



# Gene therapy for haemophilia

It is an exciting time in gene therapy for haemophilia. For a long time gene therapy has been hailed as a potential 'cure' for haemophilia, but the expectation was that viable treatment was many years away in the future. In recent years international experimental gene

therapy studies have begun to demonstrate successful results in people with haemophilia and are now conducting advanced studies in larger groups. Other experimental gene therapy trials are also commencing. What does this mean for people with bleeding disorders in Australia?

This resource has been developed to answer the questions from Australian bleeding disorders community members about gene therapy and its relevance to them now and in the future.



# What is gene therapy

Gene therapy uses genetic material – a gene, which is a piece of DNA or RNA – to treat, cure or prevent health conditions.

## Types of gene therapy

There are different types of gene therapy.

Some are aimed at health conditions caused by a mutation or alteration in a gene. This mutation affects specific proteins in the body, which then

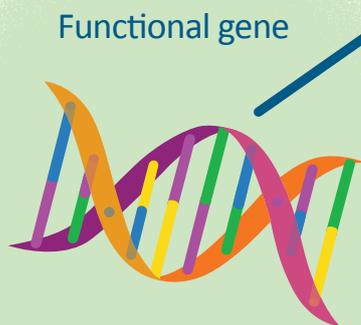
causes the health condition. All of these types of gene therapy target the gene with the mutation, but each type of therapy works differently.

There are currently gene therapy clinical trials to treat a variety of genetic health conditions, such as haemophilia, cystic fibrosis, Huntington's disease, spinal muscular atrophy or types of cancer. The types of gene therapy used in these clinical trials include two types used in haemophilia: **introducing a new functional gene** and **gene editing**.

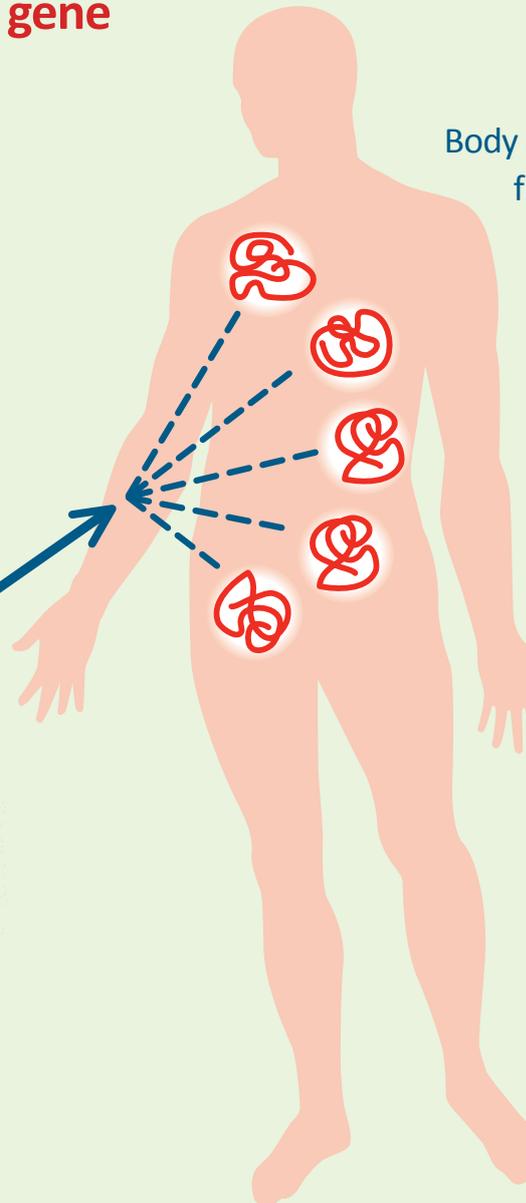
## 1. Introducing a new functional gene

Introducing a new functional (normal or enhanced) copy of the gene causing the health condition into the body, which then produces proteins that work normally.

