Peptide-targeted polymers for Duchenne muscular dystrophy gene therapy

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Advanced Delivery Science

Outline

- Background
- Approach
- Results
- Summary
- Acknowledgments

Duchenne Muscular Dystrophy (DMD)

- X-linked recessive mutation in *DMD* gene
- First signs in 2-5 y males
- Prevalence 1 in 3500 male births
- Causes decreased amount or absence of dystrophin
  - Links cytoskeleton to ECM
- Patients have short life expectancy and poor quality of life

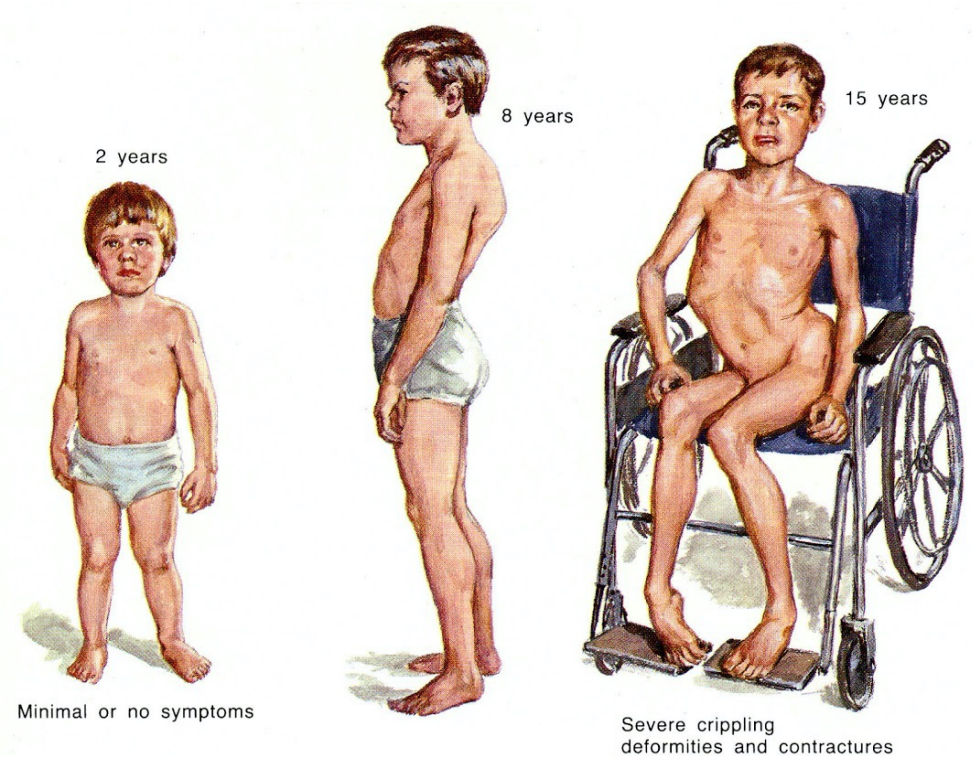
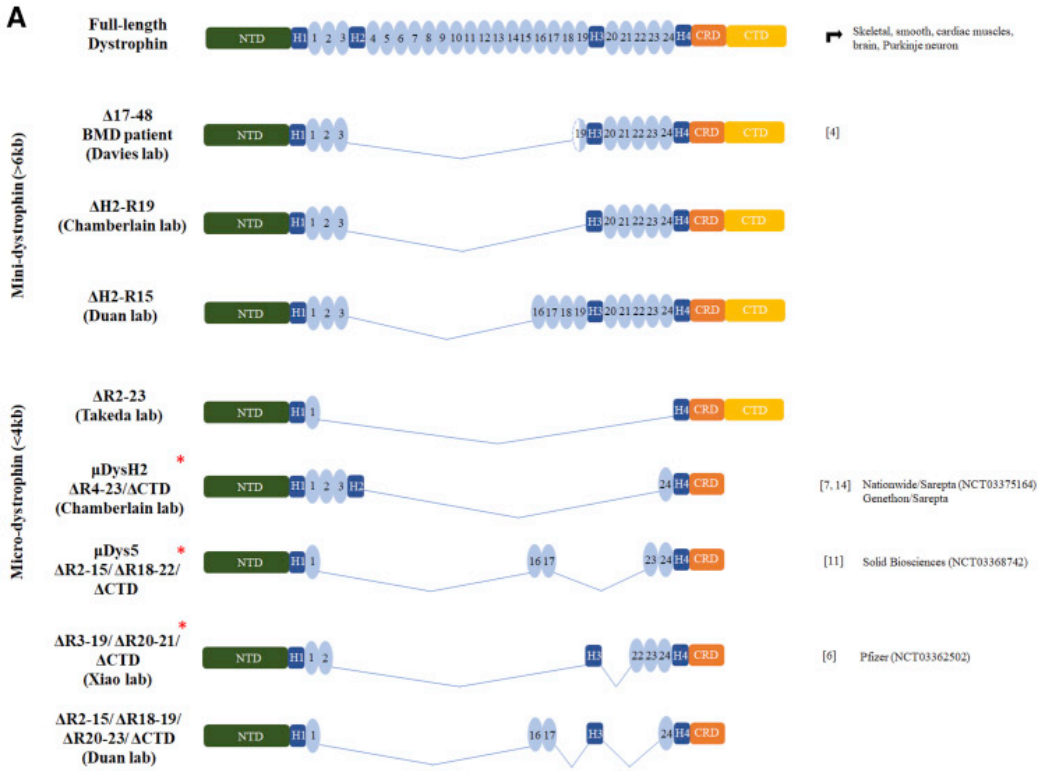


