Therapy for Duchenne muscular dystrophy in the genomic era

Dorothy Hodgkin Memorial Lecture

Kay E. Davies
MRC Functional Genomics Unit,
Department of Physiology, Anatomy and Genetics,
University of Oxford

Oxford International Women's Festival

MRC Functional Genomics Unit

University of Oxford

The many scientific achievements of Dorothy Hodgkin

Structures of:

- **Cholesteryl iodide 1943**
- **Penicillin (~25 atoms) 1949**
- **Vitamin B12 (~80 non-H atoms) 1954**
- **Insulin (829 non-H atoms [51 amino acids]) 1969**

Her work paved the way for determination of 3-D structures of biological molecules.

Nature 1934

At this stage, such ideas are merely speculative, but now that a crystalline protein has been made to give X-ray photographs, it is clear that we have the means of checking them and, by examining the structure of all crystalline proteins, arriving at far more detailed conclusions about protein structure than previous physical or chemical methods have been able to give.

J. D. Bernal.
D. Crowfoot.

Department of Mineralogy and Petrology,
Cambridge.
May 17.

Medals and Honours

- 1947 Fellowship of the Royal Society
- 1956 The Royal Society Medal
- 1960 Wolfson Society Professor
- 1964 Nobel Prize for Chemistry
- 1965 Order of Merit
- 1970 Honorary Fellow of the RS of Edinburgh
- 1971 Baly Medal of the Royal College of Physicians (RCP)
- 1974 Honorary Fellow of the RCP
- 1977 Gold Medal of the RS of Medicine
- President of the BAAS
- 1978 Longstaff Medal of the Chemical Society
- 1980 Honorary Fellow of the RSC
- 1983 Lomonosov Gold Medal of the USSR Academy of Sciences
- 1984 Dimitrov Peace Prize, Bulgaria
- 1987 International Lenin Peace Prize

Dorothy Crowfoot Hodgkin was honored on this postage stamp issued in the United Kingdom.

- Also a member of 14 foreign academies (at least one in each Continent except Antarctica!)
- Chancellor of Bristol University 1970-1988
- President of Pugwash 1975-88
- IUCr President 1972-1975.

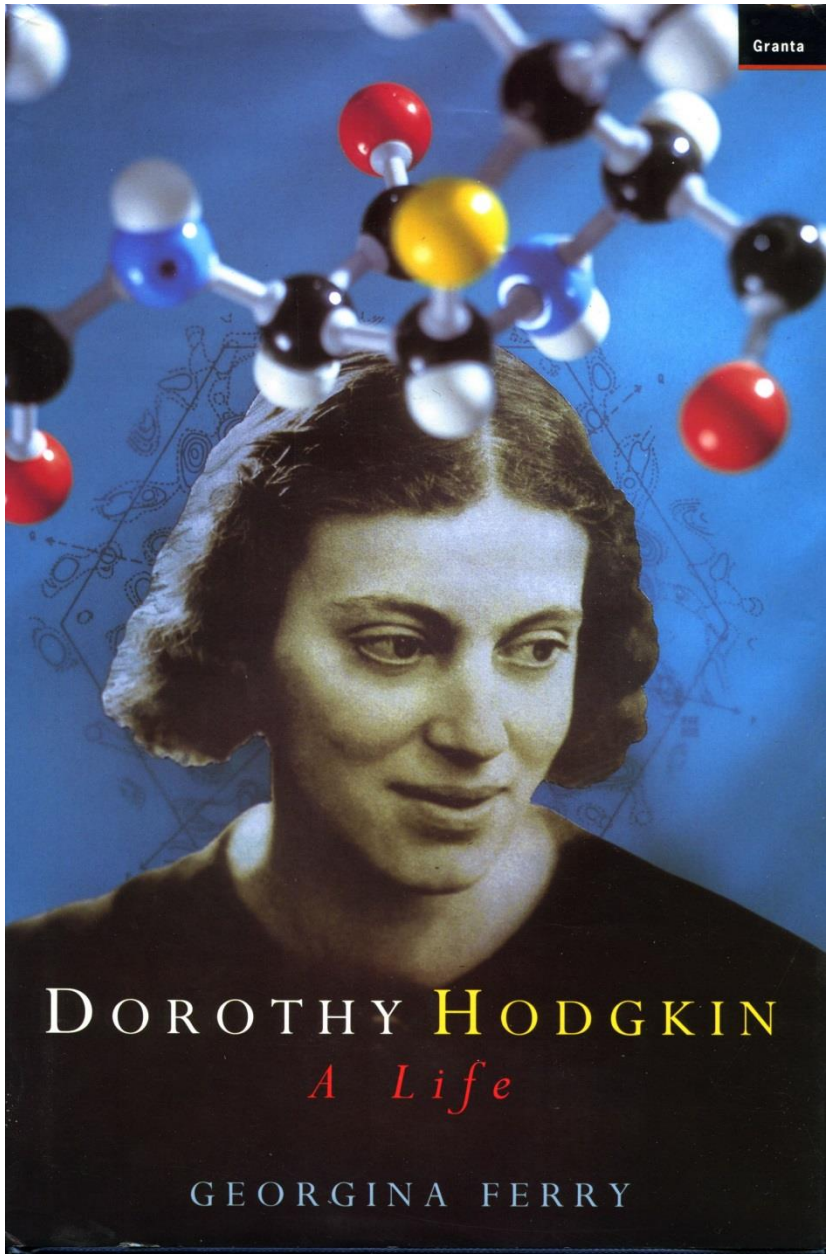
Dorothy's portrait in the National Portrait Gallery in London. Pained by Maggi Hambling in 1985. **http://www.maggihambling.com/**

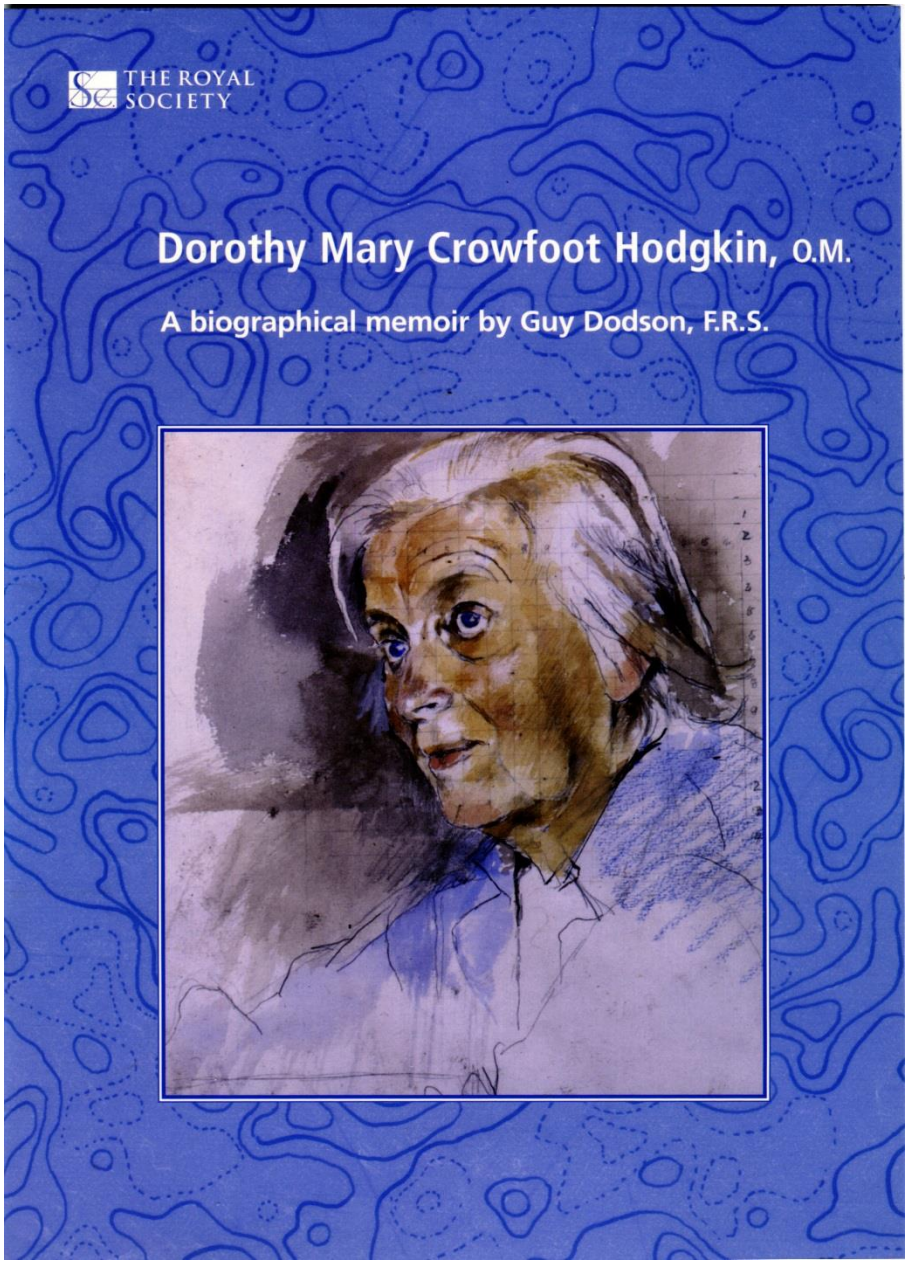
Dorothy Hodgkin

Nobel laureate for chemistry, 1964

- **Somerville supported her at a time when there was widespread opposition to married women pursuing academic careers**
- **Dorothy proactively supported other female academics and followed her students long after they left Oxford, including Margaret Thatcher**
- **Somerville, and Dorothy Hodgkin in particular, gave women the confidence to follow the career they felt most passionate about**
- **Women remain under-represented in the sciences, even 52 years after Dorothy Hodgkin's her Nobel Prize**
- **International Festival of Women in Science, organized by AWISE, the Association of Women in science and Engineering**

Biographies





Role models

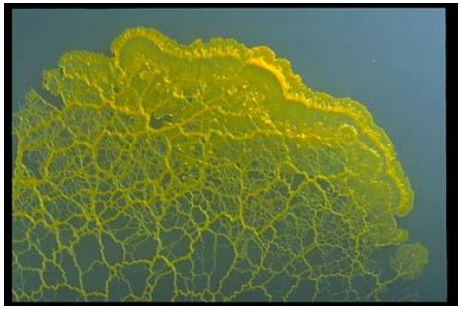
- **Dorothy Hodgkin**
- **Need a supportive environment at every stage (and a role model does not always have to be a woman)**

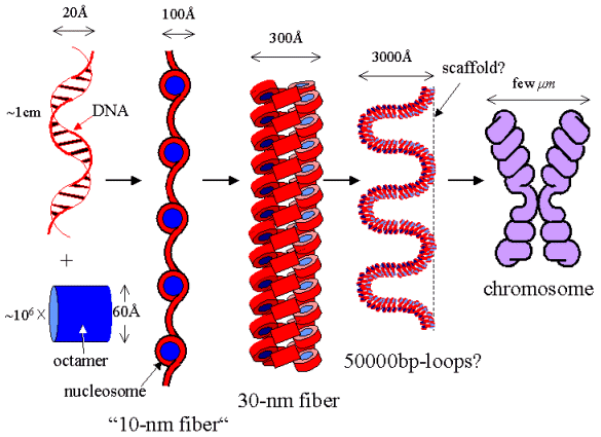
Somerville College and Chemistry 1969-1973

