Progressing Gene Therapy Products to the Clinic – a Platform Based Approach

K Mitrophanous
Head of Research

October 2011

Presentation Overview

- Introduction to lentiviral vector technology
- Production
- Quality control
- Clinical development

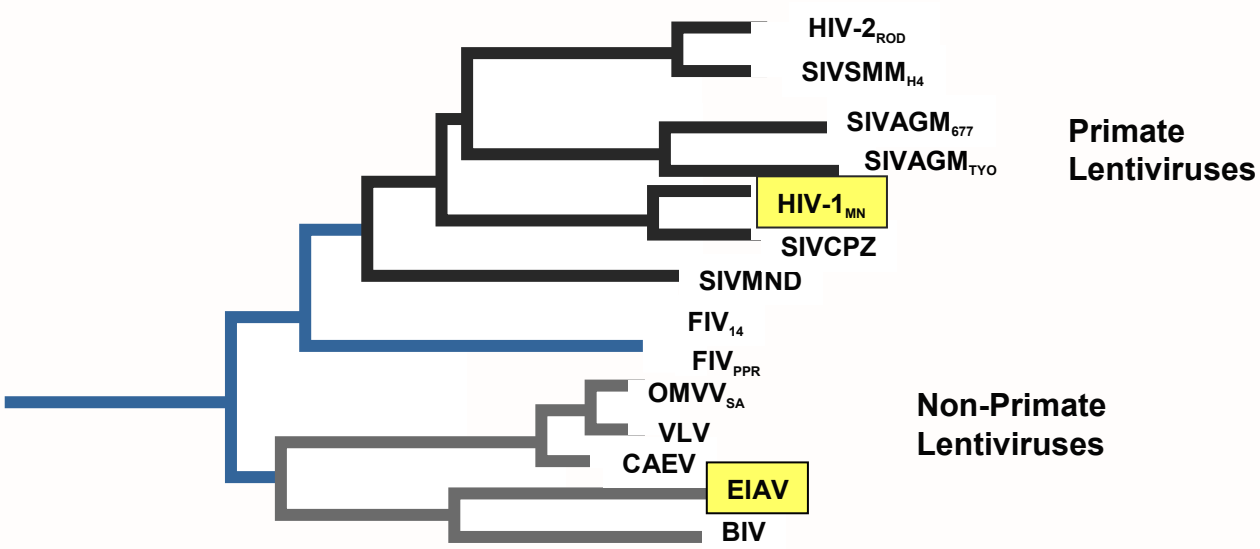
OxfordBioMedica

Lentiviral Vector Product Pipeline

Product (Partner)	Indication	Stage of Development
ProSavin®	Parkinson's disease	Phase I/II trial ongoing
RetinoStat® (Sanofi)	"Wet" age-related macular degeneration	Phase I trial ongoing
StarGen™ (Sanofi)	Stargardt disease	Phase I/IIa trial ongoing
UshStat® (Sanofi)	Usher syndrome	IND approved
EncorStat® (Sanofi)	Corneal graft rejection	Phase I/II preparation
MoNuDin®[1]	Motor neuron disease	Research