*For example, in haemophilia or cystic fibrosis*



Functional gene



Body produces functional protein

## 2. Gene editing

Gene with mutation



Gene is edited



Becomes a functional gene

'Editing' the altered gene to correct the mutation.

*For example, in cancer, sickle cell disease, inherited blindness, potentially haemophilia*

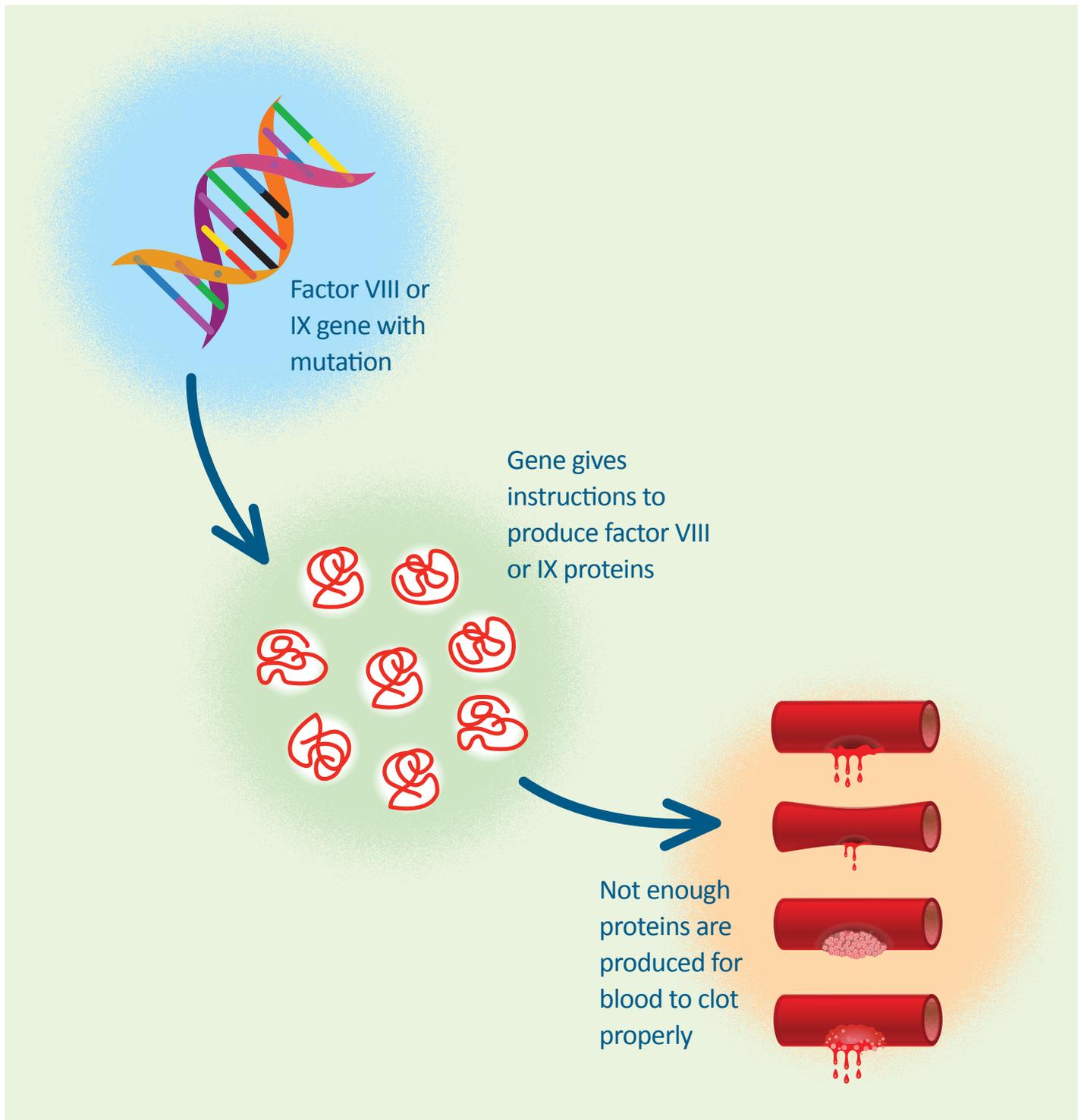
Gene editing has been trialled in humans by removing some cells from a person's body, editing the DNA, then injecting the 'edited' cells back into the body.