Organic chemistry laboratory
teaching laboratory

Highly supportive environment in spite of ~200 males and 22 females studying chemistry

Wolfson College and Biochemistry 1973-1978 (D.Phil and Fellowship)

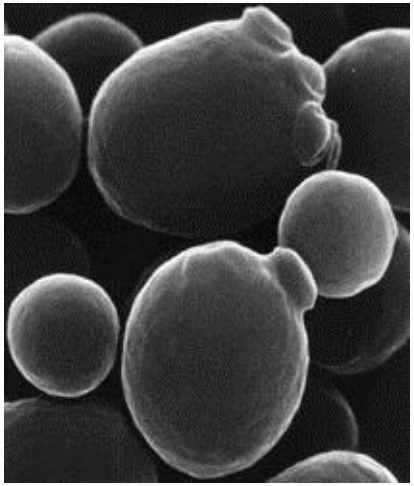




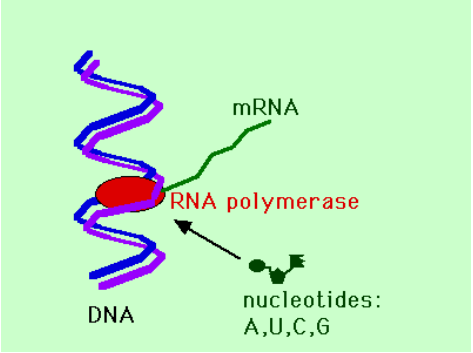
Be clear about what you want

- **Confidence in applying for fellowships**
- **Having confidence to change fields if motivated to do so**

Paris- cloning and genetics



Yeast



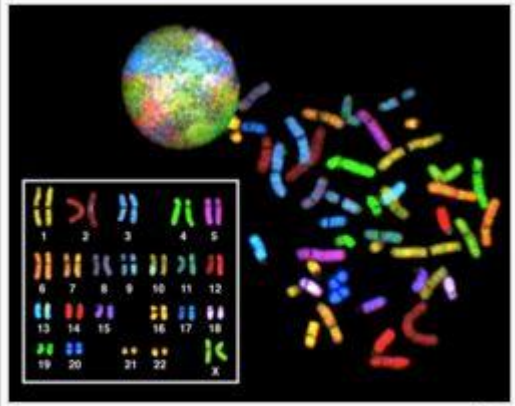
The life changing publication!

Construction of a genetic linkage map in man of restriction length polymorphisms

Botstein, D., White, R., Skolnick, M and Davis, R. Am J, Human Genetics 32: 314-331

- ***Using DNA to follow the inheritance of the disease***
- ***Pinpoints where the genetic defect is on the chromosome***
- ***Allows the identification of the gene***

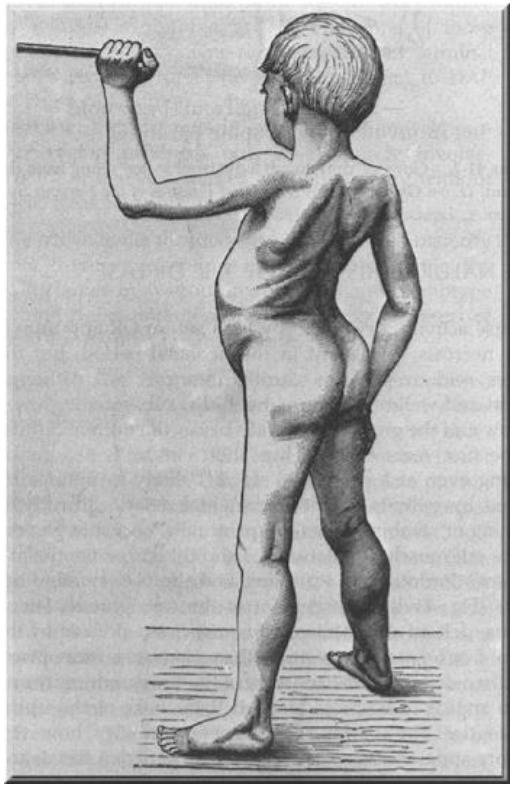
St Mary's Hospital Medical School London



Spectral karyogram of a human female

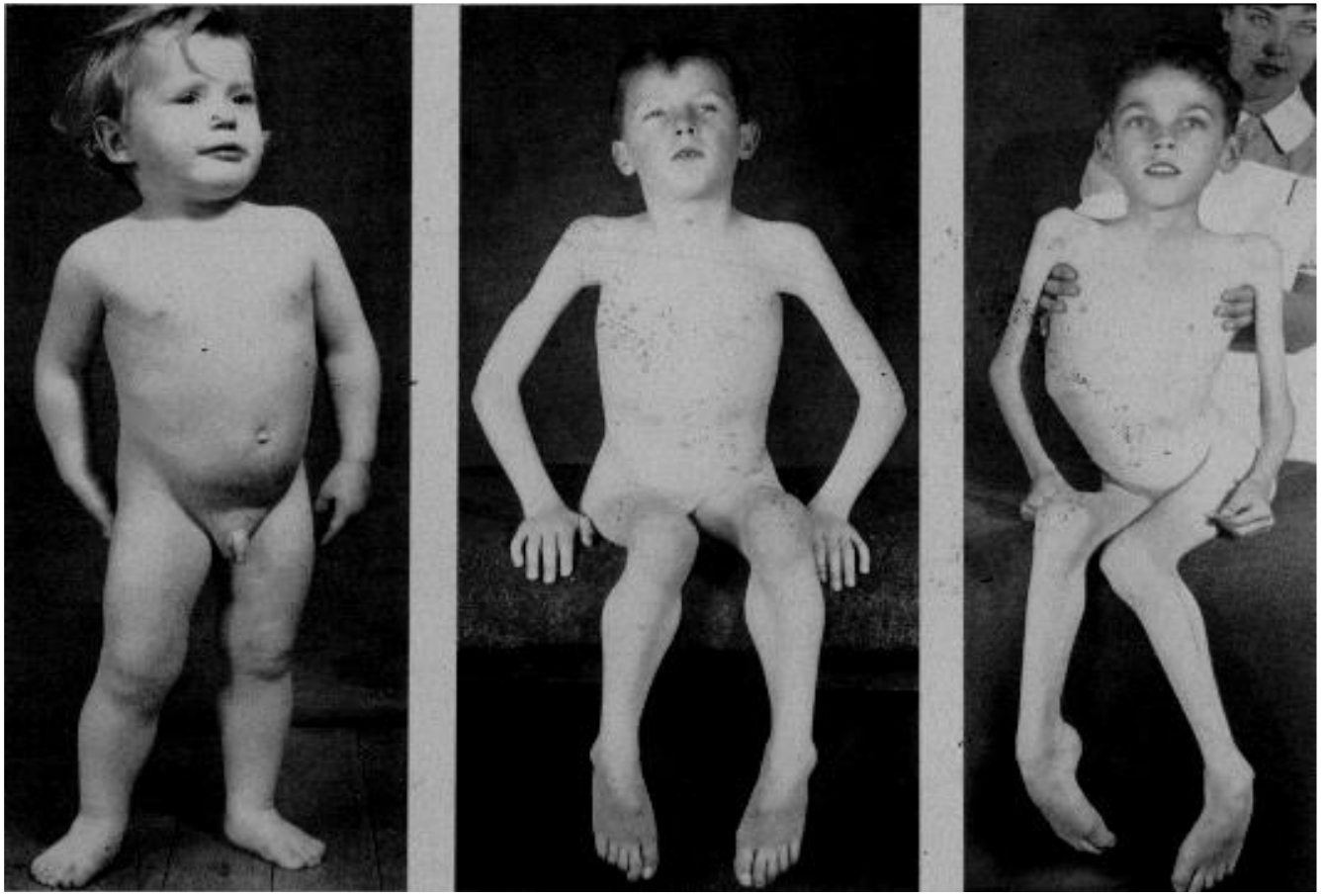
Duchenne Muscular Dystrophy (DMD)
The Facts

- ✓ *X-linked progressive muscle wasting disease*
- ✓ *Early confinement to a wheelchair (~12 years) and death in twenties*
- ✓ *Skeletal and cardiac muscle involvement, some cognitive difficulties*
- ✓ *Prevalence 1 in 5000 boys*
- ✓ *Patient population in the developed world is estimated to be 50,000. All populations around the world are affected (250,000)*
- ✓ *No curative treatment is available, only supportive approaches (mainly physiotherapy and physical aids, steroid treatment)*



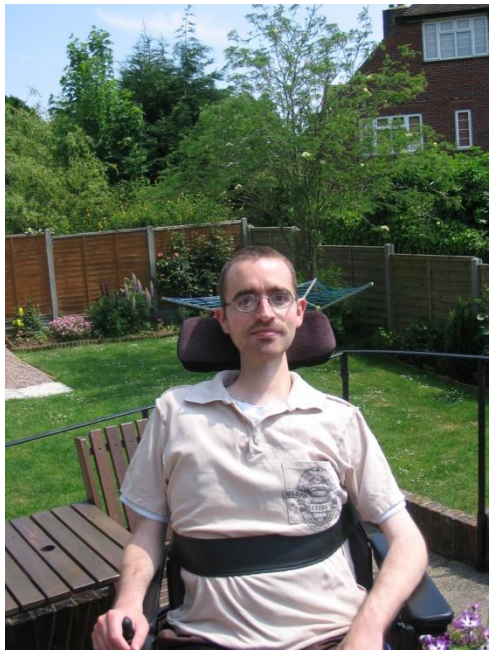
Taken from Dubowitz : Muscle Disorders in Childhood

DMD Brothers



2 years 10 years 12 years

Diagnosis may be late and there may be other affected brothers



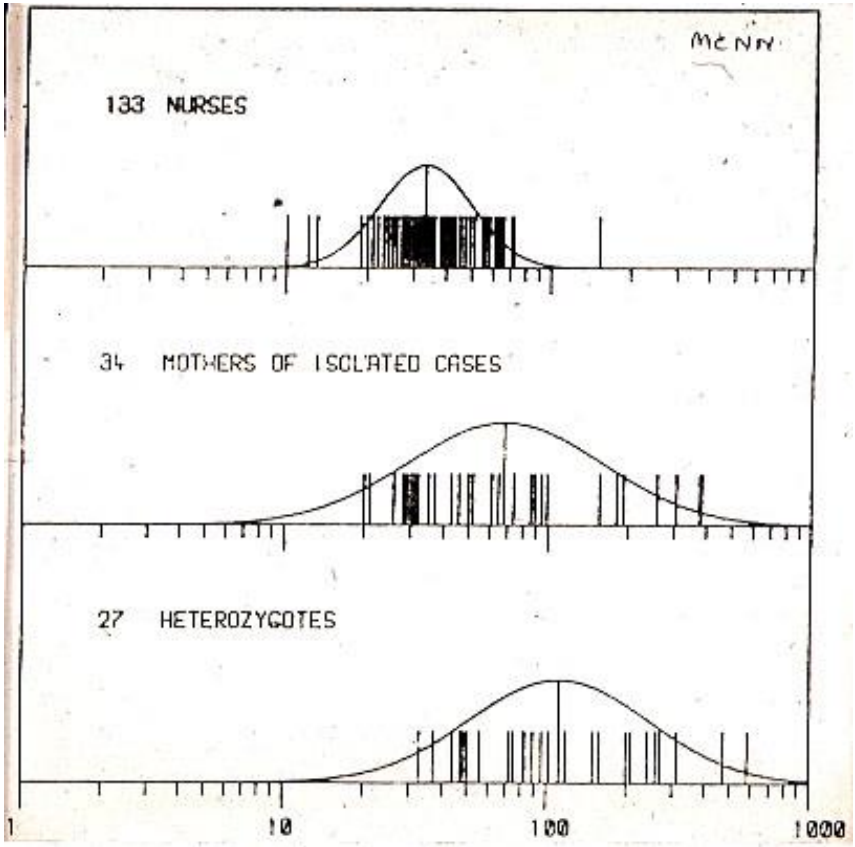
Very high new mutation rate- most cases do not have previous family history