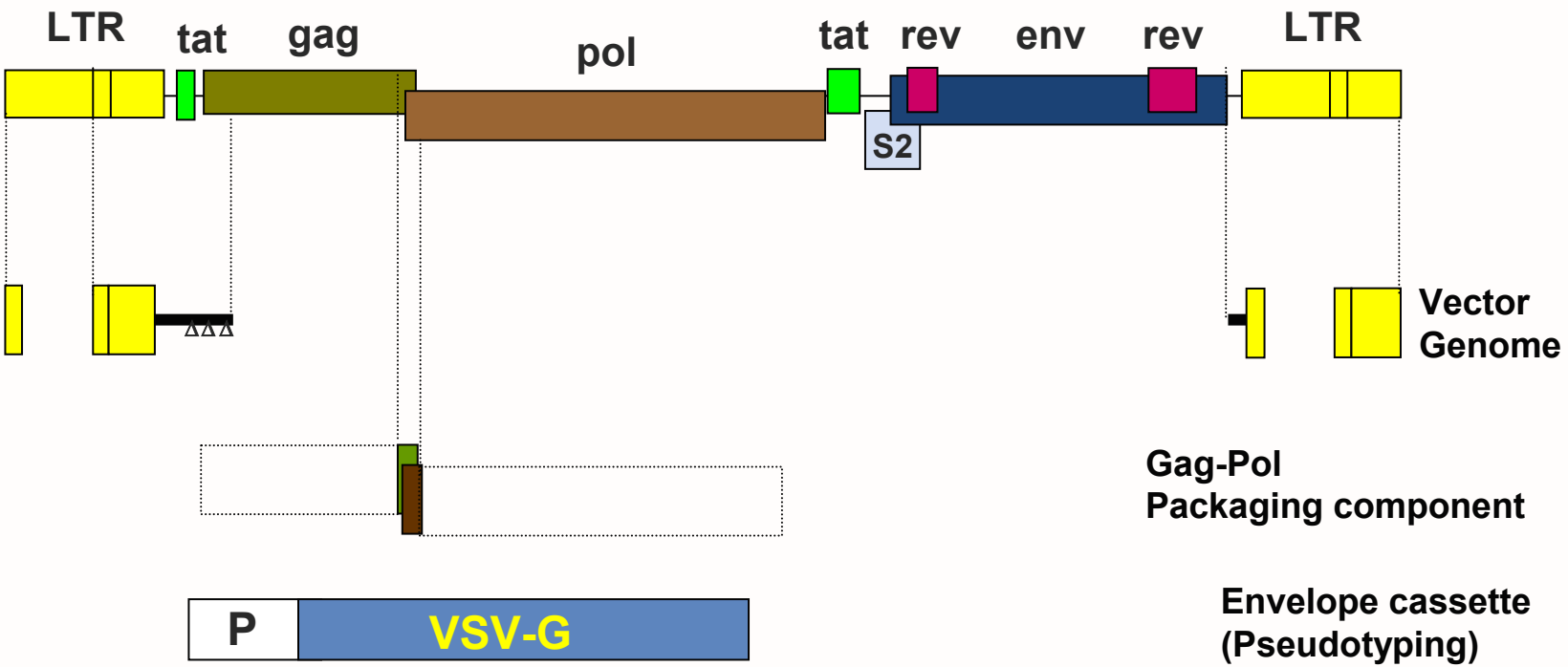
1. UK Motor Neurone Disease Association & Amyotrophic Lateral Sclerosis Therapy Development Institute

Lentiviruses - EIAV



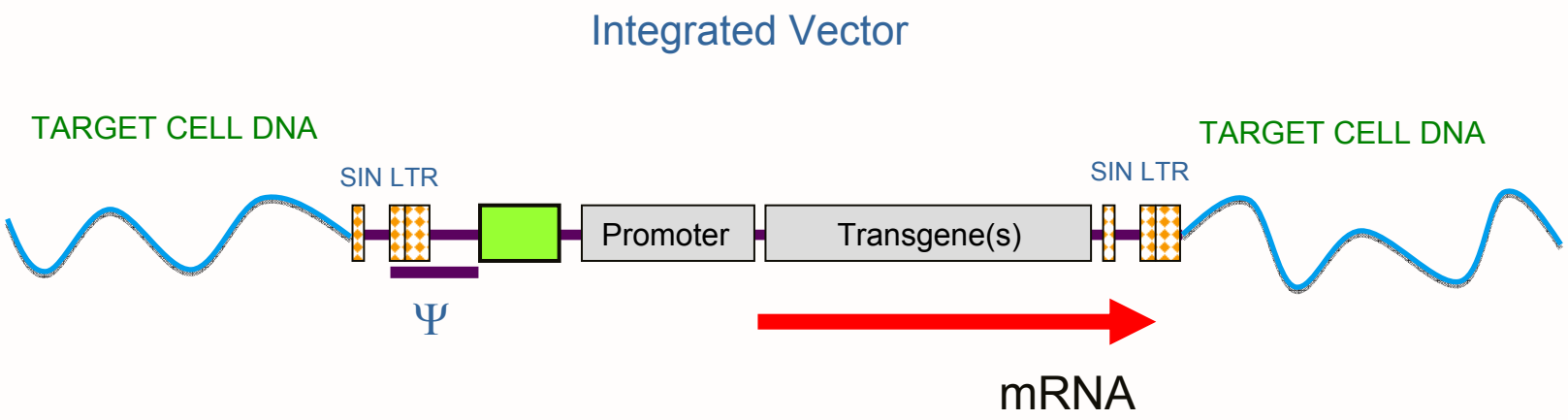
- Non-human pathogen
- Self limiting anaemia in horses
  - Does not cause immunodeficiency, restricted global distribution
- Low probability of human encountering EIAV
  - No pre-immunity, low probability of recombination

