- Much lower blood cysteamine levels after CF10 than after cysteamine
- Based on the laboratory results, the potential for less frequent and lower dosing, lack of bad breath and body odour, and no damage to the gut

The Drug Discovery and Development Pipeline

Anderson *et al.*
Lett. Drug Des. Disc.
2006, **3**:336-345

We are here



Frost *et al.*
Eur. J. Med. Chem.
2016, **109**:206-215

Next Steps - I

- **Completion of the pre-clinical development of CF10 – 2020-2022**
  - MRC DPFS Award – will be completed by July 2022
    - Bulk synthesis – **COMPLETED – 10kg**
    - Regulatory GLP toxicology and safety studies – **COMPLETED – Very well tolerated**
    - Formulation development – **COMPLETED – Oral solution and capsule formulations**
    - Application for Clinical Trial Authorisation - **PENDING**
- **Evaluation of CF10 in a new rat model of cystinosis**
  - Scheduled to be completed in 2023
  - In collaboration with Dr Jennifer Hollywood, University of Auckland

Summary of Regulatory Toxicology Studies

- **Dog** (capsule formulation) - NOAEL 200 mg/kg/d x28 oral. Gastrointestinal toxicity at 400 mg/kg/d x28
- **Rat** (solution formulation) – NOAEL 333 mg/kg/d x28 oral. Gastrointestinal toxicity at 1g/kg/d x14
- At the NOAELs no significant haematological, clinical biochemistry, micro- or macro-pathological effects were reported.
- CF10 produced no significant effects in GLP genetic (*in vitro* and *in vivo* - rat), cardiac (hERG and telemetry – dog) or respiratory (rat) toxicology studies.

Volatile Thiol Levels in Exhaled Breath in Regulatory Pre-clinical Safety Studies

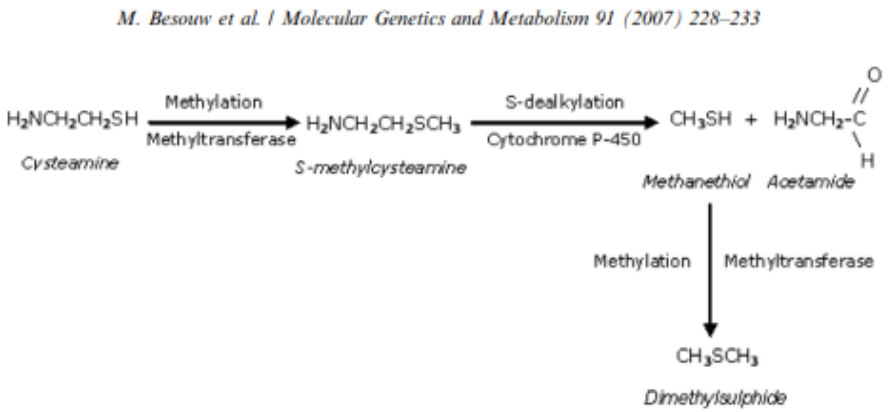
University of Sunderland

MRC Medical Research Council

CYSTINOSIS FOUNDATION UK

Halitosis and Body Odour Following Cysteamine are due to Volatile Thiols

Cysteamine is metabolised to methanethiol and dimethyl sulphide, volatile sulphur compounds with a bad smell.



Dimethyl Sulphide is the Major Thiol in Exhaled Breath

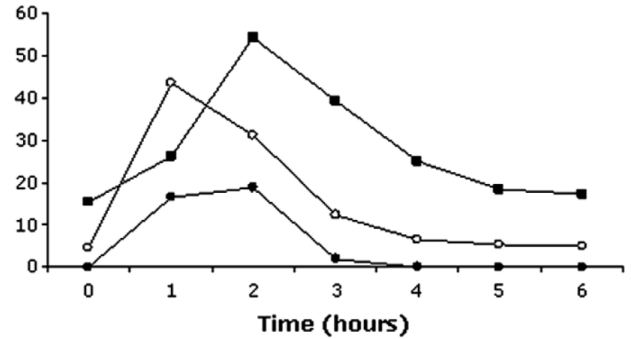
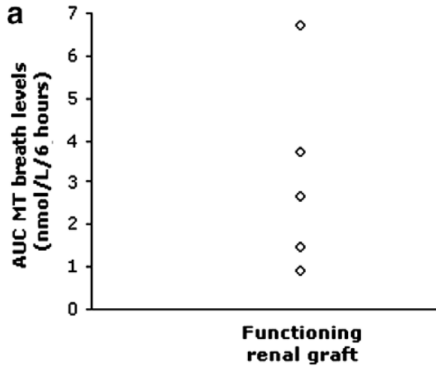
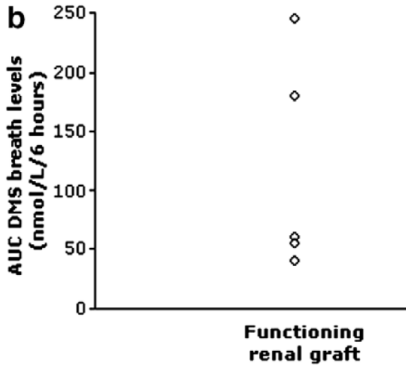


Fig. 3. Cysteamine plasma levels in μmol/L (—○—), breath MT levels × 10 in nmol/L (—■—) and DMS breath levels in nmol/L (—■—) in patient 2, before and until 6 h after cysteamine intake.