More recently there have been experiments with injecting 'gene scissors' that will edit the cell with the gene mutation in the person's body.

## Haemophilia and genes

**Haemophilia** is caused by an alteration (mutation) in the **factor VIII (8)** or **factor IX (9) gene**. Factor VIII and factor IX are proteins in the body that help blood to clot.

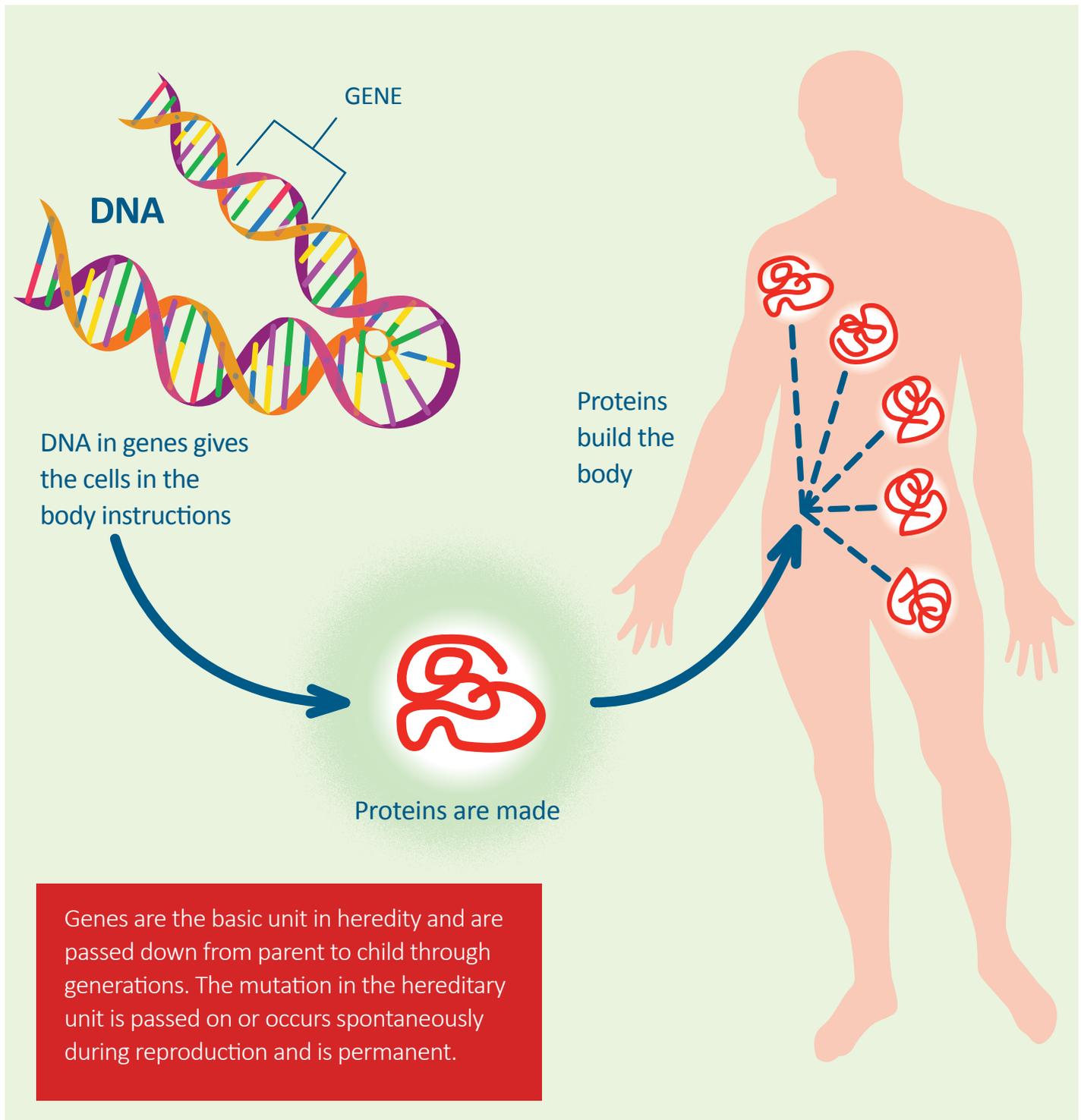
The 'haemophilia' alteration changes the **genetic code** or instructions carried by the factor VIII or factor IX gene. As a result of these changed instructions, the body does not produce enough of the normal factor VIII or factor IX proteins for blood to clot properly.



