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Gene Therapy to Control Intractable Pain in Cancer Patients:

A New Paradigm?

Michael W Graham
Chief Scientist
Benitec Biopharma Ltd

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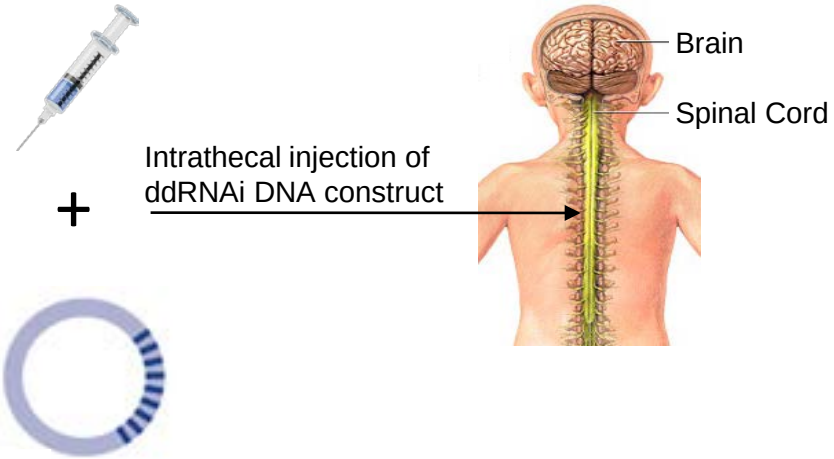
This presentation contains forward looking statements that involve risks and uncertainties.

Although we believe that the expectations reflected in the forward looking statements are reasonable at this time, Benitec Biopharma can give no assurance that these expectations will prove to be correct.

Actual results could differ materially from those anticipated. Reasons may include risks associated with drug development and manufacture, risks inherent in the regulatory processes, delays in clinical trials, risks associated with patent protection, future capital needs or other general risks or factors.

Gene Therapy to Control Cancer Pain

- Intractable neuropathic pain is a significant unmet medical need
- Genetic therapy considerations:
  - Safety – Terminal cancer patients
  - Modality – Gene silencing using ddRNAi
  - Target – Key genes shown to play key role in pain
  - Delivery – route and vector
- Introduce constructs using IT administration of lentiviral vectors
  - Integrate into cells; specify long term expression of shRNAs

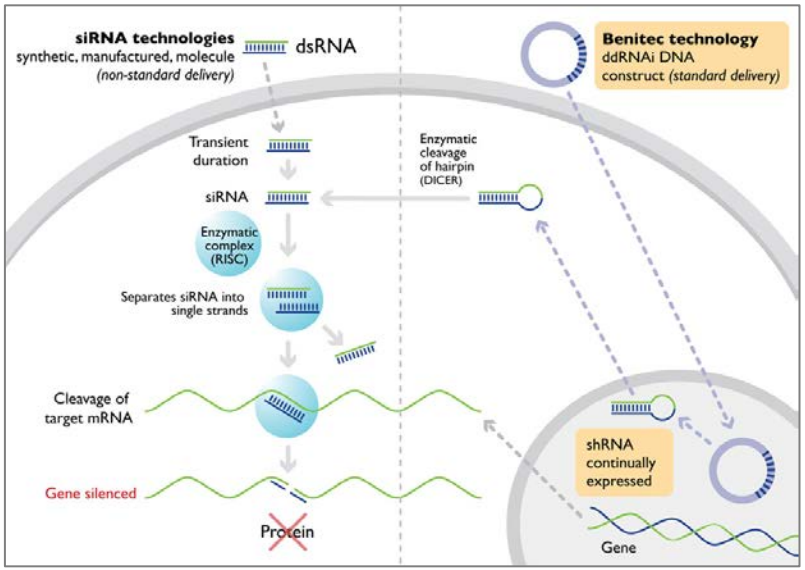


  - Potential long term pain relief with a single injection

Introduction

- Benitec Biopharma Ltd
  - Overview of company
  - ddRNAi-based therapeutics
- Pain Program
  - RNAi in pain research
  - PRKCG as a target for silencing
  - Externally validated
- Status of pain program
  - Construct design
  - Construct testing *in vitro* & *in vivo:*
    - Tetra Q, University of Queensland, Brisbane Australia
    - Dr David Yeomans, Stanford Univ., CA, USA
  - Subsequent steps

siRNA ddRNAi (shRNA)



