CRISPR-Cas9: Fulfilling the Promise of Gene Therapy

Owen Phillips, MD, MPH
Professor
Department of Obstetrics and Gynecology
UTHSC

Disclosures

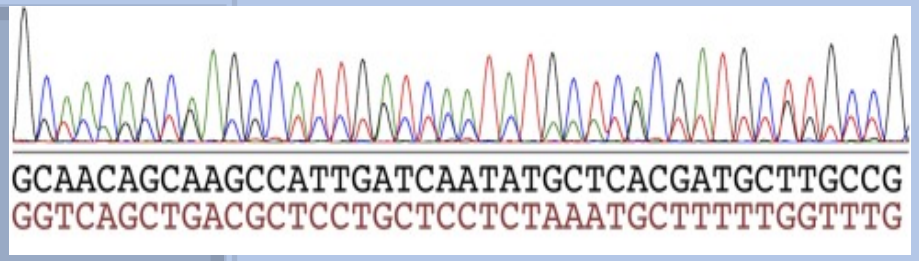
- None

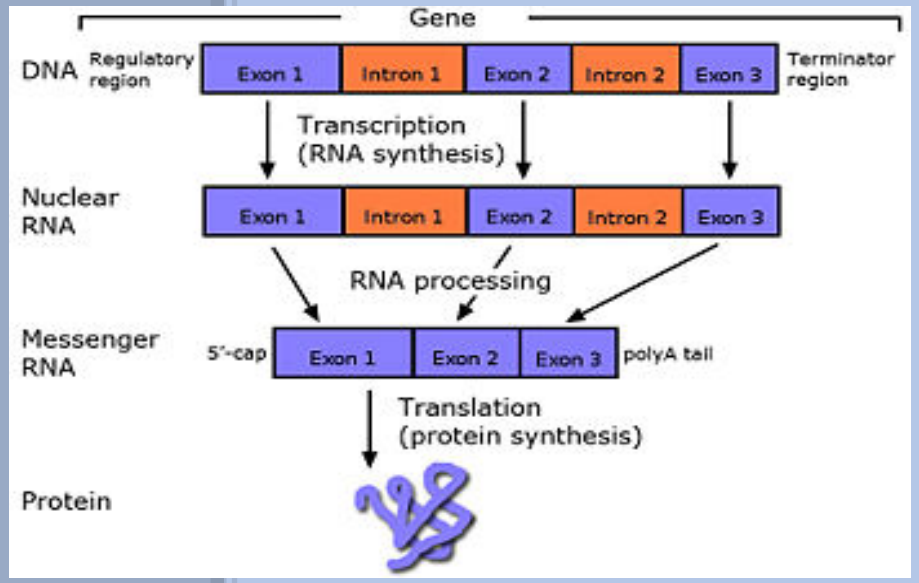
Objectives

- Discuss the technology of CRISPR- CAS 9
- Discuss diseases that gene editing is already making in-roads to cures
  - Sickle Cell Disease
  - Spinal Muscular Atrophy
  - Duchenne's Muscular Dystrophy
- The possibility of in utero genetic treatment
- The worries

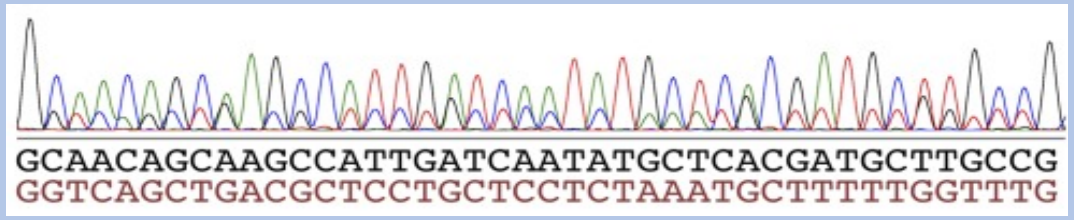
The Genome

- 3 billion base pairs
- 40,000 + genes
- Coding for our vital proteins
- The more we know, the more complicated it gets





What has changed recently?



Sequencing of entire human genome

Defining the genetic cause of many diseases

The capacity to develop gene therapies

Historically: Gene therapy

- The 1980's-- Discovery of an adenovirus
- This virus could serve as a vector for a piece of DNA attached to it that then could infect cells.
- The 'virus+DNA' would find its way into the host DNA and go to work producing a protein product
- 1989, the ability to bioproduce a recombinant adeno-associated virus (AAV) as a vector for DNA

Early successes and disasters

- A few human trials (children with lethal diseases)

Injected with AAV + gene DNA

- The percentage of cells that started to produce the enzyme or replicate a protein was inconsistent
- Where this DNA piece landed was random
- A treated child with SCID died from leukemia
- Moratorium on gene therapy followed

New discovery: CRISPR CAS9:

