Forward looking statements

This presentation contains forward-looking statements that involve substantial risks and uncertainties. All statements, other than statements of historical facts, contained in this presentation, including statements regarding our strategy, future operations, future pre-clinical and clinical trial plans, future financial position, future revenues, projected costs, prospects, plans and objectives of management, are forward-looking statements. The words “aim,” “anticipate,” “believe,” “estimate,” “expect,” “intend,” “may,” “plan,” “predict,” “project,” “target,” “potential,” “will,” “would,” “could,” “should,” “continue,” and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words.

Forward-looking statements represent our management’s beliefs and assumptions only as of the date of this presentation. We may not actually achieve the plans, intentions or expectations disclosed in our forward-looking statements, and you should not place undue reliance on our forward-looking statements. Actual results or events could differ materially from the plans, intentions and expectations disclosed in the forward-looking statements we make. The forward-looking statements contained in this presentation reflect our current views with respect to future events, and we assume no obligation to update any forward-looking statements except as required by applicable law. These forward-looking statements are subject to a number of risks, uncertainties and assumptions, including those that may be described in greater detail in the Registration Statement on Form F-1 (including the prospectus) that we have filed with the U.S. Securities and Exchange Commission. We have included important factors in the cautionary statements included in that prospectus, particularly in the Risk Factors section, that we believe could cause actual results or events to differ materially from the forward-looking statements that we make.
Research and development pipeline

- **QRX-911**: Undisclosed target
- **QRX-704**: Huntington’s disease
- **QRX-504**: Fuchs (FECD)
- **QRX-021**: Undisclosed CF target
- **QRX-812**: Undisclosed target
- **QRX-604**: Friedreich’s ataxia
- **QRX-313**: Epidermolysis bullosa
- **QRX-411**: Usher syndrome
- **QR-110**: LCA
  - >2,000 patients
- **QR-010**: Cystic fibrosis
  - >49,000 patients
QR-010 for cystic fibrosis
RNA repair of cystic fibrosis ΔF508
ssRNA oligonucleotides to improve the lives of patients with severe genetic disease

• All molecules that you will hear about today are ssRNA antisense molecules
• They do not all work via the same mechanism
• All hold the promise of restoring protein function
• ssRNA molecules are chemically modified – no vectors or envelopes
• Manufacturing is easy; cost of goods is low
• ProQR has exclusive IP
QR-010: ProQR’s lead molecule, now in clinical development for CF

- QR-010 is a 33 mer chemically modified ss antisense RNA oligonucleotide
- Demonstrated to restore CFTR function in 2 *in vitro* models and 2 *in vivo* preclinical models
- Approach is unique
- Promise of gene therapy without the barriers
- Inhaled delivery with demonstrated uptake to the airways of the lung and delivery to extrapulmonary organs affected by CF
QR-010 for cystic fibrosis

Focus on CFTR function

In wild-type cells CFTR protein reaches the membrane
QR-010 for cystic fibrosis

Focus on CFTR function

In Cystic Fibrosis cells CFTR protein does not reach the membrane

AUC AUCUUU GGU GUU
QR-010 for cystic fibrosis
Focus on CFTR function

CFTR Protein
Chloride ions

RNA
DNA

PNAS
Reversal of cystic fibrosis phenotype in a cultured Δ508 cystic fibrosis transmembrane conductance regulator cell line by oligonucleotide insertion
Paul C. Danefart*, Malay K. Raychowdhury*, David K. Tabalsadi, and Horacio F. Centella

QR-010
ΔF508
AUC AUC UUU GGU GUU
QR-010 for cystic fibrosis
Focus on CFTR function
QR-010: Demonstrated increase in CFTR activity in two *in vitro* assays

**MQAE Assay [CF PAC1 cells]**

**Ussing Chamber assay [primary HBE]**
QR-010 increases CFTR activity in two *in vivo* assays

**ΔF508 mouse model**

- Exact same mutation as in humans
- QR-010 used in mice and humans

**Two independent functional assays**

- Nasal potential difference (NPD) – diagnostic test for CF in humans
- Saliva secretion assay – specific mouse study that is a surrogate for the human sweat chloride test, another diagnostic test for CF in humans

**QR-010 dependent restoration of CFTR protein function**
Background: NPD tracing interpretation

Mouse

Human


Background: Saliva Secretion Assay

• Similar to sweat chloride test in humans
• Saliva glands of female mice are highly dependent on CFTR to produce saliva
QR-010 increases CFTR activity as demonstrated by mouse NPD

Wild-type mouse

ΔF508-CFTR mouse
Untreated
QR-010 increases CFTR activity in the saliva secretion assay
QR-010: aerosol gets through mucus and remains stable

Additional studies:
- Stable in the presence of proteases
- Stable in the presence of CF standard of care inhaled medications
- Aerosol is 3-5 micron: optimized for small and medium airways
QR-010: Clinical Trials

Phase 1b Safety and Tolerability study

- QR-010 delivered via inhalation
- 64 homozygous ΔF508 patients (> 18 yrs)
- First development study

Nasal potential difference proof-of-concept study

- QR-010 delivered topically to nasal passages
- 16 patients total, 8 homozygous ΔF508 patients and 8 compound heterozygous patients (>18 yrs)
- Proof-of-concept study
QR-010: PQ-010-001
Phase 1b Safety and Tolerability

- 64 homozygous ΔF508 CF patients (>18yrs)
- Inhalation through Pari eFlow nebulizer
- Participating sites: 20 sites in EU (CTN) and US (TDN)

Endpoints:
- Safety, tolerability and pharmacokinetics
- Exploratory efficacy (FEV1, CFQ-R, weight gain, sweat chloride)
QR-010: PQ-010-002
Proof-of-Concept Study

- Proof of Concept Nasal Potential Difference (NPD) study in ΔF508 CF patients >18yr
- 8 homozygous and 8 compound heterozygous patients in adaptive design
- Open-label case-controlled study
- Multiple dose design: 12 doses (3 per week x 4 weeks)

- Local dosing in the nose
- Up to 5 participating sites in EU (CTN) and US (TDN) all experienced NPD reference sites
- Endpoints:
  - NPD
  - Sweat chloride
QR-010: ProQR’s lead molecule, now in clinical development for CF

- On-going preclinical work continues to demonstrate consistent increase in CFTR function
- Two clinical trials actively enrolling
  - Safety and tolerability
  - NPD proof-of-concept
- No similar approach to correct CFTR function
- Strong IP
Can we do better?