Challenges of Muscular Dystrophy

- 1980:

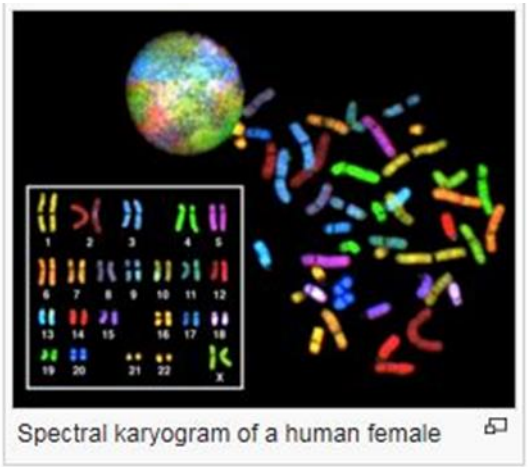
- **Unreliable carrier testing**
- **No prenatal diagnosis available.**

Carrier testing for DMD

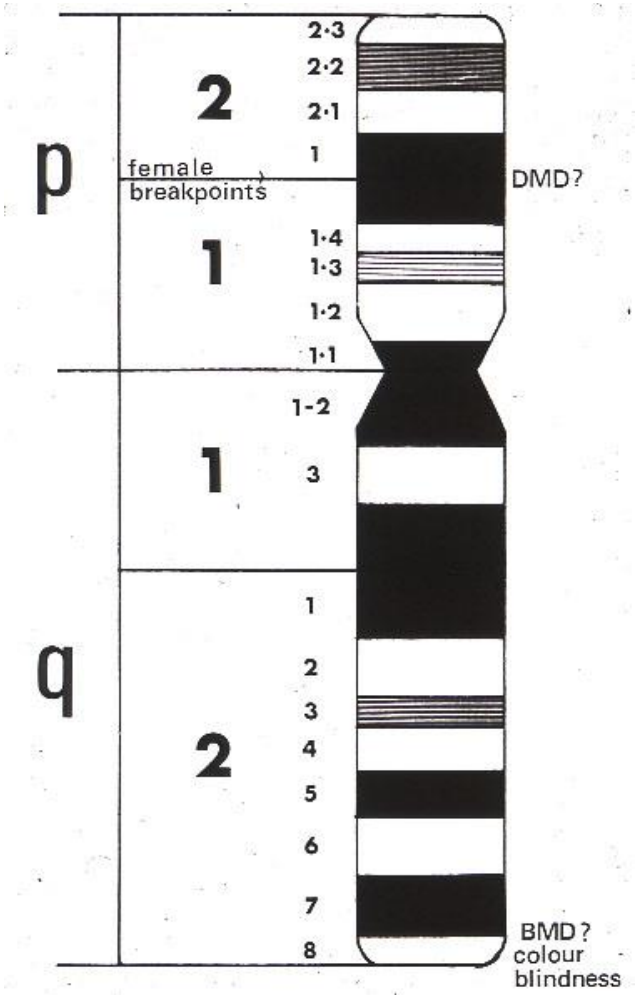


Creatine kinase levels in serum- indication of muscle damage

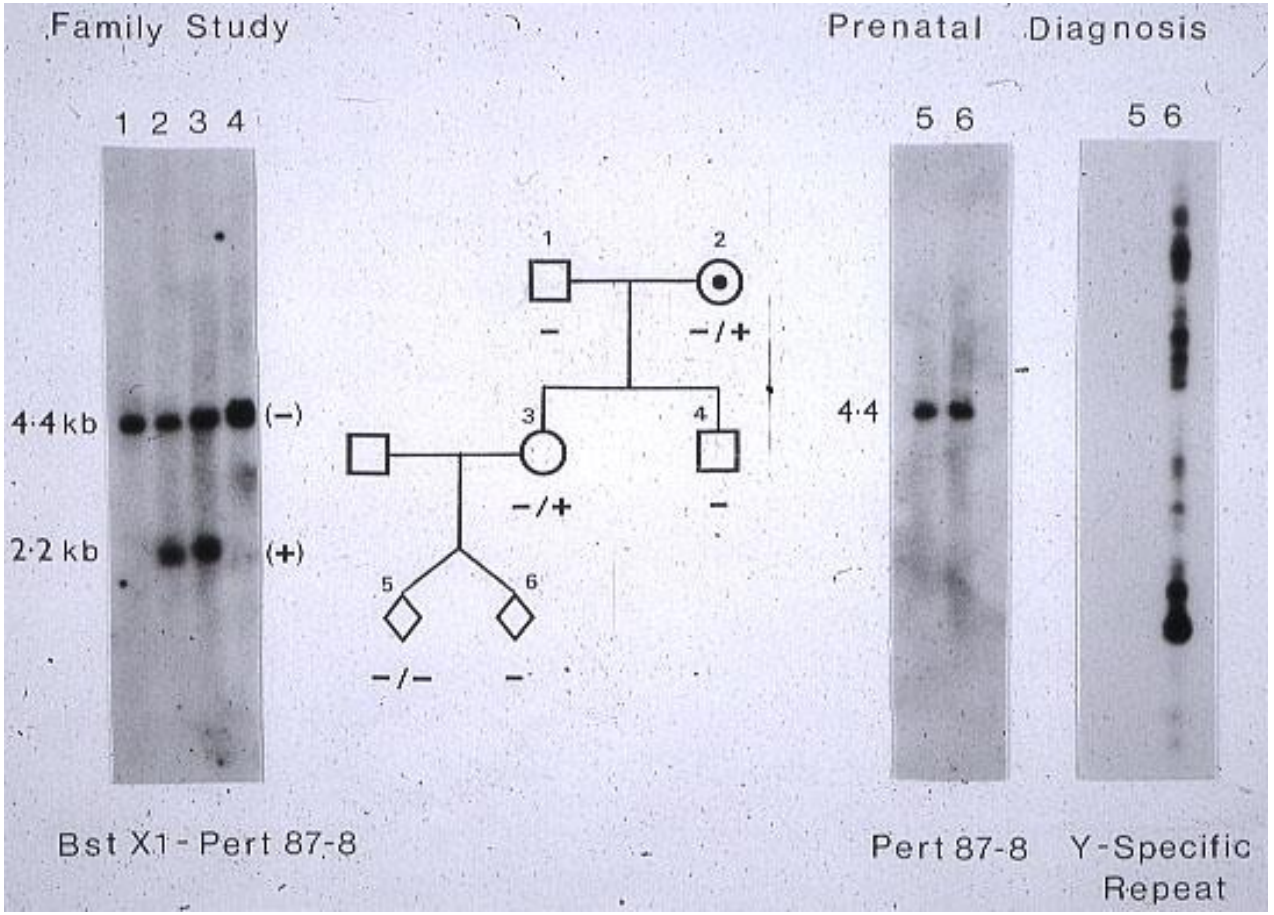
Were there any clues for the location of the mutated gene in 1980?



Spectral karyogram of a human female



Prenatal diagnosis of DMD



Diagnostic test took at least 3 weeks to perform

DMD diagnosis 2016

- **Prenatal diagnosis routine in most countries - test can now be carried out within a few hours rather that 2-3 weeks**
- **Carrier detection possible but challenging**
- **New sequencing technologies mean that many will be diagnosed at birth (muscle biopsies rarely performed)**
- **Precision diagnosis**

Key factors for success

- **A highly motivated head of lab (Bob Williamson)**
- **Excellent clinical collaborators (Peter Harper)**
- **Excellent obstetricians (Charles Rodeck)**
- **Wonderful families who donated their samples for the development of the tests**
- **And good funding from the muscular dystrophy charities and the MRC**

What next?

- **Independent group**
- **MRC Senior Fellowship**

Back to Oxford

John Radcliffe Hospital

Institute of Molecular Medicine

Be organised and manage time effectively

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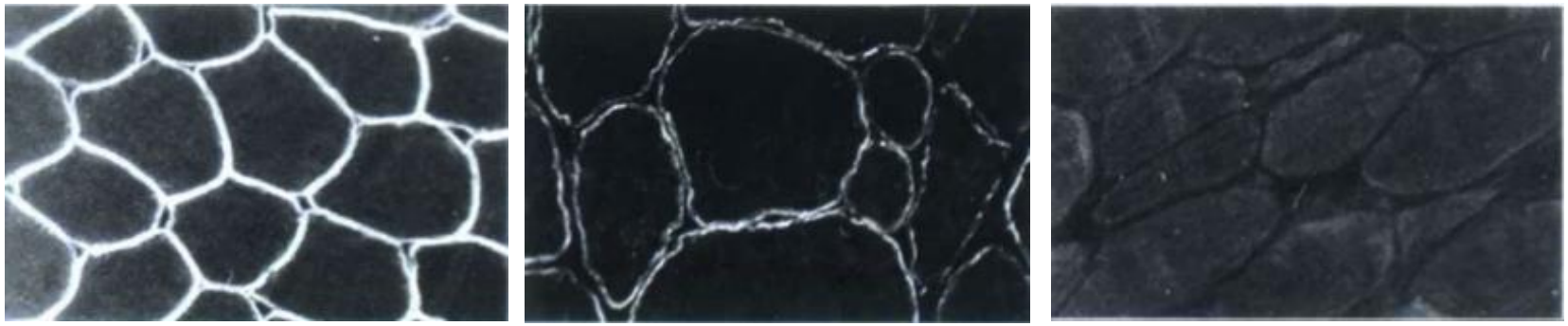
Discovery of the gene causing DMD (Kunkel and Worton, 1986)

One scientific discovery leads to other questions and challenges

How do we develop effective treatments for this disease?

What gene is mutated in DMD?

