CRISPRing Humans: Why haven't we cured all genetic diseases?

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AND

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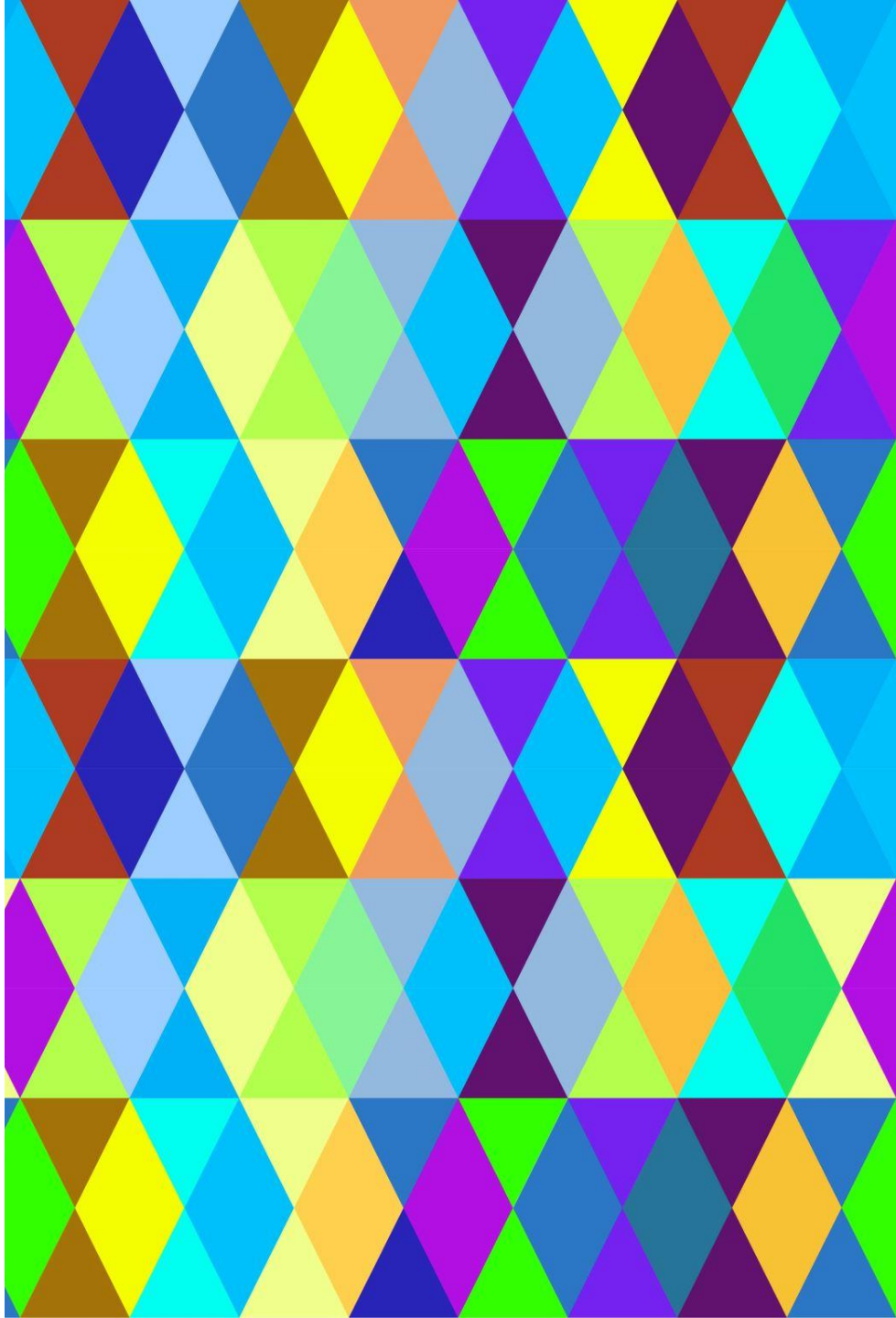
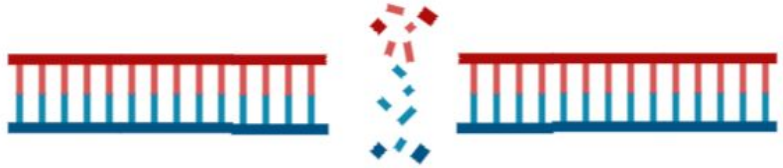


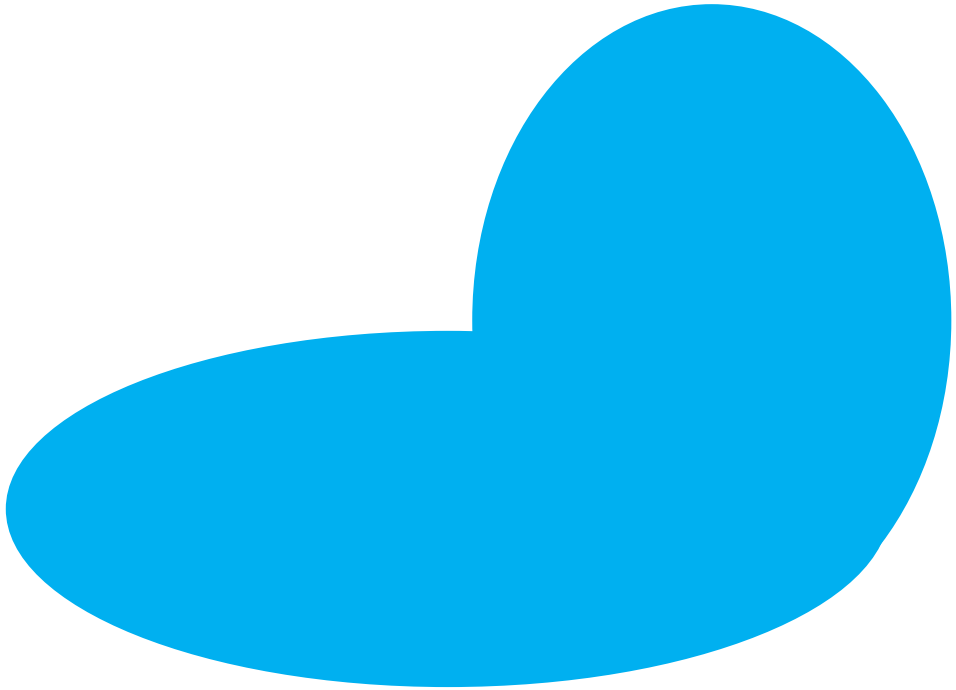
What is the CRISPR/Cas9 system?