EIAV Lentiviral Vector System



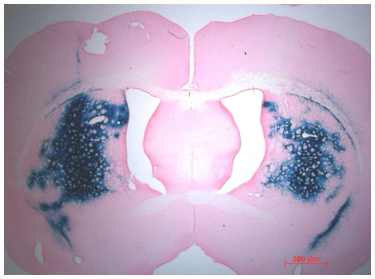
- No functional viral proteins or coding regions in vector genome
- Lentiviral vector genome contains only 0.86kb of EIAV nucleic acid
- Titres ~$10E^9$ TU/mL

Minimal Lentiviral Vector in Transduced Cells

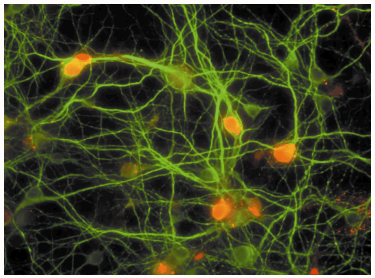


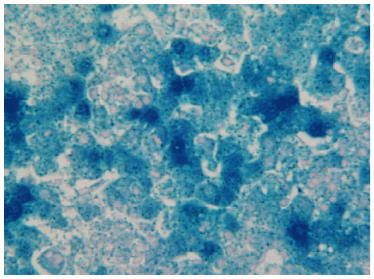
- No viral genes are transferred to target cells
- Transduction and integration process is stable and predictable
- Only the transgene is transcribed and expressed in target cells
- Genetic capacity ~9kb
- Transcription unit may be polycistronic with IRESs (e.g. ProSavin®)