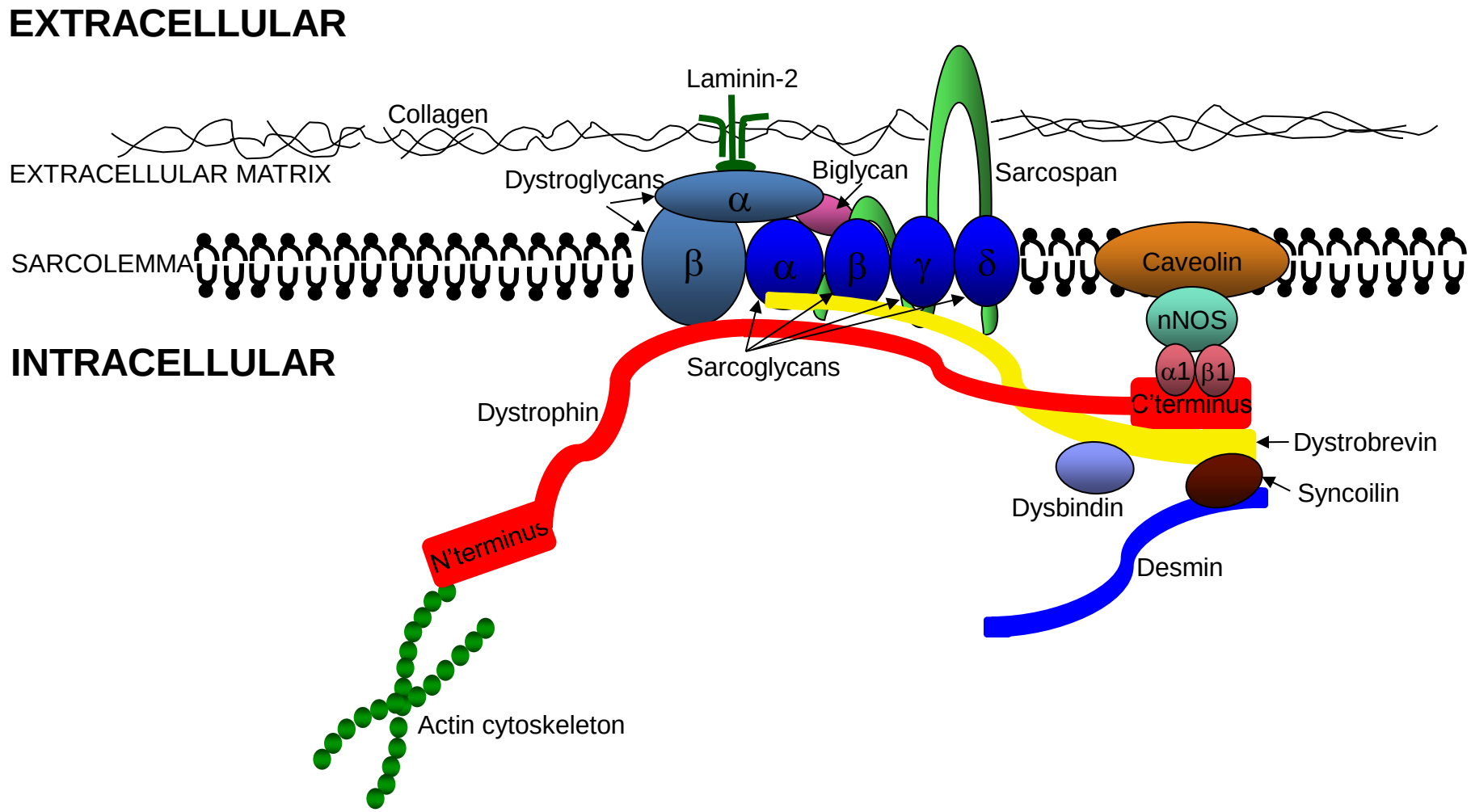
- **Largest gene in human genome codes for a large 427kdalton muscle protein, dystrophin**
- **Mutations cause altered expression of dystrophin- most are deletions**
  - **DMD (Duchenne) – no functional dystrophin produced**
  - **BMD (Becker) – truncated, semi-functional dystrophin produced (milder phenotype, ~1 in 20,000 male births)**



Normal **BMD** **DMD**

Dystrophin at the membrane

Dystrophin associated protein complex



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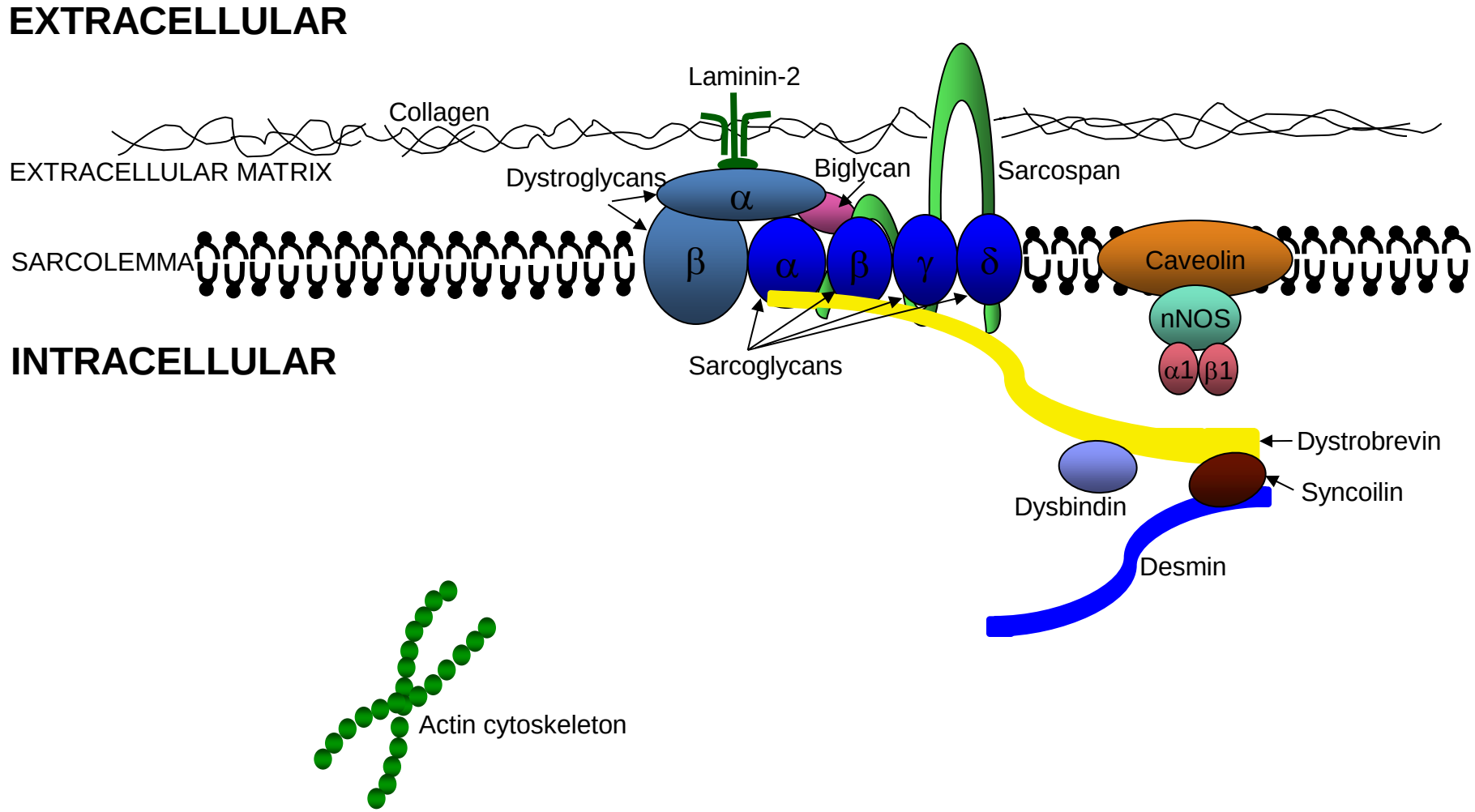
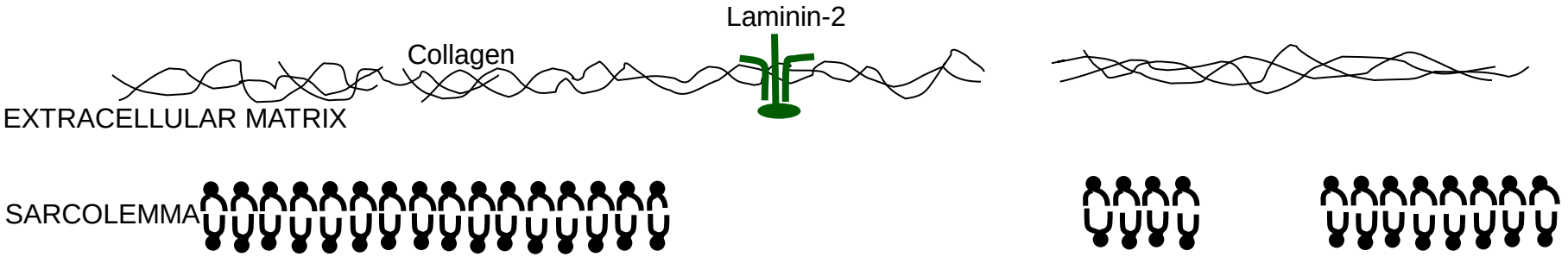


EXTRACELLULAR



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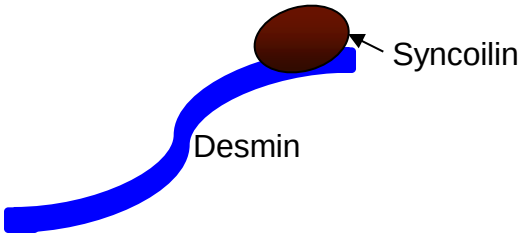
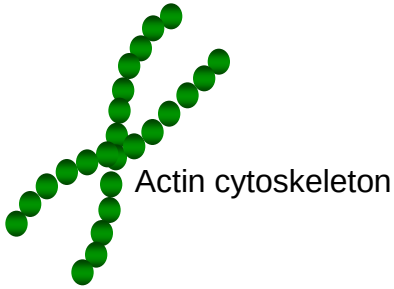
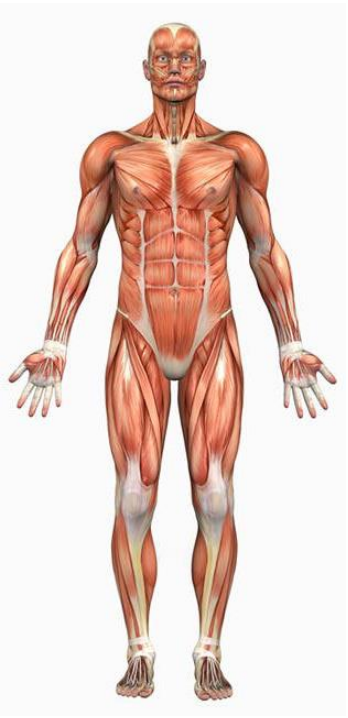