Benitec Biopharma Ltd - Overview

- ASX listed company (ASX:BLT)
  - Sydney, Australia
- Dominant IP position in ddRNAi (DNA-directed RNAi)
  - shRNA
  - IP covers therapeutic use of expressed shRNAs
- Pipeline building on IP
  - Therapeutic areas with significant unmet needs
  - Gene therapy
- Global presence
  - Collaborations with expert academic / commercial groups in Australia, US, China, UK.

ddRNAi-based programs

Indication	Discovery	Pre-clinical	Human clinical	Delivery Strategy	Market
Cancer-associated pain	Nervarna™			**Localised via lentiviral injected intrathecally**	**$2.6 billion by 2016**
Drug resistant cancer	Tribetarna™			**Systemic coupled with PEI nanoparticles**	**Leading form of cancer worldwide**
Hepatitis B	Hepbarna™			**Systemic via AAV**	**400 million globally**
Oculopharyngeal muscular dystrophy	Pabparna™			**Local injection (AAV) combined with gene therapy**	**Orphan disease affecting 1 in 100,000 in Europe**
Hepatitis C				**Systemic via AAV**	**>170 million people worldwide**
HIV/AIDS *(COH & Calimmune)*				**Ex vivo lentiviral transfection of CD34 stem cells**	**1/200 infected with HIV worldwide**
Advanced cancer *(Gradalis)*				**shRNA cassette combined with therapeutic vaccine**	**11.7 million in the US**
Retinitis pigmentosa *(Genable)*				**Local injection (AAV) combined with gene therapy**	**Orphan disease**

Benitec funded programs

Licensed program

Partnered program

External program

RNAi in Pain Research

- Both shRNAs & siRNAs widely used in research
  - validating targets / defining signaling pathways
- Potential therapeutic strategy
- Many potential targets, ion channels, receptors

Target	RNAi method	Type of pain	Reference
PRKCG	shRNA	neuropathic	Zou et al, 2011 Hum Gene Ther; 22: 465-475
NF-κBp65	shRNA	nociceptive	Sun et al 2012 Eur J Pharmacol; 682: 79-85
NMDA receptor NR1	shRNA	mechanical allodynia	Garraway et al, 2007 J Pharm Exp Therap; 322: 982-88
MMP-2 & MMP-9	siRNA	mechanical allodynia	Kawasaki et al, 2008 Nature Med; 14: 331-336
K^+ channel Kir4.1	siRNA	neuropathic pain	Vit et al, 2008 J Neurosci; 28: 4161– 4171
Capsaicin receptor TRPV1	shRNA	mechanical allodynia	Christoph et al, 2008 J. Mol Cell Neurosci; 37: 579-89
EP receptor EP4 (prostaglandin E2)	shRNA	nociceptive	Lin et al, 2006 J Pharml Exp Ther; 319: 1096-06
NMDA receptor NR2B	siRNA	spontaneous	Tan et al, 2005 Gene Ther 2005: 12: 59-66
ATP receptor P2X3	siRNA	neuropathic	Dorn et al, 2004 Nucl Acids Res 2004; 32: e49

PRKCG is a well validated target

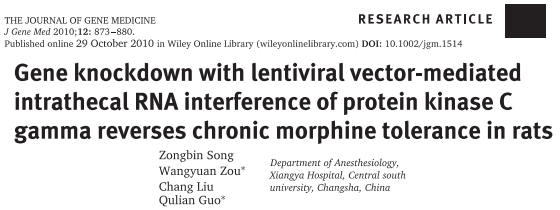
- PRKCG knockout mice
  - Reduced neuropathic pain response
    - CCI - like model
  - Preserved acute pain response

Preserved Acute Pain and Reduced Neuropathic Pain in Mice Lacking PKCγ

Annika B. Malmberg,* Chong Chen, Susumu Tonegawa, Allan I. Basbaum

SCIENCE • VOL. 278 • 10 OCTOBER 1997

- PRKCG knockdown alleviates morphine tolerance
  - Rat model
  - IT administration of PRKCG Lentivirus