ROBERT J. BEALL, PH.D.
Former President and CEO
Cystic Fibrosis Foundation
Our Goal: A Lifelong Cure For All CF Patients

Gene (DNA) → RNA → Protein → Symptoms

Permanent Repair
Gene editing
Gene delivery
Stem cell biology

Periodic Therapy
Transcription
Translation (PTCs)
RNA replacement**
RNA editing

Daily Therapy
CFTR modulation
Potentiators
Correctors

Continuous Therapy
Infection
Mucus
Inflammation
Nutrition….
1. Abnormal Potential Difference in CF Airways
Raised Transepithelial Potential Difference (PD) and Amiloride Inhibition of PD in CF vs Normal Subjects: Evidence for an Intrinsic Defect in CF Epithelial Ion Transport

Knowles, Gatzy, Boucher NEJM 1981
Development of a Working Hypothesis
1. Abnormal Potential Difference in CF Airways

2. Discovery of CF Gene in 1989
Discovery of CF Gene 1989
- Acts as a Chloride Channel
- Controls Salt and Water Balance in the Airways
1. Abnormal Potential Difference in CF Airways

2. Discovery of CF Gene in 1989

3. Small Molecules (Kalydeco) Can Partially Correct the CFTR Defect in Cystic Fibrosis Patients
2012 – FDA Approves Ivacaftor
Therapeutics Development Program

**Therapeutics Development Awards**

**Discovery**
- Basic Research
  - CFTR Corrector Consortium
  - CFTR Structural Consortium
  - Mucociliary Clearance Consortium
- High-throughput Screening
  * Abbvie
  * CFFT
  * Flatley
  * Genzyme
  * Novartis
  * Parion
  * Pfizer
  * Proteostasis
  * Reata
  * Vertex

**Development**
- IND
- Preclinical Safety Testing
- TDN
- Clinical Dosage and Efficacy
- FDA Approval

**Distribution**
- Available to CF Patients

**Our Biotech Collaborators**
- Alcresta
- AlgiPharma
- Anthera
- Bayer
- Cellexsys
- CORBUS
- Bayer
- Novoteris
- Shire
- CFFT
- Flatley
- Genzyme
- Novartis
- Parion
- Pfizer
- Proteostasis
- Reata
- Vertex
- CURx
- IONIS
- Nivalis
- NovoPro
- ORPro
- PARION
- POLYPHOR
- ProQR
- Savara
- Southern Research
- SPYROX BIOSCIENCES

Bring Existing Drugs for CF Indication
Ivacaftor Improved FEV: Most Important Proof of Concept in CF History

Ivacaftor vs Placebo
Everyone on Ivacaftor

McKone, Borowitz….Davies et al, NACFC 2013. Poster 207
Time-to-First Pulmonary Exacerbation

Modified Fuchs’ criteria

Week 24
Hazard Ratio 0.40
P = 0.0016

Week 48
Hazard Ratio 0.46
P = 0.0012

Hazard Ratio: 0.45 (0.28, 0.73) P=0.0012

Study day

Proportion of event-free subjects

Placebo
Ivacaftor

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
0 28 56 84 112 140 168 196 224 252 280 308 336 364

Effect of Ivacaftor on Small Bowel pH

Rowe et al., Am J Resp Crit Care Med, 2014

Drucey Borowitz, Daniel Gelfond, Sub-study PIs
Lumacaftor/Ivacaftor Improved FEV\(_1\)

**Absolute Change from Baseline in Percent Predicted FEV\(_1\)**

- **Placebo**
- **LUM 600 mg qd / IVA 250 mg q12h**
- **LUM 400 mg q12h / IVA 250 mg q12h**

* * P<0.025

Ramsey, Boyle, Elborn...Wainwright et al. Poster #250 NACFC 2014
Symposium 10.3, Wainwright, Friday 11:30 AM
2nd Generation Modulators Restore CFTR Activity

![Graph showing CFTR Activity (in vitro) for different treatments and conditions.]

- **G551D**
  - Untreated: Low activity
  - Kalydeco: Moderate activity
  - VX-809: High activity
  - VX-809 + Kalydeco: Normal activity
  - VX-809 + Kalydeco + 2cd Corrector: Goal activity

- **F508del/F508del**
  - Untreated: Lower activity compared to G551D
  - Kalydeco: Similar activity to G551D
  - VX-809: Higher activity than G551D
  - VX-809 + Kalydeco: Normal activity
  - VX-809 + Kalydeco + 2cd Corrector: Goal activity
Can we do better?
Corrector Therapy
• Two 2\textsuperscript{nd} Generation Correctors now in clinical studies: VX-152 and VX-440
• Each additive or synergistic with first generation correctors in vitro (i.e. VX-661 or VX-809)
• CF trial design in development

Vertex – ENaC blocker Program (with Parion)
• P-1037 / VX-371 Currently enrolling Phase 2a as monotherapy (N=120, 2 week study)
• Combination with corrector/potentiator therapy will follow
Comparison ASL Heights: Iva/Luma +/- P-1037

AUCs of ASL Images

- Vehicle
- IVA/LUMA
- 1037/IVA/LUMA
- 1037/HS/IVA/LUMA

Increased Hydration (Vehicle Corrected)

- **p<0.005, ****p<0.0005 relative to IVA/LUMA

Source: Parion 2015 NACFC Poster
Innovating for the Future

February 2016
Explosion of CFTR Modulator Trials

1. **PTC** – Ataluren – Phase 3 – Read through stop codons
2. **ProQR** – QR-010 - Phase I - RNA repair for F508del
3. **Bayer** – Riociguat – Phase 2 – Corrector
4. **Novartis** – Phase 2 - Potentiator
5. **Nivalis** – N9115 – Phase 2 - Corrector
6. **John Flatley Lab** – Phase 2 – Corrector

“Second Generation” – mid to late 2016

1. **Vertex** – VX-152 and VX-440 - Phase 2 - Correctors
2. **Galapagos/AbbVie** – GLPG2665 - Phase 1
A Lifelong Cure For All CF Patients

Gene (DNA) → RNA → Protein → Symptoms

**Permanent Repair**
- Gene editing
- Gene delivery
- Stem cell biology

**Periodic Therapy**
- Transcription
- Translation (PTCs)
- RNA replacement**
- RNA editing

**Daily Therapy**
- CFTR modulation
- Potentiators
- Correctors

**Continuous Therapy**
- Infection
- Mucus
- Inflammation
- Nutrition…. 
A Lifelong Cure For All CF Patients

QR-010 normalizes CFTR activity in CF mice
Building Science for a One Time Cure

Recruit world class investigators into CF research

Workshops
CFTR Expression (Oct 2014): increase level of RNA and protein
Gene Editing (Dec 2014): repair CFTR DNA mutations
Gene Deliver (Dec 2014): delivery DNA and editing enzymes
Stem Cells (Mar 2015): identify and “correct” target cells

Successful RFAs

<table>
<thead>
<tr>
<th>Applications received</th>
<th>Funded</th>
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<tbody>
<tr>
<td>Gene expression</td>
<td>19</td>
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<tr>
<td>Gene Editing</td>
<td>22</td>
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<tr>
<td>Gene Delivery</td>
<td>23</td>
</tr>
<tr>
<td>Stem cell biology</td>
<td>29</td>
</tr>
</tbody>
</table>

Expect to fund ~40-50 laboratories, 2 companies
~ $7M investment in 2015, increasing 2016-18

