# Combination Therapies for Human Prion Disease











## Human Prion Disease

- Always FATAL
- Signs:
  - Ataxia
  - Cortical visual symptoms (Heidenhain)
  - Progressive dementia
  - Myoclonus
  - Akinetic mutism
- Average time to death in most common strain:

## 4 months



# Cell death

- Spongiform change
- Neuronal loss
- Astrocytosis
- Accumulation of PrPres (strain dependent)

Images courtesy of G. Jansen



# Strains...



## Conformational drug resistance:

- Prevent conversion to one shape, prions adapt...
- Block strain A, strain B unaffected...

# **Overcoming Treatment Challenges**

- Rare (1-2 / million / year)
- Rapid progression (delayed treatment)
- Conformational drug resistance
- Strain-specific effects

Combination therapy

- Poor translation from animal to human
- Blood brain barrier (affects whether drugs get into the brain)

Humanized slice culture model

# Combined treatment: 3Cs

### Conversion

Cell death (apoptosis)





Clearance (autophagy)

# Conversion



- Target several distinct initiator sites on PrP<sup>C</sup> (involved in early misfolding steps)
- Block conversion into several conformations

# Combining conversion inhibitors

- FDA-approved, human-safe
- Anti-prion effect
- Distinct PrP<sup>C</sup> binding sites:
  - Promazine / chlorpromazine
  - Quinacrine
  - Doxycycline



M. James, L. Spyracopoulos

## Prelim data: combination effects



## Clearance?



# AR-12; induce autophagy

# Cellular Physiology

**Original Research Article** 

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AR-12inhibits multiple chaperones concomitant with stimulating autophagosome formation collectively

preventing virus replication<sup>†</sup>

Running Title: Chaperones, AR12 and Viruses

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## Prelim data: AR-12 clears PrP<sup>Sc</sup> in ScN2a cells

# Cell death?





Requirements for study:
 – A model of cell death
 – Candidate drugs

# The Model: Prion organotypic slice culture assay (POSCA)



- Adaptable to mice expressing human PrP<sup>C</sup>
- Infectable with different strains of prions
- PrP<sup>Sc</sup> replicates faster than in vivo
- Reproduces pathology as seen in vivo
- No blood brain barrier

## Day 35 Day 42 Day 49







### Uninfected



#### Infected





# The drugs: Bile acids





# Ursocholic acid Dehydroxylation in gut





Conjugation in liver

# Bile acids as therapy

- Upstream effects:

  Help prevent proteins from misfolding

  Downstream effects:
  - Inhibit apoptosis

#### Tauroursodeoxycholic acid, a bile acid, is neuroprotective in a transgenic animal model of Huntington's disease

C. Dirk Keene\*<sup>†</sup>

Mol Neurobiol (2012) 46:475-486 DOI 10.1007/s12035-012-8295-4

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Communicated by R

#### Tauroursodeoxycholic Acid Prevents MPTP-Induced Dopaminergic Cell Death in a Mouse Model of Parkinson's Disease

M. Castro-Caldas		Neurobiology of Aging 36 (2015) 228-240	
C. J. Henderson •			
M. J. Gama	<b>治理治治治</b>	Contents lists available at ScienceDirect	J CA
Received: 2 May 2012 © Springer Science+E		Neurobiology of Aging	NEL-ROBIOLOGY
	ELSEVIER	journal homepage: www.elsevier.com/locate/neuaging	- A man disease

Amyloid- $\beta$  pathology is attenuated by tauroursodeoxycholic acid treatment in APP/PS1 mice after disease onset



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#### ORIGINAL ARTICLE

#### Tauroursodeoxycholic acid in the treatment of patients with amyotrophic lateral sclerosis

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See editorial by A. C. Ludolph on page 11.

#### Keywords:

amyotrophic lateral sclerosis, cholic acids, tauroursodeoxycholic acid

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doi:10.1111/ene.12664

**Background and purpose:** Tauroursodeoxycholic acid (TUDCA) is a hydrophilic bile acid that is produced in the liver and used for treatment of chronic cholestatic liver diseases. Experimental studies suggest that TUDCA may have cytoprotective and anti-apoptotic action, with potential neuroprotective activity. A proof of principle approach was adopted to provide preliminary data regarding the efficacy and tolerability of TUDCA in a series of patients with amyotrophic lateral sclerosis (ALS). **Methods:** As a proof of principle, using a double-blind placebo controlled

design, 34 ALS patients under treatment with riluzole who were randomized to placebo or TUDCA (1 g twice daily for 54 weeks) were evaluated after a lead-in period of 3 months. The patients were examined every 6 weeks. The primary outcome was the proportion of responders [those subjects with improvement of at least 15% in the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) slope during the treatment period compared to the lead-in phase]. Secondary outcomes included between-treatment comparison of ALSFRS-R at study end, comparison of the linear regression slopes for ALSFFRS-R mean scores and the occurrence of adverse events. **Results:** Tauroursodeoxycholic acid was well tolerated; there were no betweengroup differences for adverse events. The proportion of responders was higher under TUDCA (87%) than under placebo (P = 0.021; 43%). At study end baseline-adjusted ALSFRS-R was significantly higher (P = 0.007) in TUDCA than in placebo groups. Comparison of the slopes of regression analysis showed

slower progression in the TUDCA than in the placebo group (P < 0.01)

**Conclusions:** This pilot study provides preliminary clinical data indicating that TUDCA is safe and may be effective in ALS.

# Advantages of bile acids

- UDCA approved for treatment of primary biliary cirrhosis, sclerosing cholangitis
- Can be safely administered to humans
- Reaches high levels in CSF (human ALS patients)
- Water soluble
- Minimal toxicity
- Crosses the blood-brain barrier





#### RML infected, untreated



500 µM TUDCA, treatment starting day 14 Cortez et al. J

Cortez et al, J Virology, 2015





#### RML infected, untreated



100 µM UDCA, treatment starting day 14

Cortez et al, J Virology, 2015

# In vivo translation



- Works in male mice if given early (day 35)
- No effect given late in disease (day 100)





## Overview:

We use a brain slice culture model, or "prion disease in a dish", which is ideal for studying how the stages of disease develop and for testing different types of treatments. We are targeting three different stages (the three "C's") of disease in order to find a cure:

a) Conversion. During disease, prion proteins change shape and then cause other prion proteins to change shape. Blocking this shape-changing process (called conversion) is one target for drugs. We are testing more than one conversion inhibitor at a time because we know that if we only prevent conversion into one shape, the prion protein is able to adapt and convert into a different shape. This is similar to the idea of antimicrobial resistance in bacteria. This shape or conformation resistance in prions could be one reason that treatments have failed to date.

b) Clearance. There are cell processes that try to get rid of the misshaped prion proteins as they form. Improving this clearance process is another way to treat disease.

c) Cell death. As misshapen prion proteins build up in the brain, they trigger brain cell death. Using drugs that help cells resist cell death is a third way to slow down the disease.

I believe it is only by targeting all three targets that we will be successful in treating prion disease. Currently we are testing compounds that are already safe in humans, so that we can quickly translate our findings to human use.