Gene therapy – is it the new frontier?

Gene Therapy 101

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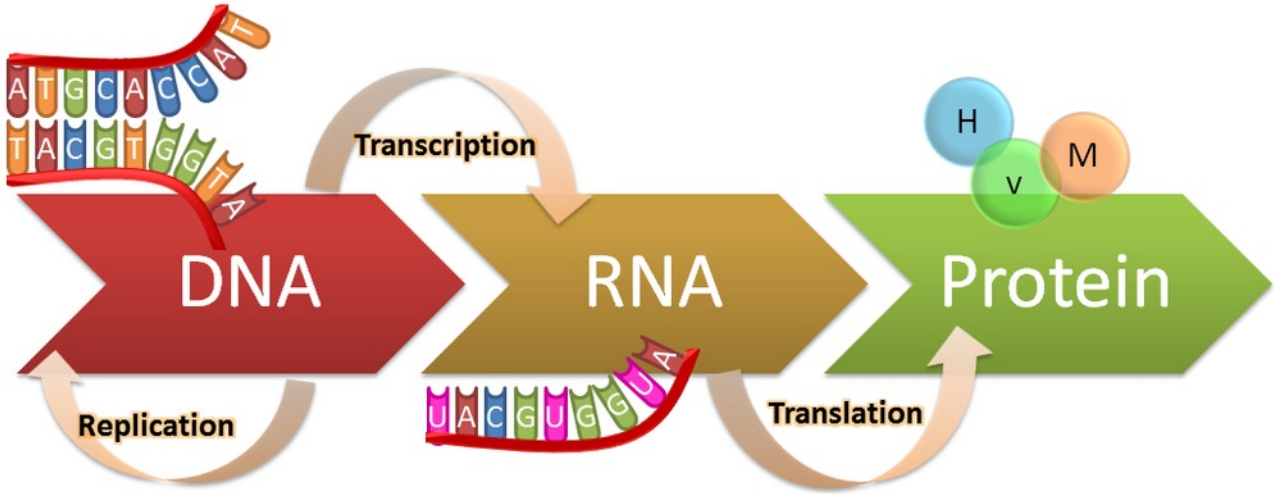
Definitions

- **Gene**
- **mRNA** → “Payload”
- **Translation**



https://genius.com/Biology-genius-the-central-dogma-annotated

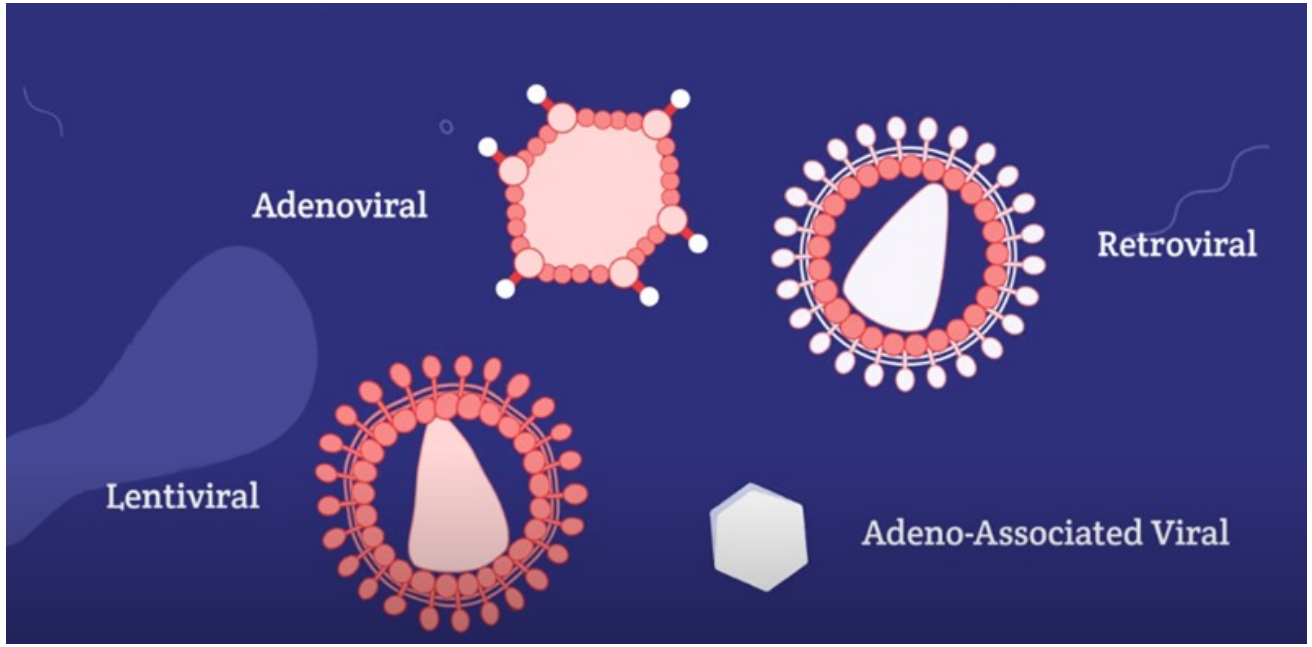
- **Vector** (“Truck”) – carries the payload to the cell, binds to cell surface, enters the cell and delivers the payload to the nucleus (for genes)
- **Non-integrating** – the gene payload remains separate from the rest of the DNA, it does not get copied when that cell divides (dilution effect)
- **Integrating** – some approaches insert the DNA into the DNA of the cell; that DNA is then copied and carries on when the cells divide
- **Gene editing** – a special form of integration where the the gene is inserted in a very specific place (this is what CRISPR is used for, but there are other methods, too