M. Besouw et al. / Molecular Genetics and Metabolism 91 (2007) 228–233

Exhaled Dimethyl Sulphide in the Breath of Dogs Treated with CF10

- CF10 was given orally once daily for 28 days at a dose of 200 mg/kg/d (4 $g/m^2/d$) in capsules – the No Observed Adverse Effect Level (NOAEL).
- Breath samples were collected on day -7, before dosing on day 1, before dosing on day 28 and 2h after dosing on day 28, and dimethyl sulphide measured using the OralChroma portable gas chromatograph.
- Peak dimethyl sulphide levels in patients following cysteamine **are ~30 ng/10ml** (50nM - Besouw *et. al.* Mol. Genet. Metab. **91**:228-233, 2007).

	Before CF10		After CF10	
	Day -7	**Day 1**	**Day 28 pre-CF10**	**Day 28 2h post-CF10**
Control – No CF10	4 (0-14)*	0.3 (0-115)	0.2 (0-0.8)	0 (0-18)
200 mg/kg/d CF10 x28	3 (0-26)	0 (0-108)	1 (0-7)	0 (0-25)

* - Data are the median and range of values in six dogs in ng DMS/10ml of breath.

Exhaled Methanethiol in the Breath of Dogs Treated with CF10

- CF10 was given orally once daily for 28 days at a dose of 200 mg/kg/d (4 g/m^2/d) in capsules – NOAEL.
- Breath samples were collected on day -7, before dosing on day 1, before dosing on day 28 and 2h after dosing on day 28 and methanethiol measured using the OralChroma portable gas chromatograph.
- Peak methanethiol levels in patients following cysteamine are **~10 ng/10ml** (20nM - Besouw *et. al.* Mol. Genet. Metab. **91**:228-233, 2007).

	Before CF10		After CF10	
	Day -7	**Day 1**	**Day 28 pre-CF10**	**Day 28 2h post-CF10**
Control – No CF10	0.2 (0-0.9)*	0.3 (0-0.7)	2.2 (0.5-5)	0.6 (0-2)
200 mg/kg/d CF10 x28	0.5 (0.1-2.4)	1.5 (0.6-16)	2.5 (1.3-6)	0.7 (0-2)

* - Data are the median and range of values in six dogs in ng MT/10ml of breath.

Dimethyl Sulphide and Methanethiol Levels in Exhaled Breath following CF10

- In comparison to pre-treatment levels, and levels in control untreated dogs, at the CF10 NOAEL dose (200 mg/kg/d or 4g/m^2/d x28) there is **no increase in dimethyl sulphide or methanethiol in the exhaled breath**.
- Dimethyl sulphide and methanethiol levels in the breath of dogs after treatment with the Toxic Dose Low (400 mg/kg/d or 8g/m^2/d x28) or 50% of the NOAEL (100 mg/kg/d or 2g/m^2/d x28) also showed no consistent CF10-related increases.
- Individual dogs, both control untreated and CF10-treated, have occasional high dimethyl sulphide, methanethiol **and hydrogen sulphide** concentrations which are most likely due to endogenous metabolism and/or the gut microbiome of the animal.
- As designed, CF10 is a cysteamine prodrug with markedly reduced potential for conversion to the volatile thiols dimethyl sulphide and methanethiol, and hence risk of halitosis and poor body odour.