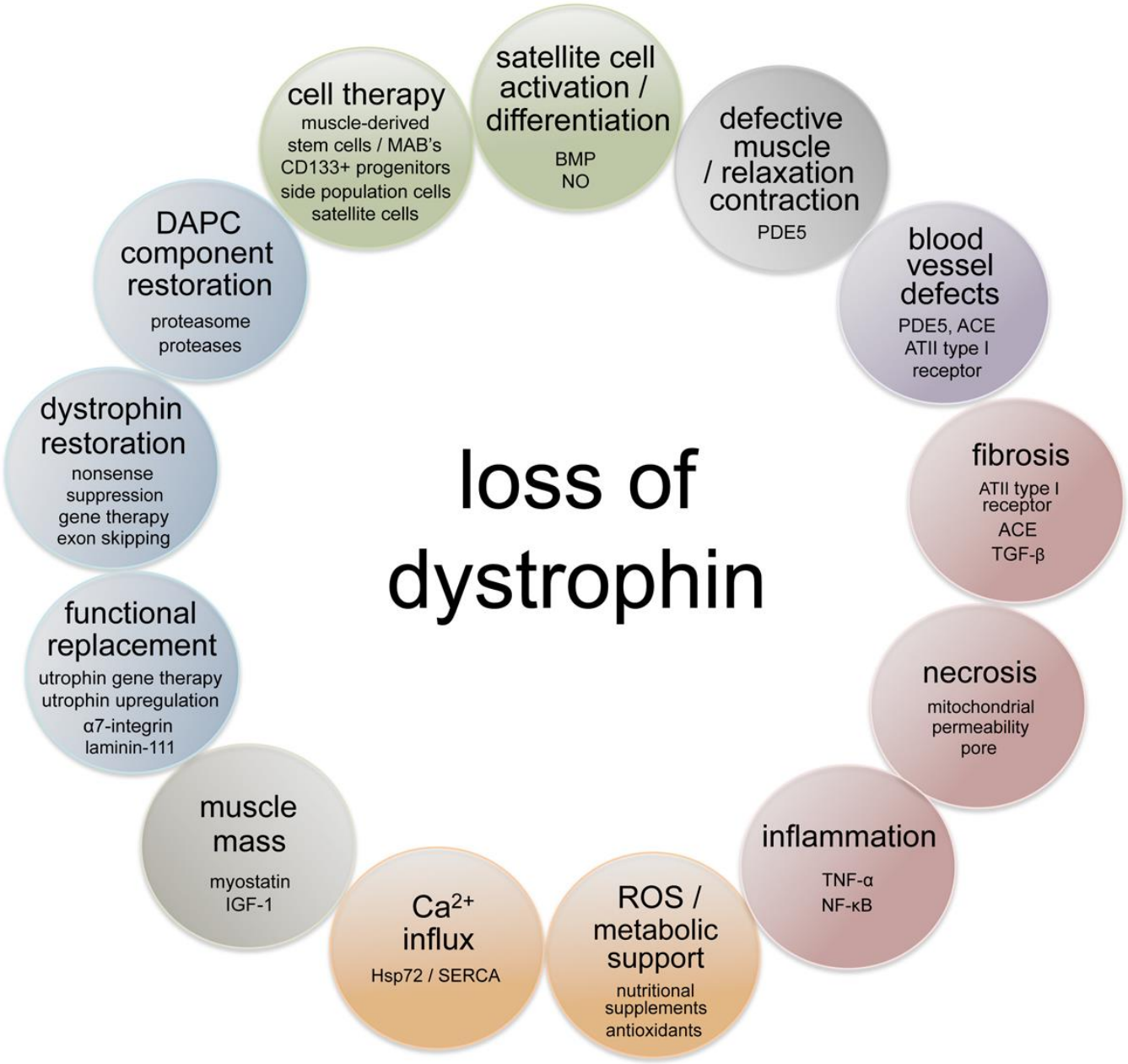
Therapy of DMD – the Challenges

Large protein

- ***Need to replace at least 20% of normal levels***
- ***Need to target all muscles (heart, skeletal muscle, diaphragm)***
- ***Muscle can make up 40% of a person's body mass therefore need systemic delivery***
- ***Need to avoid immune response***



Life long treatment needed
Natural history variable



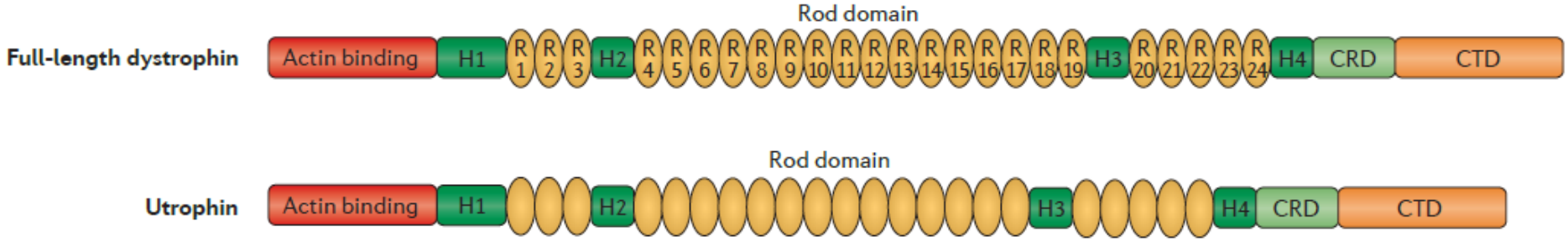
Taken from Fairclough, Perkins, Davies. Pharmacologically Targeting the Primary Defect and Downstream Pathology in DMD Current Gene Therapy 2012

Approaches to therapy for DMD

- **Advantages of pharmacological approaches**
  - **Targets all muscles**
  - **Cheaper to deliver**
  - **No immune response**

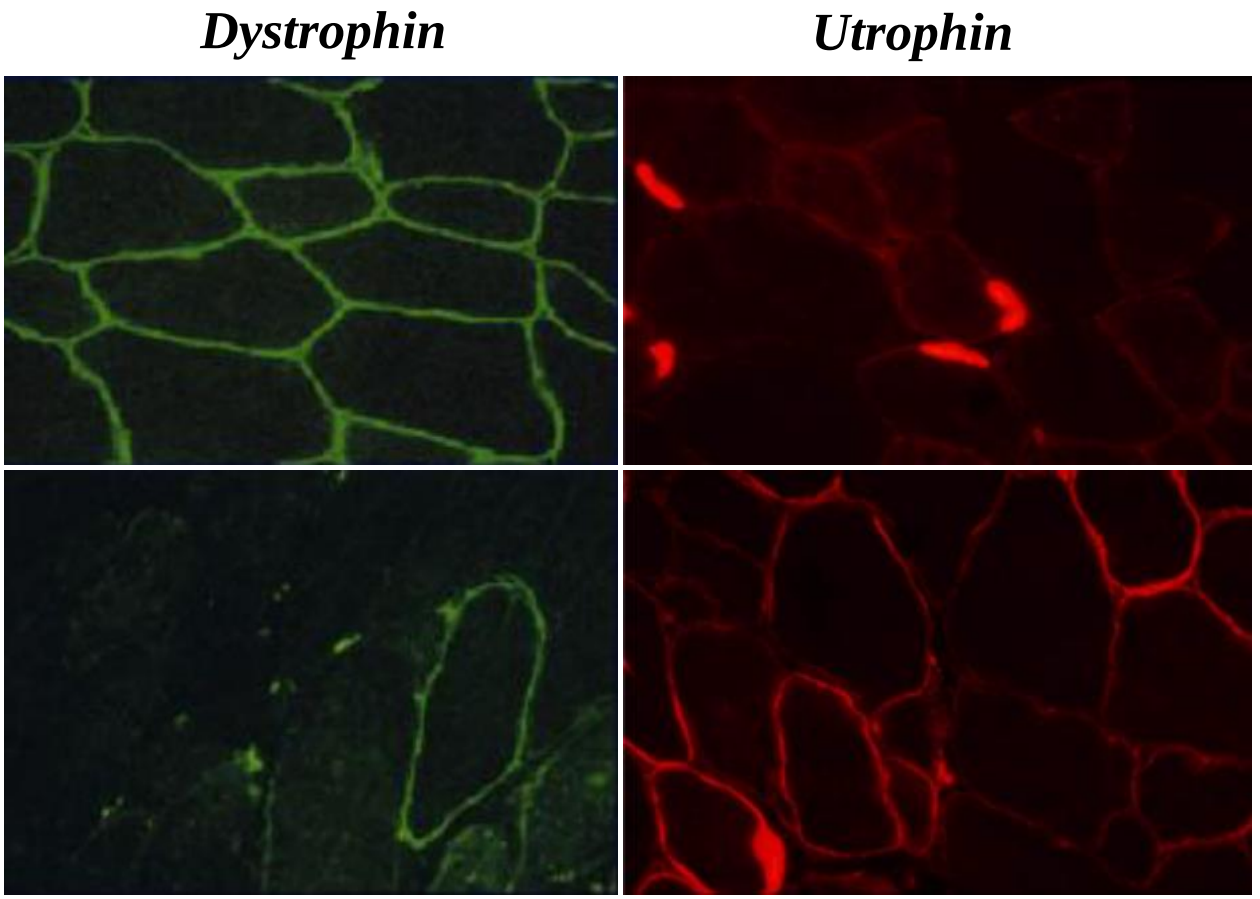
Dystrophin related protein utrophin

- *Dystrophin related protein in skeletal muscle with homology to dystrophin is discovered (Love et al, Nature 339: 55-8)*
- *The associated protein shares structural similarities (80%) with dystrophin*



Fairclough RJ, Wood MJ, Davies KE.
Nat Rev Genet. 2013

Utrophin is naturally increased in DMD muscle



Kleopa et al Hum Mol Genet. 15: 1623-8 (2006)
Naturally occurring utrophin correlates with disease severity in DMD