Cas9 is an enzyme that causes double stranded breaks in DNA (endonuclease)

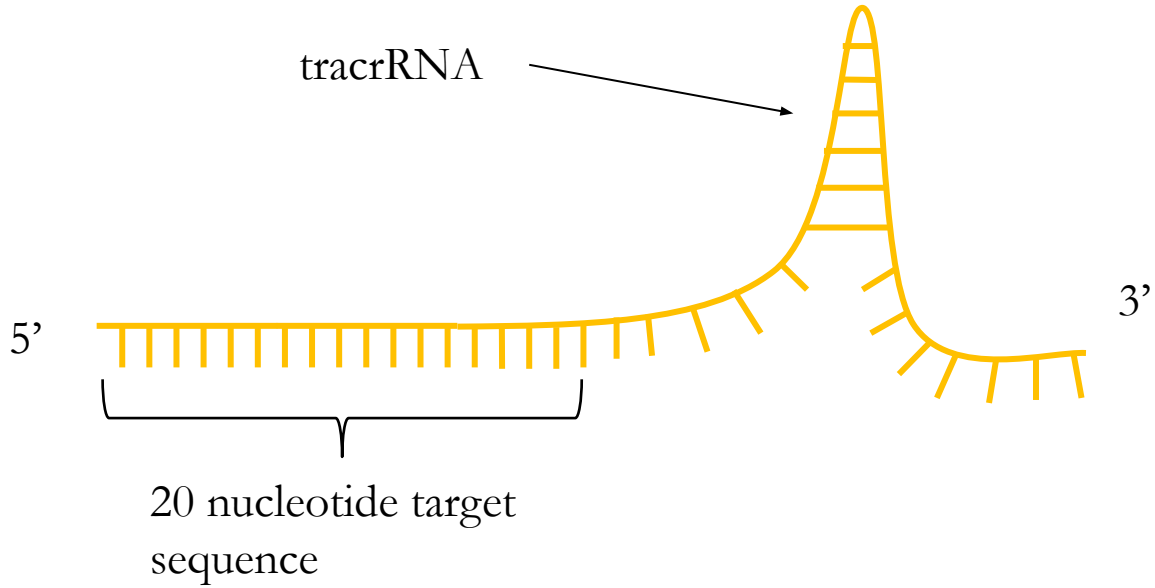


Source: https://international.neb.com/tools-and-resources/selection-charts/alphabetized-list-of-recognition-specificities

What is the CRISPR/Cas9 system?

