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NC3Rs

National Centre for the Replacement, Refinement and Reduction of Animals in Research

DRGNET

Enabling access to primary human DRG to facilitate drug discovery and basic research

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The problem…

Most therapies fail in the clinic through lack of efficacy

Will more predictive *in vitro* models increase success in clinical translation?

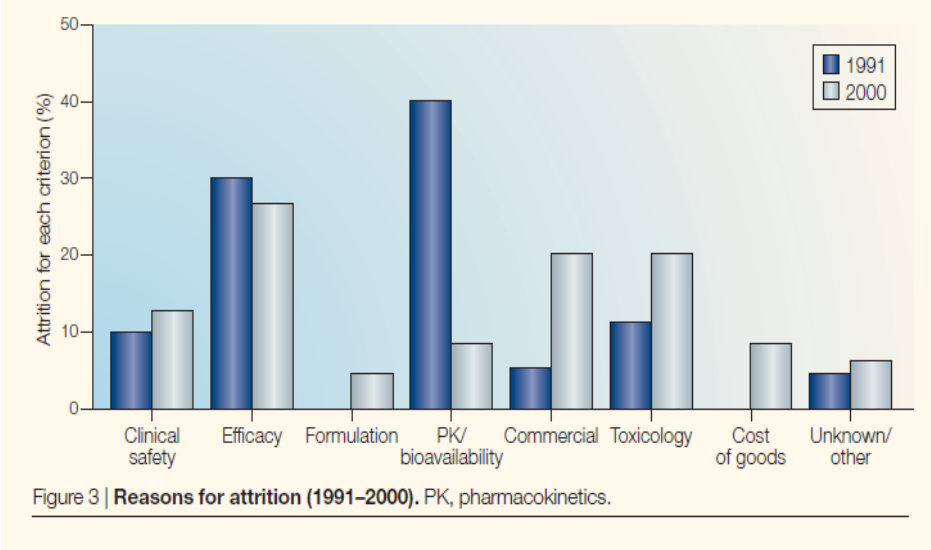
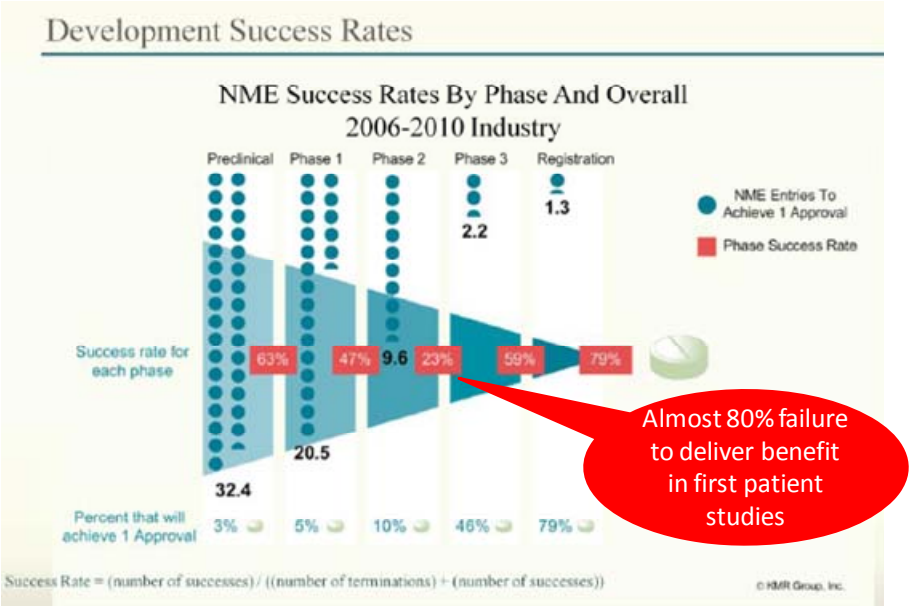