Lentiviral Vectors: Broad Tissue Tropism

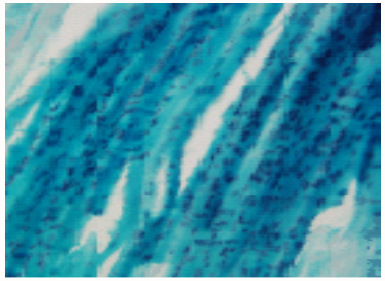


Neurons

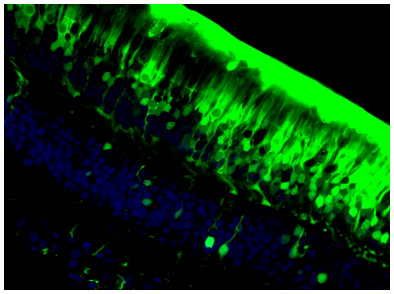




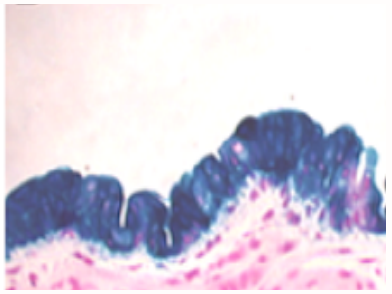
Liver



Skeletal muscle



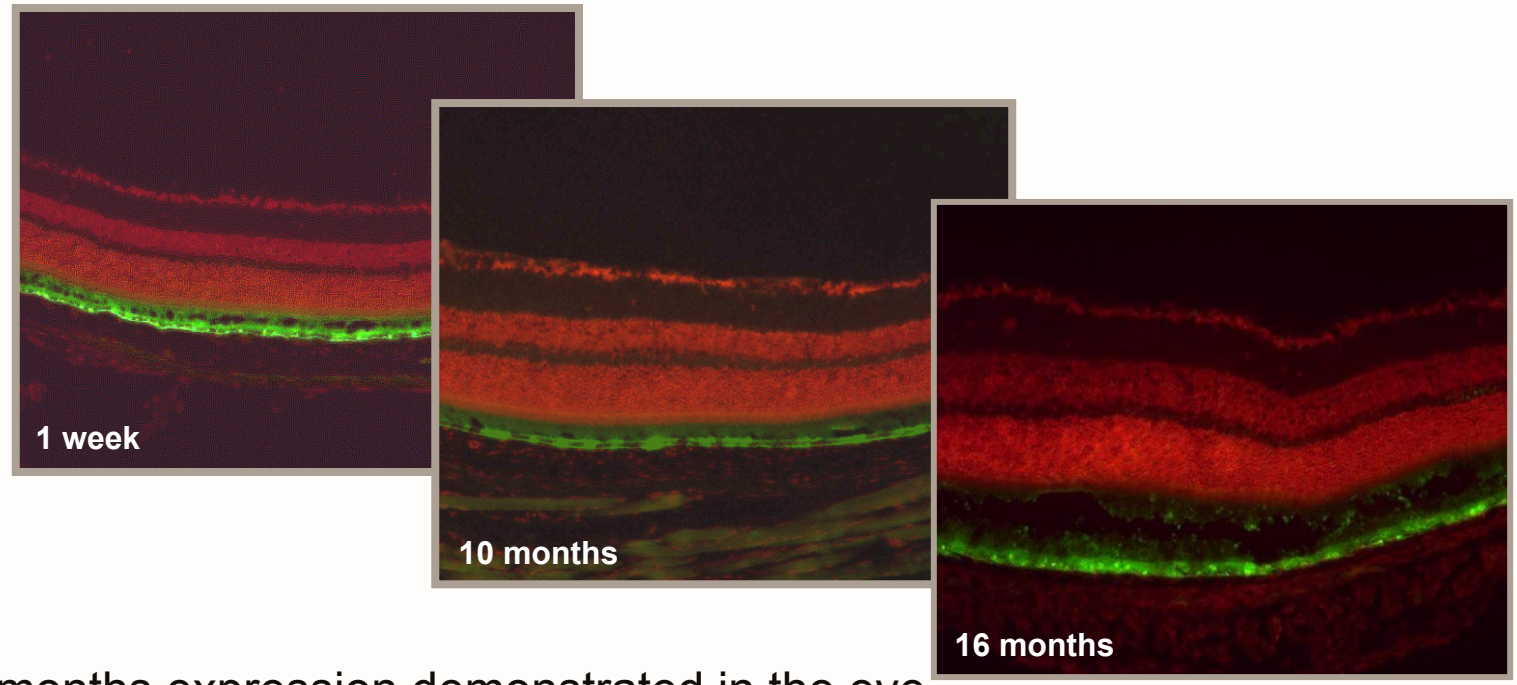
Eye (RPE/photoreceptors)



Respiratory Epithelium

- Most tissues can be transduced with high efficiency

Lentiviral Vector Platform Technology: Long-Term Expression



- 16* months expression demonstrated in the eye
- ~4yrs expression also observed in brain in Parkinson's NHP model
- No "shut down" in expression
- Potential for single administration products

* Experiment stopped at 16 months

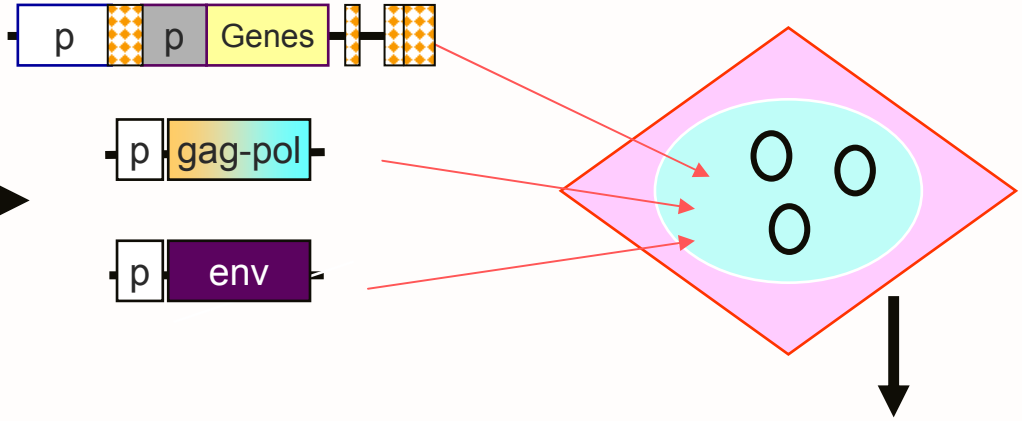
Presentation Overview

- Introduction to lentiviral vector technology
- Production
- Quality control
- Clinical development