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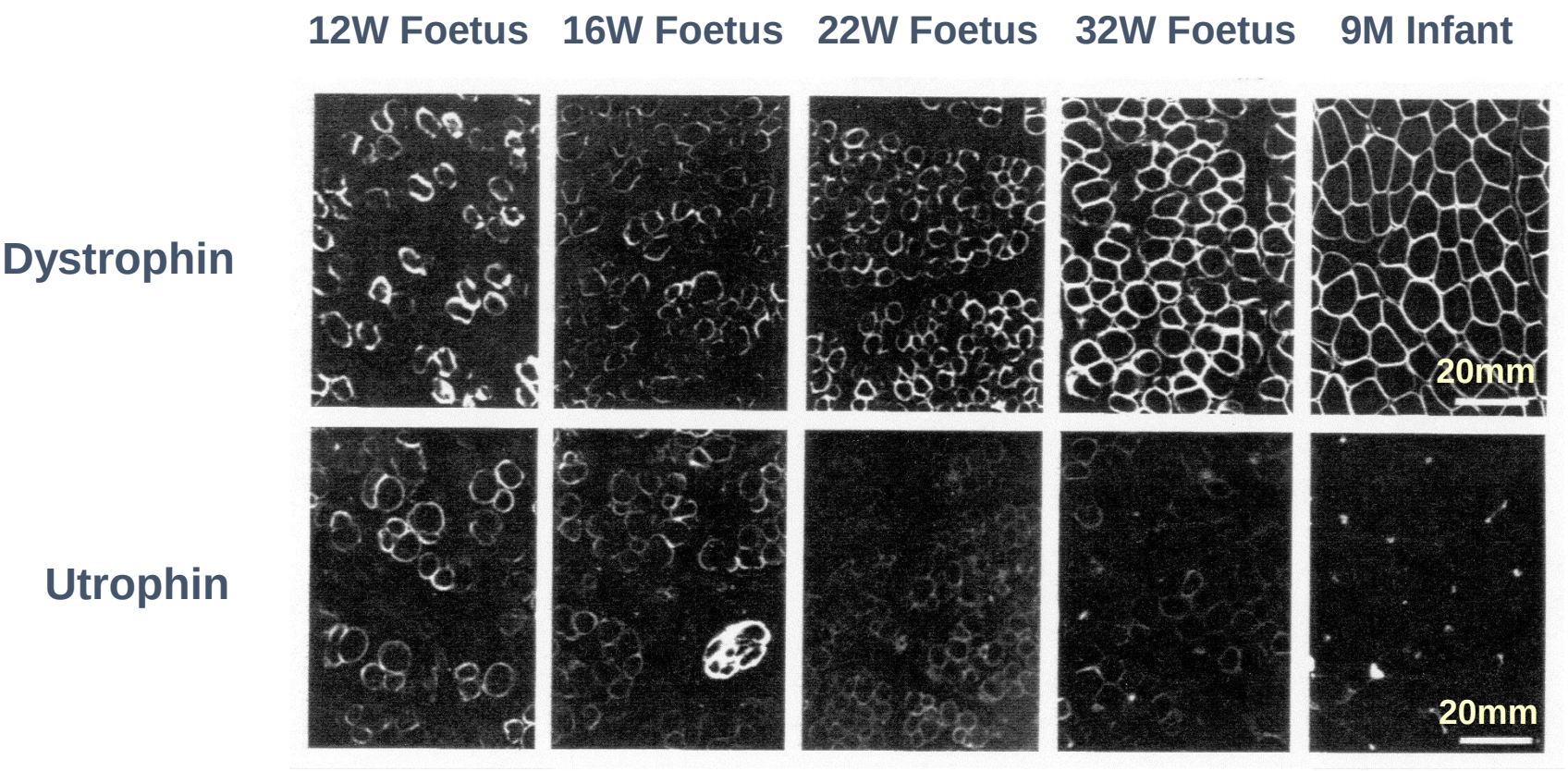
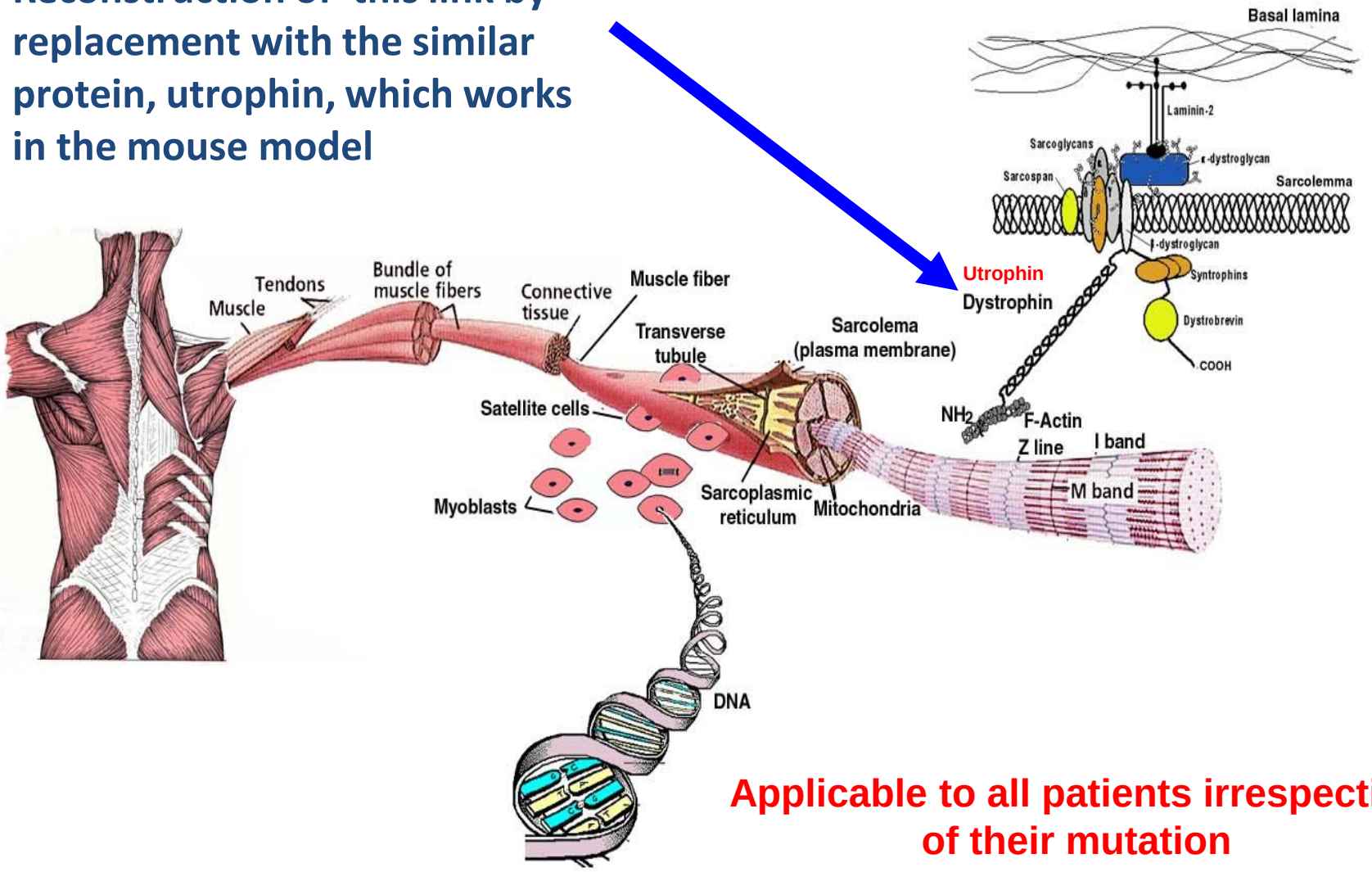
Dystrophin and utrophin in human development



taken from Tome et al. Neuromuscular disorders 1994 v4 p343-8

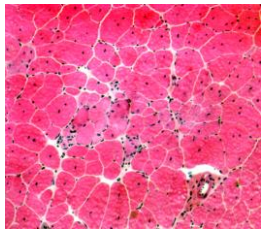
Reconstruction of this link by replacement with the similar protein, utrophin, which works in the mouse model



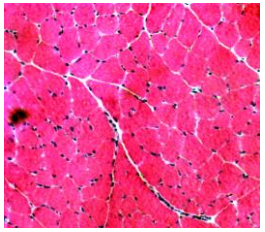
Applicable to all patients irrespective of their mutation

Evidence that utrophin modulation can be potentially therapeutic

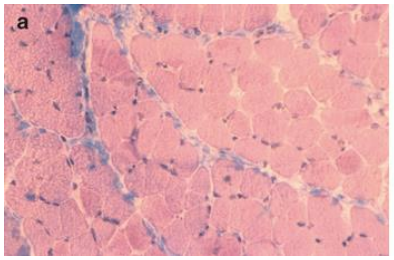
- *Can we replace dystrophin with increased utrophin?*
  utrophin transgene prevents dystrophic phenotype in mdx mouse and DMD dog
- *How much utrophin do we need?*
  2-3 fold increase (may be less for signinficant clinical benefit)
- *Does increased utrophin throughout the body have any side effects?*
  over expression in mouse did not have deleterious effects



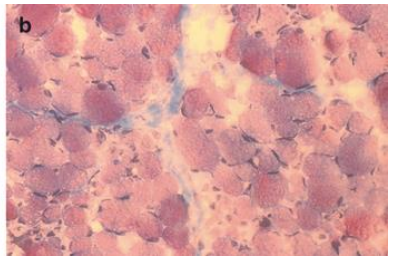
DMD mouse



DMD mouse + utrophin transgene



DMD dog + utrophin -AAV



DMD dog

Cerletti et al Gene Therapy 2003

Tinsley et al Nature 1998

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Time for a family

Nicholas born 11/02/1988

Never a good time to have children but very rewarding- main time is not in first few years but often in the teens

1990 Future at Oxford?

Institute of Molecular Medicine

A move to the MRC Clinical Science Centre (1992-4)

A multidisciplinary Centre to bring basic science together with clinical applications

Always take advice and attend courses- they teach you a lot about yourself

Always work with others whenever possible

Recognise your allies in delivering the job

MRC Functional Genetics Unit

Implications of genetics and influencing policy

- **Chairman of Expert Working Group on the Human Genome Mapping Project (OST) 1993**
- **Chairman of Wellcome Trust Molecular Cell Panel, 1992-95**
- **Member of the Genetics Advisory Group, WT 1996-1999**
- **Basic Science Interest Group, WT 1992-98**
- **Chairman Advisory Committee on Genetic Modification HSE 1996-2000**

Only possible to be committed to many aspects of biomedicine with an excellent PA, Helen Johnson (27 years!)

Future at Oxford?

Institute of Molecular Medicine

Life after the IMM

Professor of Genetics,
Department of Human Anatomy and Genetics
1996-1998
Sir George Radda

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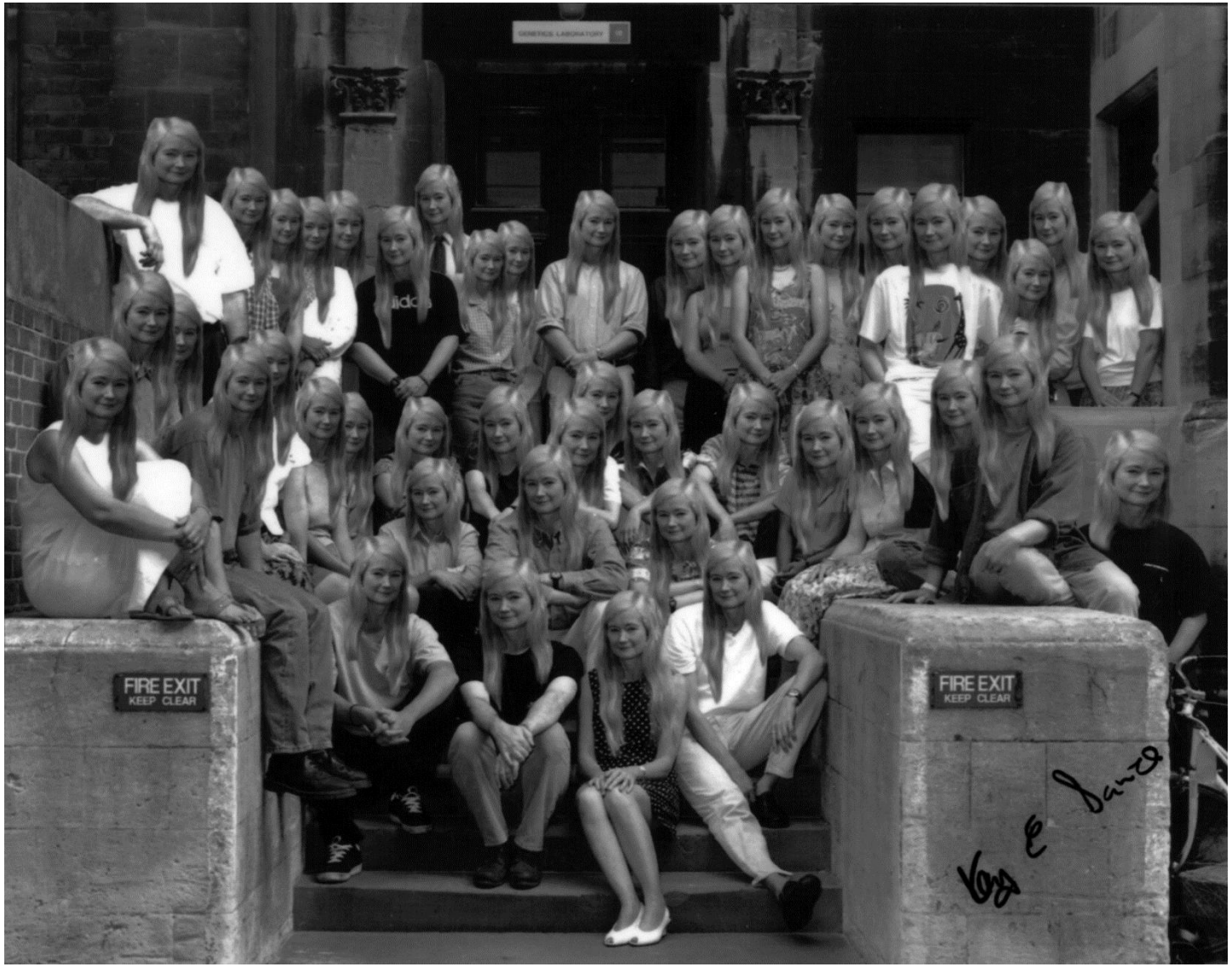
Genetics Laboratory Oxford - 1996