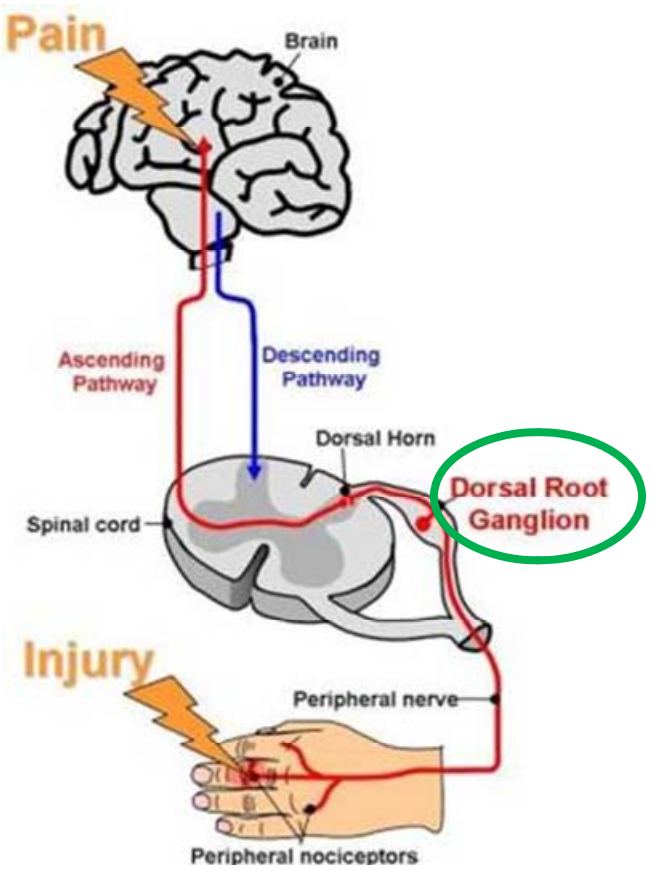
Figure 3 | **Reasons for attrition (1991–2000).** PK, pharmacokinetics.

Kola and Landis Nature Reviews Drug Discovery (2004) 3 711-715



Targeting pain

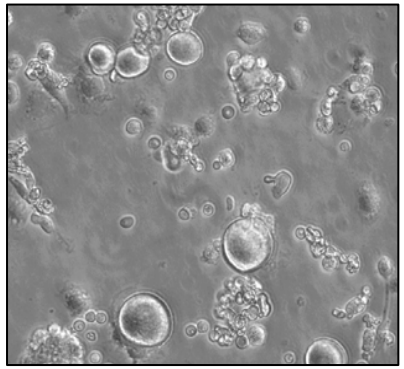
- Functional heterogeneity in sensory system
- Pain conducted by specialised nociceptive sensory neural population
- Selective expression of key receptors and ion channels in nociceptive population
  - Key targets for development of novel pain therapeutics
- Limited access to native human DRG material
  - Extrapolation from preclinical species



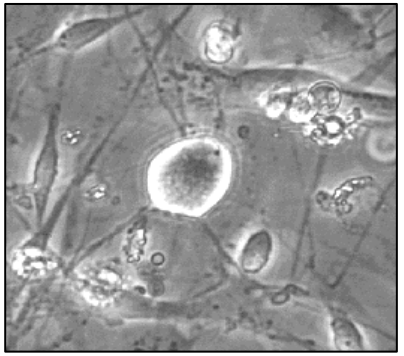
Primary cultured neurons from dorsal root ganglion in preclinical species

Neonatal rat

DRG Cells



Cultured DRG cells



- Phenotypic diversity retained in culture
- Amenable to short and longer term culture (<2 weeks)
- Material restricted by initial dissection ($5x10^5$ cells/ rat neonate)
- Heterogenous culture – Schwann cells, fibroblasts, satellite glia
- Viable cells can be cultured from adult
- Potential species differences

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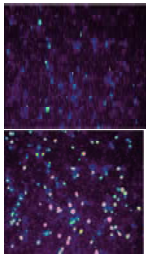
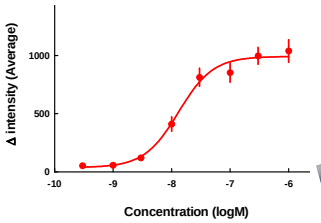
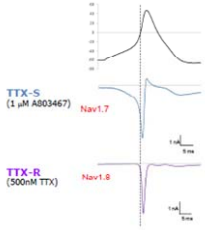
Physiology, pharmacology and disease modelling in primary DRG