Getting the therapy to the cells that need it

- Liver is a common target so there is a lot of research to target liver cells
- Eye and bone marrow have advantages
  - Eye – can inject the therapy directly into the area that it's needed
  - Bone marrow – can treat in the lab, then do a bone marrow transplant with the patients own corrected cells
- Special challenges of the brain
  - Getting vectors past the blood brain barrier requires direct injection
  - Since the vector is not in the blood supply, it may not get to all cells as easily

Vectors

- Viruses – all are altered to remove the components needed to copy themselves
  - AAV (Adeno-associated virus) – small, non-disease causing, typically little inflammation
  - Adenovirus – larger, so can carry a bigger gene, but also more likely to cause significant inflammation
  - Lenti- and retro-viruses carry RNA, more likely to be used outside of the body, for example to treat bone marrow cells
- Nanoparticles – less immune response, may allow for re-treatment if needed

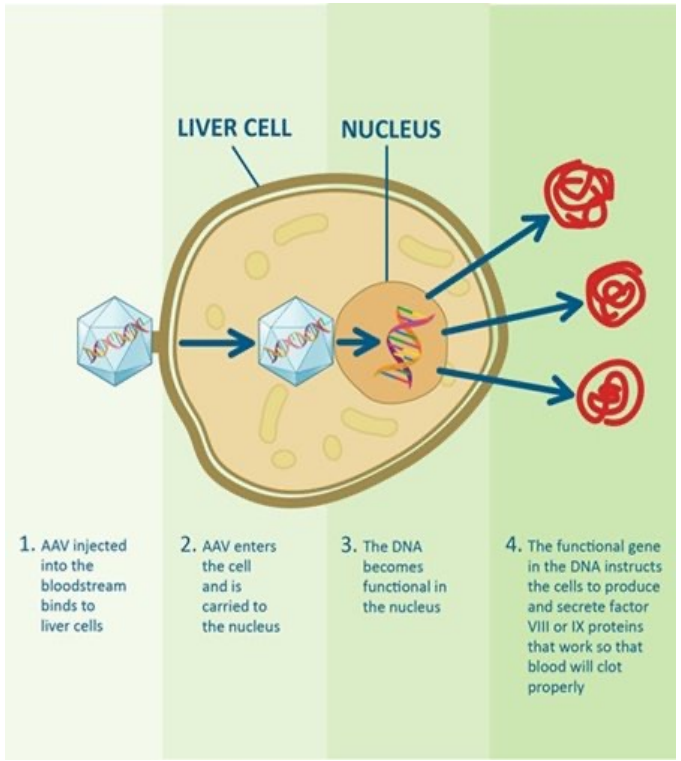


https://patienteducation.asgct.org/gene-therapy-101/vectors-101

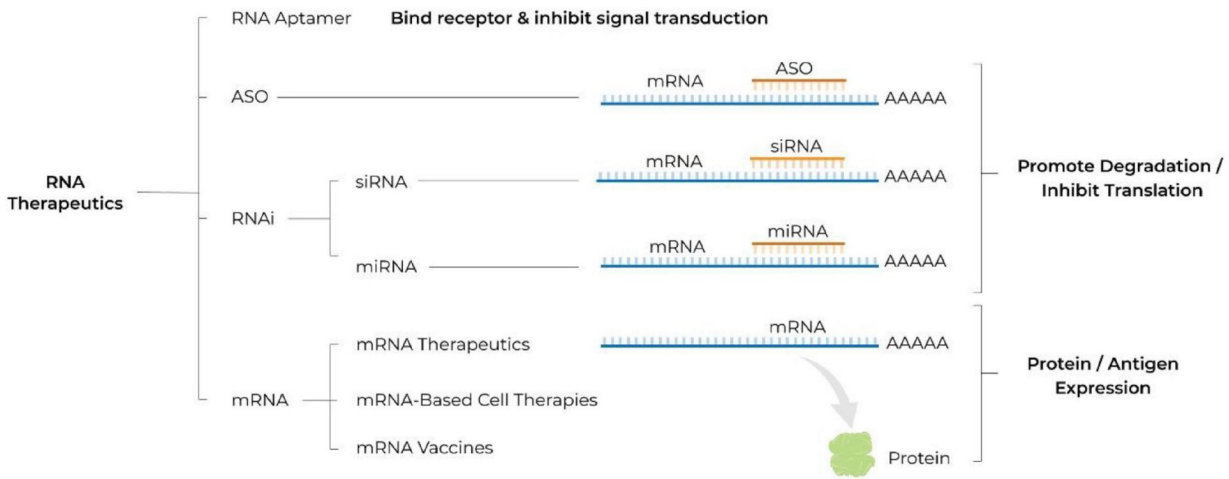
AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
Muscle CNS	Kidney Eye Brain Liver Joint Lung Muscle	Brain	Eye CNS	Eye CNS Liver	Muscle	Muscle	Heart Liver Pancreas Muscle	Liver Muscle Lung