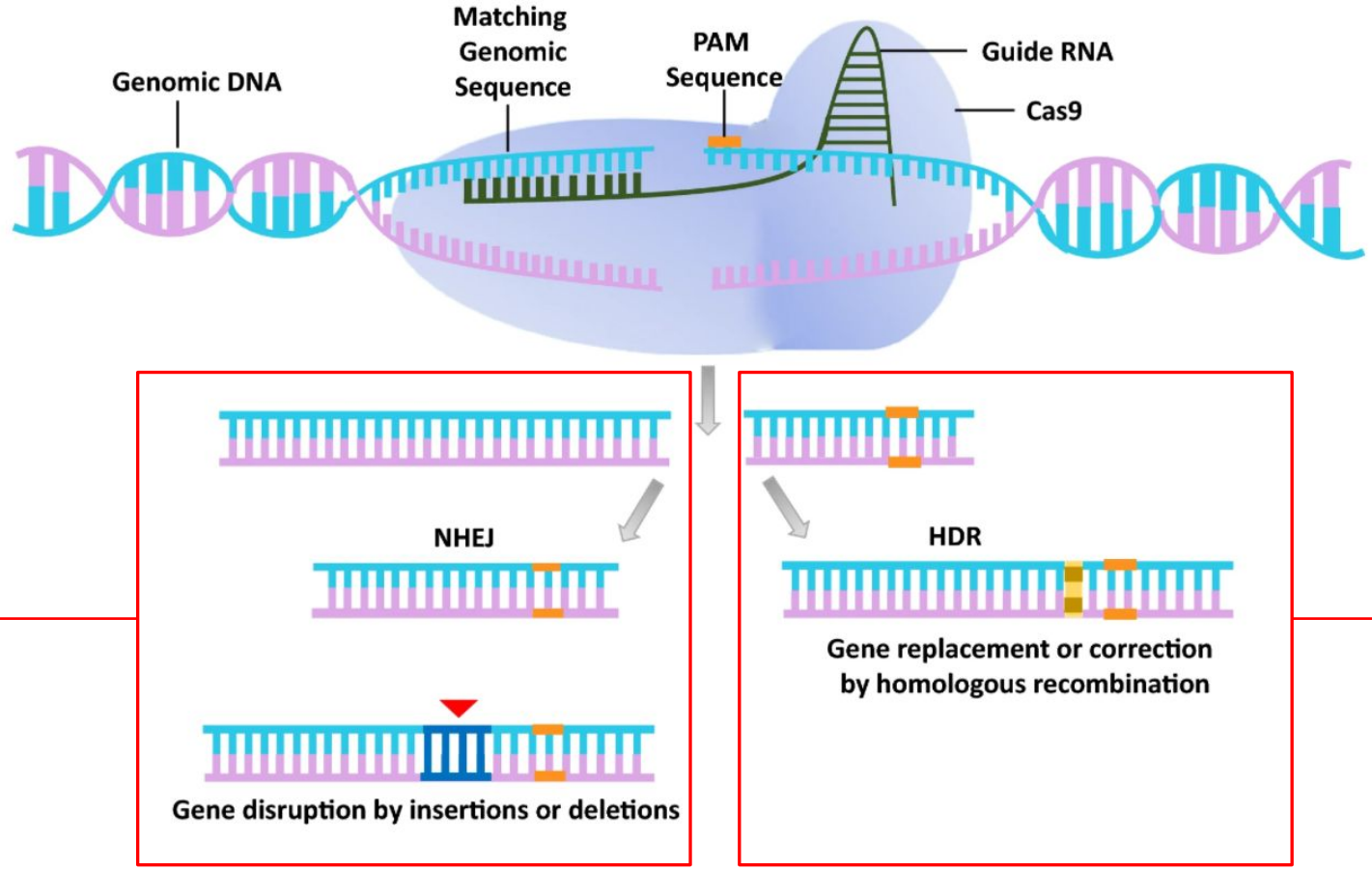


Cas9 nuclease



Single-guide (sg) RNA

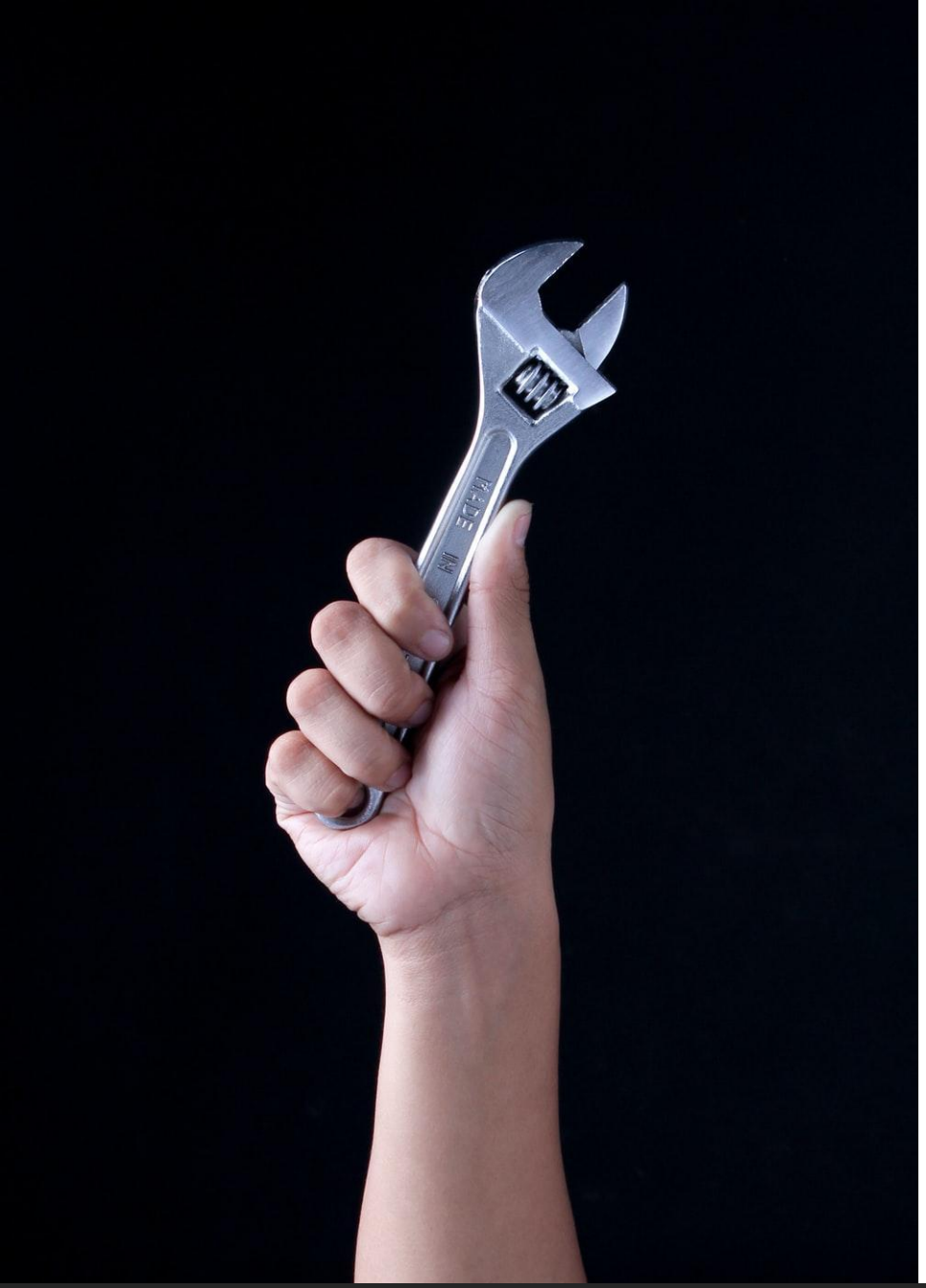
What is the CRISPR/Cas9 system?