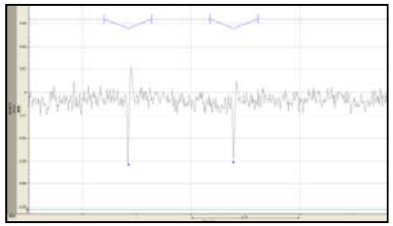




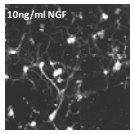
HCS – kinetic imaging

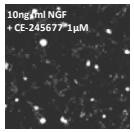


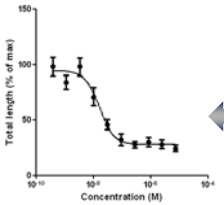
Ephys



Multielectrode Arrays



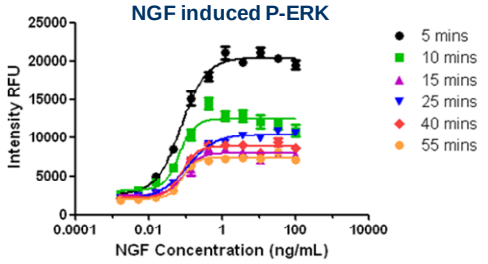


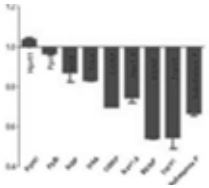


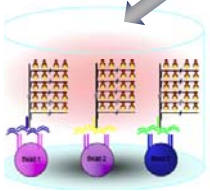
Morphometric analysis

Primary DRG neurones

Signalling pathways

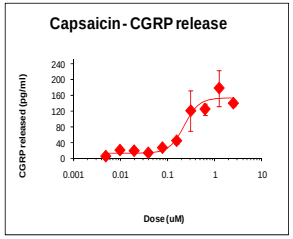






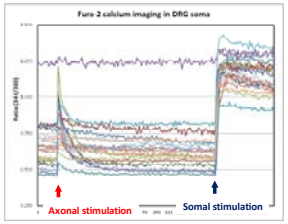
Multiplex RNA quantification

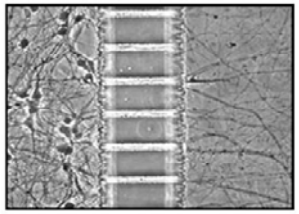
Neurotransmitter release

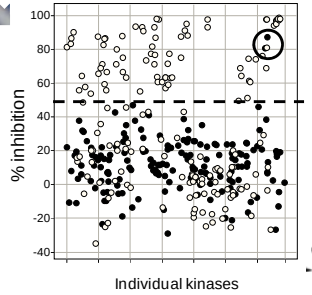


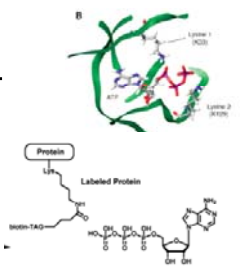
Microfluidic chambers

Chemical proteomics









Development of stem cell based models

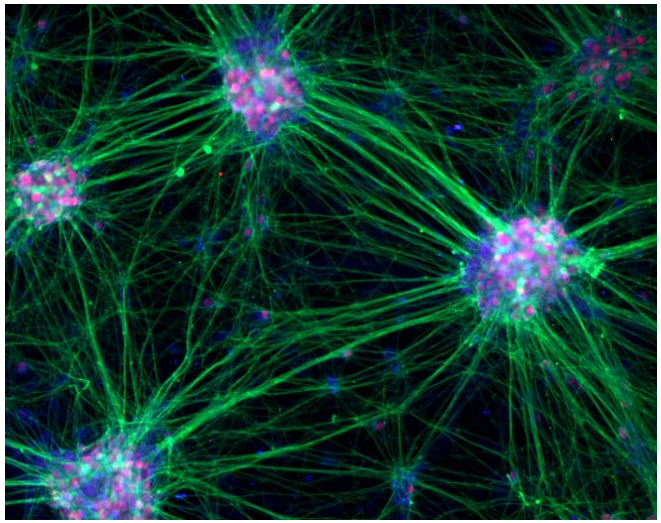
- Recent data indicates sensory-like neurones can be derived from human stem cells
- Lack of comparator studies with native human DRG material