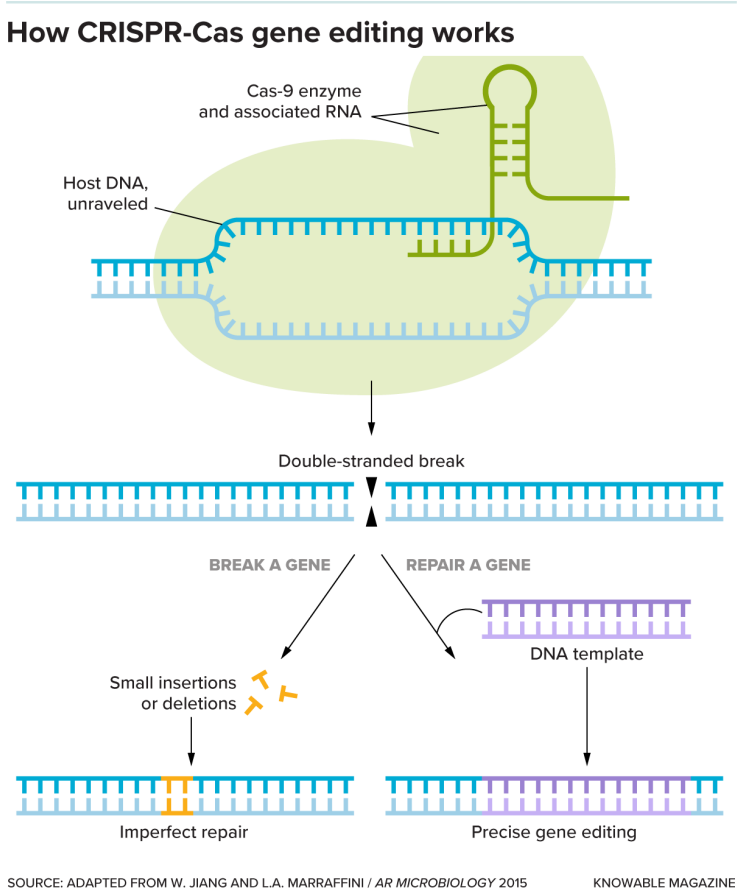
https://biovian.com/news/beginners-guide-to-the-production-of-aav-vectors-for-gene-therapy-mentored-by-a-cdmo/

How does gene/mRNA therapy work?

