CRISPR/Cas9 based therapies for Duchenne muscular dystrophy

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CRISPR/Cas9 gene editing

- Cut and replace with new piece of DNA
- Cut and seal up without replacement
General principles of CRISPR/Cas9 editing

Flag = guide RNA

Scissors = Cas9, cuts DNA
How It Works- Puts the Puzzle Back Together

Non-mutated DNA

Duchenne DNA

Mutation=puzzle pieces do not fit together (no dystrophin)

Treated Duchenne DNA

After CRISPR=puzzle pieces fit together (dystrophin made)
Dystrophin protects the muscle membrane

- **Dystrophin**
- **glycoprotein complex (DGC)**
- **laminin**
- **sarcolemma**
- ** ECM**
- **sarcoglycans**
- **actin**
- **nNOS**
- **Healthy**
- **DMD**
- **BMD**

Images show localization of Dystrophin/DAPI in different muscle conditions.
DMD exons 45-55 represents a hot spot of mutations

Cartoon courtesy of Sarepta webinar
CRISPR/Cas9 to delete exons 45-55

Out-of-frame

Intron 44 gRNA

~708kb

In-frame

After deletion

Intron 55 gRNA

Young et al, Cell Stem Cell, 2016
Young et al, J. Neuromuscular Dis, 2017
Proof of principle that CRISPR platform functions *in vivo*

Normal mouse muscle

Untreated hDMD mouse

Treated hDMD mouse

Dystrophin is shown in red

hDMD del45/mdx

Human=null
Mouse=null

AAV generated by Jeff Chamberlain, UW
AAV-CRISPR mediated dystrophin expression in mdx heart 5 weeks after systemic injection

AAV CRISPR
Heart
31% dys+

PBS ctrl
Heart

Dystrophin
Revertant dystrophin
48/50

Dystrophin western

PBS AAV Ladder (HiMark) hDMD (wt)/mdxD2

Dystrophin
Ponceau S (loading)

AAV generated by Jeff Chamberlain, UW
How does gene therapy with CRISPR/Cas9 differ from AAV microdystrophin?

• AAV-microdystrophin involves the use of a virus to deliver an extra copy or more of a miniaturized dystrophin.
  • Does not integrate-stays in the nucleus.

• CRISPR/Cas9 leads to permanent changes to the DNA.
Issues to consider in AAV mediated delivery of CRISPR/Cas9

• Small capacity of AAV (~4.7kb);
  • Sp Cas9 needs two vectors, which impacts the vector dose;
• Immune response to virus may prevents re-administration of the same serotype;
• Immune response to Cas9 will trigger myositis (muscle inflammation);
• AAV has been reported to integrate into the cut site.
Clinical status of nanomedicine

- First nano-carrier was FDA approved in 1995 (liposomal doxorubicin)

- >50 approved nanomedicines and >75 in clinical trials

(Bobo et al 2016)
Benefits of nanoparticles

- Can be modified to increase functionality
- Can be targeted
- Many are biodegradable
- Can be re-administered
- Colloidal stability (stability in blood)
- Crossing endothelial barrier
- Cellular Trafficking
Summary

1. CRISPR platform that targets 50% of patients is functional *in vitro* and *in vivo*;

2. We are developing methods of direct delivery as well as cellular engraftment to translate the platform;

3. Nanoparticles can be iteratively optimized for systemic delivery in vivo.