Image by F. Netter, MD

DMD has no cure, but current therapies include:

- Physical therapy and braces
- Ventilatory support
- Glucocorticoids
- Gene therapies
  - Exon skipping (Eteplirsen)
  - Gene Replacement using *microdystrophin*

Image from Hopkins Medicine

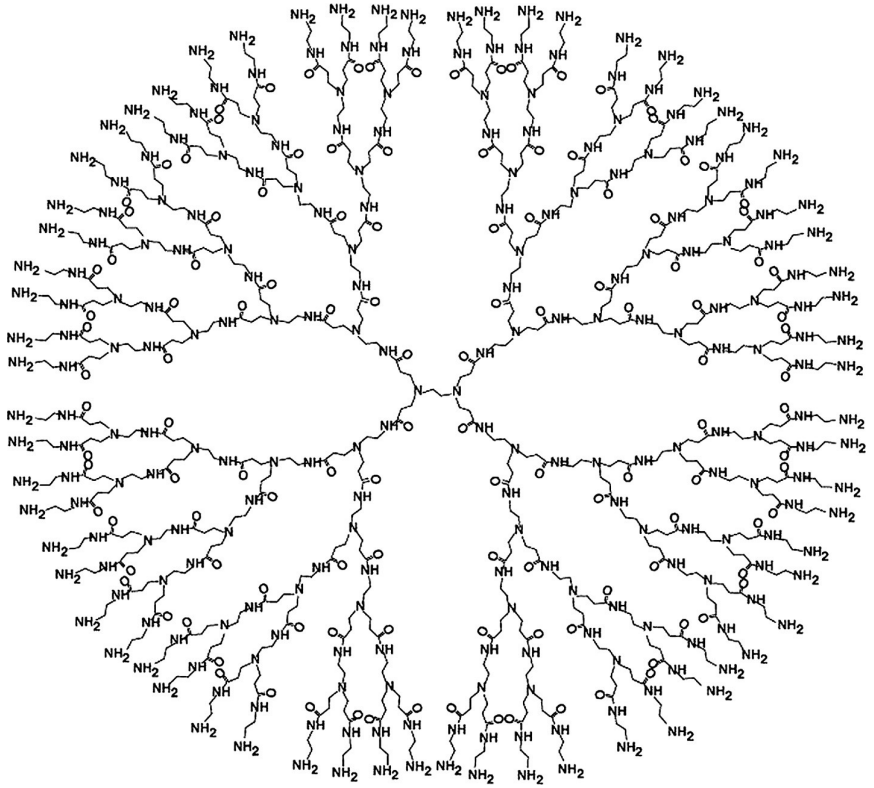


Davies et al, 2019

Non-Viral Delivery Vehicles

- Cationic polymers and liposomes
- Electrostatically bind genes
- Protect from enzymatic degradation
- Improve cellular uptake
- Promote endosomal escape
- Minimize immunogenicity