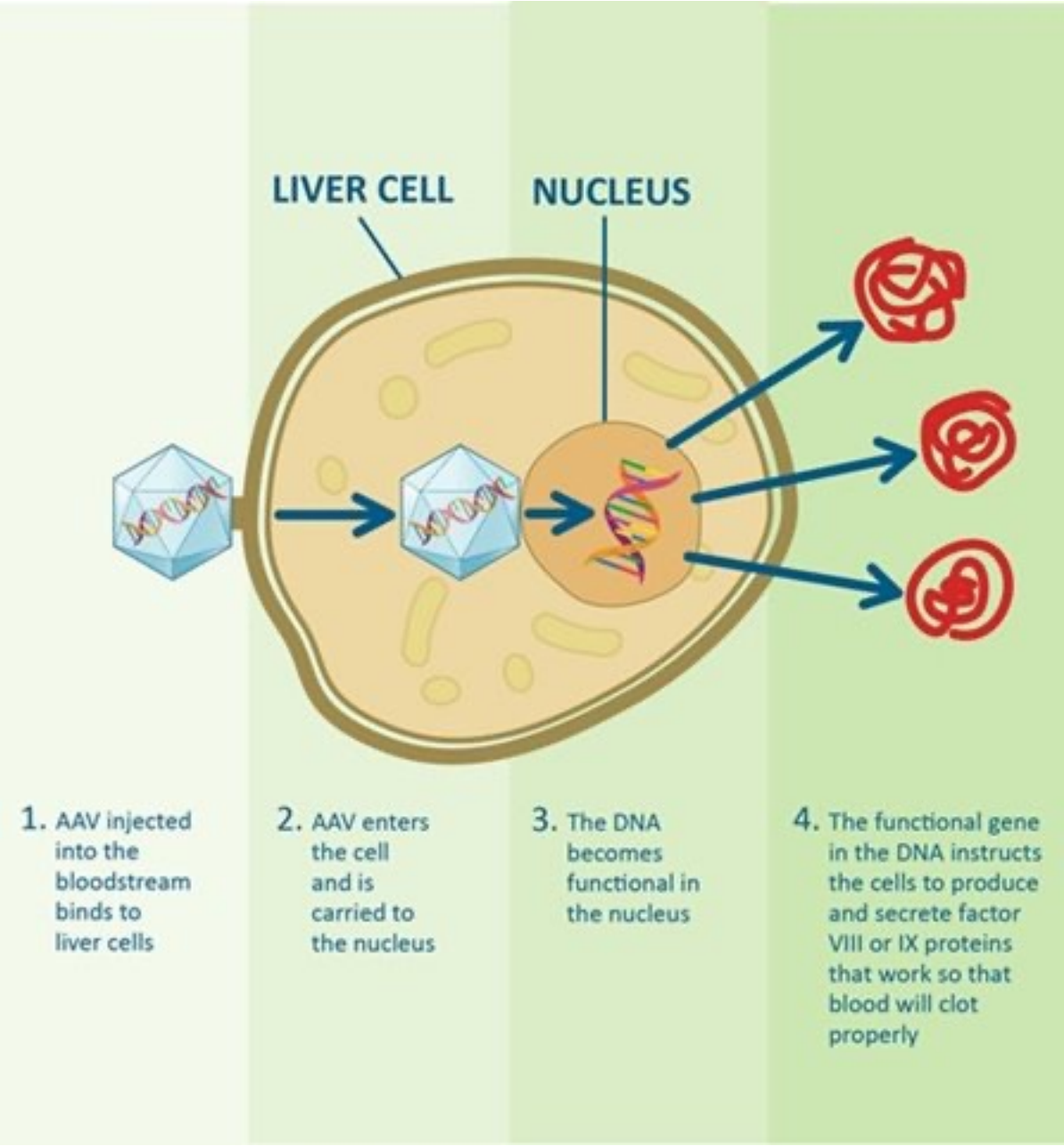


https://www.haemophilia.org.au/bleeding-disorders/living-with-a-bleeding-disorder/gene-therapy/how-gene-therapy-works/