Additional discussions on gene delivery: viral & nanoparticle technologies.
Drug Discovery / Preclinical Pipeline

2nd Gen CFTR Modulators: (9 projects)
- Vertex, Pfizer, Genzyme, PTI, Reata, Parion

Nonsense (PTC) mutations: (4 projects, 3 ongoing, 1 in development)
- Southern Research Institute/UAB collaboration
- PTC completing Ataluren phase III trial
- Negotiating an additional large Pharma screen
- Novel oligonucleotide approaches

RNA directed therapy: (3 projects, 2 ongoing, 1 in development)
- Shire: direct RNA delivery, ProQR: RNA repair
- Splicing, Expression

Gene editing and delivery: (3 projects; 1 funded, 2 in development)
- CRISPR/Cas9
- Zn Finger nuclease
- novel delivery technologies
Thank you!
Innovation unit

In-house discovery engine
Research and development pipeline

- **QRX-911**: Undisclosed target
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- **QRX-313**: Epidermolysis bullosa
- **QRX-411**: Usher syndrome

**Innovation**

**QR-110**
- LCA
- >2,000 patients

**QR-010**
- Cystic fibrosis
- >49,000 patients

**Pre-clin development**  **Phase 1 & clinical PoC**  **Pivotal studies**
Research and development pipeline

**QRX-911**
Undisclosed target

**QRX-704**
Huntington’s disease

**QRX-504**
Fuchs (FECD)

**QRX-021**
Undisclosed CF target

**QRX-812**
Undisclosed target

**QRX-313**
Epidermolysis bullosa

**QRX-411**
Usher syndrome

**QRX-604**
Friedreich’s ataxia

**QRX-203**
Alzheimer’s disease

**QR-110**
LCA
>2,000 patients

**QR-010**
cystic fibrosis
>49,000 patients

Innovation

Pre-clin development

Phase 1 & clinical PoC

Pivotal studies
Innovation platform
targeting genetic disorders at the RNA
RNA space

- Breakthrough in antisense
- First therapeutics emerge
- RNA editing

- 1970
- 1980
- 1990
- 2000
- 2010

Antisense & knockdown
Exon skipping
Restoring functionality
Approach

Well understood causality
Genetic defect leading to disease manifestation well understood

Patient specific
High unmet needs

Intellectual property
Aggressive patenting strategy
Broad IP portfolio

Feasible delivery
Feasible delivery route to target organ

Technology based
RNA modulation to restore wild-type functionality
Promising programs in 5 therapeutic areas

CNS
- Huntington’s disease
- Alzheimer’s disease

Ophthalmology
- LCA10
- Usher syndrome
- Fuchs (FECD)

Respiratory
- Cystic fibrosis

Dermatology
- Dystrophic EB

Neuromuscular
- Friedreich’s ataxia
Selecting the best programs

Well understood causality
Unmet need
IP position
Strong proof-of-concept
Feasible delivery
QR-110

Splice correction for p.Cys998X causing Leber’s congenital amaurosis (LCA10)
Leber’s congenital amaurosis disease background

• LCA is a broad set of diseases
  • 18 types
  • Caused by many mutations

• Different phenotypes
  • LCA2: RPE65
  • LCA10: CEP290 (a ciliopathy)

• **LCA10** - p.Cys998X: ~2,000 LCA patients in the Western world

• No treatments available
Most severe form of early childhood blindness

Very early severe vision loss with onset in the first months of life

Symptoms include sensory nystagmus (involuntary eye movement), amaurotic pupils, oculo-digital signs, and absent electrical signals on electroretinogram (ERG).

Is associated with a cone-sparing macular presentation
LCA10 Clinical Phenotype

• Most severe form of early childhood blindness

• Very early severe vision loss with onset in the first months of life

• Symptoms include sensory nystagmus (involuntary eye movement), amaurotic pupils, oculo-digital signs, and absent electrical signals on electroretinogram (ERG).

• Is associated with a cone-sparing macular presentation
In wild-type cells CEP290 maintains cilium structure and enables normal protein transport.
In p.Cys998X-LCA10 cells protein transport is hampered and the outer segment degenerates.

Exon 26  Exon 27

mRNA

Exon 26  Exon 27

pre-mRNA
QR-110 for LCA10

RNA
DNA
Exon 26
Exon 27
mRNA
pre-mRNA

QR-110

Antisense Oligonucleotide (AON)-based Therapy for Leber Congenital Amaurosis Caused by a Frequent Mutation in CEP290

Rob WJ Collin*, Anneke I den Hollander**, Saskia D van der Velde-Visser*, Jeannette Bennicelli†, Jean Bennett‡ and Frans PM Cremers§
Restoration of mRNA and functionality in patient fibroblasts

mRNA profile restoration (patient fibroblasts)

Functional restoration (patient fibroblasts)
Restoration of mRNA in eye-cups

Eye cup model of iPSC

Emerging eye cup with retinal pigment epithelium in red

Red = rhopospin pigment only in photoreceptors which sense light.

Zhong et al., 2014
Restoration of mRNA in eye-cups

- Eye cup model of iPSC
- Emerging eye cup with retinal pigment epithelium in red
- Red = rhopospin pigment only in photoreceptors which sense light.

Control

Mutant
Wild-type

GAPDH

Zhong et al., 2014
Restoration of mRNA in eye-cups

Control | Treatment
---|---
Exon 26 | Exon 27
Mutant | Wild-type
Exon 26 | Exon 27
GAPDH

Zhong et al., 2014

Eye cup model of iPSC
Emerging eye cup with retinal pigment epithelium in red
Red = rhopospin pigment only in photoreceptors which sense light.
Intravitreal delivery

- Eye well validated target for oligo's
- Routine procedure (IVT)
- Infrequent dosing expected

- Long retinal half-lives
- A number of marketed therapeutics including intravitreal oligonucleotides

\[
T_{1/2} \text{ in NHP retina} = 2 \text{ months}
\]

Henry et al., 2001
IOVS 42 2646
Efficient delivery to retinal Outer Nuclear Layer

100ug 6FAM-QR-110 IVT 14d and 60d mouse

14d

60d

X40

X40

14d

60d

ONL

ONL

X63

X63

6FAM only 14d mouse

14d

RPE

OS/IS

ONL

INL

X40

14d

6FAM-QR-110 (green) or FAM only (green)

DAPI (blue)

100ug in mouse well tolerated for 60 days
QR-110 for Leber’s congenital amaurosis

Clinical program to start in 2016

Preliminary study outline:
- Phase 1b (no placebo/sham injection)
- 8+ patients with residual ONL (observable retinal structure)
- Repeated doses in one eye (intravitreal injection)

Primary endpoints
- Safety
- Tolerability

Secondary endpoints
- Electroretinogram (ERG)
- Full-field stimulus test (FST)
- OCT (retinal degradation area)
- Visual acuity
- Patient reported outcome
- Mobility testing
QRX-411
Splice correction for Usher’s syndrome
QRX-411 for Usher’s syndrome

Leading genetic cause of deafness & blindness

- Usher type II
- Retinitis pigmentosa
  - onset: childhood
  - (almost) complete blindness in the 3rd or 4th decade of life
- Congenital hearing impairment

Most common mutations in *USH2A* gene

- USH2A required for transport across the connecting cilium
- Lack of USH2A leads to slow degeneration of the photoreceptors
- AON treatment for PE40 mutation, potential to expand to other mutations.