Gene knockout

Repairing a mutated gene

Source: Ghosh, D., Venkataramani, P., Nandi, S. *et al.* CRISPR–Cas9 a boon or bane: the bumpy road ahead to cancer therapeutics. *Cancer Cell Int* **19,** 12 (2019). https://doi.org/10.1186/s12935-019-0726-0:

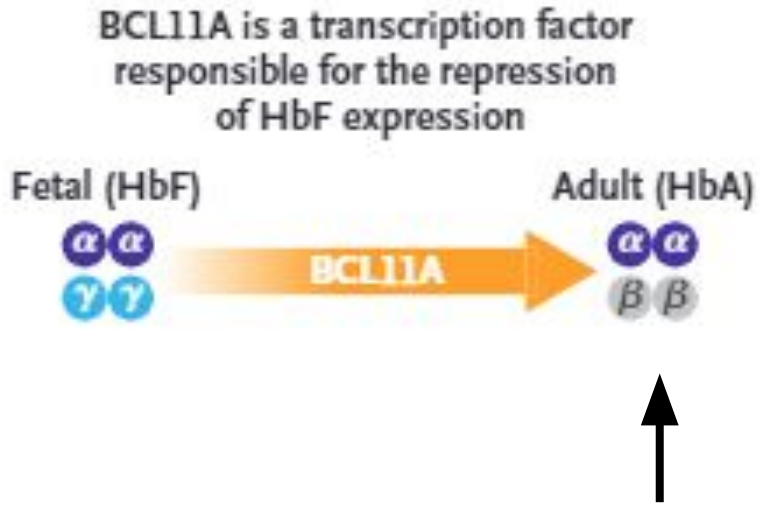


Potential of CRISPR/Cas9

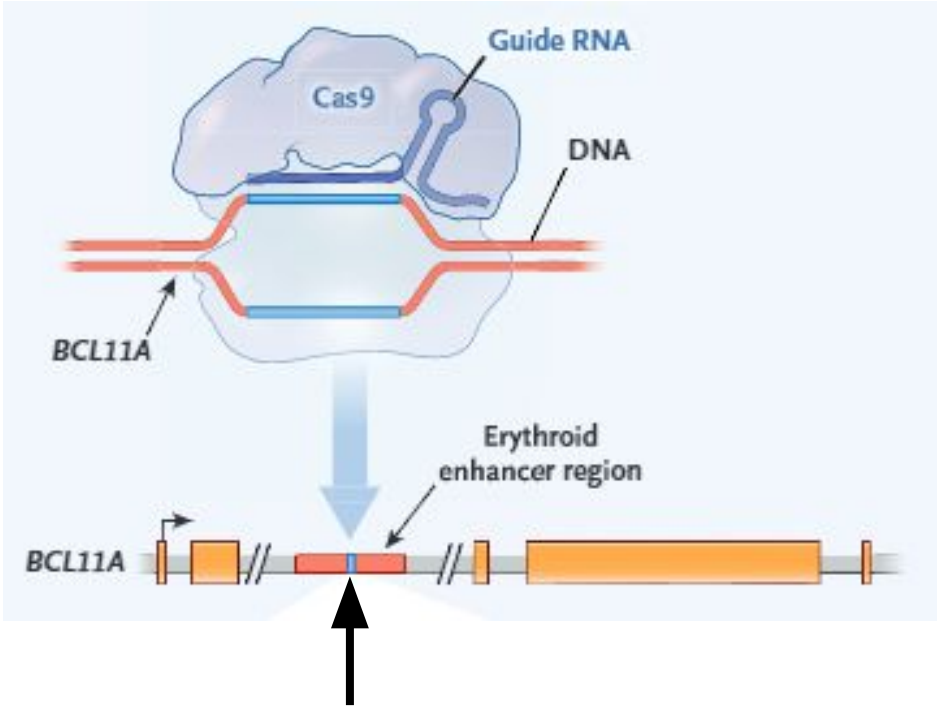
- Over 3,000 mutations have been linked to disease phenotypes in humans (Cox et al. 2015)

- CRISPR/Cas9 can repair problematic genes

Clinical Potential – Sickle Cell Disease (SCD)