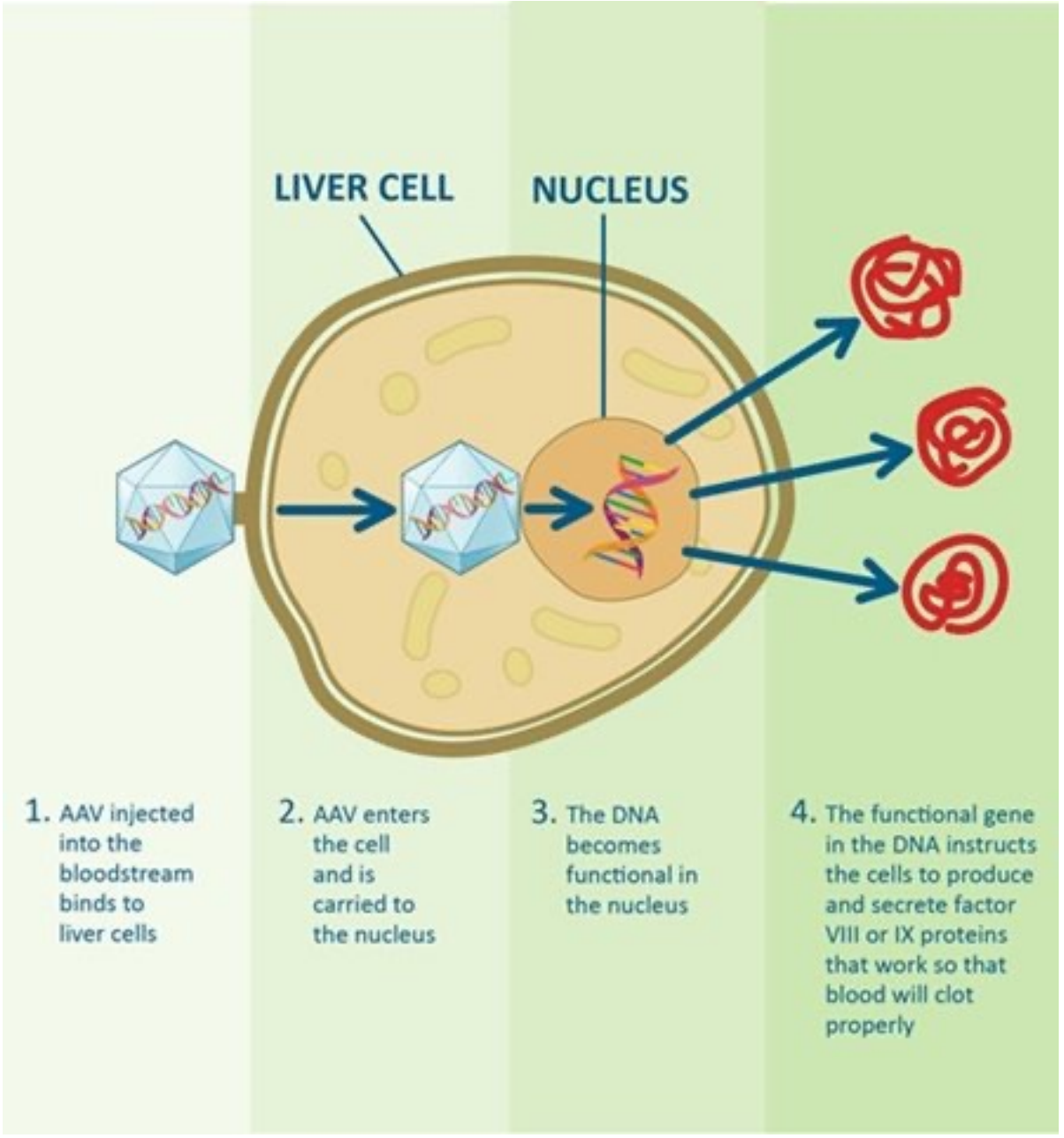




How does gene therapy work?



https://www.haemophilia.org.au/bleeding-disorders/living-with-a-bleeding-disorder/gene-therapy/how-gene-therapy-works/



https://www.haemophilia.org.au/bleeding-disorders/living-with-a-bleeding-disorder/gene-therapy/how-gene-therapy-works/

How does gene therapy work?

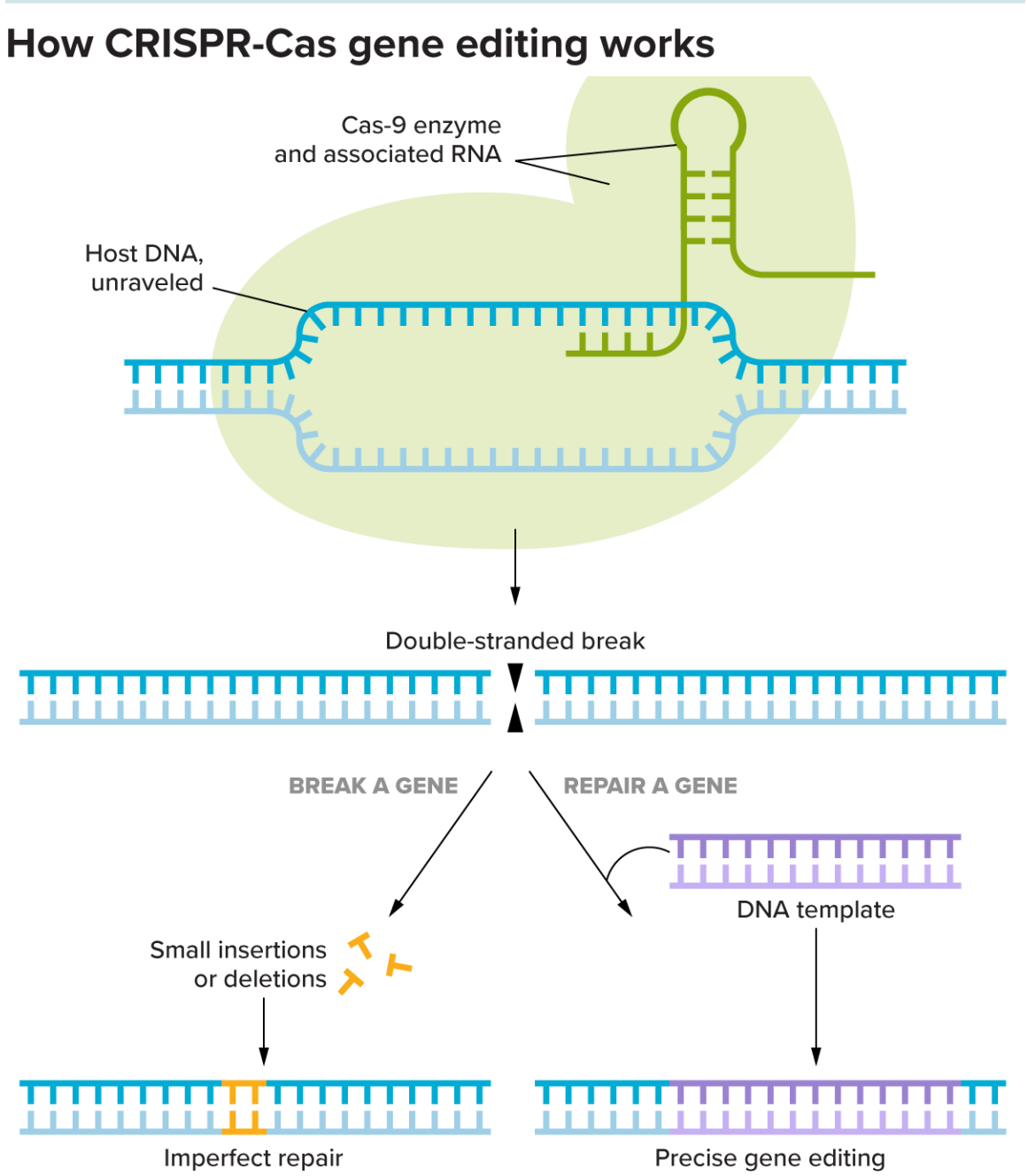
Treatment requires LOTS of copies of the vector/gene