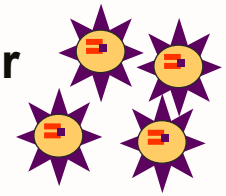
GMP Manufacturing Process

Cell Factory containing HEK293T cells

Three-plasmid co-transfection



Crude vector harvest



Ion exchange purification, Concentration

Bulk Drug

Concentration, Formulation, Sterilisation, Vial

Drug Product (recovery ~30%)

Production of Lentiviral Vectors

- CMC support for a ‘Platform’ Phase I/II process for all OXB Lentiviral vector products
  - Manufacturing process
  - QC
  - QA/QP
- Regulatory review
  - AFSSAPS (2007)
  - MHRA
  - FDA

Production Summary

- Current scale of process amenable to early stage clinical trials
  - Phase I and II
  - Currently outsourced to a CMO
  - Majority of the process utilises disposables
  - Care in selection and development of materials, APIs etc
- OXB recently purchased a manufacturing facility in Oxford
  - To provide additional flexibility & minimise self-competition (Phase I/II)
  - To facilitate process development and scale-up (Phase III, Market supply)
  - On track for operational readiness Q1 2012

Presentation Overview

- Introduction to lentiviral vector technology
- Production
- Quality control
- Clinical development

Quality Control - Strategy

- Quality control scheme designed to;
  - Evaluate manufacturing process performance and guide improvements
  - Demonstrate consistency between batches
- Product specification enables translation from non-clinical to clinical studies
  - Efficacy (not necessarily final process material)
  - Toxicology
  - Biodistribution
  - Shedding

Quality Control - Implementation

- ‘Standard’ QC assays

- A series of custom lentiviral vector QC testing assays
  - Validated to support Phase I/II development of ProSavin®, other IMPs, and in preparation for Phase III
  - Core series of ‘generic’ platform assays for IMP characterization
  - Two product-specific assays (identity, potency)

Quality Control - Implementation

Drug Substance

- Bioburden
- Endotoxin (LAL)
- Mycoplasma – cult and non-cult
- Adventitious agents (*in vivo*, *in vitro*)
- RCL (end of production cells)
- Strength (Transducing Units, TU/mL)
- RNA copy number (copies per mL)
- Particle to infectivity ratio (RNA copies / strength)

Drug Product

- Residual Sodium Butyrate*
- Residual Benzonase*
- pH
- Sterility
- Endotoxin (LAL)
- Total protein content
- SV40 Large T DNA (293T cells only)
- Total Residual DNA (Picogreen)
- DNA characterisation (HEK293T, plasmids)
- Residual BSA
- Appearance
- RCL
- Strength (Transducing Units, TU/mL)
- Potency (product specific assay e.g. HPLC, angiogenesis assay)
- Identity – PCR
- PERT (RT units)
- Particle to infectivity ratio (RNA copies / strength)
- Specific Activity (strength/total protein)

Quality Control - Conclusions

- Quality control should be based on knowledge of key characteristics of the IMP
    - Biology of lentiviral vector (RNA genome, RT activity, integration)
    - Manufacturing process (e.g. impurities)
    - Platform assays provide an advantage for future IMPs
    - Knowledge guides method evaluation, qualification and validation
    - Product-specific potency sometimes challenging