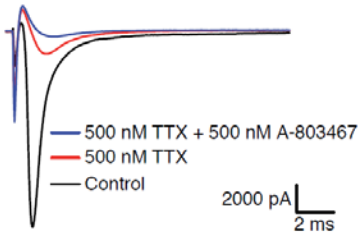
nature biotechnology

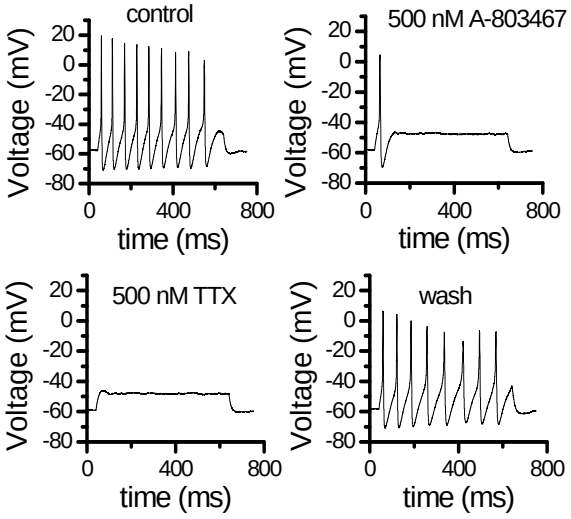
LETTERS

Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors

Stuart M Chambers[1,2], Yuchen Qi[1–3], Yvonne Mica[1,2,4], Gabsang Lee[1,2], Xin-Jun Zhang[2], Lei Niu[2], James Bilsland[5], Lishuang Cao[5], Edward Stevens[5], Paul Whiting[5], Song-Hai Shi[2] & Lorenz Studer[1,2,6]







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The issue...

- Pain is a significant unmet clinical need
  - Recent PhII failures highlight gaps in preclinical – clinical translation
- Access to primary human DRG material limited
  - Small number of publications on primary human DRG physiology
  - Knowledge extrapolated from preclinical species
- Post-mortem human DRG material not of sufficient quality for physiological study
- Gap in translation from preclinical species to human physiology
- Previous initiatives (Anabios in US) have successfully sourced viable DRG cells from transplant clinics
  - Limited to fee-for-service use

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Aims of the DRGNET project

Understand human DRG physiology by enabling access to primary human DRG material

- Enable access to primary human DRG material for the pain research community
- Link basic and industrial researchers with clinical scientists to provide material
- Pfizer Neusentis and Grunenthal are supporting this challenge to deepen understanding of human sensory physiology

3Rs Benefits

- Reduction in the use of animal DRG (rat, mouse, NHP) by enabling use of human DRG for preclinical research and drug development
  - Reduction in culling of animals for Ephys
  - Reduction in culling of animals for plate based pharmacology
- Replacement of *in vivo* exploratory target identification with robust *in vit*ro models
  - Replacement of *in vivo* animal efficacy models with *in vitro* modelling
  - Replacement of target identification strategies (eg microarray) in animals with the use of primary human cells

Better understanding of human DRG physiology will facilitate development of robust pluripotent stem cell derived modelling to further decrease reliance on preclinical species.

Top-line deliverables of the DRGNET project

- Build a sustainable platform for supply of viable human DRG material for academic and industrial research
  - Enable wide access to cells rather than fee-for-service biological testing
- Ethically source material; provision as viable cells and/or frozen material
- Build infrastructure for sample tracking and shipment logistics
- Cost base suitable for academic and industrial use

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Phased approach

Phase 1:

- Demonstrate the ability to provide a consistent supply of human DRG material which is ethically sourced, viable and suitable for physiological and pharmacological testing;
- This will include consideration of (i) where the primary cells would be obtained from, i.e. transplant clinic networks, and (ii) the training requirements for surgeons to harvest the best quality DRGs.

Phase 2:

- The supply would need to meet varying research demands depending on the platforms used. For electrophysiology, 1-2 DRGs per week would be sufficient; this would increase for transcript profiling or plate based pharmacology;
- A key component of delivery will be to evaluate the possibility of shipping cells either as viable material at ambient temperature or as frozen cryopreserved stocks;
- Costing of material must not be prohibitively expensive. A tiered costing system for access to the resource by multiple sectors/users should be considered with an estimated maximum of £650 per DRG or per microwell plate's worth of cryopreserved cells;
- There must be a sustainable business model to allow supply to continue after seed funding is exhausted;
- The Challenge also considers long term and scalable solutions to replace the human DRGs, such as development and validation of pluripotent stem cell-derived sources for human sensory neurones as a next horizon for pain research.

Potential in-kind contributions

Phase 1:

- Advice and guidance on industry requirements for harvested DRGs and what criteria are used for assessing cell viability and suitability for physiological and pharmacological testing.

Phase 2:

- Guarantee of minimum spend for 2-3 years;
- Optimisation of dissociation and cryopreservation protocols;
  - Molecular profiling of DRGs;
  - Comparison of heterogeneity between DRGs from individual humans;
  - Comparison of human DRG with pluripotent stem cell derived sensory neurones;
- Comparison of animal and human DRGs;
  - Single cell sorting and transcript analysis;
  - Microarray studies;
  - Electrophysiology.