THE JOURNAL OF GENE MEDICINE
J Gene Med 2010;12: 873–880.
Published online 29 October 2010 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/jgm.1514

RESEARCH ARTICLE

Gene knockdown with lentiviral vector-mediated intrathecal RNA interference of protein kinase C gamma reverses chronic morphine tolerance in rats

Zongbin Song
Wangyuan Zou*
Chang Liu
Qulian Guo*

Department of Anesthesiology, Xiangya Hospital, Central south university, Changsha, China

- PRKCG has been implicated in other pain models
  - Cancer pain (Xiaoping *et al* 2010. *Brain Research* **1335:** 83- 90)
  - Formalin-induced colon inflammatory pain (Zhang *et al* 2011. *Neurosignals* **19:** 142-150)
  - Trigeminal neuropathic pain (Nakajima *et al* 2011. *Journal of Dental Research* **90:** 777-781)
  - Alcohol withdrawal (Shumilla et al, 2005. *Journal of Pain* **6:** 535-549)
  - Pertussis toxin (Wen *et* al, 2003. *Brain Research* **963:** 1-7)

ddRNAi to PRKCG - “proof of concept”

- Rat - CCI model
- Single IT administration
  - lentiviral vector
  - PRKCG shRNAs
- Key findings:
  - Effective on mechanical allodynia
  - Effective on thermal hyperalgesia
  - Dose response
  - Long term effects

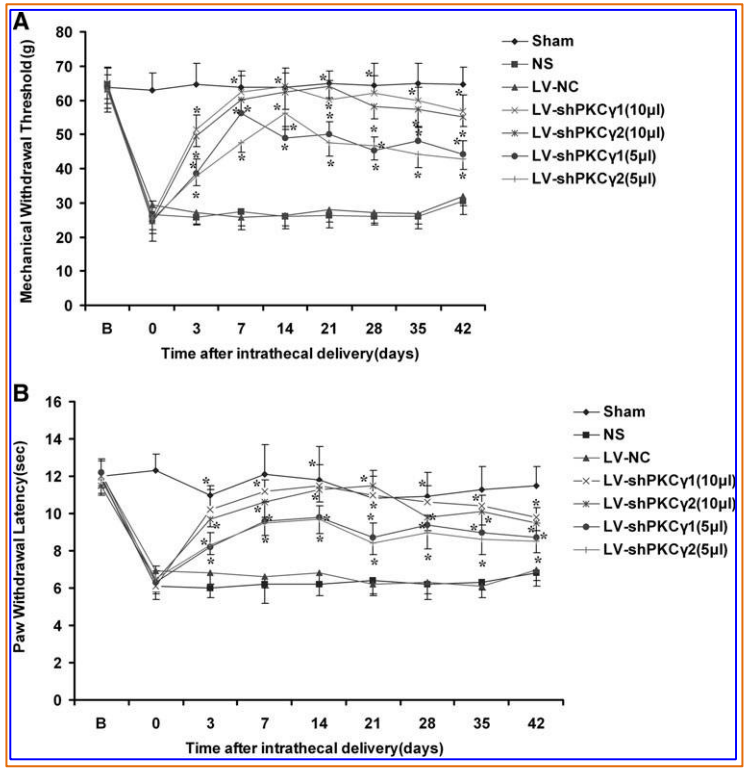
HUMAN GENE THERAPY 22:465–475 (April 2011)

DOI: 10.1089/hum.2010.207

Intrathecal Lentiviral-Mediated RNA Interference Targeting PKCγ Attenuates Chronic Constriction Injury–Induced Neuropathic Pain in Rats

Wangyuan Zou, Zongbin Song, Qulian Guo, Chang Liu, Zhong Zhang, and Yanfeng Zhang

Department of Anesthesiology, Xiangya Hospital, Central South University, Changsha, 410008, China.



ddRNAi to PRKCG - “proof of concept”

- Constructs defined by Zou *et al* target rat-specific sequences
  - Target sites not conserved in human, or other pre-clinical test species
  - Disrupt “seed” sequences; unlikely to silence PRKCG in these species

shPKCγ1

Rat PRKCG (NM_012628)	AGTTTGAGGCCTGTAATTA
Human PRKCG (NM_002739)	..........T.....C..
Macaque	..........T.....C..
Dog (XM_541432)	

shPKCγ2

Rat PRKCG (NM_012628)	CTCTATGCCATCAAGATAC
Human PRKCG (NM_002739)	.....C..........CT
Macaque	.....C........A..C.
Dog (XM_541432)	.....C..........C.