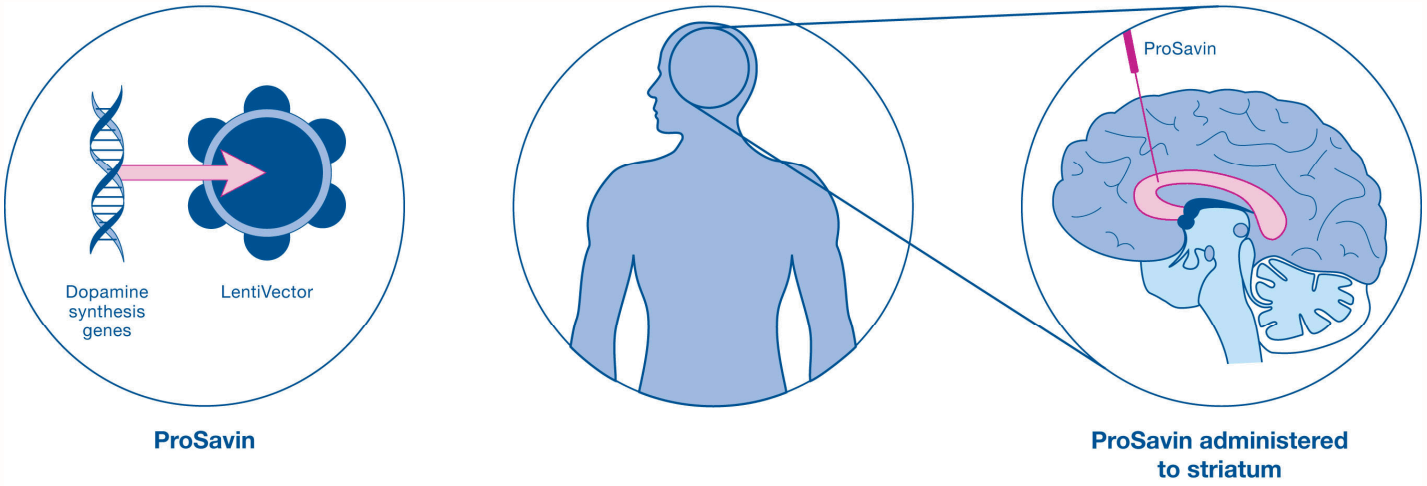
- Define critical assay parameters as early as possible
    - Enables cross comparison with historical data
    - Reference standards for trending

Presentation Overview

- Introduction to lentiviral vector technology
- Production of lentiviral vectors
- Quality control
- Clinical development

ProSavin®: a Gene-Based Treatment for PD

- ProSavin® delivers genes for 3 enzymes required for DA synthesis
  - Administered locally to the *striatum*, where DA is normally released
  - Converts non-dopaminergic cells to DA factories
  - Aims to restore a constant dopamine supply to striatum and smooth the fluctuations cause by oral therapies
  - Improvement in motor function, decrease in motor side effects



ProSavin® Non-Clinical Summary

- Proof of concept demonstrated in preclinical NHP studies
  - NHP locomotor and behavioural recovery sustained for ~4 years
- No acute or chronic toxicity (Rat 6 month, NHP 9 month)
- Histology confirmed expression of enzymes and dopamine synthesis
- Biochemical products limited to target tissue
- No biodistribution or immunogenicity issues
  - ProSavin® largely confined to striatum

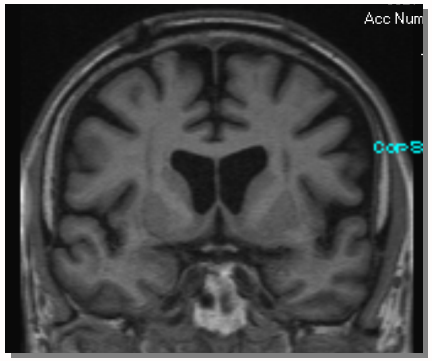
ProSavin® Phase I/II Trial Design

- A Phase I/II open-label study of safety and efficacy of ProSavin® in subjects with bilateral, idiopathic Parkinson's disease
- Regulatory approval for study in France and UK
  - Neurosurgery at Henri Mondor Hospital in Créteil, France (Professor Stéphane Palfi)
  - UK site opened Q1 2011 at Addenbrookes Hospital, Cambridge (Professor Roger Barker)
- Dose escalation in cohorts of 3-6 patients (at 1x, 2x, 5x doses)
- Primary efficacy endpoint:
  - Motor function (UPDRS III OFF score) at six months
- Secondary endpoints include:
  - Reduction of L-DOPA therapy
  - Time spent in ON vs. OFF state (patient diaries)
  - Activity of daily living (PDQ-39 index)