Polyamidoamine (PAMAM) dendrimers

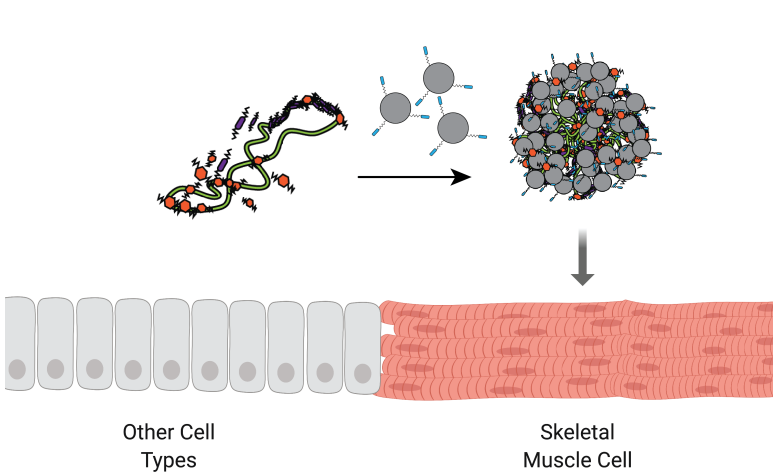


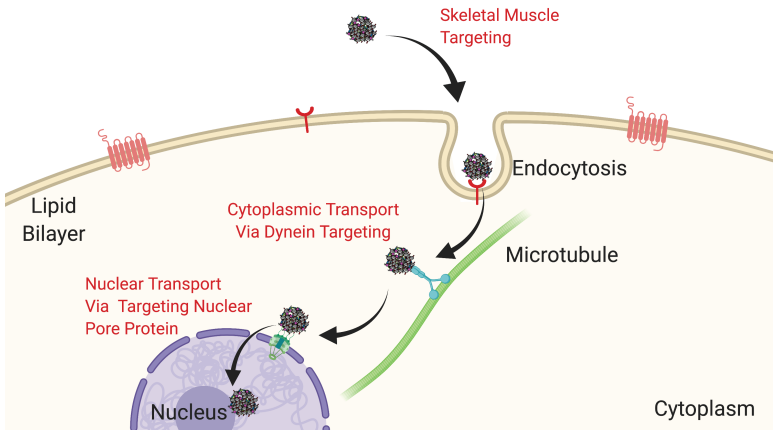
Kesharwani et al, 2015

Targeting Delivery Barriers with Functional Peptides

Barriers to Gene Delivery

1. Specific cell targeting
2. Cellular uptake
3. Intracellular transport
4. Nuclear delivery



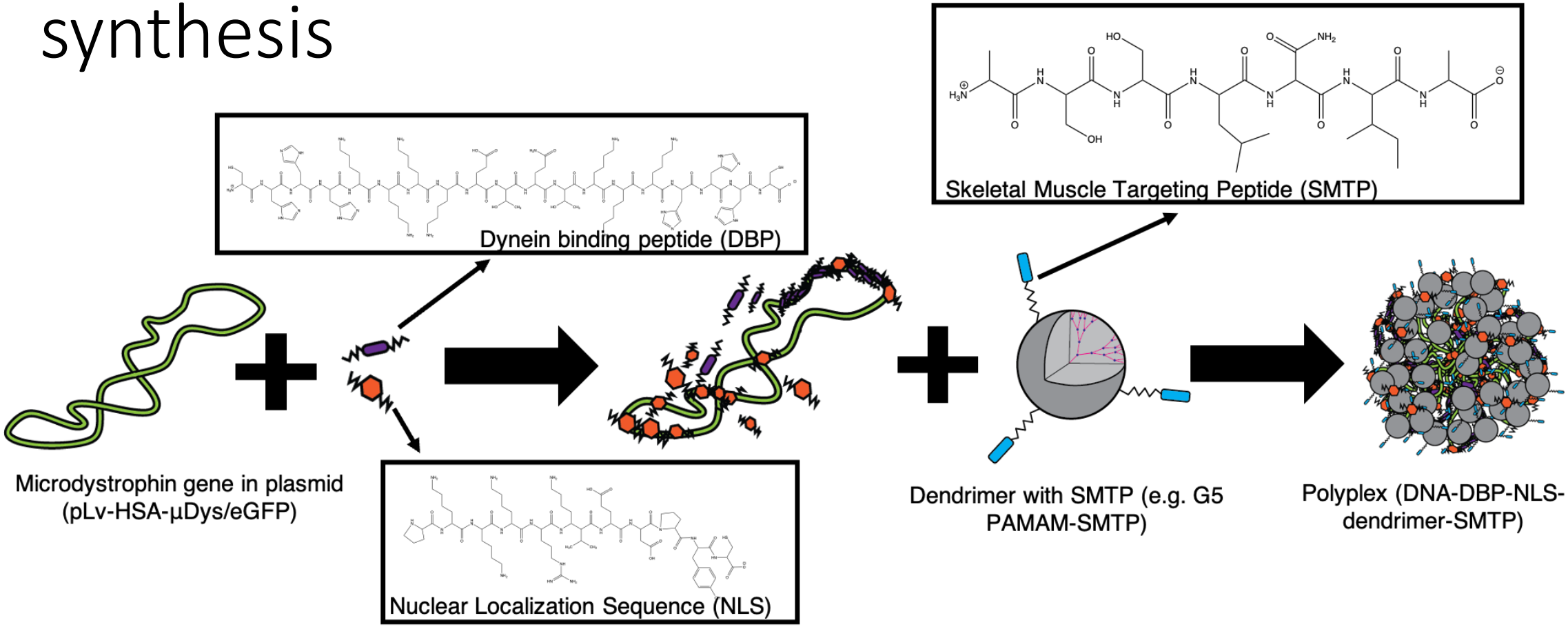


Hersh et al, 2021

Approach

Develop a non-viral nanocarrier that targets barriers to gene delivery through the incorporation of functional peptides to improve the delivery efficiency of the µDys gene