High unmet need

- >15,000 USH2A patients in western world

Lu et al., 2010
QRX-411 for Usher’s syndrome

In wild-type cells Ush2a protein enables protein transport through the connecting cilium.
QRX-411 for Usher’s syndrome

In cells with the mutation Ush2a protein is not active hampering protein transport over the cilium.

- Outer segment
- Connecting cilium
- Inner segment

Exon 40 PE40 Exon 41 mRNA
Exon 40 PE40 Exon 41 pre-mRNA
QRX-411 for Usher’s syndrome

QRX-411 for Usher’s syndrome

Exon 40  PE40  Exon 41
pre-mRNA

mRNA
Strong proof of concept
RNA restoration after AON treatment

Patient cells (PE40 heterozygote) | Healthy control
---|---
untreated | untreated

Radboud University

Exon 40 PE40 Exon 41
Exon 40 Exon 41
Strong proof of concept

RNA restoration after AON treatment

Patient cells (PE40 heterozygote)  |  Healthy control
---|---
QRX-411(T) untreated | untreated

Radboud University

Exon 40  PE40  Exon 41
Exon 40  Exon 41
QRX-411 status and overview

✓ Single stranded oligo nucleotide resulting in WT mRNA
✓ Delivery through intravitreal administration
✓ Two lead compounds selected
QRX-504

RNA modulation for Fuchs endothelial corneal dystrophy (FECD)
QRX-504 for Fuchs Endothelial Corneal Dystrophy

Progressive degeneration of the cornea
- Reduced or loss of vision due to loss of function of corneal endothelial cells or loss of corneal endothelial cells
- ~5% of middle-aged Caucasians have guttae, a hallmark of FECD. A subset of that group develops a severe phenotype
- Disease is also associated with painful corneal blisters

FECD3 caused by mutations in TCF4 gene
- 75% of population with guttae have TCF4 expansions
- Formation of nuclear RNA foci that sequester splicing factors
- Foci lead to loss of function of endothelium cells

Unmet Need
- Eye disorder, leading to blindness, 15,000 corneal transplants performed annually in the US due to Fuchs
- High unmet medical need
In wild-type cells, MBNL1 protein regulates splicing of many RNAs.
Mutated TCF4 RNA and MBNL1 form aggregates (foci), and splicing is disrupted.
QRX-504 for FECD3

mutant TCF4

free MBNL1

pre-mRNAs

correctly spliced mRNAs
Fuchs patients with mutations in TCF4 have RNA foci
FECD3 is an RNA toxicity disease
QRX-504 reduces RNA foci in FECD CEC

FECD CEC

Untreated (control oligo)

QRX-504 200 nM

Cell Profiler outlines

*P<0.0001

200nM

Number of foci per nucleus

0
1
2
3
4 or more

0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Untreated

Treated

ProQR Therapeutics - R&D day

March 14, 2016
Oligo delivery to corneal endothelium
IVT administered QRX-504 shows robust uptake

Cy3-labelled-QRX-504
QRX-504 status and overview

✓ Single stranded oligo nucleotide resulting in reduction of RNA foci in FECD CEC cells

✓ Delivery to corneal endothelium through intravitreal administration

✓ Lead compound selected
Macula

1.5mm
Macula

1.5mm

Macula

Fovea

1.5mm
The Retina
Graft Installation

Slit cut in eye, vitreous body flushed and
In 2010 WHO estimated that 265 million people worldwide were visually impaired.
In the UK today, 2 million people suffer from sight loss.
Over 250 genes have been mapped to retinal disease (and we have discovered more than any other lab).
Major Causes of Blindnesss

- Macular Degeneration
- Posterior Pole
- Other incl. Corneal
- Cerebrovascular
- Optic Atrophy
- Hereditary Retinal
- Diabetes
- Glaucoma

Causes of Blindness (England and Wales 1999-2000)
Bunce Wormald BMC Public Health 2006
Advances in Innovation

Visual assessment and imaging

New Technologies & Devices

Genotyping, Phenotyping and Informatics

Causes of Blindness (England and Wales 1999-2000)
Bunce Wormald BMC Public Health 2006
Imaging the Eye

Colour Photography

Fluorescence

OCT
The Cost of Vision Problems

$139 billion in direct and indirect costs

The 2013 Burden Estimate (in $ billions)

- Productivity Loss - $48.4
- Long Term Care - $20.2
- Other Indirect - $3.5
- Other Direct - $1.7
- Medical - $65
Phase 0: De-risking failure

Fibroblast or Blood cells

Somatic Cells

Reprogram with Embryonic Transcription Factors

Patient specific iPS cells

In vitro Disease Model

Patient

Differentiate

Patient Specific RPE Cells

the london project
to cure blindness
Phase 0: De-risking failure

Fibroblast or Blood cells

Somatic Cells

Reprogram with Embryonic Transcription Factors

Patient specific iPS cells

In vitro Disease Model

Patient

Differentiate

Patient Specific RPE Cells

Therapeutic Targets
Phase 0: De-risking failure

Fibroblast or Blood cells

Somatic Cells

Reprogram with Embryonic Transcription Factors

Patient specific iPSC cells

Differentiate

Patient Specific RPE Cells

Drug Discovery
Phase 0: De-risking failure

- **Fibroblast or Blood cells**
- **Somatic Cells**
  - Reprogram with Embryonic Transcription Factors
- **Patient specific iPSC cells**
- **In vitro Disease Model**
- **Patient**
- **Therapeutic Targets**
- **Drug Discovery**

**Flowchart**

1. **Fibroblast or Blood cells** to **Somatic Cells**
2. **Somatic Cells** → **Patient specific iPSC cells**
3. **Repogram with Embryonic Transcription Factors**
4. **Patient specific iPSC cells** to **In vitro Disease Model**
5. **In vitro Disease Model** to **Patient**
6. **Patient** to **Therapeutic Targets**
7. **Therapeutic Targets** to **Drug Discovery**
Phase 0: De-risking failure

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<th>Gene</th>
<th>Disease</th>
<th>iPSC Lines</th>
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<td>Best 1</td>
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<tr>
<td>CFH</td>
<td>AMD</td>
<td>✔</td>
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<td>Retinitis Pigmentosa</td>
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<td>Retinal Cone Dystrophy</td>
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<td>Choroideremia</td>
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<tr>
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<tr>
<td>Lrat</td>
<td>Retinal Cone Dystrophy</td>
<td></td>
</tr>
<tr>
<td>Rdh5</td>
<td>Fundus Albipuntatus</td>
<td></td>
</tr>
<tr>
<td>Timp3</td>
<td>Sorsby Fundus Dystrophy</td>
<td></td>
</tr>
<tr>
<td>RPE65</td>
<td>Retinitis Pigmentosa</td>
<td></td>
</tr>
</tbody>
</table>