Genetics Laboratory - 1998



Head of Department

Appointed Dr Lee's Professor of Anatomy 1998

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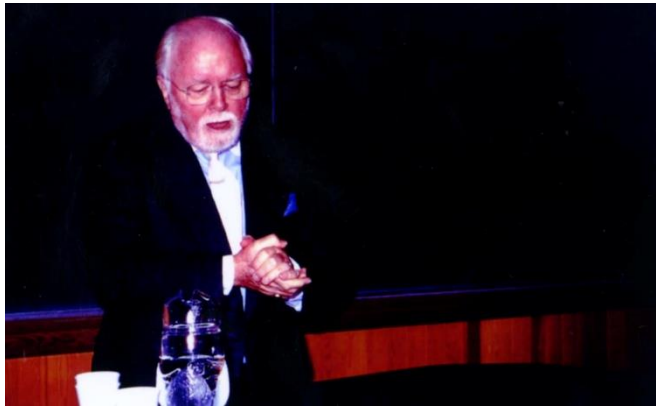
Department of Human Anatomy becomes Human Anatomy and Genetics

- **New challenge**
- **Lots of support from staff**
- **Develop new strategy**
- **Setting up of the MRC Functional Genetics Unit**



The Lord Attenborough CBE
opened the
MRC Functional Genetics Unit
on
Wednesday 5th July 2000

Long term funding- at Least 5 years

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Strategy to find ways to up-regulate utrophin

- ***High throughput screening for a chemical drug***
- ***Collaboration with the chemists***

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Help can come from unexpected quarters

- **Help from Jim Watson and OSI Pharmaceuticals**
- **How could we persuade a company to take it on?**

Foundation of VASTOXplc

MRC Functional Genomics Unit

summit plc

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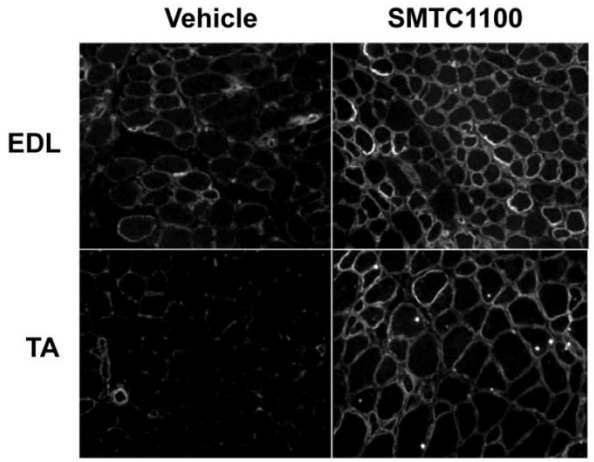
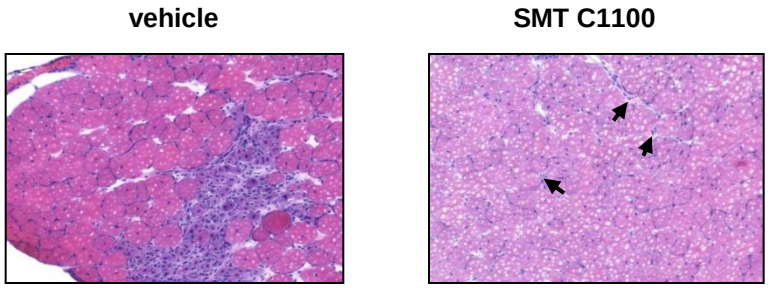
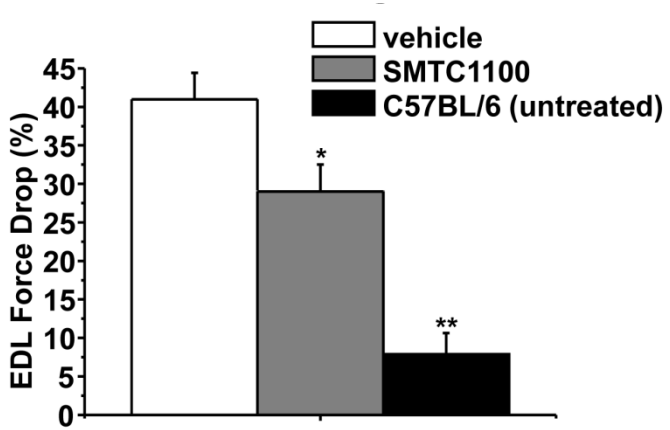
SMT C1100: first in-class drug for utrophin modulation in DMD therapy



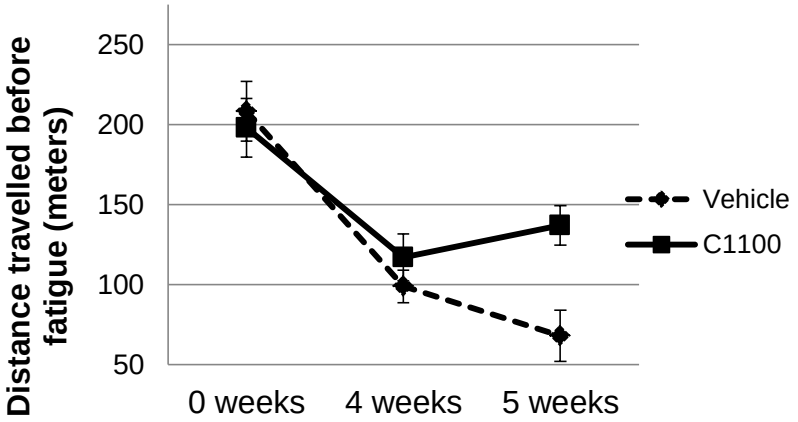
Up to 2-fold ↑ in utrophin protein levels



↓ fibrosis/degeneration



25 % ↓ in stretch-induced damage

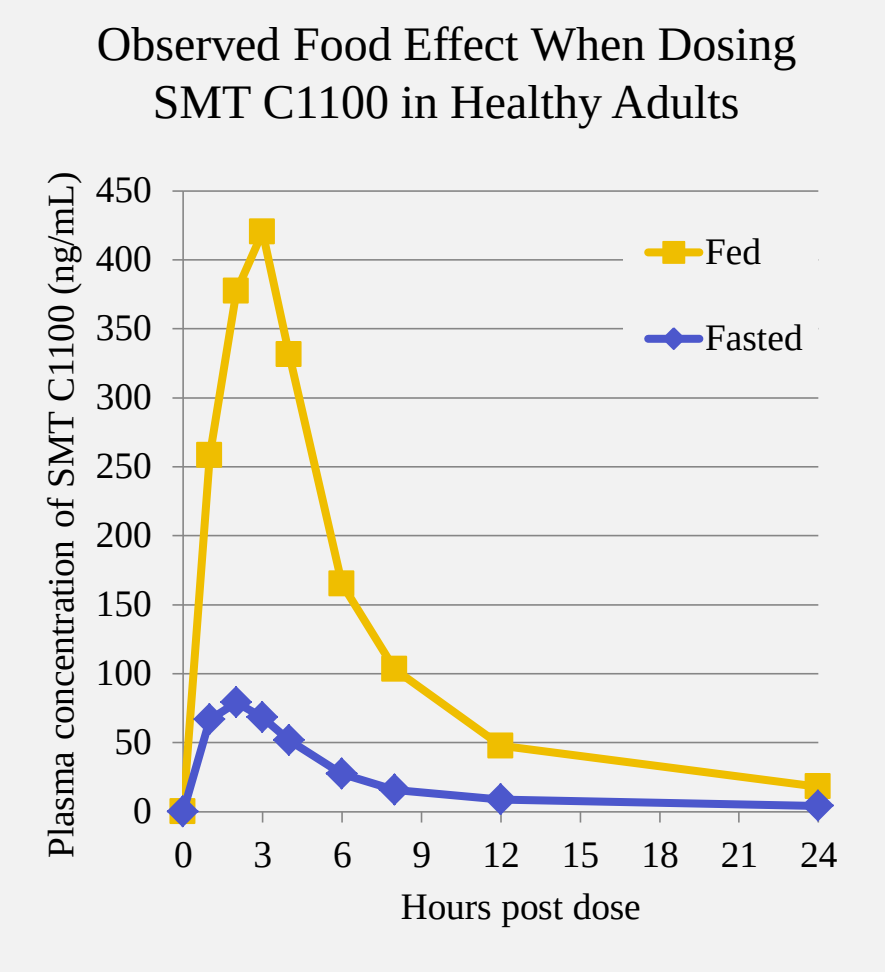


↑ distance run before fatigue

Tinsley et al PLoS One 2011

Phase 1 (Trial 01):
Safe and Well Tolerated in Healthy Volunteers

- **SMT C1100 was safe and well tolerated at all doses tested**
- **Achieved levels expected to increase utrophin expression for at least 14 hours/day**
- **Strong food effect with higher plasma levels achieved when SMT C1100 taken with meals**



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SMT C1100 Overview

Molecule: First-generation, orally administered small molecule utrophin modulator

Status:

- Progressing into Phase 2 proof of concept trial – received UK regulatory and ethics approval on trial design
- Drug exposure levels in patients expected to provide therapeutic benefit
- Well-tolerated in ~100 healthy volunteers and 24 DMD patients

- Orphan drug designation granted in US and Europe
- Strong IP: Granted composition of matter patent through 2029 in US, and 2027 in EU & Japan

>>> Next Milestone: Commence patient dosing in Phase 2 proof of concept trial

Next Steps: Phase 2 Proof of Concept Trial

> Trial to evaluate clinical benefit of SMT C1100 in patients with DMD
> Plan to report interim data periodically beginning H2 2016
> First patient anticipated to be dosed soon