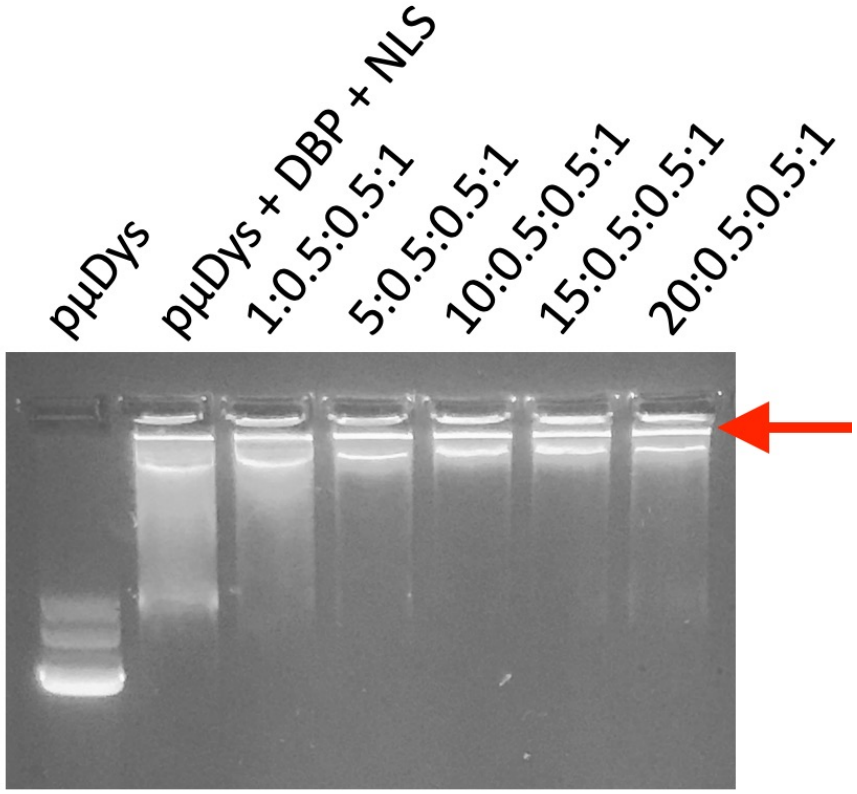
G5 PAMAM-SMTP-DBP-NLS-*pμDys* polyplex synthesis



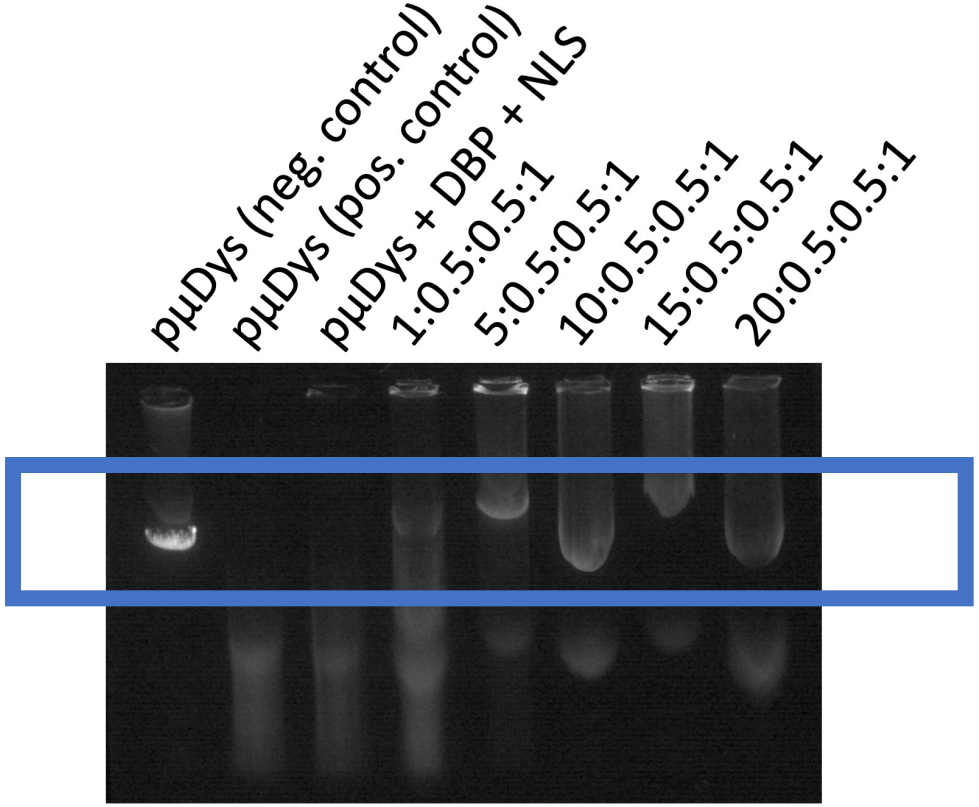
Charge Ratio G5 PAMAM-SMTP : DBP : NLS : pμDys