Sample label | Y402H | I62V | E936D | L9H | intron 10 | A69S | R130C | R176C | Isotype
---|-------|------|-------|-----|-----------|------|-------|-------|--------
HF 081309  | YY    | VI   | EE    | LL  | TG        | AA   | TT    | CC    | e3e3
HF 082809 (20wk) | HH  | VI   | EE    | LL  | TG        | AA   | TT    | CC    | e3e3
HF 030411  | YY    | II   | EE    | LL  | TG        | AS   | TT    | CC    | e3e3
HF 031611-1 | YY  | II   | EE    | LL  | TG        | AA   | TC    | CC    | e3e4
HF 032411  | YY    | II   | EE    | LL  | GG        | AA   | TT    | CC    | e3e3
HF 091511  | HH    | VV   | EE    | LL  | GG        | AS   | TC    | CC    | e3e4
HF 110211  | HH    | VV   | EE    | LL  | GG        | AA   | TT    | CC    | e3e3
HF 110912  | YY    | VI   | DE    | LL  | GG        | AA   | TC    | CC    | e3e4
hF 120111  | HH    | VV   | ?     | TT  | GG        | GT   | ?     | ?     | ?

Table 1. Current bank of induced pluripotent stem cell lines expressing relevant genes associated with AMD: Y402H – CFH, L9H – CFB, A69S – ARMS2/HTRA1 and R130C/176C – ApoE.
Phase 0: De-risking failure

Induced pluripotent stem cells (iPSCs) → Generate embryoid bodies in V-bottomed 96-well plates → Dissect out transparent neuroepithelium → Long term suspension culture

Control

Patient

Week 21

the london project
to cure blindness
Clinical Outcome

EDTRS VS Snellen
George Freeman MP – Minister of Life Sciences
December 2015 MEH/IoO Visit

Faster Cures
Early stage remuneration
Questions from the audience
Lunch Break

Presentations start again at 12:50 PM EST
Epidermolysis Bullosa

The Worst Disease You've Never Heard Of
In 2014, there was an updated consensus on the classification of EB subtypes to further add clarification to the major types and subtypes. This approach took into account many factors including the level of skin cleavage, the phenotypic characteristics, the mode of inheritance, the targeted protein, and the gene involved with mutation present, to name a few.

<table>
<thead>
<tr>
<th>EB type</th>
<th>Mutated protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBS suprabasal</td>
<td>Transglutaminase 5</td>
</tr>
<tr>
<td>EBS basal</td>
<td>Plakoglobin</td>
</tr>
<tr>
<td></td>
<td>Plakophilin 1</td>
</tr>
<tr>
<td></td>
<td>Desmoplakin</td>
</tr>
<tr>
<td>JEB</td>
<td>Keratin 5/14, plectin, BP230, exophillin 5, kindlin-1</td>
</tr>
<tr>
<td>KS</td>
<td>Integrin α6β4, integrin α3, collagen XVII, laminin 332</td>
</tr>
<tr>
<td>DEB</td>
<td>Collagen VII</td>
</tr>
</tbody>
</table>

[Image: EB Definition – Onion Skin Approach]

J Am Acad Dermatol [http://dx.doi.org/10.1016/j.jaad.2014.01.903]
EB Definition – **Simplex**

If only it was easy enough to say there were two subtypes, suprabasal and basal.

---

**Suprabasal**

Suprabasal EBS has 6 subtypes
There are 7 structural proteins potentially affected
Example:
EBS – Suprabasal - Acral peeling skin syndrome
Transglutaminase 5
Known mutations on TGM5 gene
Missense, deletion, small deletion/insertion

**Basal**

Basal EBS has 11 subtypes
There are 6 structural proteins potentially affected
Example:
EBS – Basal - Generalized Severe
Keratin 5 or Keratin 14
Known Mutations on K5/K14 genes
Missense, deletion, splice, nonsense, small deletion/insertion, insertion
**EB Definition – Dystrophic**

2 major subtypes, 14 subtypes – 1 affected protein – Collagen VII

**Dominant**

Dominant has **6 subtypes**
Known mutations on COL7A1 gene
Missense, splice and deletion

**Recessive**

Recessive has **8 subtypes**
Known mutations on COL7A1 gene
Missense, nonsense, deletion, splice, insertion, small deletion/insertion
Epidermolysis Bullosa - Definition

EB has been called a skin disease (because of it’s main symptom) and it’s been called a group of disorders (because there are 4 major types and a large number of subtypes). Yet, neither properly defines the disease.

What is Epidermolysis Bullosa?

Epidermolysis Bullosa (EB) is a rare, genetic connective tissue disorder. There are many genetic and symptomatic variations of EB, but all share the prominent symptom of extremely fragile skin that blisters and tears from minor friction or trauma. Internal organs and bodily systems can also be seriously affected by the disease.

EB is always painful, is often pervasive and debilitating, and is in some cases lethal before the age of 30. The list of secondary complications can be long and may require multiple interventions from a range of medical specialists.

EB affects 1 out of every 20,000 live births and affects both genders and every racial and ethnic background equally. Those born with it are often called ‘Butterfly Children’ because as the analogy goes, their skin is as fragile as the wings of a butterfly.

There is no treatment or cure. Daily wound care, pain management and protective bandaging are the only options available.

By the numbers…..

1 out of every 227 people has a defective gene that causes EB
There are about 25,000 people in the US with EB
There are about 30,000 in Europe and 500,000 worldwide
About 200 children are born each year in the US with a form of EB
Complications – Frequency in RDEB

The following samples of frequencies of secondary complications show profound individual impairment and demonstrate the cavernous unmet need.

<table>
<thead>
<tr>
<th>Frequency from total RDEB population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
</tr>
<tr>
<td>54.4%</td>
</tr>
</tbody>
</table>

**Frequency of pseudosyndactyly**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 yrs</td>
<td>25.64%</td>
</tr>
<tr>
<td>2 – 6 yrs</td>
<td>59.26%</td>
</tr>
<tr>
<td>6 – 10 yrs</td>
<td>77.42%</td>
</tr>
<tr>
<td>10 – 18 yrs</td>
<td>74.19%</td>
</tr>
<tr>
<td>&gt; 18 yrs</td>
<td>73.47%</td>
</tr>
</tbody>
</table>

**Frequency of contractures**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 yrs</td>
<td>18.42%</td>
</tr>
<tr>
<td>2 – 6 yrs</td>
<td>33.33%</td>
</tr>
<tr>
<td>6 – 10 yrs</td>
<td>64.52%</td>
</tr>
<tr>
<td>10 – 18 yrs</td>
<td>70.97%</td>
</tr>
<tr>
<td>&gt; 18 yrs</td>
<td>77.55%</td>
</tr>
</tbody>
</table>

**Frequency of cutaneous scarring**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 yrs</td>
<td>84.62%</td>
</tr>
<tr>
<td>2 – 6 yrs</td>
<td>100%</td>
</tr>
<tr>
<td>6 – 10 yrs</td>
<td>96.77%</td>
</tr>
<tr>
<td>10 – 18 yrs</td>
<td>100%</td>
</tr>
<tr>
<td>&gt; 18 yrs</td>
<td>100%</td>
</tr>
</tbody>
</table>

25.5% of those with RDEB have Cardiovascular issues
Complications – Frequency in RDEB-GS

When narrowing the scope to RDEB-GS, the frequency rates become horrific. Compare these frequency rates to the general population and the burden of disease is staggering.