# What is a gene?

Genes are tiny structures made up of DNA. They are segments of DNA that are located on chromosomes. Genes give the body instructions when it is being formed that decide individual characteristics such as the colour of a person's hair or eyes.

This is because the DNA in genes gives the body instructions for making proteins. Proteins are the building blocks that form the body and make it work. Blood is made up of proteins, including clotting factors.



Genes are the basic unit in heredity and are passed down from parent to child through generations. The mutation in the hereditary unit is passed on or occurs spontaneously during reproduction and is permanent.

# Gene therapy in haemophilia

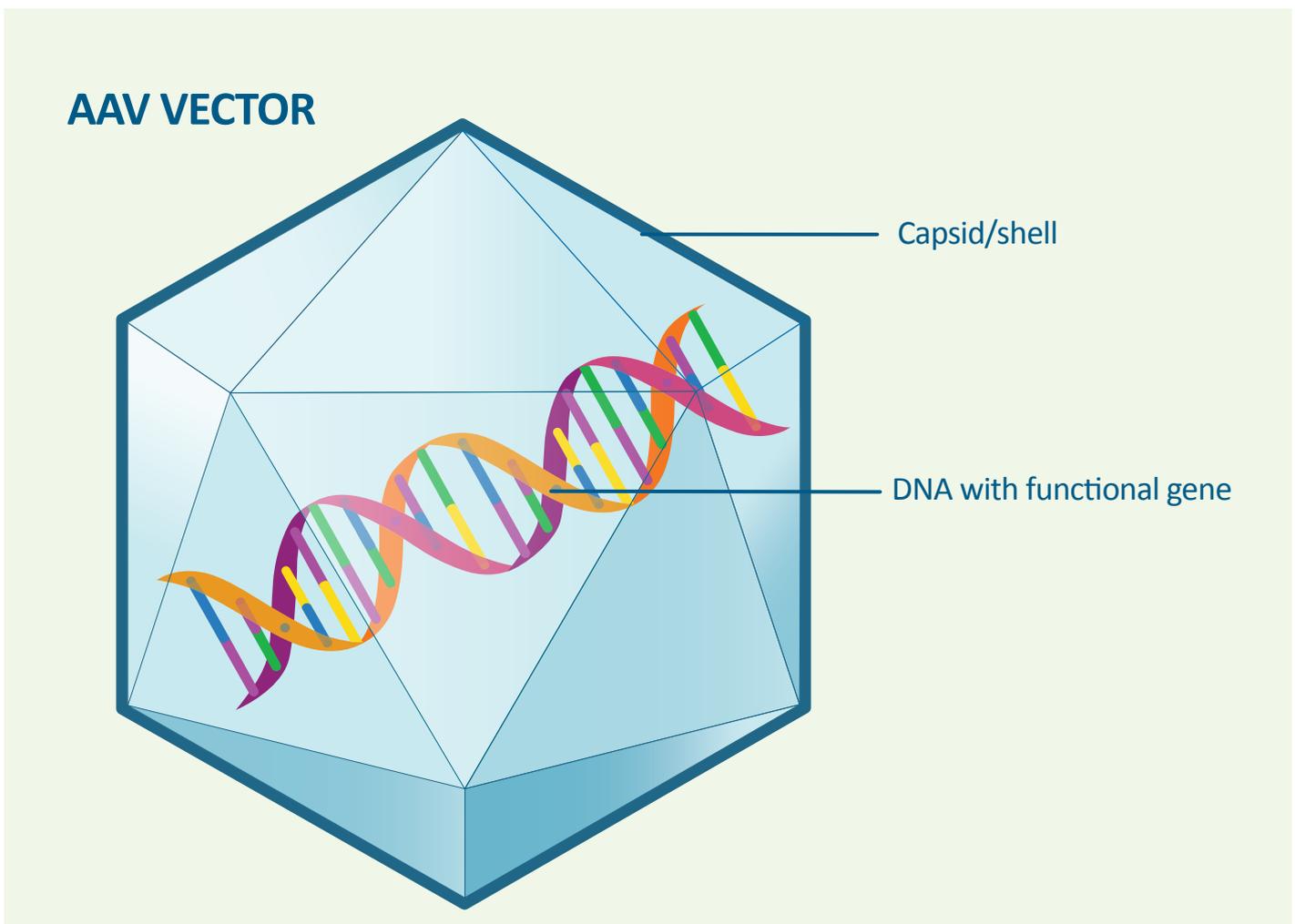
The most advanced clinical trials for gene therapy in haemophilia are using a particular type of carrier, called a **vector**, to deliver the gene therapy via the bloodstream to liver cells in the body. One important type is known as the **AAV** (adeno-associated virus) **vector**.