More HbA = worse symptoms



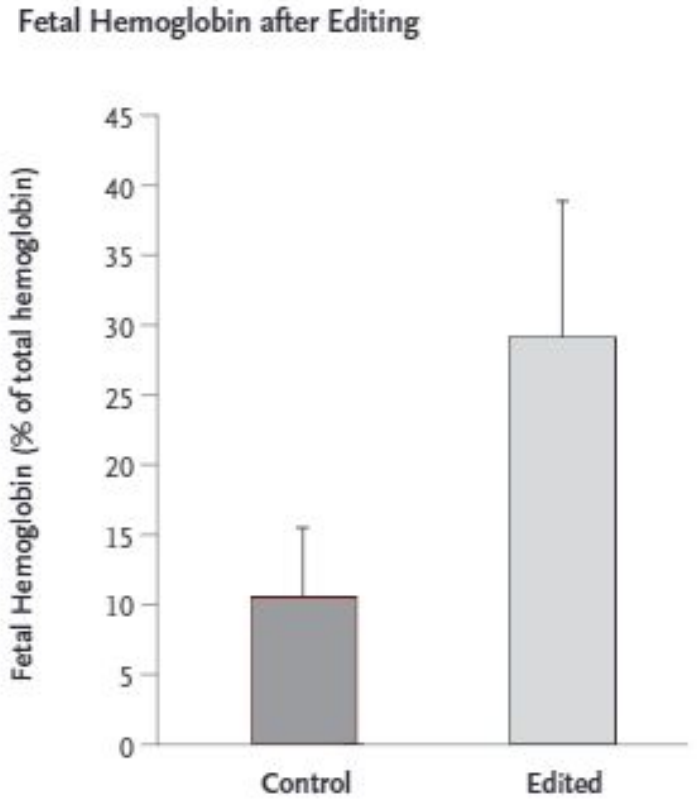
Knockout enhancer to lower expression of *BCL11A*

Study + Source: Frangoul, H., Altshuler, D., Cappellini, … Corbacioglu, S.. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. *New England Journal of Medicine*, *384*(3), 252–260. https://doi.org/10.1056/nejmoa2031054

Gene Editing Success for SCD

- Lowering expression of *BCL11A* through enhancer knockout worked

- A higher percentage of fetal hemoglobin alleviated symptoms



Genetic Diseases are Costly

- Pediatric patients were estimated to be charged between $14 to $57 billion for treating genetic diseases in 2012 (Gonzaludo et al. 2019)

- **Why hasn't CRISPR/Cas9 cured all genetic diseases??**

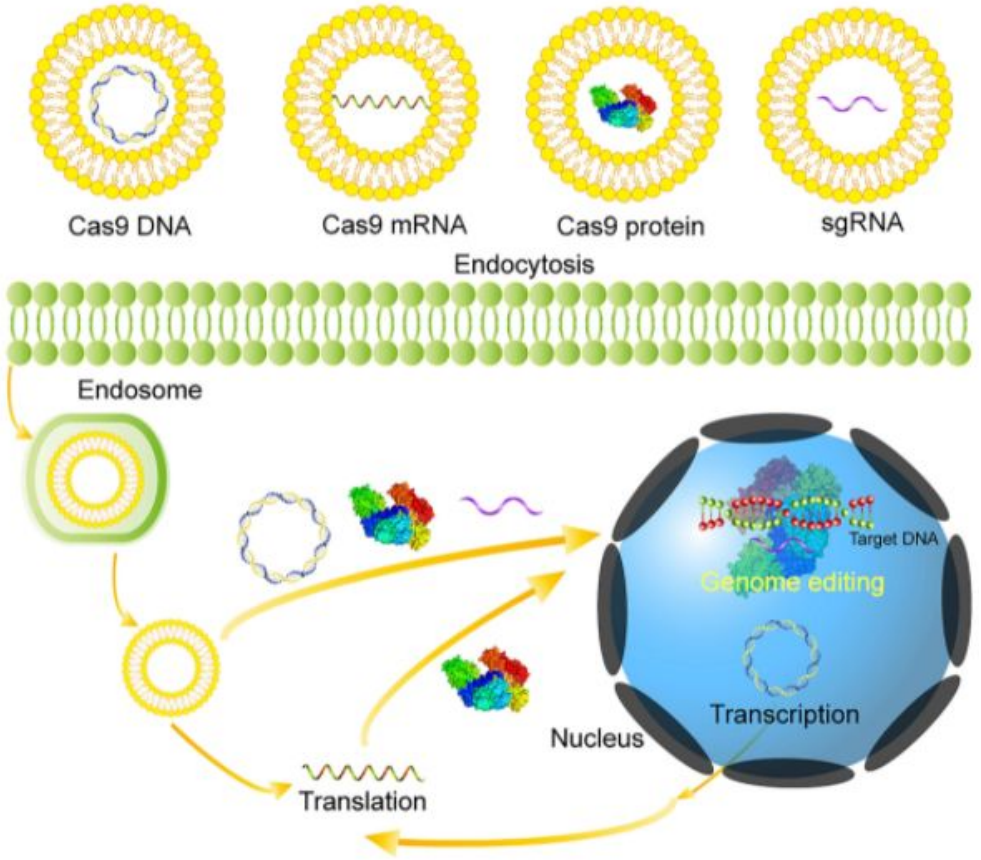
Source: Gonzaludo, N., Belmont, J.W., Gainullin, V.G. *et al.* Estimating the burden and economic impact of pediatric genetic disease. *Genet Med* **21,** 1781–1789 (2019). https://doi.org/10.1038/s41436-018-0398-5

Challenges of Using CRISPR/Cas9 in Humans

1. Lack of safe and efficient delivery systems
2. Off-target effects
3. Ethical considerations

Delivery systems

- How do we deliver in vivo?
- Physical delivery systems
  - Coat the CRISPR/Cas9 plasmid or protein/sgRNA complex so that it can enter the cell
  - e.g. Lipid nanoparticles (LNPs) or gold nanoparticles
- Fewer safety concerns than with viral vectors