### Frequency of musculoskeletal, hematologic and constitutional complaints

<table>
<thead>
<tr>
<th>Anemia</th>
<th>Growth Retardation</th>
<th>Pseudosyndactyly</th>
<th>Other Contractures</th>
</tr>
</thead>
<tbody>
<tr>
<td>79.8%</td>
<td>78.8%</td>
<td>86.3%</td>
<td>74.3%</td>
</tr>
</tbody>
</table>

### Frequency of GI complaints

<table>
<thead>
<tr>
<th>Dysphagia</th>
<th>Esophageal Web, Stricture or Stenosis</th>
<th>Constipation</th>
</tr>
</thead>
<tbody>
<tr>
<td>83.2%</td>
<td>59.3%</td>
<td>60.6%</td>
</tr>
</tbody>
</table>

### Frequency of Ocular complaints

<table>
<thead>
<tr>
<th>Corneal Scarring</th>
<th>Corneal Abrasions or Blisters</th>
<th>Impaired Vision</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.4%</td>
<td>56.4%</td>
<td>19.6%</td>
</tr>
</tbody>
</table>

### Frequency of Oral complaints

<table>
<thead>
<tr>
<th>Microstamia</th>
<th>Ankyloglossia</th>
<th>Gingival Erosions &amp; Blisters</th>
<th>Abnormal Enamel or Dysplastic Teeth</th>
<th>Excessive Caries</th>
<th>Premature Tooth Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>71%</td>
<td>80.8%</td>
<td>89.9%</td>
<td>31.3%</td>
<td>54.7%</td>
<td>47.9%</td>
</tr>
</tbody>
</table>

### Frequency of select additional physical findings in a longitudinal follow-up of randomized sample of RDEB-GS

<table>
<thead>
<tr>
<th>Scarring</th>
<th>Milia</th>
<th>Nail Dystrophy</th>
<th>Alopecia</th>
<th>Hypotrichosis</th>
<th>Pseudosyndactyly</th>
<th>Other Contractures</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.3%</td>
<td>78.4%</td>
<td>97.3%</td>
<td>35.1%</td>
<td>21.9%</td>
<td>93.2%</td>
<td>85.1%</td>
</tr>
</tbody>
</table>
RDEB-GS – Cancer & Life Expectancy

Squamous Cell Carcinoma is curable in the general population, not in RDEB. People who suffer from RDEB are 26.6 times more likely to suffer at least one incidence of SCC.

CANCER

21.67%  Chance of developing SCC, if patient lives to 25 years old

39.57%  Chance of developing SCC, if patient lives to 30 years old

53.00%  Chance of developing SCC, if patient lives to 35 years old

Life Expectancy

10%  Lost their battle before they were 10 years old

40%  Succumbed by the age of 20

72%  Passed away by the age of 30
Given the incidence rate of 1 in 20,000 live births and prevalence percentages, debra of America estimates the below numbers of patients.

<table>
<thead>
<tr>
<th></th>
<th>Incidence (# born per year)</th>
<th>Prevalence (# at any given time in population)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EB</strong></td>
<td>200</td>
<td>21,107</td>
</tr>
<tr>
<td><strong>Simplex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized</td>
<td>69</td>
<td>9,550</td>
</tr>
<tr>
<td>All Others</td>
<td>40</td>
<td>4,440</td>
</tr>
<tr>
<td><strong>Junctional</strong></td>
<td>21</td>
<td>1,338</td>
</tr>
<tr>
<td>Severe Generalized</td>
<td>4</td>
<td>213</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
<td>1,125</td>
</tr>
<tr>
<td><strong>Dystrophic</strong></td>
<td>50</td>
<td>5,779</td>
</tr>
<tr>
<td>DDEB</td>
<td>29</td>
<td>3,011</td>
</tr>
<tr>
<td>RDEB</td>
<td>21</td>
<td>2,768</td>
</tr>
<tr>
<td>- Severe Generalized</td>
<td>4</td>
<td>1,277</td>
</tr>
<tr>
<td>- Other</td>
<td>17</td>
<td>1,490</td>
</tr>
</tbody>
</table>
As of today there is no cure or FDA approved treatment. Pain management, wound care, and preventative bandaging are the only options.

**There is HOPE**

Currently under investigation

- RNA Repair
- Gene Editing
- Gene Therapy
- Gene Transfer
- Grafting of Autologous Skin
- Protein Replacement
- Stem Cell Transplantation
- Topical Creams for Wound Healing
Burden of Illness – Financial Burden

EB, and particularly the more severe forms, are incredibly expensive. Wound care supplies, hospital visits, surgeries, medications all are factors.

**Wound Care Supplies**
The specialized wound care dressings can cost more than $15,000 per month. The average cost of these supplies are approximately $125,000 per annum.

**Hospital Visits**
A child may need to receive blood transfusions every eight weeks to treat the anemia, and may require quarterly esophageal dilations to swallow liquid.

- Assuming costs are:
  - Blood Transfusions - $8,000
  - Esophageal Dilations - $15,000

Annual cost = $108,000

**Drugs**
The list of daily medications is extensive. It is difficult to calculate the cost but a list of medicines for an RDEB child could be:

- Protonix, Methadone, Gabapentin, Carvedilol, Enalapril, Hydroxizine, Lexapro, Lorazepam, Ferrous Sulfate, Zinc, Vitamin D

1 Month Supply of Wound Care Supplies for an 8 year-old
Burden of Illness—Bandage Changes

It’s impossible to truly understand what a person with EB undergoes daily. Bath and bandage changes can last 3 or more hours, are incredibly painful, and are likened to parents torturing their child.

I know Mom doesn’t want to hurt me.
Thank You ProQR From All Of Us Living with EB
QRX-313
RNA modulation for dystrophic epidermolysis bullosa
Skin morphology

Stratum corneum
Epidermis
Dermis
Subcutaneous fat

Healthy skin
DEB skin

Location Collagen type VII
QRX-313 for DEB

In healthy skin collagen VII forms anchoring fibrils that link skin layers.

- Exon 72
- Exon 73
- Exon 74
QRX-313 for DEB

In DEB skin anchoring fibrils are absent or dysfunctional.