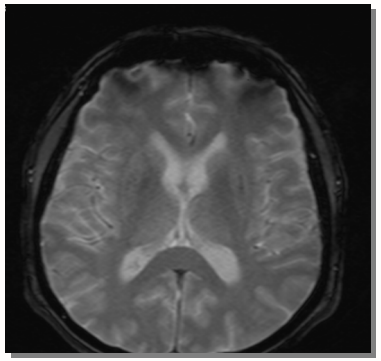
ProSavin®: Safety Profile

- Patients ambulatory 24 hours after surgery
- No dyskinesia exacerbations in OFF state
- No changes on MRI or PET
- No evidence of adverse immune responses
- No surgical or ProSavin®-related SAEs
- No vector/transgene antibody detection
- No vector particles (RNA) detected in blood and urine
- No integrated vector (DNA) detected in white blood cells

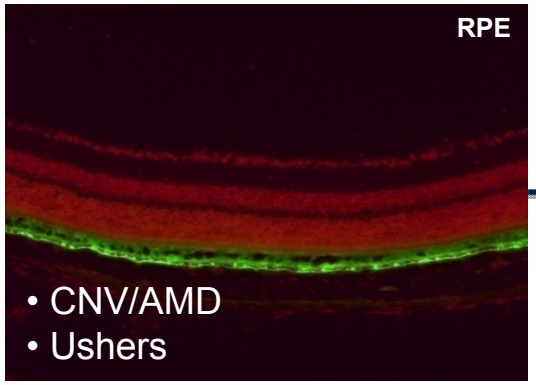
T1 ws



T2 ws

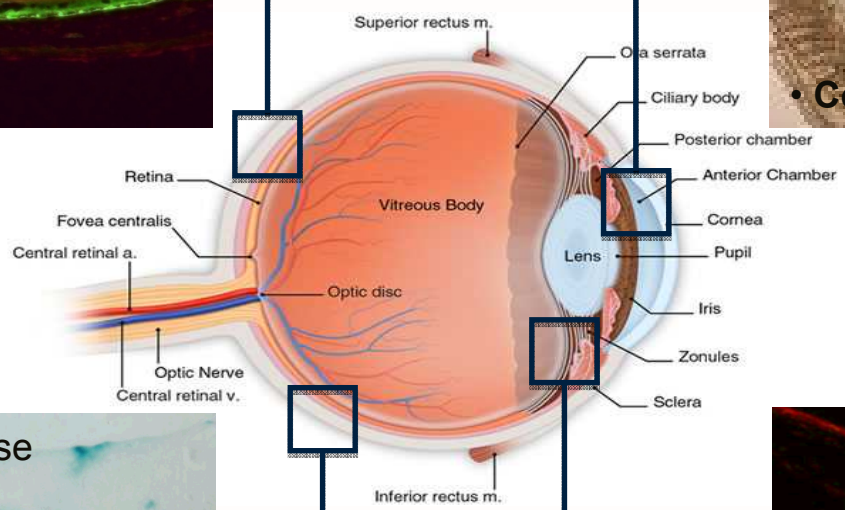


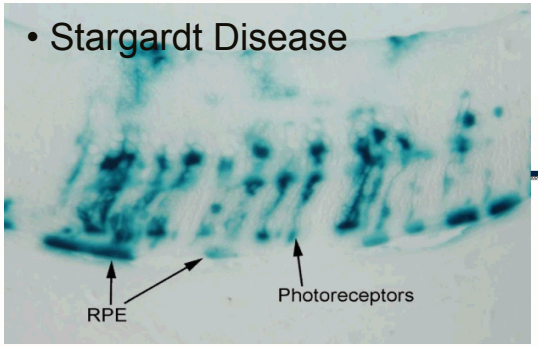
Ocular Gene Therapy

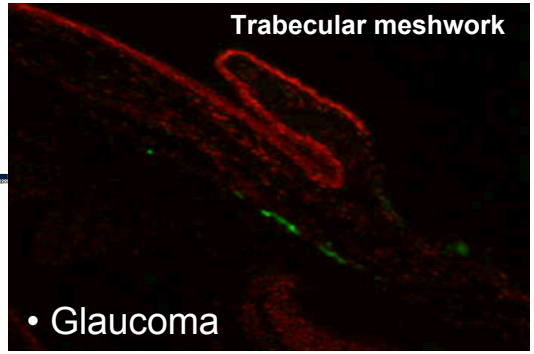


Subretinal

Anterior Chamber







Ocular Gene Therapy

RetinoStat®

- Addressing retinopathies caused by aberrant blood vessel growth
- Genes for two anti-angiogenesis proteins are delivered using lentiviral vectors