Hersh et al, 2021

Polyplexes bind and protect *pμDys*



pDNA bound to dendrimers remains in wells

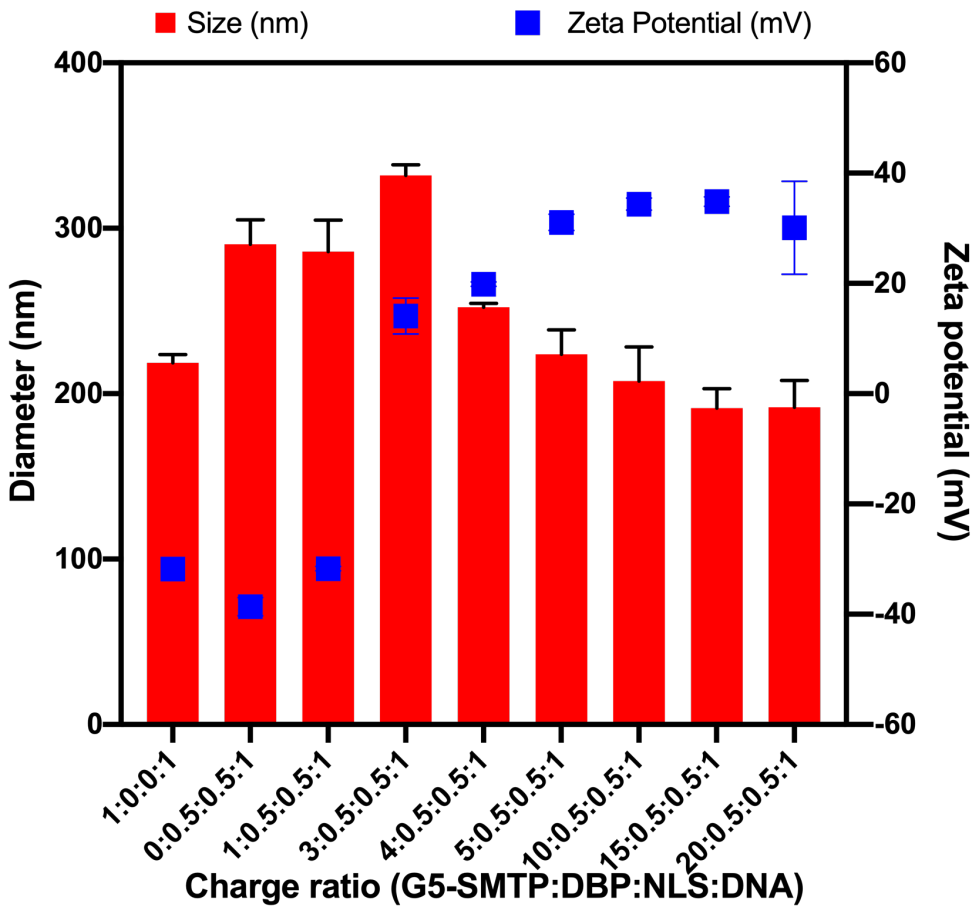


Protected pDNA leaves streak at top of gel

Hersh et al, 2021

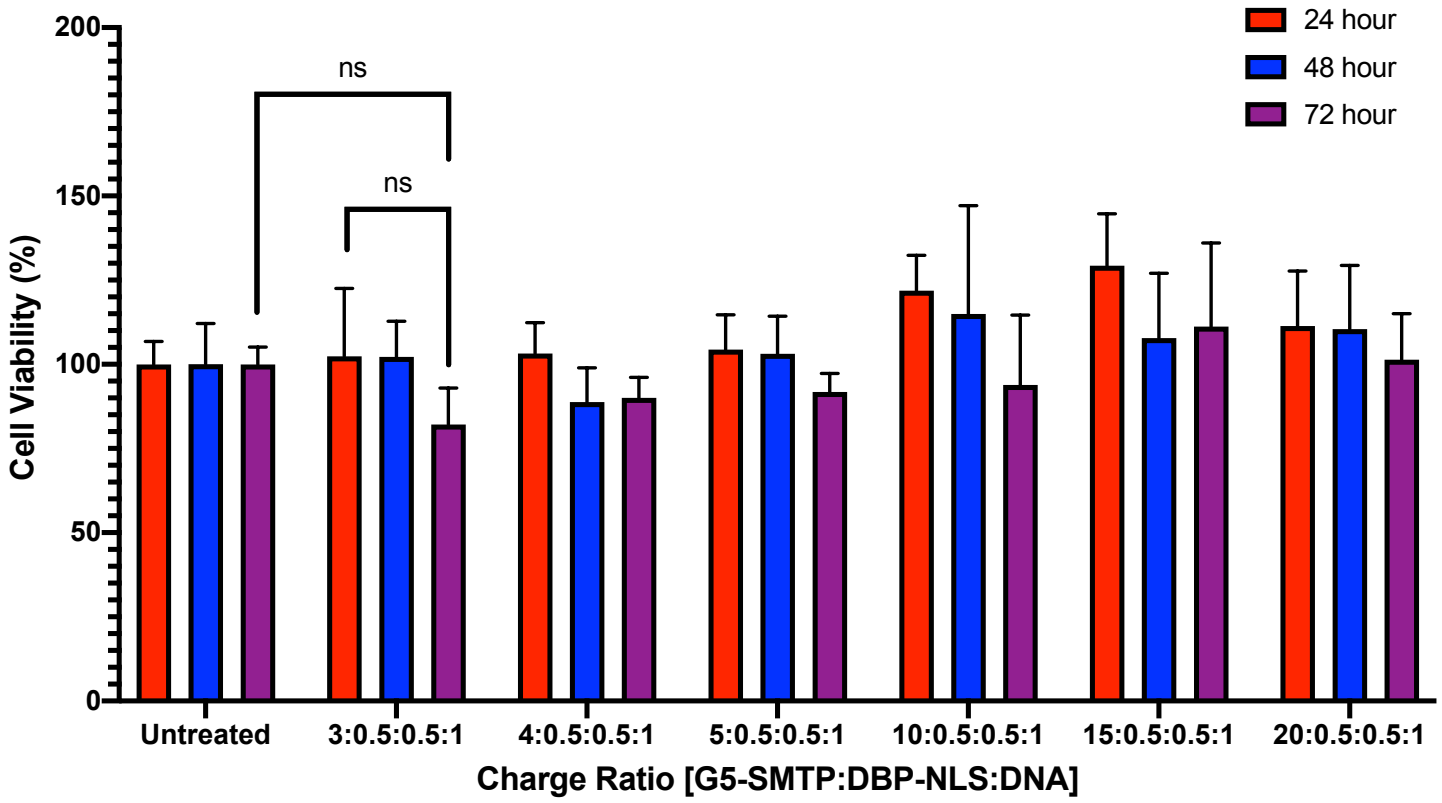
Polyplexes form at ideal size and surface charge