$1X10^{13}$ vg (viral genomes)/kg

10,000,000,000,000

Muscle diseases may use $1X10^{14}$ or 10^{15}

How does gene editing therapy work?

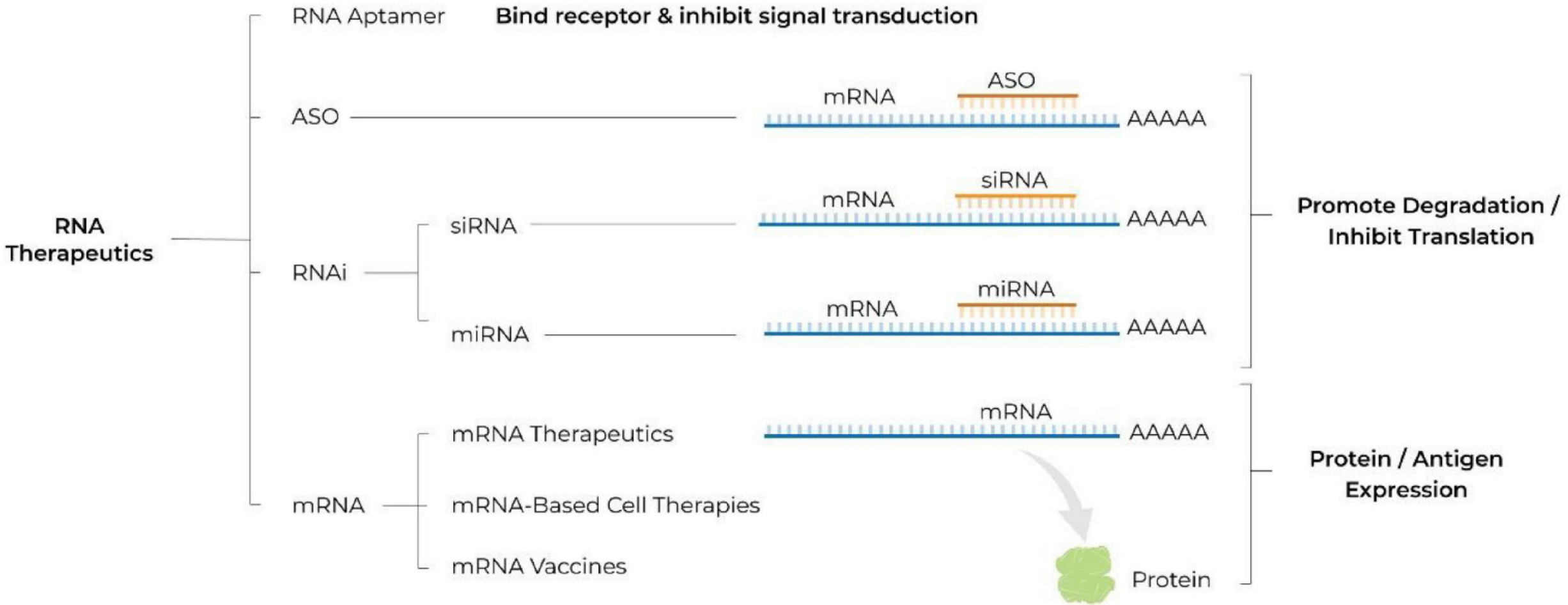


https://knowablemagazine.org/content/article/living-world/2024/crispr-gene-editing-therapy-systems-eukaryotic-cells