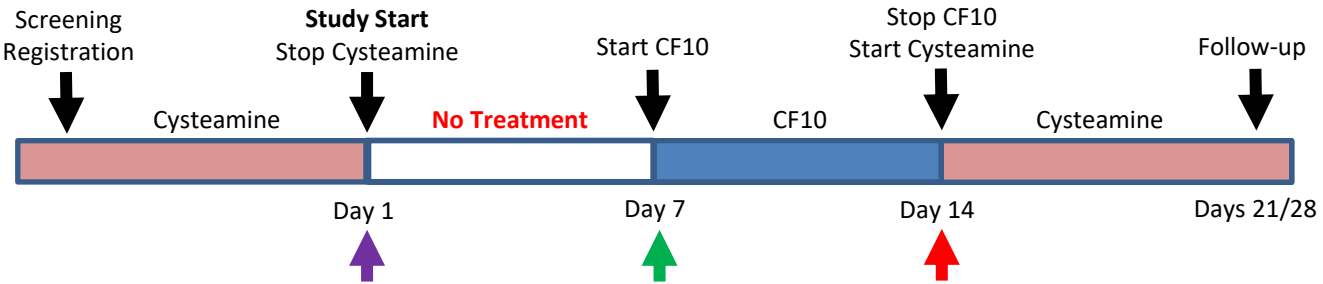
Next Steps - II

- **Pilot "run-in" clinical study in cystinosis patient volunteers receiving cysteamine, the Cysteamine PALS study – 2022-2023**
  - Close interaction with and input from the cystinosis community
  - Clinical Research Facility, University Hospitals Birmingham – PI Graham Lipkin
  - Standard cysteamine treatment. Pharmacokinetic and pharmacodynamic studies including developing and piloting exhaled breath methanethiol/dimethylsulphide and skin cystine measurements. Clinical evaluation as planned for the CF10 clinical trials
- **CF10 clinical trials - 2024-**
  - Funding application to the UK Medical Research Council - July 2022
  - Close interaction with and input from the cystinosis community
  - Manufacture and formulation of CF10 GMP material
  - Phase I trial in the UK cystinosis patient volunteers – CR UK Clinical Trial Unit, University Hospitals Birmingham – PI Graham Lipkin

Proposed CF10 Phase 1a Dose-Finding Trial Design

-

Objective: To identify the dose of CF10 that depletes cystine to <1nmol ½ cystine/mg protein



Day 1 - Before (PD - WBC and skin biopsy cystine) and after the **last dose of cysteamine** (PK - 0, 0.5, 1, 2, 4, 6 hour blood samples, urine and exhaled methanethiol and dimethyl sulphide).

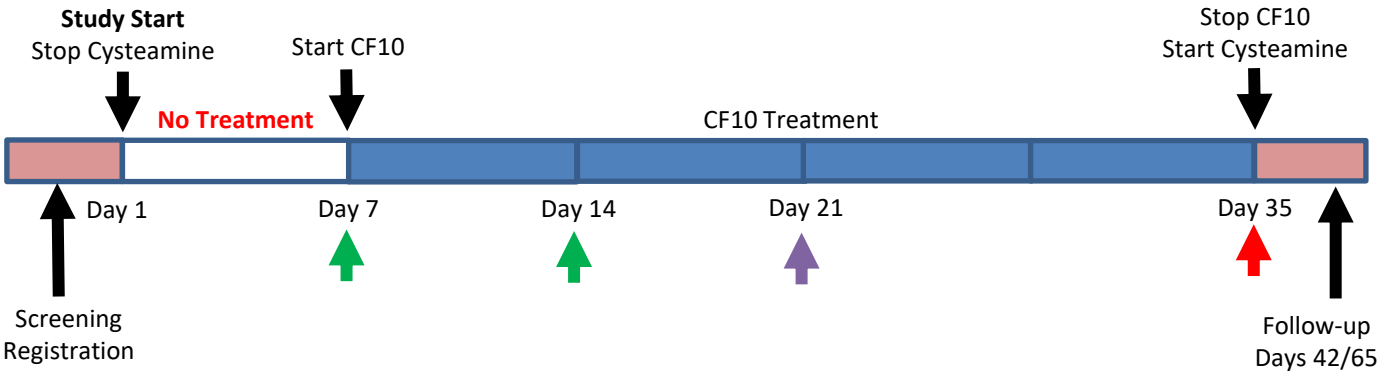
Day 7 - Before (PD - WBC and skin biopsy cystine) and after the **1st dose of CF10** (PK - 0, 0.5, 1, 2, 4, 6 hour blood samples, urine and exhaled methanethiol and dimethyl sulphide).

Day 14 - Before (PD - WBC and skin biopsy cystine) and after the **last (7th) dose of CF10** (PK - 0, 0.5, 1, 2, 4, 6 hour blood samples, urine and exhaled methanethiol and dimethyl sulphide).

Proposed CF10 Phase 1b 28-Day Trial Design

-

Objective: To confirm the safety of CF10 given daily at a dose that depletes cystine to <1nmol ½ cystine/mg protein



Day 7 and 14 - Before (PD - WBC and skin biopsy cystine) and after the **1st and 7th doses of CF10** (PK - 0, 0.5, 1, 2, 4, 6 hour blood samples, urine and exhaled methanethiol and dimethyl sulphide).

Day 21 - Before (PD - WBC cystine) and after the **14th dose of CF10** (PK - 0, 0.5, 1, 2, 4, 6 hour blood samples, urine and exhaled methanethiol and dimethyl sulphide).