1987 Atsuo Nakata in Osaka, Japan

CRISPR: “Clustered regularly interspaced palindromic repeats”, sites along DNA

Discovered in E.Coli

Natural way for bacteria to disable viral invasions

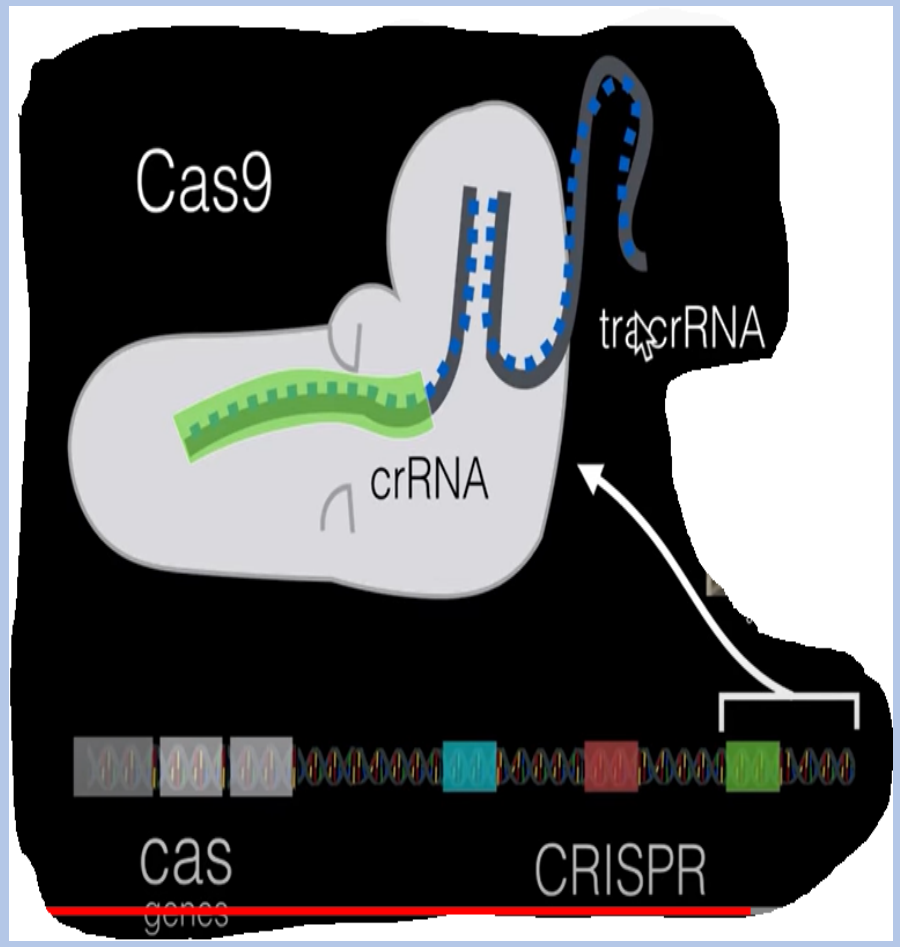
When bacteria are exposed to a virus, it creates a piece of RNA that matches the viral sequences. This ‘guide or cr’ RNA links with CAS 9 protein to search for virus in the cell- cut it and disable the virus

In the bacteria

CAS 9 protein has cutting properties (endonuclease)

crRNA- the viral DNA the system is looking for to chop up

Tracer RNA holds it all together



C (clustered) **R** (regularly) **I** (interspaced)
S (short) **P** (palindromic) **R** (Repeats)

- In bacteria, all that happens is DNA or RNA is chopped up in a specific location by matching a tracer RNA to a viral DNA segment and cutting with the endonuclease (CAS9)

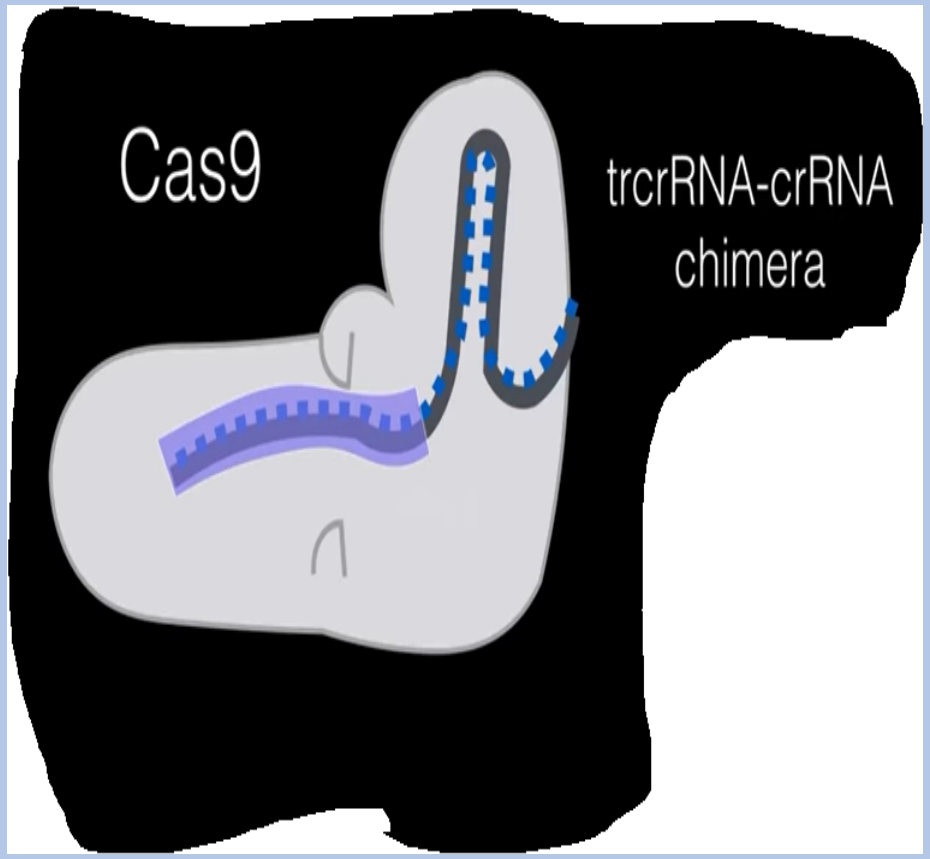
- What if it were possible in humans to target a specific piece of DNA and disable it ?!?