- Stability in zeta potential at charge ratios above 5:1 (PAMAM:pDNA)
- Target sizes at charge ratios above 5:1 (PAMAM:pDNA)



Hersh et al, 2021

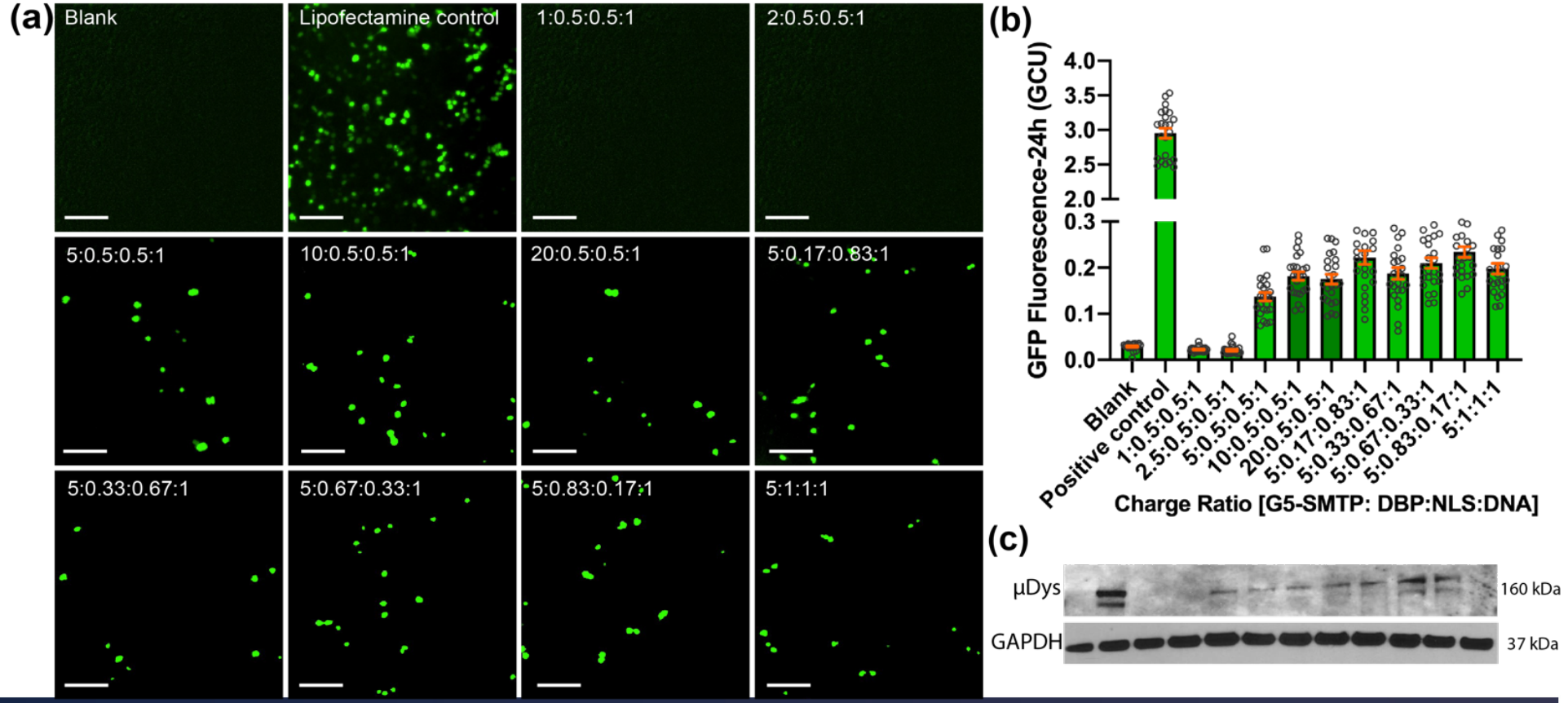
Polyplexes are non-toxic to skeletal muscle cells