Keratinocytes

Interstitial collagen fibers

Blister

mRNA

Exon 72 Exon 73 Exon 74

Pre-mRNA

Exon 72 Exon 73 Exon 74

ProQR Therapeutics - R&D day

March 14, 2016
QRX-313 for DEB

Functional Collagen VII Protein

Keratinocytes

Anchoring fibrils

Interstitial collagen fibers

mRNA

QRX-313

Pre-mRNA

Exon 72
Exon 73
Exon 74

Exon 72
Exon 74

ProQR Therapeutics - R&D day
March 14, 2016
134
Restoration of anchoring fibrils after exon exclusion

Wild type

Epidermolysis Bullosa

Epidermolysis Bullosa + exon exclusion

Goto et al, 2006
Exon 73 exclusion with QRX-313
In vitro proof of concept at the RNA level
QRX-313 in formulation penetrates blister-like human skin equivalents

<table>
<thead>
<tr>
<th>PBS formulation</th>
<th>Gel formulation</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact skin</strong></td>
<td><strong>“Blister” edge</strong></td>
<td><strong>“Blister” bed</strong></td>
</tr>
<tr>
<td>epidermis</td>
<td>epidermis</td>
<td>epidermis</td>
</tr>
<tr>
<td>dermis</td>
<td>dermis</td>
<td>dermis</td>
</tr>
<tr>
<td>epidermis</td>
<td>epidermis</td>
<td>epidermis</td>
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<td>epidermis</td>
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<td>epidermis</td>
</tr>
<tr>
<td>dermis</td>
<td>dermis</td>
<td>dermis</td>
</tr>
</tbody>
</table>

QRX-313
Cell nuclei
QRX-313 on human skin equivalents induces Δ73 mRNA in the dermal fibroblasts

- PBS
- gel

Full length

Δ exon 73

Exon 72 Exon 73 Exon 74

Exon 72 Exon 74
QRX-313 status

✓ Single stranded oligo nucleotide resulting in removal of mutated exon
  • Well understood mechanism of action
  • Strong pre-clinical PoC
  • Lead compound selected

✓ Efficient delivery through topical administration

✓ Potential to expand to other subsets of patients
QRX-203
RNA modulation for Alzheimer’s disease
QRX-203 for Alzheimer’s Disease

Most prevalent form of dementia
- Progressive neurodegenerative disease
- Impairments of memory, learning ability, language and judgement

Amyloid related disorder
- Toxic amyloid-beta peptide causes plaque formation in brain
- Potential to treat other amyloid-beta related disorders

QRX-203

Alzheimers disease

Cerebral Amyloid Angiopathy
Katwijks’ disease

Geographic Atrophy in Dry
Age related Macular Degeneration
Preventing Aβ inclusion into mature APP

Enzyme inhibitors

Plaque removing antibodies

QRX-203
QRX-203 for Alzheimer’s disease
APP processing: Non-Amyloidogenic pathway

The “healthy” cleavage of APP is by α-secretase
QRX-203 for Alzheimer’s disease
APP processing: Amyloidogenic pathway

When APP is cleaved by β-secretase. Toxic Aβ is released and aggregates.
QRX-203 for Alzheimer’s disease

Modulates RNA and prevents A\(\beta\) formation

Protein

\[
\begin{align*}
\text{APP} & \rightarrow \alpha\text{-secretase} \\
\Delta \text{APP} & \rightarrow \\
\text{mRNA} & \rightarrow \\
\text{QRX-203} & \\
\text{Pre-mRNA} &
\end{align*}
\]
Strong proof of concept
Efficient skip on RNA level

- NT
- QRX-203

Exon 16 | Exon 17 | Exon 18

Full length

Δ exon 17

Exon 16 | Exon 18
Strong proof of concept
Removal of exon 17 results in protein without Aβ segment
Validated delivery methods for CNS
Exploring several routes of administration
Alzheimer’s Disease: Disease Pathogenesis, Diagnosis and Treatment Landscape

Thomas Wisniewski MD
Professor of Neurology, Psychiatry and Pathology
March 14th, 2016
Alzheimer’s disease is the 6th deadliest disease in the USA

- Only Cause of death among top 10 with no effective treatment(s)
- Affects ~13% of people >65 years old (~1 in 8)
- Affects ~40-50% of people >85 years old
- In 2015 direct costs of AD in the USA are ~$226 Billion
- Costs will rise to ~1.1 Trillion in 2050, if no treatments are developed

Alzheimer’s Disease - most common dementia

Worldwide

- Today: ~47 million
- 2050: ~131.5 million
Alzheimer’s disease is the only cause of death among the top 10 in the USA without an effective way to prevent, cure or significantly slow its progression!
Costs for Dementia Care in the USA versus Research Funding

- Estimates suggest that the monetary costs of care for dementia patients in the USA is significantly greater than all other major medical conditions including cancer and heart disease.*
- This contrasts with federal research spending: estimated 2015 Federal Research Spending in 2015:
  - Cancer: $5.4 Billion
  - HIV/AIDS: $3 Billion
  - Heart Disease: $2.0 Billion
  - Diabetes: $1 Billion
  - Alzheimer’s disease: $586 million

Neuropathology of Alzheimer’s Disease

- Neuritic Plaques
- Neurofibrillary Tangles
- Congophilic Angiopathy
- Synaptic Loss
Alzheimer’s Pathology

Amyloid plaque shown by Immunohistochemical labeling With anti-amyloid β antibody

Neurofibrillary Tangle shown By immunofluorescent labeling with Anti-phosphorylated tau antibody
Congophilic Angiopathy

H & E Stain

Aβ Immunoreactivity

Congo Red Staining under polarized light
Aβ immunostaining in Neuritic Plaques in Red

Abnormally phosphorylated tau (PHF1) Immunostaining in Neurofibrillary Tangles And Dystrophic Neurites in Black
Accumulation of toxic oligomeric Aβ species is driven by either over production or impaired clearance.
Alzheimer’s disease

**Familial**
Onset <60y
~1%

Inherited abnormalities of:
- presenilin 1 (PS1)
- presenilin 2 (PS2)
- amyloid precursor protein (APP)

**Sporadic**
Onset >65y
~99%

Risk factors increasing likelihood of Alzheimer’s

Environmental
- Age
- Head trauma
- High blood pressure
- High cholesterol
- Diabetes
- Stroke

Inherited
- Apo E isoform
- First degree relative
- ~20 GWAS identified
- Genetic risk factors

**Risk factors increasing likelihood of Alzheimer’s**
Apolipoprotein E Isotypes

Residues:

112  | Cys  | Cys  |
    |      |      |
158  | Cys  | Arg  |
    |      |      |
      |      | Arg  | Arg

E2   |      |      |
     |      |      |
E3   |      |      |
     |      |      |
E4   |      |      |
Apolipoprotein E isoform profile is the strongest risk factor for sporadic AD.