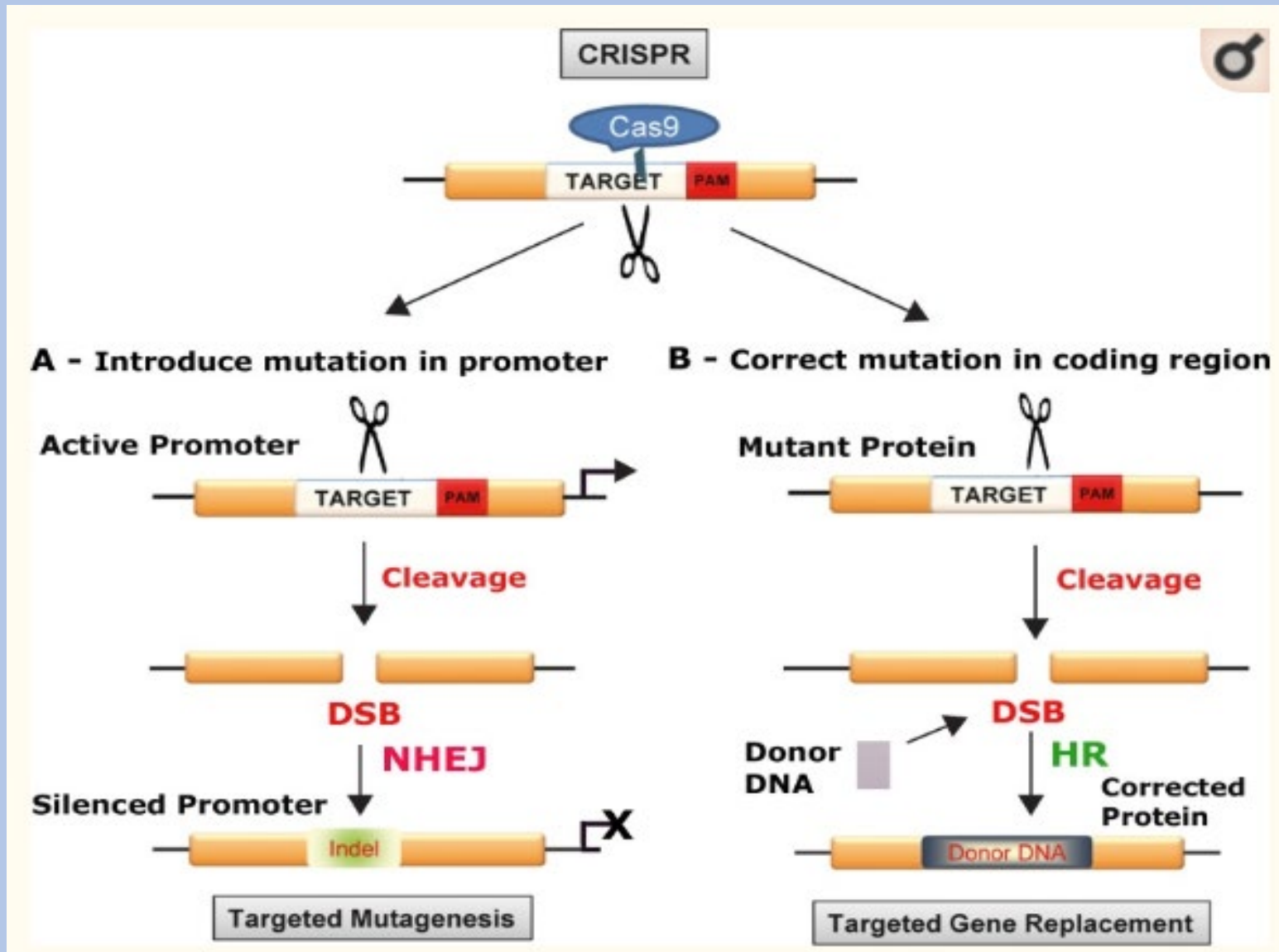
- Or what if it were possible to cut at a specific site and introduce a corrected or normal piece of DNA

Emmanuelle Charpentier and Jennifer Doudna
Accepting Nobel Prize for Chemistry, 2020

System for introducing a new piece of DNA

- CAS9 endonuclease
- Purple now is new piece of DNA
- trcrRNA- tracer (guide) RNA looking for the right spot in the DNA sequence to cut and insert
- And ‘snip’




CRISPR
Cas9
TARGET
PAM
A - Introduce mutation in promoter
B - Correct mutation in coding region
Active Promoter
TARGET
PAM
Cleavage
DSB
NHEJ
Silenced Promoter
Indel
Targeted Mutagenesis
Mutant Protein
TARGET
PAM
Cleavage
DSB
Donor DNA
HR
Corrected Protein
Donor DNA
Targeted Gene Replacement