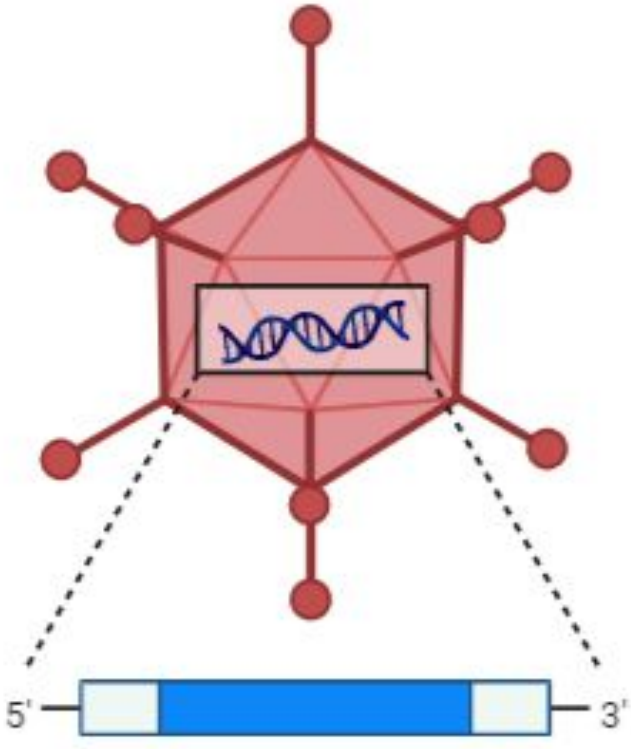


Source: Xu, X, Wan, T, Xin, H, Li, D, Pan, H, Wu, J, Ping, Y. Delivery of CRISPR/Cas9 for therapeutic genome editing. *J Gene Med*. 2019; 21:e3107. https://doi.org/10.1002/jgm.3107

Viral Delivery System

- Viral delivery systems: most efficient systems to deliver plasmid-based nucleic acids to mammalian cells in vitro/in vivo (Lee and Kim 2019)

- e.g. Lentivirus, adeno-associated virus (AAV)

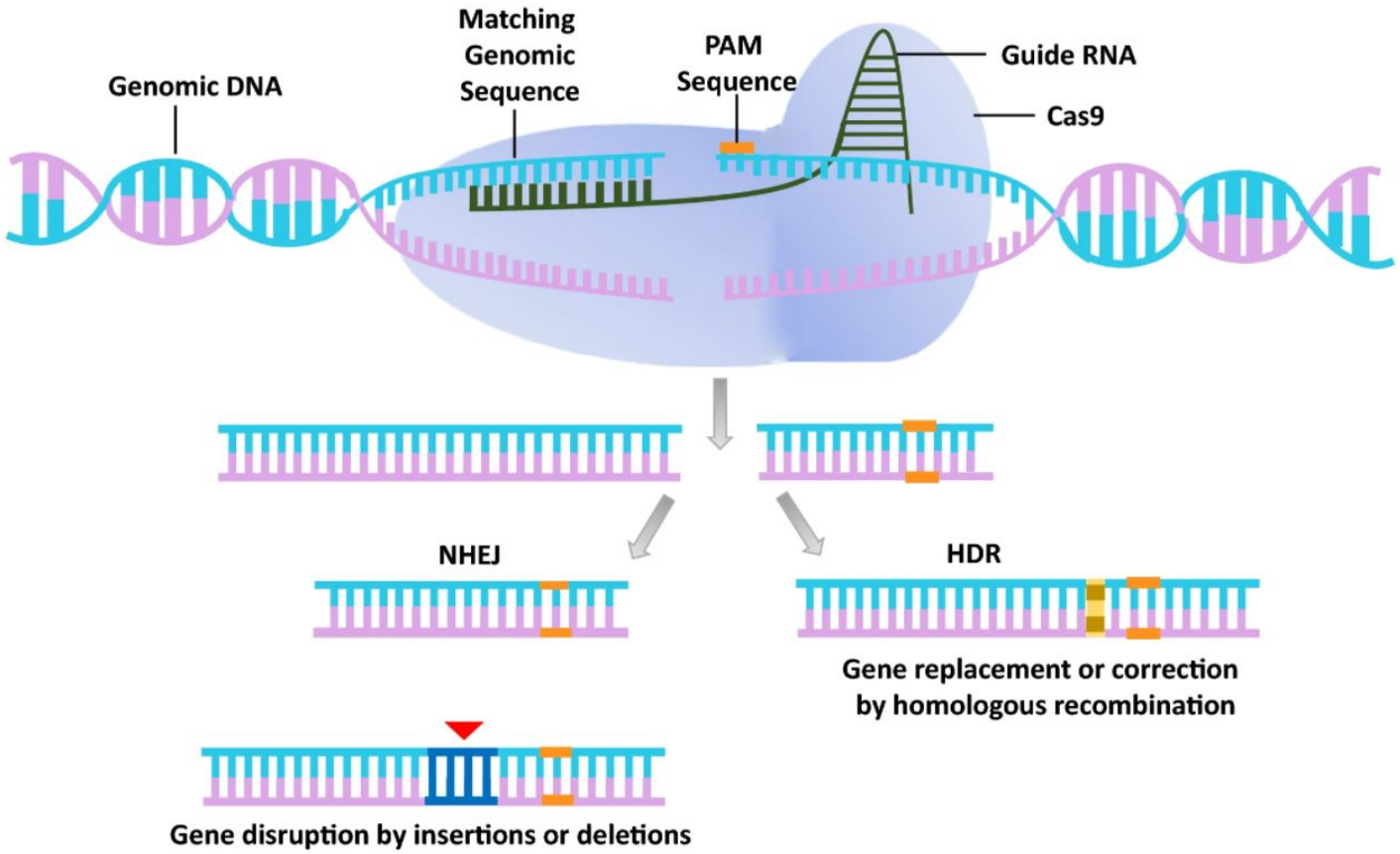


Challenges of Delivery Methods

	Physical Delivery	Viral Delivery
Immune Response	Yes (Lee et al. 2017)	Yes (Lee and Kim 2019)
Delivery efficiency	Poor in vivo (Lino et al. 2018)	Good, but can remain in cell for a long time (Deyle and Russell 2009)
Carrying capacity	No limit	Limited (Lino et al. 2018)

Off-Target Effects

Off-Target Effects



What happens when the wrong target is hit?