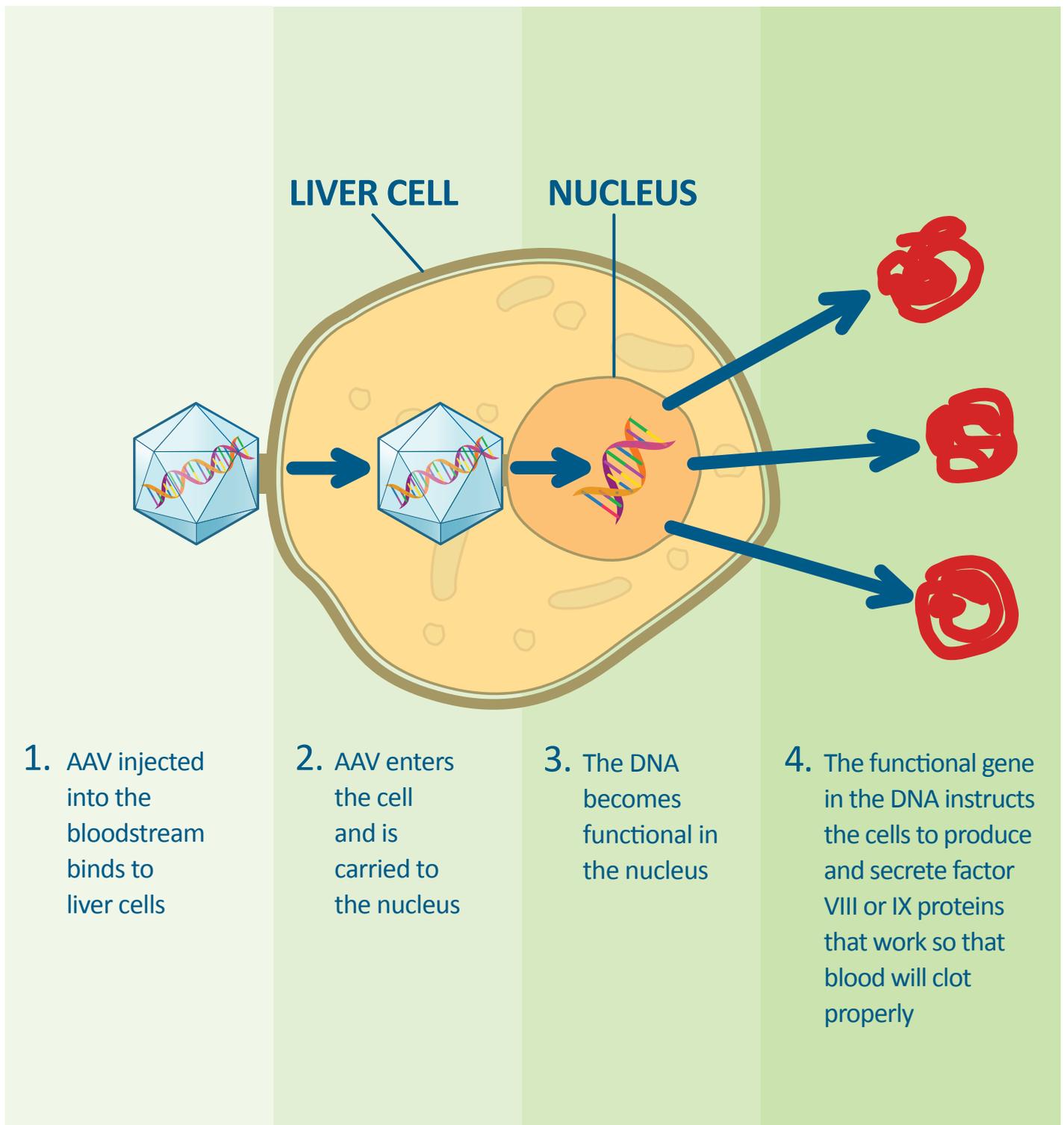
The aim is to deliver a **therapeutic** or functional version of the **factor VIII** or **IX gene** into the body, so that it then gives the right target cells in the body directions to produce factor VIII or IX that works properly.