- https://www.youtube.com/watch?v=UKbrwPL3wXE

“First Molecular Disease”: Sickle Cell Disease

SCD affects 100,000 Americans

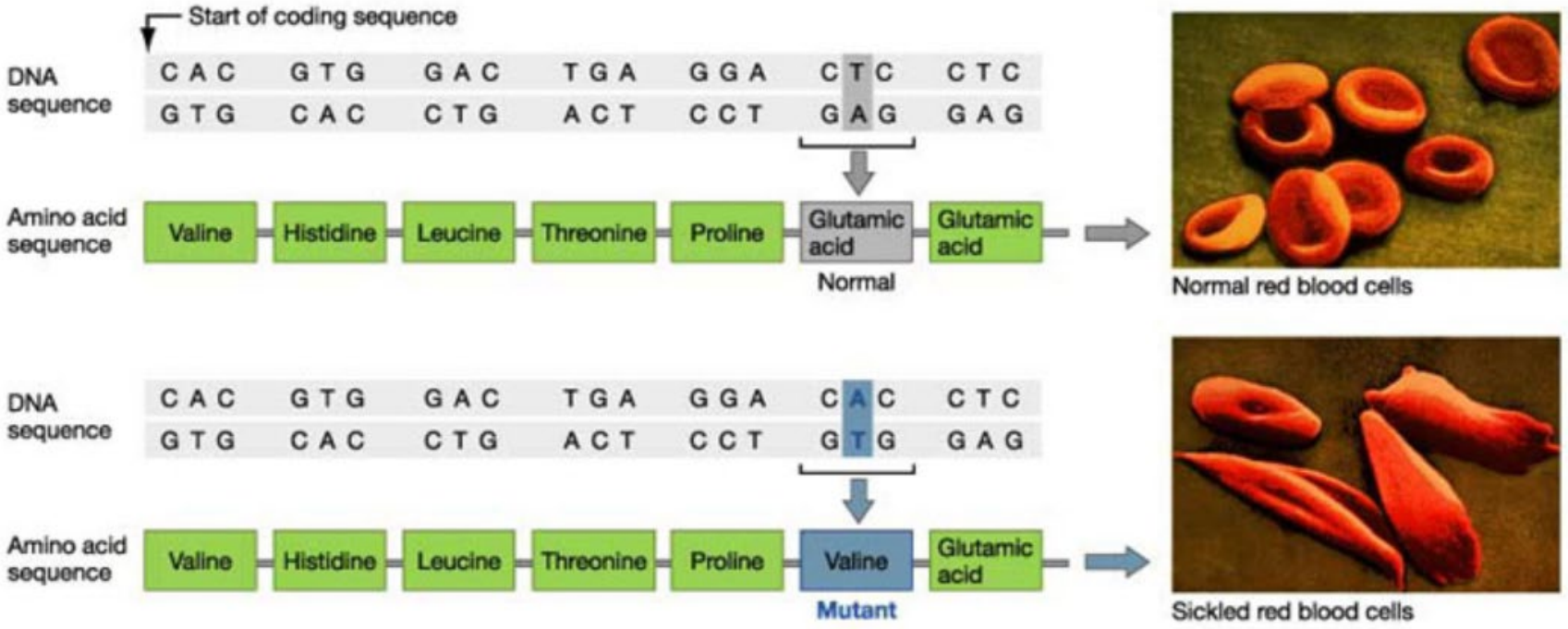
Pain, low quality of life, shortened life spans

Recent improvements in treatments

- Hydroxyurea
- Therapies aimed at preventing sickling
- Bone marrow transplant (many obstacles and complications)

Now, CRISPR

Sickle cell disease from abnormal beta hemoglobin



Gene therapy for SCD

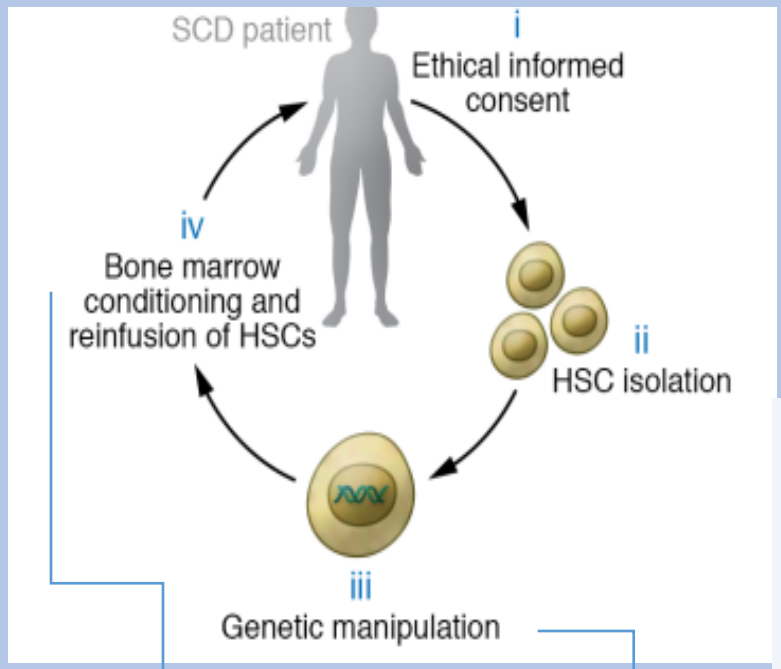
- One approach to improving symptoms is to boost production of fetal hemoglobin (HbF) over HbS.