Planned Study Design:	• Open label trial expected to enroll up to 40 ambulatory DMD patients aged 5-10 years old • 48 week trial • Sites in Europe and US (subject to FDA regulatory approval)

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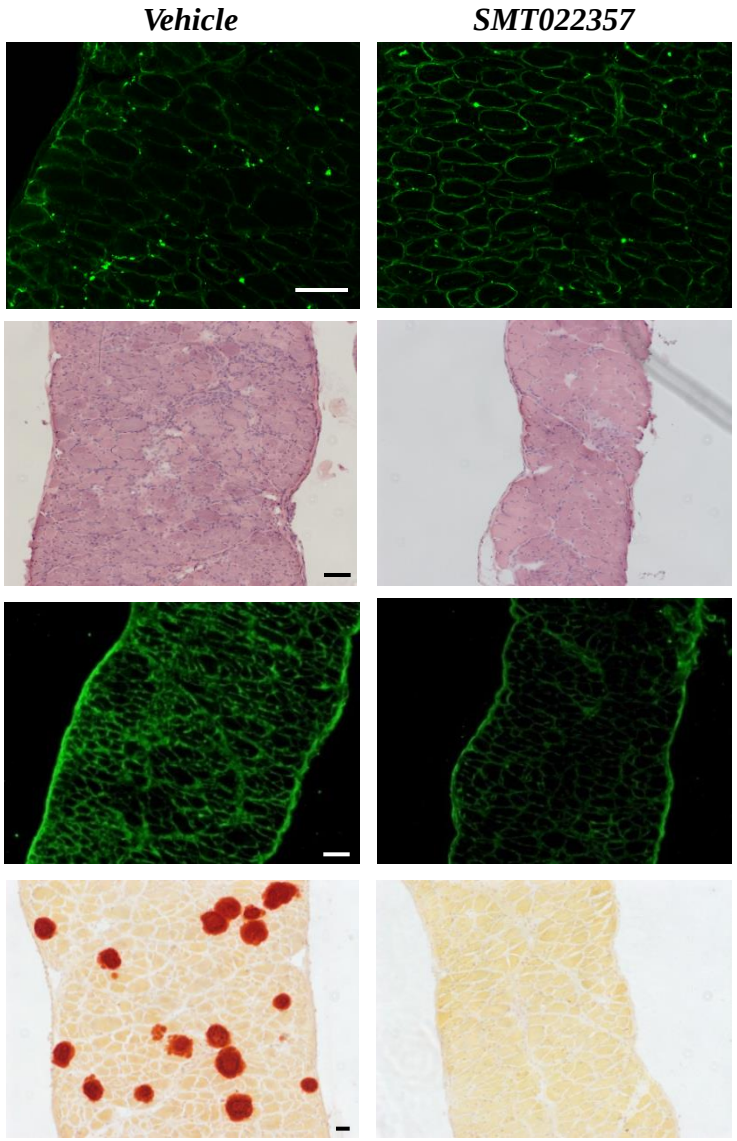
Second generation utrophin modulators

- **Compounds structurally related to the first generation utrophin modulator (SMT C1100),**

- **Present favourable chemical physical properties and a more robust metabolism profile.**

Second Generation Utrophin Modulators

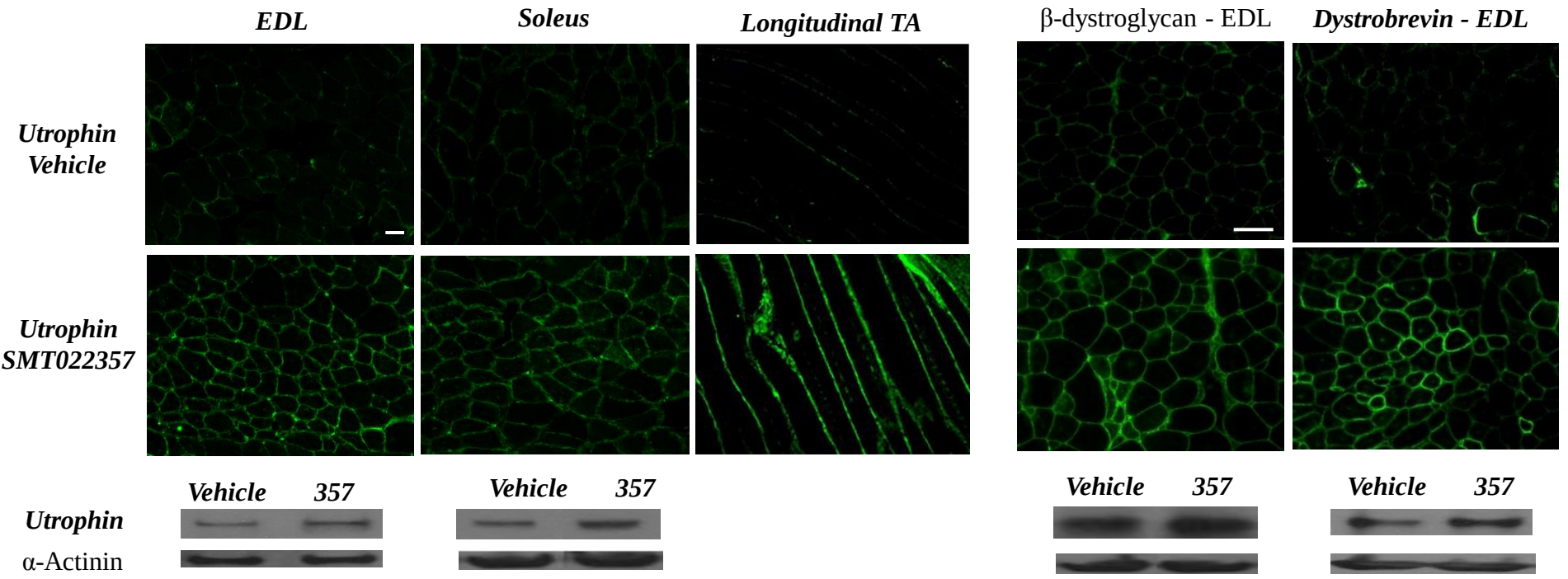
- ***SMT022357 improves the pathology in the diaphragm***



- ✓ ***Increase in number of utrophin positive fibers in diaphragm***
- ✓ ***20% increase of utrophin protein level***
- ✓ ***SMT022357 decreased fibre regeneration as determined by reductions in centrally nucleated myofibres (-36%, p<0.001),***
- ✓ ***SMT022357 decreased necrosis by 56.6% (p=0.04)***
- ✓ ***SMT022357 reduces the collagen I content and fibrosis phenomenon by 15%***
- ✓ ***Excessive calcium influx fundamental to initiating disease pathology and muscle failure.***
- ✓ ***SMT022357 treatment prevented the accumulation of these deposits demonstrating***

Second Generation Utrophin Modulators

- ***Increase in number of utrophin positive fibers in skeletal muscle,***
- ***Returns of dystrophin protein complex and improved fiber membrane stability***

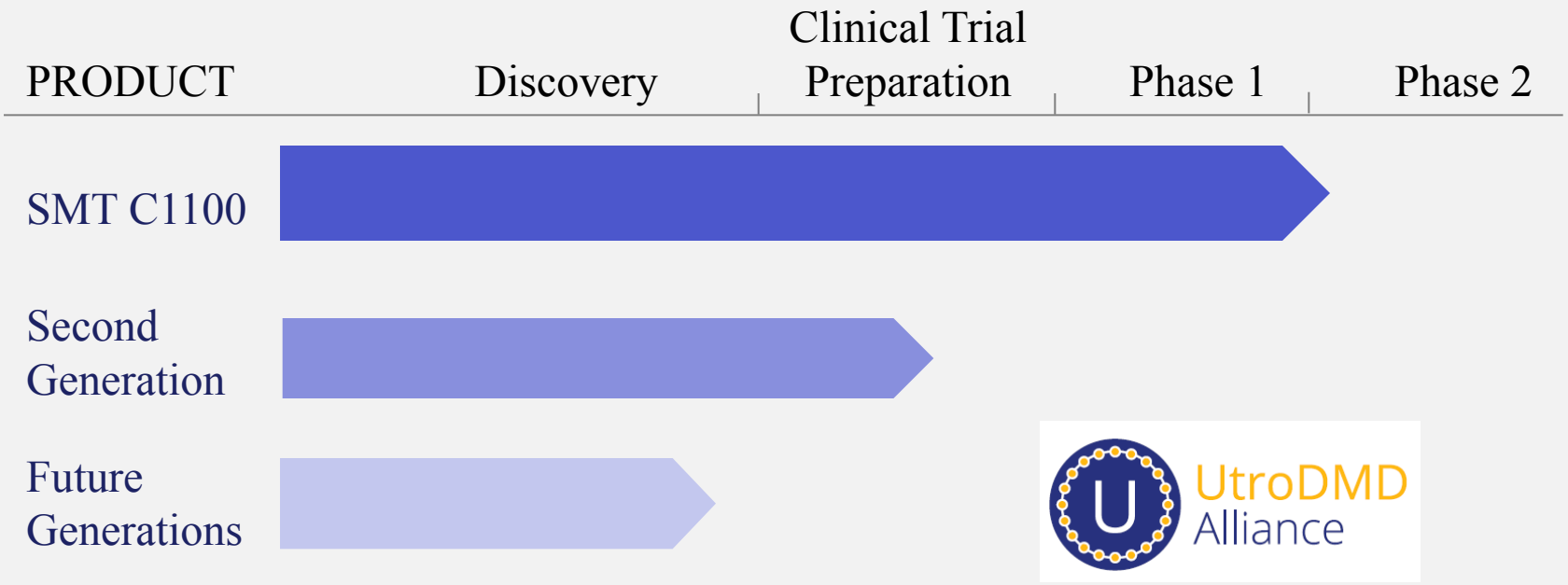


- ***The modulation of utrophin is independent from regeneration and change in fiber-type composition,***
- ***In skeletal muscle, SMT022357 conducts to a similar increases in fiber-type I (SOL, 1.9x) and IIb (EDL, 1.8x) muscle,***

- ***Utrophin localized along the entire length of the membrane with SMT022357 treatment.***

Utrophin Modulation Development Pipeline

- Disease modifying approach
- Potential to treat all DMD patients regardless of the dystrophin mutation



Milestones in DMD Research

- **1981** — Prenatal diagnosis using DNA markers
- **1986** — Identification of the dystrophin gene
- **1980-1999** — Introduction of dystrophin into muscle using viral vectors
- **1996** — Demonstration that utrophin may compensate for dystrophin
- **2004** — Initial clinical trials with gene delivery (plasmid)
- **2016** — Clinical trials viral delivery, exon skipping, read through of stop codons, utrophin modulation

Newborn screening for DMD- is now the right time?

Effective treatment

No effective treatment

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DEPARTMENT OF CHEMISTRY

MRC Functional Genomics Unit

- *Staff of the Animal House*
- *The mice*



- *All the DMD patients and their families*

MRC Functional Genomics Unit

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MDA Muscular Dystrophy Association www.mdausa.org

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Getting closer to an effective treatment for DMD

Very exciting time in science!

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