DNA cannot be delivered orally, for example, in a tablet or syrup. When it is delivered through an injection or drip into a vein, the DNA needs to be protected or it will be destroyed in the bloodstream.

Some viruses are often used as vectors in gene therapy because they can deliver the new DNA by entering cells. They are very efficient at this, which is why so many viruses cause infections in us. Scientists have tested several viruses and removed any virus genes to insert the factor VIII or factor IX genes. The adeno-associated virus (AAV) has proven to be very effective in delivering new factor VIII or factor IX genes to animals and humans.

The viruses have a shell or **capsid** which protects the DNA and also targets particular body tissues. Factor VIII and IX are produced in the liver, so the AAV vector used in gene therapy for haemophilia is designed to target liver cells.

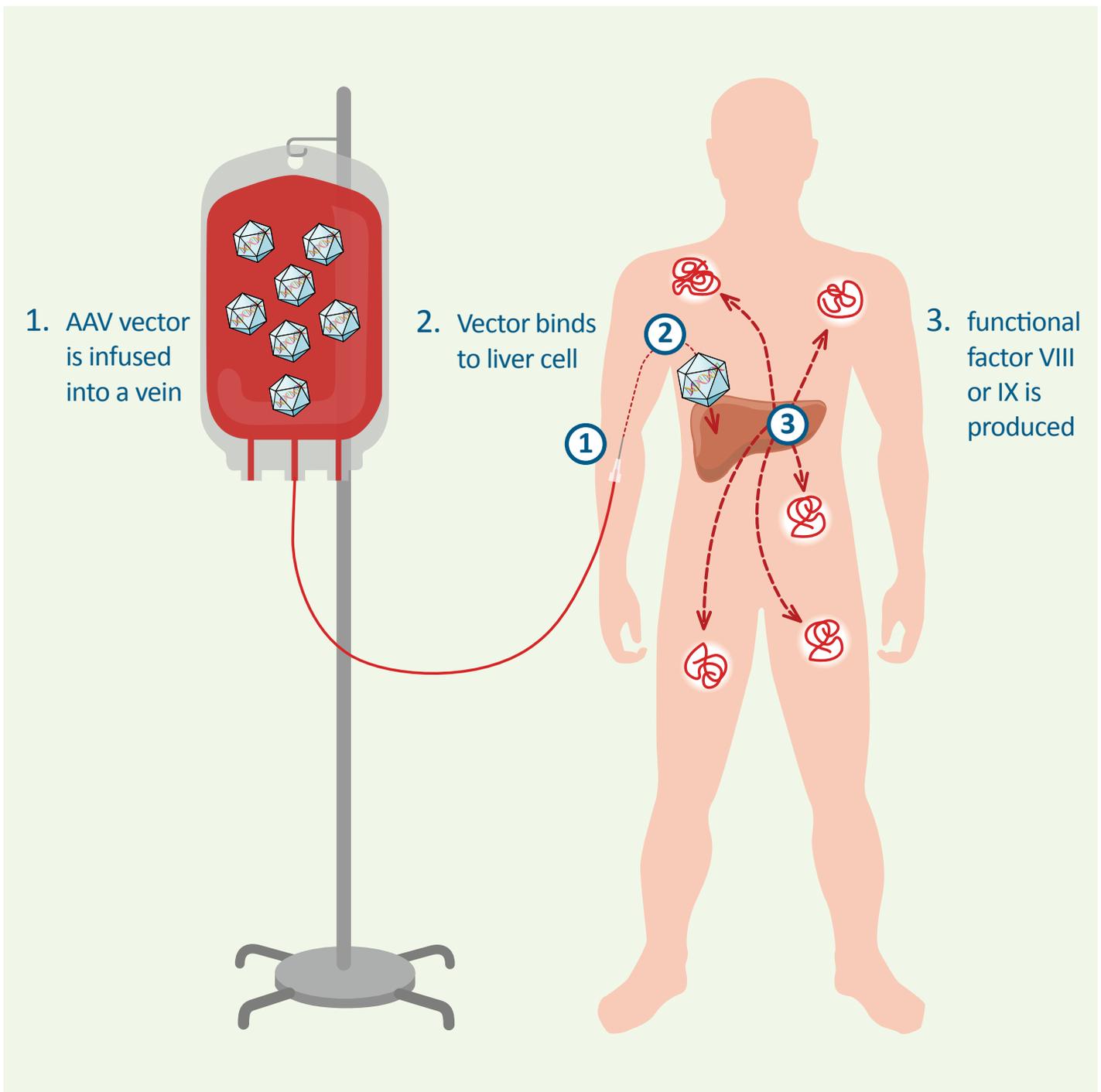




## Will using the AAV vector give me a disease?

The virus (AAV) does not cause illness in humans. The virus is also modified in the laboratory to replace the AAV DNA with the DNA containing the therapeutic gene.

This means the AAV vector is very unlikely to cause disease when used in people, but instead will help to produce working factor VIII or factor IX.



## How will I have gene therapy?

Gene therapy is often given in a single infusion (drip or slow injection) of fluid that contains the AAV vector with the functional gene. If you are having gene therapy, you will visit the clinic administering your clinical trial and have the infusion into a vein.

When the AAV vector is in your blood-stream, it will travel to your liver. There it will bind to

liver cells. The vector DNA will enter the nucleus of the cell. Then the functional gene in the DNA will give your body instructions to produce factor VIII or factor IX proteins that work so that your blood clotting will improve.

Your body will take several months to increase the amount of functional factor VIII or factor IX in your blood and stabilise.

## How effective is it?

Gene therapy for haemophilia has been studied in humans for more than 20 years. Early clinical trials in haemophilia were not successful but addressed many technical problems that needed to be overcome.

More recent clinical trials with AAV vectors have only involved small numbers of people. To see what would improve results the trials tested a range of doses and different variants of functional factor, such as the factor IX Padua variant, which occurs naturally. This variant has enhanced factor IX activity.