- How: disable BCL11a which switches hemoglobin production from HbF to adult hemoglobin

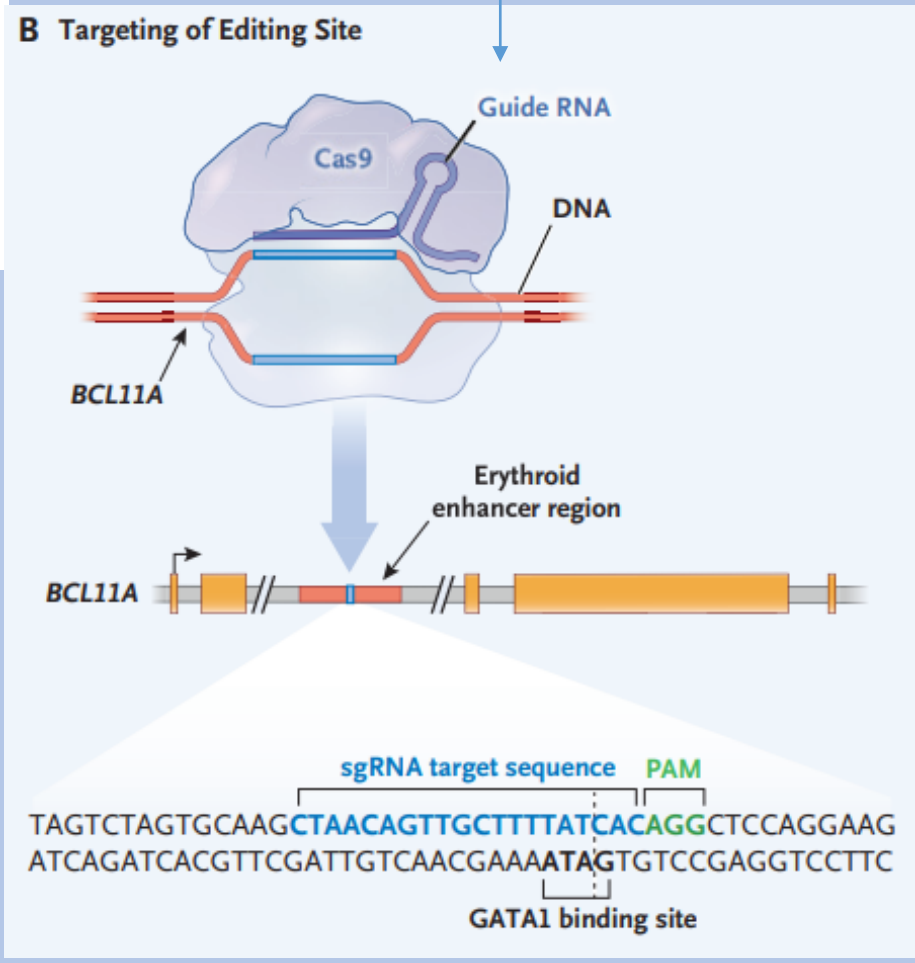
- Aspirate bone marrow (with hematopoetic stem cells)

In lab, using CRISPR Cas9, now 95% of cells treated have enhanced HbF production

- After conditioning of the bone marrow, treated cells are then reintroduced



AAV



Use of Busulfan (or other high-dose chemo) to ablate the bone marrow

- Prolonged hospitalization
- Immunosuppression
- Infections

Notes on outcomes in first trials

Cells with original SS hemoglobin	10-20%
Cells with new genetic variant	50-80%
Other	15-20%

Cardiac, renal, pulmonary function improved

Improved anemia and total hemoglobin

Did not always completely ameliorate crises

But a game changer

Sharon Gray

- First patient to be treated with gene therapy for SCD
- She says ‘cured’

FDA approval 12/8/2023: First gene therapy to be approved

Casgevy: works by increasing HbF by inactivating BCL11A

--Nakata discovered the CRISPR CAS9 system in E.Coli 1987

--BCL11A discovered 2008 by Vijay Sankaran

- Doudna and Charpentier first published 2012

Concerns

- Complications of the myeloablative process: infections, death
- Off target effects
- Warning of increased risks of leukemia
- Cost- $2.2 million
- Doudna: ‘a one-time delivery approach, with in vivo injection will be possible one day’