- Complicates pre-clinical test programs
- Requires testing of multiple constructs in toxicology / bio-distribution studies

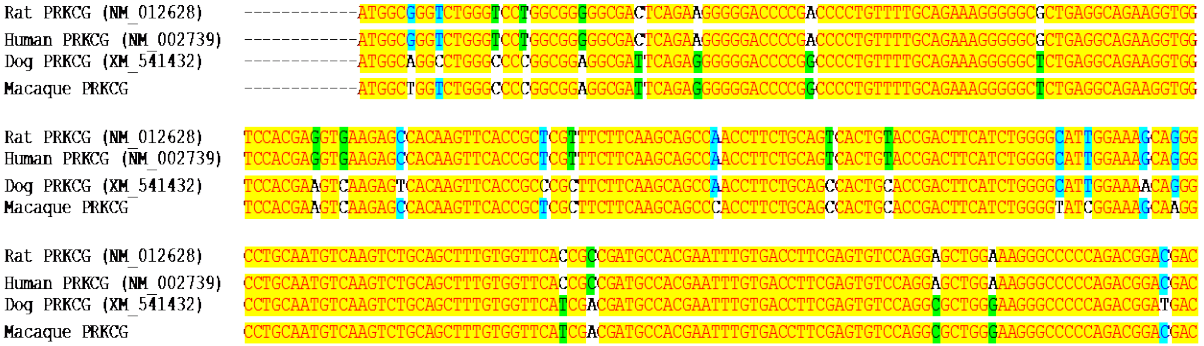
PRKCG silencing - Side Effects

- PRKCG expressed specifically in neuronal tissues.
- PRKCG knockout mice display only minor developmental, behavioral and cognitive defects:
  - Slight ataxia, modest cognitive impairment & some motor incoordination
  - Decreased anxiety, increased alcohol tolerance
  - Consequence of complete inactivation throughout development
- PRKCG mutations are causative for Spinocerebellar Ataxia Type 14.
  - *trans* dominant mutations
  - Consequence of mutant phenotype throughout development
- PRKCG plays a protective role against cerebral ischemia
  - similar role in protecting retina and lens against hypoxic shock
  - IT delivery unlikely to silence PRKCG in these tissues

Conclusion: Specific silencing of PRKCG in intrathecal compartment is unlikely to have serious side-effects

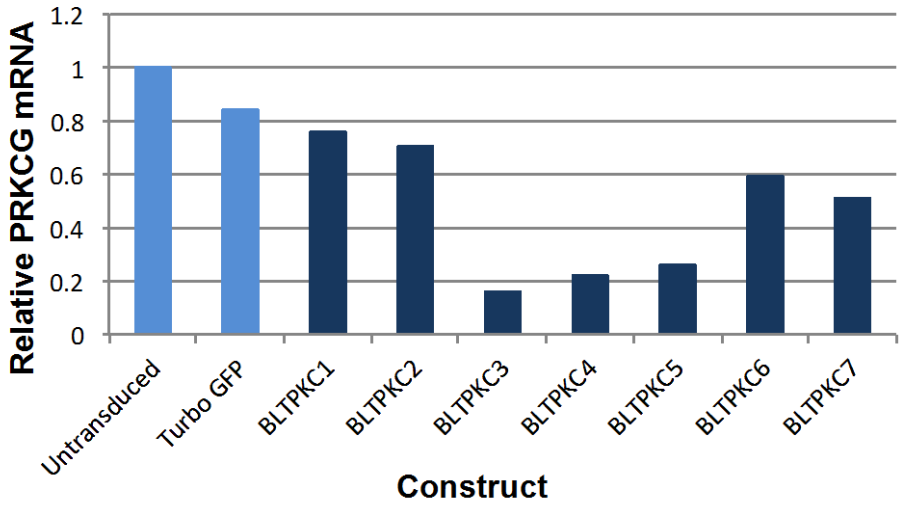
Improved Constructs to Silence PRKCG

- Define DNA constructs that target absolutely conserved sequences
  - Should silence rat, human, dog (&NHP) PRKCG
- Permit progression of a single construct through all stages of pre-clinical testing
- Several absolutely conserved regions in PRKCG CDS