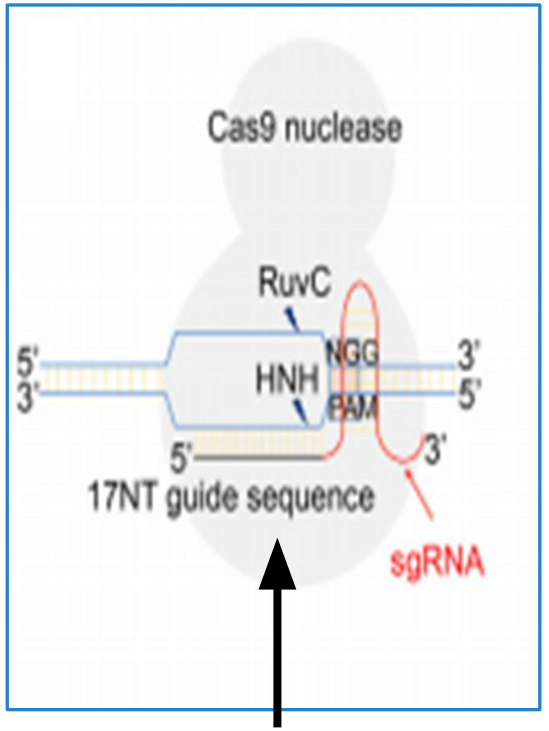
Could CRISPR/Cas9 lead to unintended deleterious effects?

Source: Ghosh, D., Venkataramani, P., Nandi, S. *et al.* CRISPR–Cas9 a boon or bane: the bumpy road ahead to cancer therapeutics. *Cancer Cell Int* **19,** 12 (2019). https://doi.org/10.1186/s12935-019-0726-0:

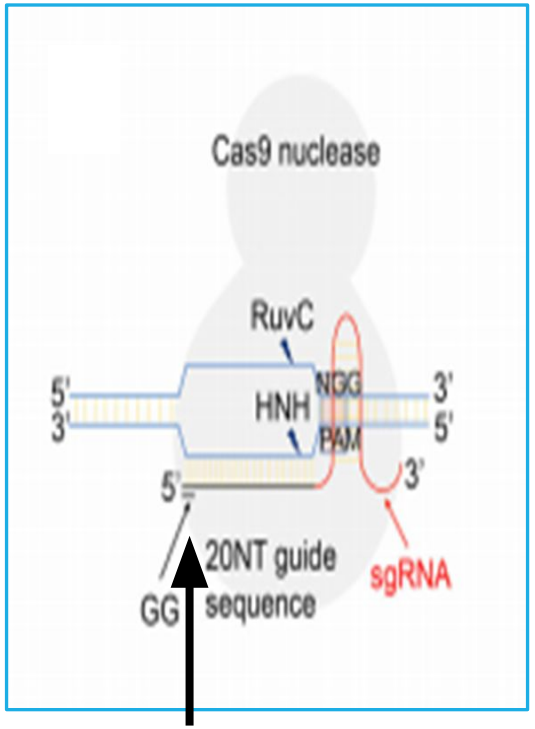
How to Minimize Off-Target Effects

- Alter the sgRNA

1. Shorten the gRNA
2. Add guanine and cytosine nucleotides



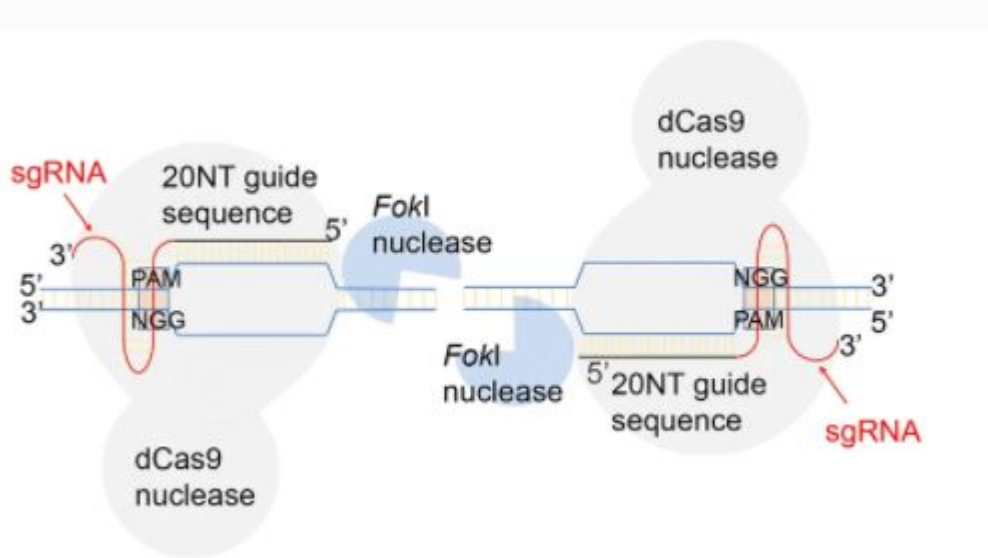
17-18 nucleotides instead of 20-23



Two guanine nucleotides at the 5' end

Minimize Off-Targets by Changing Cas9

- Only cleaves when there are two *Cas9/FokI* complexes next to each other
  - 40 nucleotides instead of 20 for gRNA!
- But, need two PAM sites



Lots of Cas proteins to Choose From!