SMA Type 1

"Werdig Hoffman syndrome": AR disorder: Incidence 1/6000

Floppy infant presentation

Fail to achieve 'free-sitting' milestone

Progressive

Death by age 2 from respiratory failure

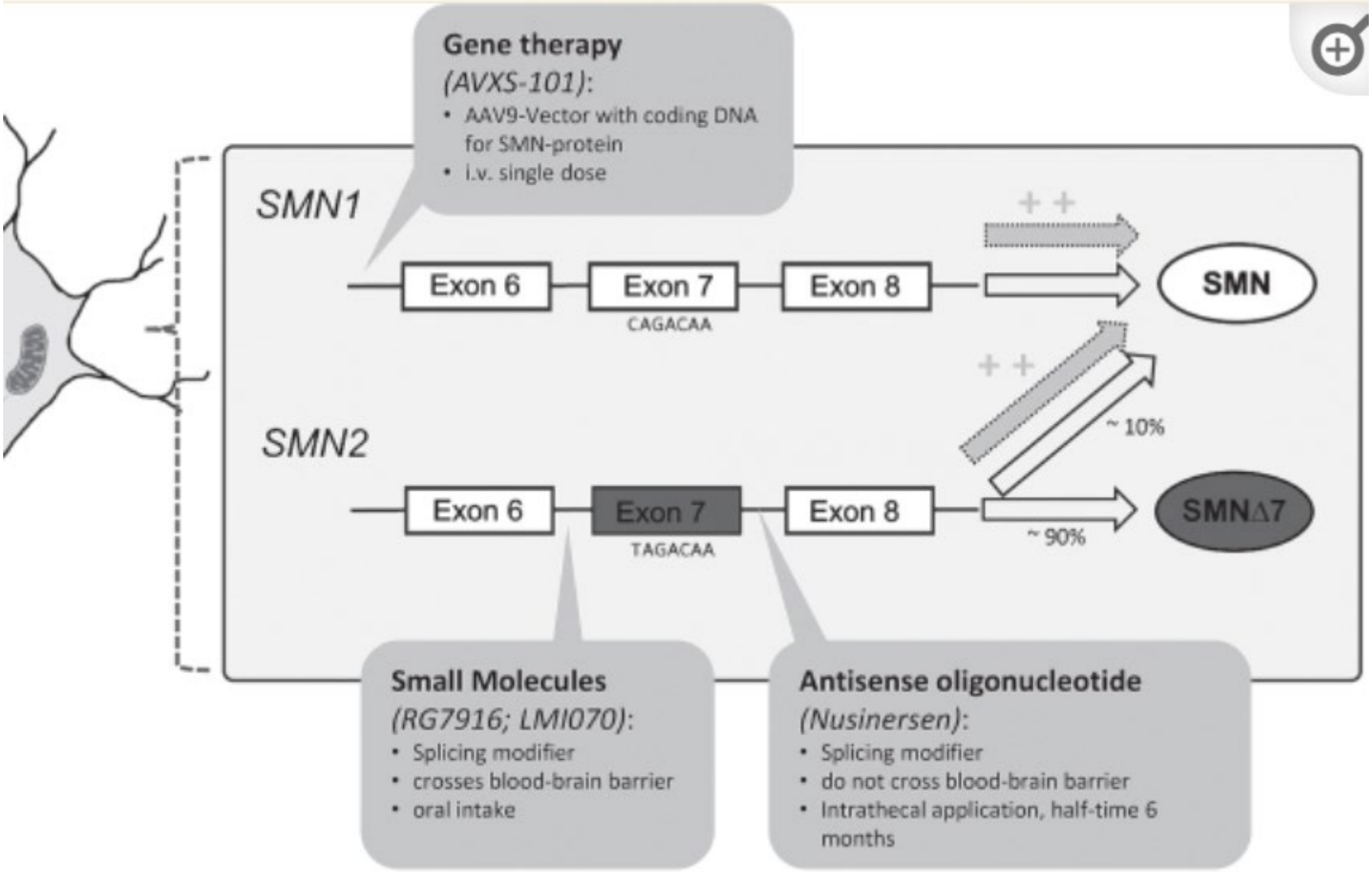
Genetics: deletions in the SMN1 gene (the gene promotes survival of motor neurons)

SMA type 1

- Now, recommended that all women be screened for SMA. If found to be a carrier, partner should be tested.

- Carrier frequency
  - Caucasian 1/30
  - AA 1/80

- Treatments
  - Neuroprotective agents (Olesoxime): now discontinued
  - Gene therapies


Gene therapy
(AVXS-101):
• AAV9-Vector with coding DNA for SMN-protein
• i.v. single dose
SMN1
Exon 6
Exon 7
Exon 8
CAGACAA
SMN
SMN2
Exon 6
Exon 7
Exon 8
TAGACAA
~ 10%
~ 90%
SMNΔ7
Small Molecules
(RG7916; LMI070):
• Splicing modifier
• crosses blood-brain barrier
• oral intake
Antisense oligonucleotide
(Nusinersen):
• Splicing modifier
• do not cross blood-brain barrier
• Intrathecal application, half-time 6 months

Gene therapies for SMA type 1

- AAV vector by CRISPR-CAS9 delivers an intact copy of the normal SMN 1 gene
- The vector and DNA fragment is given IV (single dose), crosses the blood brain barrier
- Brand Name: Zolgensma
- STRIVE study: High-dose and Low-dose regimens

Studies on SMA

- Best result: Injection before symptoms appear in a known affected child (6 months)

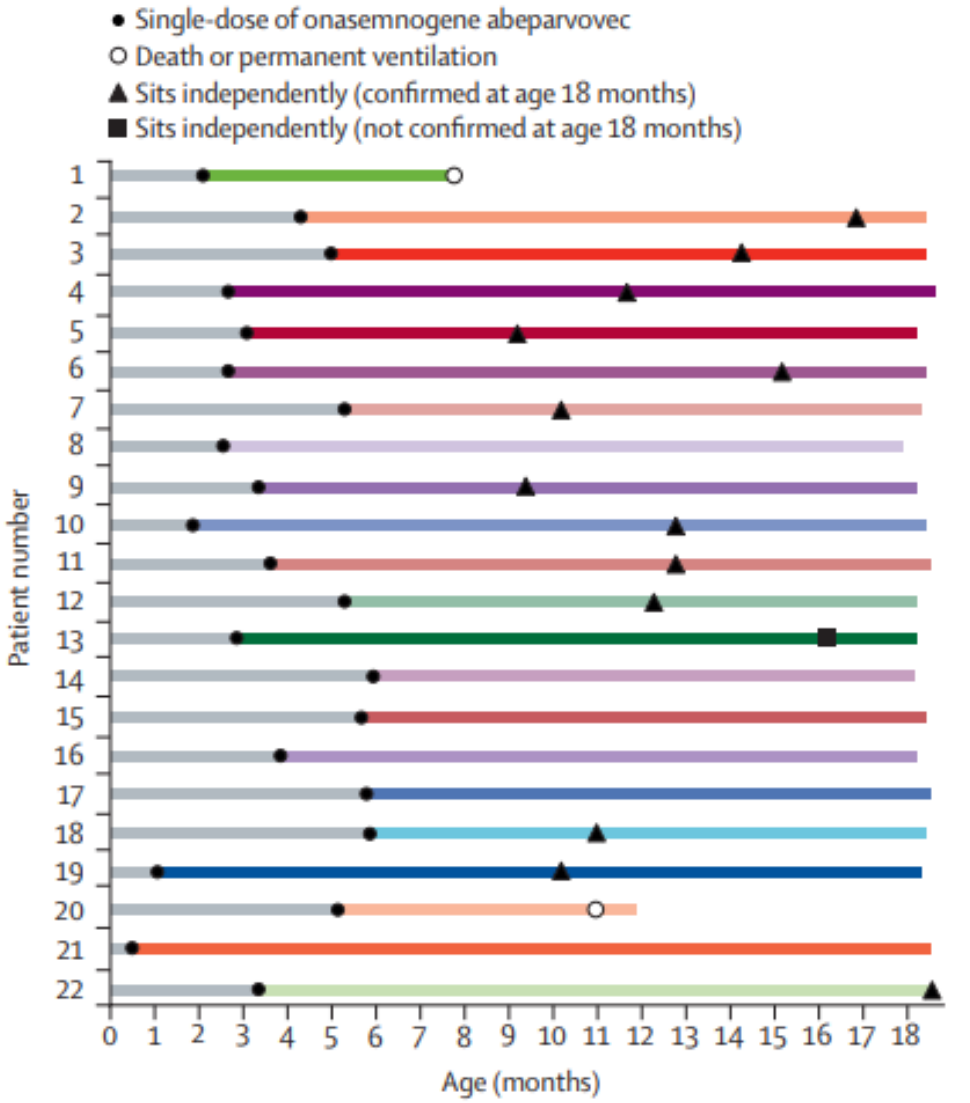
- Denervation progresses rapidly during the first 6 months