StarGen™

- Stargardt disease is caused by a mutation of the ABCR gene which leads to the degeneration of photoreceptors in the retina and vision loss

UshStat®

- Usher syndrome type 1B is caused by a mutation of the gene encoding myosin VIIA (MY07A), which leads to progressive vision loss combined with a congenital hearing defect

EncorStat®

- Despite being of the most successfully transplanted tissues, a significant number of corneal grafts are rejected due to neovascularisation
- Lentiviral vector used to *ex vivo* modify donor corneas to express anti-angiogenesis proteins prior to transplant

Ocular Non-Clinical Toxicology: Summary

- Ocular profile assessed in:
  - RetinoStat®: rabbit and NHP for 6 months
  - StarGen™: rabbit and NHP for 3 months
  - UshStat®: NHP for 3 months
- Overall well tolerated with minimal inflammation
- Similar in profile to neutral, sterile buffer
- No changes in:
  - Clinical signs, IOP, ERG, histological findings
- Histology confirmed expression of transgenes

Ocular: Non-Clinical Pharmacology/PK

Key findings:

- Studies with reporter genes indicate vector enables delivery and expression primarily in RPE and photoreceptors (PR) over the long term (up to study termination, [16 mths])
- Administration of ocular vectors leads to correction of animal models of the disease
- Long term expression has no impact on normal ‘resident’ vasculature or observed negative impact in the non-target cells

Ocular: Non-Clinical Biodistribution Analysis

- Vector Shedding/dissemination (RNA)
  - No vector shedding in urine, saliva, and contralateral eye tear swabs
  - Vector particles found in vitreous fluid but gone by day 29
  - No vector dissemination in plasma or cerebrospinal fluid

- Vector Biodistribution (DNA)
  - Long term vector found only in retina (>95%)
  - Vector absent from other tissue or where present was below the lower level of quantification

Large number of tissues and fluids were sampled (at days 2, 3, 5, 29, 92, 186) including: urine, CSF, ovary, testis, liver, heart, lung, spleen, kidney cortex, kidney medulla, right lacrimal gland, manidbular lymph node, right lower/upper eyelids, ventral rectus, optic chiasm, right optic nerve, white blood cells, superior oblique, nictitating membrane

Ocular Regulatory Summary

- IND approval for RetinoStat® secured Nov 2010, first US approval for direct administration of a lentiviral vector
- StarGen™ IND approval Mar 2011 and CTA approval Aug 2011
- UshStat® IND approval Oct 2011

Product	RetinoStat®	StarGen™	UshStat®	EncorStat®
Proof of concept	✓	✓	✓	✓
Orphan Drug Status (EU/FDA)	n/a	✓	✓	n/a
Pre-IND	✓	✓	✓	✓
Manufacture	✓	✓	✓	Planned
IND/CTA	✓(IND)	✓ (IND/CTA)	✓ (IND)	Planned
Phase I/II initiation	✓	✓	H2 2011	Planned
First results	H1 2012	H2 2012	2013	Planned

Overview - Clinical Development of Lentiviral vector ATMPs

- Ensure technology ‘fits’ with unmet clinical need
- Proof of concept studies
- Identify and engage with appropriate clinical centre(s)
- Regulatory
  - Opened dialogue with CBER and ITF/CAT on the development strategies for the lentiviral products
  - EU and US regulators receptive to data generated from common platform (endorsed truncated toxicology studies)
  - ProSavin® approved for clinical trials in France and UK
  - RetinoStat® and StarGen™ approved for clinical trial in US
  - StarGen™ approved for clinical trial in France
  - UshStat® approved for clinical trial in US

Future Challenges for Lentiviral Vector Development

- Process Scale-up (ready for Phase III and market supply)
  - Comparability program (across manufacturing sites and processes)
- Agreeing an appropriate ‘minimal’ product specification to support product launch
- Specialist centres, training etc.

Acknowledgements

- Oxford BioMedica stakeholders
  - Employees
  - Shareholders
  - Patients & clinical centres
  - Advisors and consultants
- Sanofi
- Novasep-Henogen
- BioReliance, CRL, Covance
- Collaborators and charity groups