Day 35 - Before (PD - WBC and skin biopsy cystine) and after the **last (28th) dose of CF10** (PK - 0, 0.5, 1, 2, 4, 6 hour blood samples, urine and exhaled methanethiol and dimethyl sulphide).

CF10 – Potential benefit for those living with cystinosis

- Palatable and easy to take therapy
- Reduced side effects - damage to the stomach and gut, bad breath and unpleasant body odour
- Improved tissue cysteamine delivery, and hence a lower risk of kidney failure and reduced need for dialysis and transplantation
- Fewer doses required each day, possibly only once or twice each day
- Improved quality of life and reduced psychological burden
- Greater treatment adherence and reduced risk of failure

Additional Small-Molecule Therapies for Cystinosis

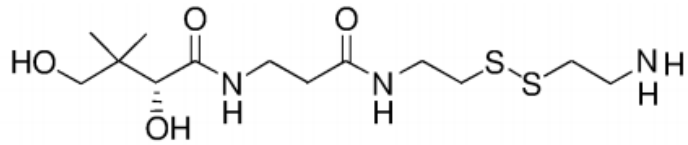
University of Sunderland

PROCYSBI® - Delayed-release cysteamine bitartrate capsules

- FDA and EMA approved
- Twice daily dosing, *versus* 4x daily administration for immediate-release cysteamine, and consequently:
  - Improved adherence
  - Improved quality of life
- Equivalent pharmacodynamics to immediate-release cysteamine
  - White blood cell cystine depletion
- Equivalent safety and tolerance to immediate-release cysteamine **but** halitosis and body odour remain problematic
- Drug price is limiting uptake in some countries

TTI-0102

- Cysteamine-pantetheine disulphide designed to prevent oxidation



(R) TTI-0102

- Converted into cysteamine in the gastrointestinal tract
- Progressively absorbed from the stomach to the colon, resulting in low C_{max} and long minimal therapeutic exposure
- Phase 1 trial in healthy volunteers completed but no clinical trials currently open
- www.thiogenesis.com/science.html / www.cystinosisresearch.org / www.clinicaltrials.gov

Additional Cysteamine Prodrugs

- **Carbohydrate-cysteamine prodrugs**
  - *In vitro* evaluation. Ramazani Y *et al.* Carbohydr. Res. 2017 **439**:9-15
- **Disulphide cysteamine-glutaric and -succinate derivatives**
  - *In vitro* evaluation. Omran Z *et al.* Bioorg. Med. Chem. 2011 **19**:3492-6
- **PEGylated derivatives of cysteamine**
  - *In vitro* evaluation. Omran Z *et al.* Bioorg Med Chem Lett. 2011 **21**:45-7
- **Disulphide cysteamine-folate derivative**
  - *In vitro* evaluation. Omran Z *et al.* Bioorg. Med Chem. Lett. 2011 **21**:2502-4

Professor Roz Anderson – 1962-2018

- BSc (Hons)
- PhD
- FRSC
- CChem
- Csci
- Woman Entrepreneur Award winner 2016

CF10 Project Team

Cystinosis Foundation UK
- Will Newman and all the CF UK Trustees and CF UK members

University of Sunderland
- Sally Burtles
- Stephen Feasey
- Dianne Hutchinson
- Adrian Moore
- Herbie Newell
- Katie Redhead
- Doreen Reveley
- Stephen Waldek

University Hospitals Birmingham
- Graham Lipkin
- Clinical Research Facility
- CR UK Clinical Trials Unit

Sundara Pharmaceuticals
- Jo Graham
- Misha Engineer
- Samer Taslaq
- Paul Watson

Fine Organics/Lianhetech
- Neil Barnwell and colleagues

High Force Research
- Neal Sim
- Stuart Penny and colleagues

Labcorp
- Melissa Purdy
- Vicky Sherwood
- Neal Hughes **and many others**

HGF
- David Selby

University of Sunderland

NHS University Hospitals Birmingham NHS Foundation Trust

CYSTINOSIS FOUNDATION UK

Co-workers, Collaborators and Funding

- **CF10 Discovery**
  - Professor Roz Anderson, Dr Lisa Frost and Mr Paul Hambleton
  - Previous PhD students, research technicians and staff
- **Cystinotic cell line and zebrafish larvae studies**
  - Professors Elena Levtchenko and Bert van den Heuvel (Leuven)
- **Cystinotic mouse model**
  - Professor Corinne Antignac (Paris)
- ***In vitro* intestinal disposition studies**
  - Professor Patrick Augustijns (Leuven)
- **Cystinotic rat model**
  - Dr Jennifer Hollywood and Professor Alan Davidson (Auckland)
- **Funding**
  - Cystinosis Foundation UK
  - Cystinosis Research Network
  - Medical Research Council UK