Muscle-specific CRISPR/Cas9 dystrophin gene editing

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Gene Therapy for DMD/BMD

- **Goal:** Develop methods to *replace* or *repair* dystrophin gene

- **Gene replacement:** AAV/micro-dystrophin

- **Gene editing:** CRISPR/Cas9
  - Must be adapted for each mutation
  - Can lead to more functional dystrophins – *Depending on the mutation*
  - Best way to deliver remains uncertain

- Gene replacement with micro-dystrophins – in human trials
- Gene editing with CRISPR/Cas9 - future potential?
**Pros and cons of micro-dystrophins**

- Clinical trials of systemic AAV/µDys delivery have begun by 3 groups (Solid Biosciences, Jerry Mendell/Sarepta, Pfizer)

- Micro-dystrophins can be delivered bodywide with AAV; One treatment lasts for years; Highly protective against muscle wasting; One vector for all patients

- However, micro-Dys is about one-third the size of the full protein

- Not fully functional, size constraints limit delivery all domains of the protein

- **Gene editing could produce larger and more functional dystrophins**
Potential for gene editing in DMD?

CRISPR/Cas9 mediated gene editing:

- Dystrophin gene editing: Produce a dystrophin missing parts of the normal protein
  - Mutation modification - to make a highly functional ‘mini-dystrophin’
  - Some mutations can generate sub-optimal dystrophins (stability/function)
- Dystrophin gene repair: Requires insertion of a new piece of DNA
  - Potential to make completely normal dystrophin (M. Spencer)

Our focus:

- Restrict Cas9 nuclease expression to muscle cells
  - Prevent gene editing in non-muscle and dividing cells
  - Minimize immune response vs bacterial Cas9
  - Prevent induction of cancer from ‘off-targeting’
- Develop editing/repair methods for multiple types of mutations
Making proteins only in muscle using MCK

- The M-CK gene is only active in muscle
- Inactive in dividing immune and cancerous cells
- Powerful on/off switches developed by Steve Hauschka (U Washington) being used in all 3 µDys trials
  - CK6/ MHCK7/ CK8/ tMCK etc

- Adapt to CRISPR/Cas9?
Injection of AAV6:CK8-Cas9 into mdx\textsuperscript{4cv} muscle

Exon skipping (2 exons)  
Gene repair  
none

Dystrophin Staining

Up to 25% normal dystrophin levels (variable)

Niclas Bengtsson et al, Nat Comm 2017
Dystrophin production following *bodywide* delivery of AAV-CK8-CRISPR/Cas9

Removal of 2 exons: <10% normal amounts of dystrophin
AAV-mediated dystrophin expression: \( \muDys \) vs CRISPR/Cas9

- canine DMD model, removal of 3 or 4 exons

AAV/CRISPR-Cas9 works similarly in the canine model of DMD
Muscle-specific dystrophin gene editing

- No Gene editing in non-muscle cells
- Production of large dystrophins (depends on mutation)
- Works moderately well in mice and large animals

Room for improvement:
- Muscle stem cell targeting is inefficient (i.e. not permanent)

Safety concerns
- Editing methods are possibly linked to immune rejection (and cancer?)
- Problems with unwanted editing of other genes

Need to limit persistence of the gene editing machinery
- Short duration (non-AAV delivery methods are needed)

Ultimate goal is to improve the efficiency of complete gene repair
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