- No significant toxicity for any charge ratio at any time point
- Toxicity increases slightly over time

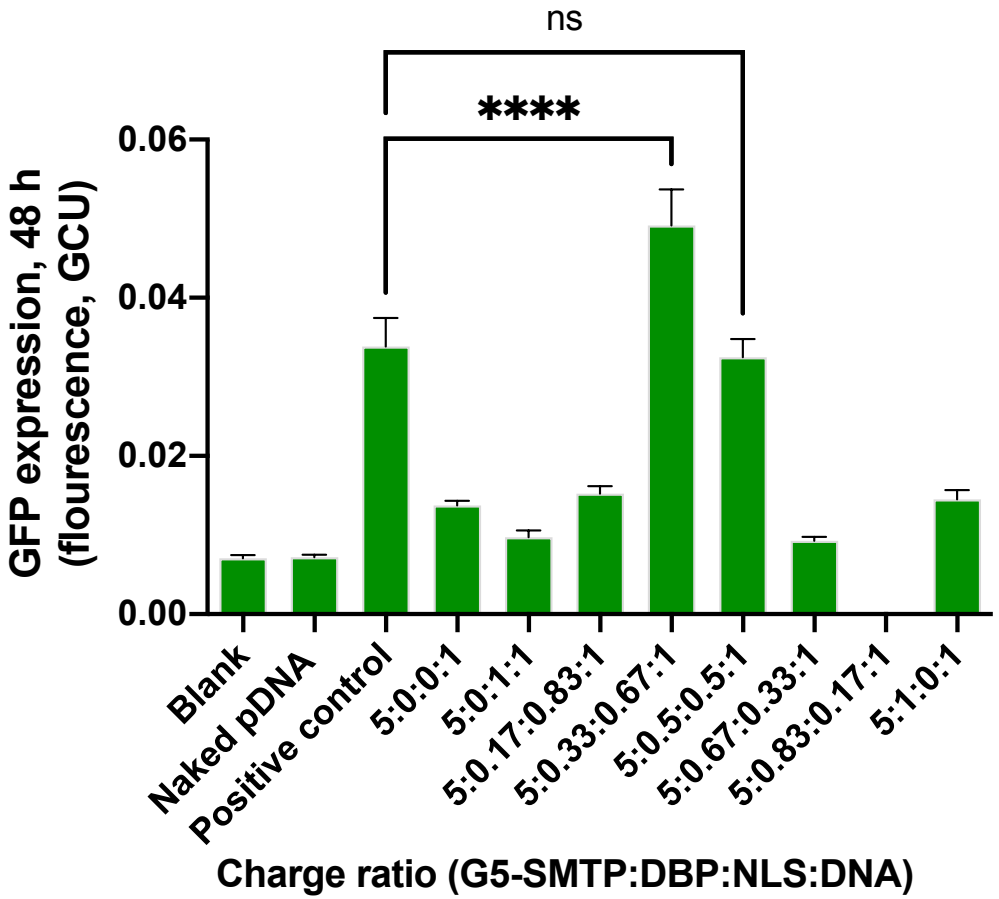
Hersh et al, 2021

Polyplexes can deliver *pμDys* to HEK 293T cells



Hersh et al, 2021

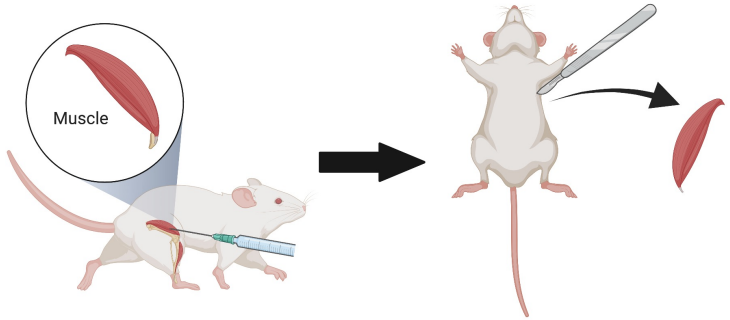
Polyplexes can deliver *pμDys* to C2C12 cells