- STRIVE: under 2 years of age IV; goal was independent sitting at 18 months.

- Still following children:

Standing, walking, eating independently

Strive study. Lancet Neurol 2021 Day, et al



Downsides/concerns

- Add SMA to newborn screening?

(It is a recommended prenatal screen by ACOG)

- Some very serious cases have a prenatal onset and will not be helped even with postnatal therapy started early

- Over 2 years of age: intrathecal application

(Intrathecal may allow lower doses in younger children)

- The earlier the treatment, the greater the clinical benefit

“Almost normal motor development”

- Cost: $3.2 million

“The most expensive drug in the world”

Duchenne's Muscular Dystrophy

- Lethal wasting disease that affects 1/5000 boys

- X-linked DMD gene: deletions in gene associated with lack of production of dystrophin

- Diagnosis at age 2 with muscle weakness
- Wheelchair bound as a teen
- Ventilation in 20's
- Death in 20-30

- Progressive muscle wasting
- Myofibers replaced with fatty or fibrotic tissue

DMD gene editing therapies on going

- Gene-therapy: to edit gene so as to produce a full-length dystrophin protein
- Elevidys: IV drug for preliminary FDA approval for 4-6 y/o
- DMD: Chronic, body-wide disorder (cardiac muscle, e.g.). Will require repeat and high-dose therapies
- Risks of complications. Including immunity to the AAV

NYT editorial: 2/17/2024

“Before Eliot received his treatment, he had difficulty going up stairs. Hopping on one foot, a milestone for a 4-year-old, was impossible.

On Aug. 29, he finally received the one-time infusion. Three weeks later, he was marching upstairs and able to jump over and over. After four weeks, he could hop on one foot. Six weeks after treatment, Eliot’s neurologist decided to re-administer the North Star Ambulatory Assessment, used to test boys with D.M.D. on skills like balance, jumping and getting up from the floor unassisted.

In June, Eliot’s score was 22 out of 34. In the second week of October, it was a perfect 34 — that of a typically developing, healthy 4-year-old boy. ”

What's next?

- Every genetic disease?
- At what cost?
- In utero treatment?