**Apolipoprotein E isoform frequency**

**General population**
- E3, 77%
- E2, 9%
- E4, 14%

**Sporadic AD population**
- E3, 48%
- E4, 45%
- E2, 7%

E4/E3 or E2 - 2-3 fold increased risk
E4/E4 8-10 fold increased risk
Apolipoprotein E Co-Localizes with Aβ in Plaques

ApoE detection in Plaques by immunodetection and Direct Sequencing

The Lancet 345: 956-958, 1995
The Amyloid (Aβ) Cascade

APP

Aβ monomers

Aβ oligomers

Aβ fibrils

Aβ plaques and NFTs

Neurotoxicity

Neuronal death

Cognitive decline

Apolipoprotein E4

Promoting Aβ aggregation

Poor Aβ brain clearance
What Recent Gene Wide Association Studies (GWAS) Tell Us about the Cause(s) of Late-onset Alzheimer's Disease

Late-onset Alzheimer's Disease
(~98% of cases)

Early-Onset Familial AD
(<60 yrs at onset, ~2% of cases)

Genes Associated With synaptic and cell membrane function: PICALM, BIN1, CD33, CD2AP, EPHA1

Genes in Aβ Metabolism: APOE, CLU, ABCA7

Genes regulating Cholesterol Metabolism: APOE, CLU, ABCA7

Genes in innate and Adaptive Immunity: TREM2, CLU, CR1, ABCA7, MS4A, CD33, EPHA1

Amyloid Cascade Hypothesis
Genes: APP, PSEN1, PSEN2
Aβ and Tau Conformational Changes in AD

Chronological relationships among pathology, clinical symptoms and biomarkers

Clinical Symptoms

Functional Impairment
Cognitive Impairment
Dementia
MCI

Clinical Diagnosis
Preclinical

Biomarkers
sMRI
FDG PET
CSF Tau
Amyloid PET/CSF Aβ
fMRI neuronal network dysfunction

Pathological Changes

Aβ
Tau

Estimated Years from symptom onset

-30  -20  -10  0
Hippocampal/Entorhinal Cortex Atrophy in MCI/AD
FDG-PET in AD

Characteristic Biparietal and Bitemporal Hypometabolism With sparing Of sensorimotor cortex
PIB *in vivo* Amyloid Imaging

Alzheimer’s Disease Patient Showing extensive amyloid binding (In red)

Control Age Matched Patient with No amyloid binding
Florbetapir (Amyvid) for Direct Amyloid Imaging

florbetapir was approved by the FDA as a diagnostic imaging agent on April 9th, 2012

Control

AD Patient

+ve amyloid uptake

negative scan

Florbetapir F 18
PET AMYLOID AND TAU TRACERS

HISTOLOGY

[THK-5117
PiB
MERGED IMAGE

[3H]THK-5117
[3H]PiB
Merge image (THK-5117/PiB)

de Leon, Li et al NYU 2015
Treatment Approaches for AD

- **Amyloid Precursor Protein**
  - **Amyloid β monomer**
    - **Amyloid β oligomers**
      - Amyloid Plaques
      - NFTs
      - Inflammation
      - Oxidative stress
  - Neuronal loss, neurotransmitter loss, cognitive deficit

- **Secretase Inhibitors**
  - Selective Aβ lowering agents
  - Anti-aggregation
  - Anti-fibril
  - Passive Immunization
  - Active Immunization
  - Innate Immunity Stimulation

- **Antioxidants**
  - Anti-inflammatory agents
  - Anti-tau aggregation/phosphorylation

- **Symptomatic Drugs**
  - (anti-cholinesterases and a NMDA antagonist)
Inhibition of $\gamma$- and $\beta$-Secretase as a Treatment for AD

$\gamma$- and $\beta$- secretase inhibitors have off target substrates such as Notch and neuregulin 1, respectively. Notch regulates cell proliferation and differentiation. Neuregulin regulates myelination of neurons. There have been significant side effect issues. BACE inhibitor trials are on-going.
Problems to Overcome for Developing a Successful AD Vaccine

For immunotherapy approaches need to overcome tolerance without inducing excessive cell mediated inflammation.

Effectively reduce Vascular Amyloid without inducing hemorrhages or ARIA.

Address tau related pathology in addition to Aβ deposition concurrently

Specifically target the most toxic oligomeric species of Aβ and tau

Targeting of Concomitant pathologies: α-synuclein and TDP-43 aggregation
## Past Vaccination Clinical Trial Failures

<table>
<thead>
<tr>
<th>Name</th>
<th>Active or Passive</th>
<th>Epitope</th>
<th>Phase</th>
<th>Company</th>
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<td>Wyeth &amp; Elan</td>
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Immunotherapeutic Approaches for AD

(A) CNS fibrillar amyloid clearance with anti-Aβ antibodies

- Aβ disaggregation by anti-Aβ Abs
- Fc-mediated phagocytosis

(B) fibrillar amyloid clearance via a "peripheral sink" with anti-Aβ antibodies

- Reduced Aβ plaque deposition
- Reduced sAβ pool

Potential for encephalitis and/or microhemorrhages

Effectively only very Early in Disease Progression?
Early proof of concept for the therapeutic approach, but issues remain

- Biogen’s Aducanumab showed improvement in objective biomarkers and clinical end-points
  - Validates the approach of targeting aggregated Aβ
- However, major issues remain
  - It is associated with significant side effects (ARIA) due to non-selective targeting of both fibrillar and oligomeric Aβ
Aducanumab (Biogen) is a human IgG1 anti-Aβ monoclonal antibody

**Aducanumab** is derived from a naturally occurring antibody isolated from human memory B cells.
Microglia-mediated clearance of amyloid plaques

- Immunostaining of Tg2576 mouse brain sections demonstrating recruitment of microglial cells around the parenchymal amyloid plaques upon BIIB037 treatment
Promise from the Adcanumab (Biogen) Trial?

- The 3 and 10mg/kg doses of Aducanumab produced significant improvements in MMSE in the Phase 1b trial, in association with amyloid burden reduction on PET as reported at the AD/PD meeting in March 2015.

- However the 6mg/kg dosage failed to show clinical benefits as reported at the AAIC meeting in July 2015.

- The incidence of ARIA-edema (ARIA-E) was high at 5%, 43%, 55% in the 1-3, 6 and 10mg/kg, respectively in apoE4 carriers and 9%, 22% and 17% in the apoE4 non-carriers.
QRX-203 for Alzheimer’s disease

APP processing: Non-Amyloidogenic pathway

The “healthy” cleavage of APP is by $\alpha$-secretase
QRX-203 for Alzheimer’s disease

APP processing: Amyloidogenic pathway

When APP is cleaved by β-secretase. Toxic Aβ is released and aggregates.
QRX-203 for Alzheimer’s disease
Modulates RNA and prevents Aβ formation

Protein

APP α

α-secretase

△APP

mRNA

Exon 16
Exon 18

QRX-203
Pre-mRNA

Exon 16
Exon 17
Exon 18
ProQR R&D Day
March 14, New York
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LUMC

Radboud
Questions from the audience