Integrating vs non-integrating

- Non-integrating
  - The truck (vector) delivers the payload (the gene) to the cell
  - The gene delivered remains separate from the cells own DNA
  - When the cell divides, the gene is not copied, and it may be lost entirely, so that, over time, the gene therapy may wane
    - The faster the cells divide, the faster this happens
    - Liver cells divide relatively slowly
- Integrating
  - The truck (vector) delivers the payload (the gene) to the cell
  - Another truck delivers the machinery to incorporate the gene into the nuclear DNA, where it can be copied and carried into new cells when the cells divide
    - Some of the “trucks” (for example lentivirus) already include the mechanism for gene incorporation
  - If the cells that are corrected are healthier, they may survive better so that the therapeutic effect can actually increase over time

How does mRNA therapy work?



https://www.frontiersin.org/files/Articles/628137/fbioe-09-628137-HTML/image_m/fbioe-09-628137-g001.jpg

Biological Challenges

- Immune response to the vector
  - Pre-existing immunity to the vector (~25% for AAV8)
    - Currently, people that have evidence of pre-existing immunity are not eligible for the clinical trials using that vector
  - Rapid immune response to the treatment with “memory”
    - B cells make antibodies, so once treated the body now will mount a destructive response if you try to give that vector again
    - T cells may cause delayed inflammatory response and reduce effectiveness of treatment (now many protocols give steroids after infusion to blunt this)
  - Future options for those with pre-existing antibodies
    - Temporary immune modulation (similar to what we sometimes do when starting enzyme replacement therapy)
    - Use different vector (same payload, different truck)
    - Use of lipid nanoparticles

Biological Challenges

- Inflammatory response (to the vector, payload or the protein that is made as a result of the treatment) – rare but important
  - TMA – thromobotic microangiopathy
    - Diffuse micro-blood clots, anemia, low platelets, organ damage (esp. kidney)
  - Cytokine Response Syndrome (“cytokine storm”)
    - Innate immune system – inflammatory molecules that lead to a rapid response
    - Loss of control or regulation of this system can lead to massive and deadly inflammation developing rapidly
    - This appears to be very rare, but is important for sites providing gene therapy to have a plan for identifying and intervening
  - Liver inflammation and injury – more likely wih pre-existing liver injury (not just a having a metabolic disorder)
- Cancer risk – so far hypothetical in AAV treatment of liver disorders
  - Likely due to low rate (2-3%) of insertion of AAV DNA into the genome, and disruption of a cancer related gene – this appears to happen with naturally occurring infections with AAV, too
  - Probably less concern for UCDs due to less propensity to liver cancer compared to other inborn errors of metabolism

Cost

- Determinants include
  - Cost of drug development
  - Cost of creating drug production facilities
  - Cost to produce drug
  - How many potential patients
- Range in 2024
  - ~$500K for some eye disorders to over $2M for SMA and DMD (just for the drugs)
- Are there market forces that will bring the price down?
- May not eliminate the need for scavenger therapy

What does a gene therapy clinical trial involve?

1. Travel
2. AAV antibody test, and lots of other screening tests
3. May include some overnight stays
   For example: Round the clock ammonia sampling
4. IV infusion of drug (most likely with a 50% chance that it's placebo)
5. Regular in person visits (may be 8-12 the first year), plus home nurse visits and phone calls
6. After a year, second infusion (when people who got placebo the first time get the gene therapy)
7. Regular visits the second year, similar to first year
8. Expect at least one or two visits per year, starting in year 3, for up to 10 years total

What does a gene therapy clinical trial involve?

1. Expect to be treated with steroids (high doses) for a month or two after the infusion of gene therapy
2. Some studies may require liver biopsies on several occasions
3. You must let the study doctors know if you have any illness during the study
4. There may be unanticipated problems

If we knew for sure that the treatment was safe and effective, we wouldn’t need to do the study

What does an mRNA therapy clinical trial involve?

1. Similar screening and preparation
2. Treatment likely IV, may eventually be given by single injection
3. mRNA therapies will need to be repeated at regularly intervals (1-4 weeks, most likely)
4. Eventually may be able to switch to home infusions

Disadvantage is need to repeat regularly

Advantage is that if there is a problem, the mRNA goes away

What does a therapy with an approved product involve?