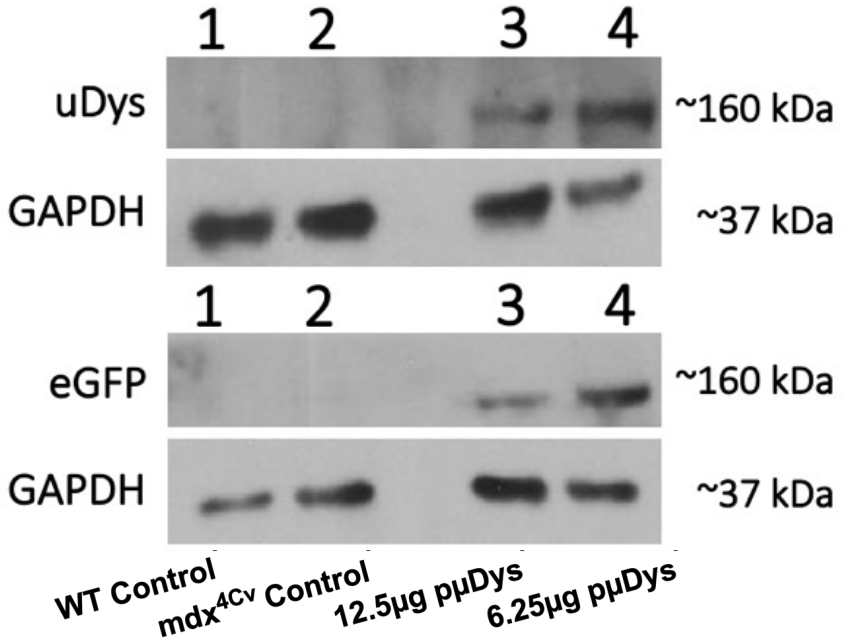


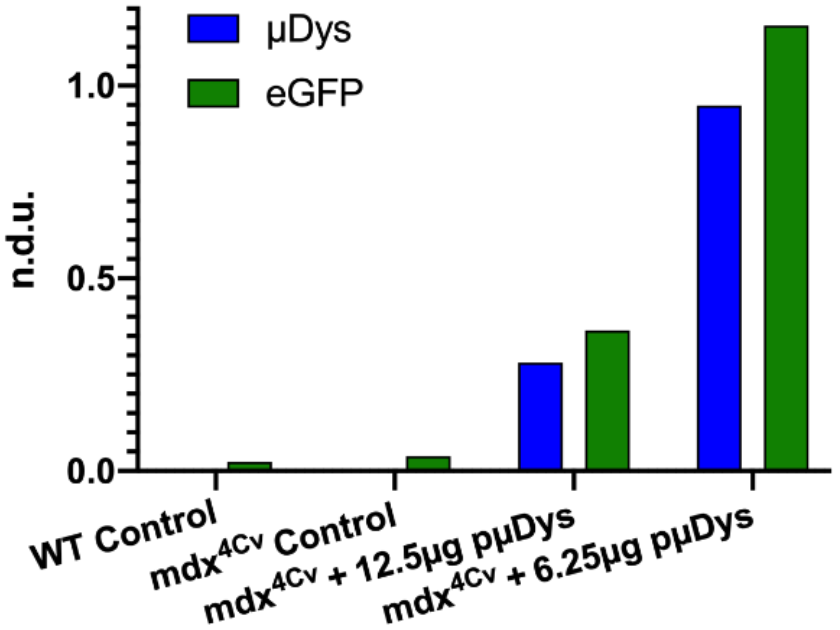
- Polyplex can transfect at levels greater than that of the positive control

Hersh et al, 2021

Polyplexes can induce *in vivo* protein expression







Hersh et al, 2021

Summary

- Developed peptide-functionalized non-viral nanocarrier gene delivery vehicles
- Capable of *in vitro* transfection in cells of interest
- Capable of *in vivo* transfection in DMD mouse model
- Potential delivery vehicle for *µDys* gene replacement therapy

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References:

1. Davies KE, Guiraud S. Micro-dystrophin Genes Bring Hope of an Effective Therapy for Duchenne Muscular Dystrophy. Mol Ther. 2019;27(3):486-488. doi:10.1016/j.ymthe.2019.01.019

2. Kesharwani P, Banerjee S, Gupta U, Amin M, Padhye S, Sarkar F, Iyer A. PAMAM dendrimers as promising nanocarriers for RNAi therapeutics. Mat Today. 2015 18(10):565-572. doi.org/10.1016/j.mattod.2015.06.003.

3. Hersh J, Condor Capcha JM, Iansen Irion C, Lambert G, Noguera M, Singh M, Kaur A, Dikici E, Jiménez JJ, Shehadeh LA, Daunert S, Deo SK. Peptide-Functionalized Dendrimer Nanocarriers for Targeted Microdystrophin Gene Delivery. Pharmaceutics. 2021 Dec 15;13(12):2159. doi: 10.3390/pharmaceutics13122159.