Yep

Congenital Deafness

- Aissam Dam; age 11

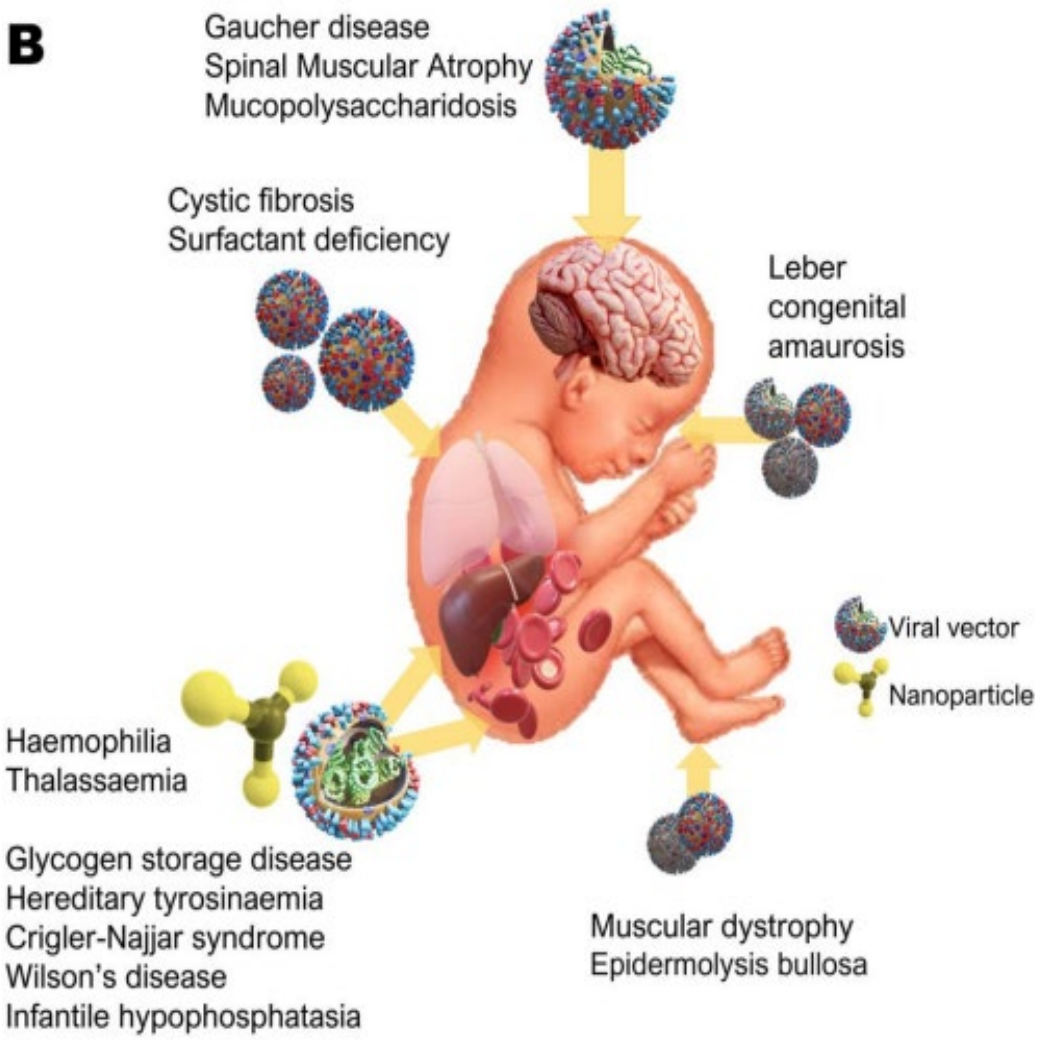
- Mutations in gene ‘Otoferin’

Treated with CRISPR-CAS9

“there’s no sound I don’t like”

In utero gene editing: experiments in mice

- Many diseases have their detrimental effects during the critical developmental period
- The vector to mass ratio higher (lower doses/cost)
- Fetal immune system more tolerant of viral vector
- Blood brain barrier easier to overcome



Mattar, et al; Prenatal diagnosis, 2022

Summary: Two potential target cell types

1. Somatic cells- all we have been discussing.
   - Brain cells
   - Muscle cells
   - Blood cells
   - Cochlear cells

2. Germ line (embryonic or eggs/sperm)

Altering these cells would affect every cell in body

Would be passed onto every subsequent generation

The Cautionary Tale: 11/2018

- He Jiankui; China

Announced use of CRISPR-CAS9 in humans and a Germline treatment

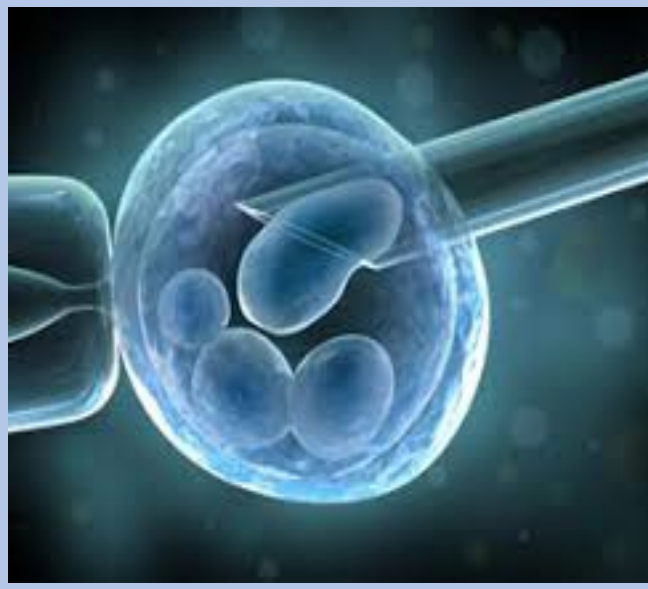
Dr. He trained in the US

Preimplantation Genetic Diagnosis

Current practice in
infertility clinics world-wide with IVF

Diagnosis from one cell from
a 4-8 cell embryo

Or with a diagnosis, could
introduce a gene with CRISPR/CAS9



This is the ethical (and medical) frontier

Introduce a gene at this stage of embryological development that would essentially be a germline engineering feat (as opposed to a somatic one)

For the purposes of disease cures or enhancements

Without the individuals' (the person to be) consent

Without enough knowledge to know what harms may occur

Dr. He's case

IVF, twin gestation, father with HIV

- Gene known to impart immunity for HIV inserted in embryos at early stage
- Twin girls born
- Dr. He currently in prison in China
- Surprise and outrage from US colleagues and experts world-wide

Summary of international working group at ASHG, October 2019

- One twin apparently has no HIV-immune gene detectable
- The other had significant misplacement with concerns that telomeric approximation of insert may cause decreased life expectancy
- Experts in the room in now feel the only way to have stopped Dr. He would have been to call the New York Times

But all agreed…..

- Chinese and Russians have ongoing CRISPR/CAS9 programs running as we speak

- And unlikely ethics boards centered in US can do much about it now

Questions?

- Where are the limits?
- Who is going to pay?
- Who is going to decide who gets treated.
- How is in utero experimentation look post Dobbs?