shRNAs Targeting Conserved Sites

- Benitec designed / tested 7 constructs targeting conserved regions
  - Constructs / LV particles (Sigma Aldrich)
  - Transformed rat C6 cells (glioma) , express PRKCG - Tetra Q
    - Assayed PKCγ mRNA levels in transformed cells
    - qRT PCR relative to GAPDH



Design "rules"
- Processing is "sloppy"
- Predominant 22nt phase
- 5' G (U6 start)
- 5' A or U for Ago2 loading
- no internal UUUU
- duplex thermostability

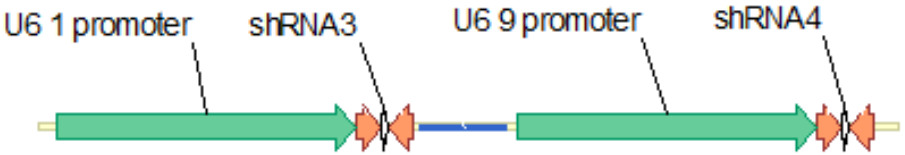
- Defined two potent shRNAs, BLTKC3 and BLTPKC4
  - Target sequences for shRNAs 3 & 4 are conserved in rat, human, dogs & NHP

Defining Lead Compound

- Testing two constructs

  - BLTPKC4 (expresses U69 shRNA 4)

  - BLTPKC3&4 (expresses U61 shRNA3 & U69 shRNA 4)
  - Likely to confer near complete silencing



- Lentiviral particles prepared by GLP / GMP manufacturer
- Scrambled insert controls (BLTPKC4cont & BLTPKC3&4cont)
- Define activities; progress a single construct

Current Experiments - *in vivo*

In vivo testing of constructs

- CI: Dr. David Yeomans, Stanford University
- Lentiviral particles from GLP / GMP manufacturer

Spared Nerve Injury rat model

- PWT
- Thermal hyperalgesia
- Four constructs
  - BLTPKC4
  - BLTPKC4 cont
  - BLTPKC3&4
  - BLTPKC 3 & 4 cont

Spinal cord injection of high titre “GLP” particles

Experiments underway

Current Experiments - *in vitro*

- *In vitro* testing of constructs
  - Tetra Q, University of Queensland, Brisbane, Australia
- Test knockdown of constructs in C6 glioma cells
  - Cloned cell lines
  - mRNA by qRT PCR
  - Protein by Western blots
- Demonstrate specificity for PRKC isotypes (RNA & protein)
  - shRNA 3 targets conserved regions in PRKCA (& PRKCB)
  - shRNA 4 target is not conserved

Human PRKC isotypes

```
PRKCG human CDS   GACTTCAGCTTCCTCATGGT
PRKCA human CDS   -------At-----------
PRKCB human CDS   --t--t-A------A-----

PRKCD human CDS   A------T-----A--A---
PRKCE human CDS   --G----A----A---A---
PRKCH human CDS   A----tgAG---A--CGA--
PRKCQ human CDS   --t--t-T---G-A--AAA-
PRKCZ human CDS   -----tgA-C-AA---GA--
PRKCI human CDS   --t--tgAt--G---CG---
```

Next Steps

- **Toxicology / Biodistribution studies**
  - CROs
  - Study plans well advanced
  - Progress best construct
- **Clinical trials**
  - Targeting 2013

Acknowledgements

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    - Dr Bruce Wyse
    - Dr Ai-Leen Lam
  - Stanford
    - Dr David Yeomans

Contact Information

For further information, please contact:

Dr Michael Graham
Chief Scientist
Benitec Biopharma Ltd.

www.benitec.com

E-mail: mgraham@benitec.com