- Many CRISPR associated protein (Cas) variants/modifications have been researched
  - Some have higher fidelity (fewer off-targets)
  - e.g. SpCas9-HF1, HypaCas9, eSpCas9

Source: Chen, J., Dagdas, Y., Kleinstiver, B. *et al.* Enhanced proofreading governs CRISPR–Cas9 targeting accuracy. *Nature* **550,** 407–410 (2017). https://doi.org/10.1038/nature24268

Ethical Concerns

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What are some ethical concerns of gene editing?

1. Unintended health consequences

Unintended Health Consequences

- Gene therapy was used to cure X-linked Severe Combined Immunodeficiency (SCID-X1) over 20 years ago

- The trial was successful, but lead to the development of leukemia

https://cnx.org/contents/5CvTdmJL@4.4

Source: Howe, Steven J., et al. "Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients." *The Journal of clinical investigation* 118.9 (2008).

Ethical Concerns

What are some ethical concerns of gene editing?

1. Unintended health consequences

2. Inheritance of the gene edit

3. The 'Slippery Slope' argument

Conclusion – What now?

Main barriers for gene editing in humans

I. Delivery systems

II. Minimizing off-targets

III. Regulatory frameworks

 Addressing these challenges may lead to future successful therapeutic applications

Acknowledgements

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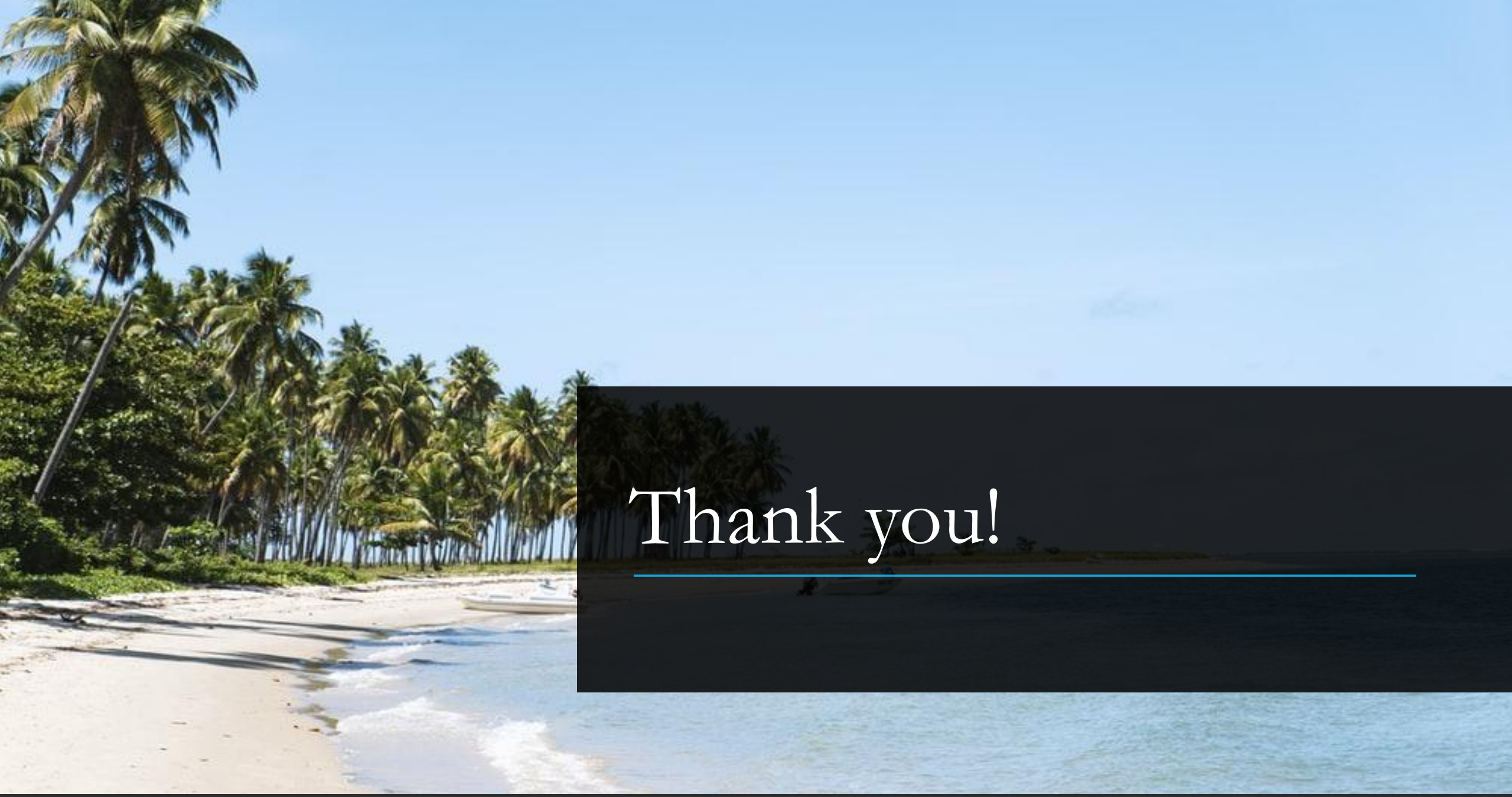

Thank you!