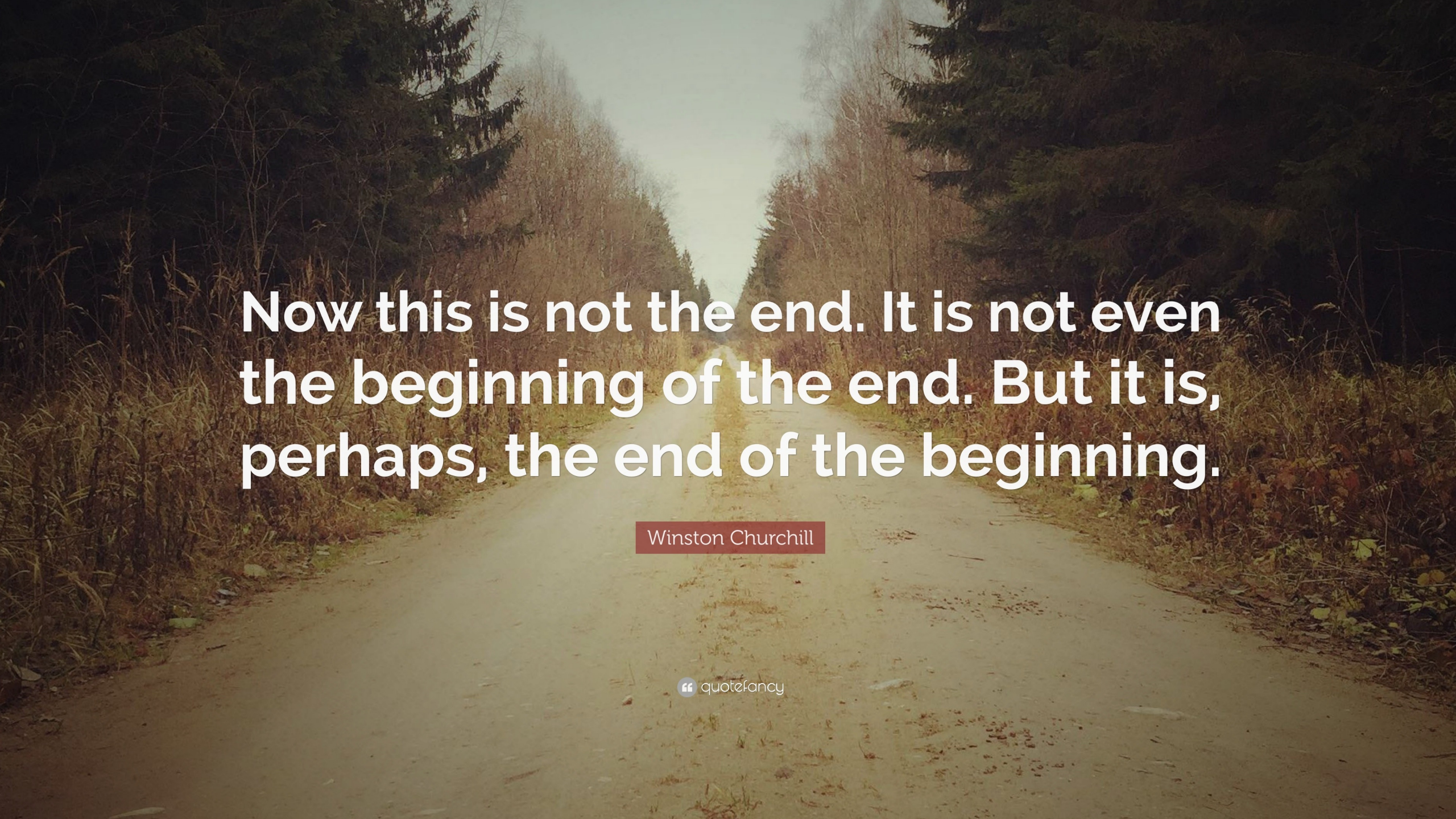
1. Evaluations to establish baseline and to discuss potential risks and benefits
2. Insurance prior authorization
3. Treatment protocols will be similar, but likely fewer visits and less monitoring than a clinical trial
4. Many therapies will only be available at a limited number of “certified” centers – you may have to travel for treatment
5. More options for follow up monitoring closer to home

Urea Cycle Specific issues

- How many cells need to be corrected to eliminate symptoms?
  - Are female livers similar to males in response to treatment?
  - Would delivery of therapy through the portal vein be more effective
- Argininosuccinic aciduria
  - To fully correct symptoms need to treat other tissues besides liver
  - AAVs are not entirely specific, so may get by with the same vector or may need a different vector
- Potential to treat with mRNA until patient is ready for gene therapy or until gene integration expands to enough liver cells to do the job alone

Realistic expectations

- These treatments are new to all of us – we may not always know the best way to manage your disease after gene therapy
  - We are learning together
- These are not cures (at least for now) – the hope is to stabilize or improve disease and reduce risk of bad outcome from the UCD
  - The treatments may work better for some than for others, and we may not know why
- It seems likely that re-treatment will be possible, if needed in the future, but that is not the case today
- Liver transplant is still available if needed after gene therapy
- After gene therapy, you will not likely be able to do another gene therapy trial, but if another therapy is clinically approved later, and you would benefit from it, you will likely be able to get it


Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning.
Winston Churchill
quotefancy