The AAV vector trials for haemophilia A and haemophilia B were testing to see what sustained results could be seen 12 months after the infusion. The most successful trials had results showing that at this point:

- most participants with severe haemophilia (less than 1% of normal factor activity) had increased their factor activity levels to the mild haemophilia range (5-40%)
- some had increased their factor activity levels to the normal range (more than 40%)
- most had been able to stop prophylaxis
- nearly all had few if any bleeds in the last 12 months.

Many questions remain, including how long this effect will last and how long to monitor for safety. These clinical trials have been following up the people who had gene therapy for between 1 and 8 years.

Current clinical trials in Australia may be testing these or different treatment approaches. If you are considering taking part in a clinical trial for gene

therapy, it is important to find out what it is testing and what the previous trial results have shown.

## What about immunity to AAV?

A challenge with using AAV to deliver the gene is that it is a virus and people's immune systems may attack it and destroy it. Many strains of the virus AAV already exist in the community and many people have already been exposed to it at some stage during their life, although they would not know because it does not cause illness in humans. If they have been exposed to AAV, their body will have produced antibodies and immune cells to make them immune to AAV. The antibodies will recognise AAV and attack the AAV capsid/shell, so that the treatment doesn't work.

Most current clinical trials exclude people who have pre-existing immunity to AAV. However, researchers are looking at ways to overcome this, for example, with higher doses or by engineering the AAV capsid so that it can hide from the immune response. Some new international clinical trials are testing this.

## What are the side-effects?

A common side-effect of gene therapy with the AAV vector is an immune reaction where ALT (alanine transaminase) levels in the liver are raised. There are not usually other immune-related symptoms and the reaction is treated with corticosteroids, which may also have side-effects.

Other side-effects have included:

- lethargy, tiredness
- anaemia (low levels of red blood cells)
- back pain.



## What is my role in the clinical trial?

When you decide to participate in a clinical trial, you are agreeing to take part in a carefully controlled study that is seeking answers to specific questions. The study will have a plan (protocol) with very particular requirements that need to be followed to provide the evidence to prove these answers. To play your part in the study, you are agreeing to complete all of the requirements so that the researchers can demonstrate that the protocol was followed.

## How safe is it?

Much of the research into gene therapy is relatively recent and there will need to be many more years of clinical trial follow-up to have more certainty about the long-term effects and safety.

As yet no major safety problems have been identified with gene therapy using AAV vectors. They are unlikely to affect a person's genome (the complete set of DNA present in your cells that define how your body works) or to cause cancer. Although DNA from AAV can be found in body fluids for up to a year after infusion, they do not appear to be infectious. Nevertheless, to be absolutely sure, the current clinical trials are monitoring this and asking participants to take precautions to make sure no pregnancies occur in the 12 months following the infusion.

Researchers are also concerned to find out if any participants have a response where their factor activity levels raise to the higher end of normal, eg over 100% of normal, and if this will create a risk of thrombosis. Where possible they are aiming to avoid a response that is too high.

The current gene therapy trials in haemophilia are investigating important questions about gene therapy:

- How well does this gene therapy treatment approach work?
- How safe is it?
- What are the effects of the treatment? Are there side-effects?
- How long does the effect last?

If you agree to participate in one of these gene therapy trials, you will make a commitment to follow the protocol and take part in intensive monitoring, often face-to-face, for more than 12 months.

## What is involved for a participant?

People participating in gene therapy clinical trials are making a commitment to frequent study visits over the first 12 months, with ongoing visits for the long-term follow-up, which is often around 5 years.

The first step in the trial is the selection process. Each gene therapy clinical trial will have its own inclusion and exclusion criteria. You will need to fit all the criteria. To check this, you may need to undergo tests or produce previous test results and will need to provide comprehensive records of your treatment history.

The second step is being available to start. Gene therapy clinical trials for haemophilia in Australia are part of worldwide studies which are administered by experienced experts in gene therapy often working with local Haemophilia Treatment Centres (HTCs) and clinics. Places in the trial are offered internationally to all the sites when they become available. There are often small numbers of places and you would need to be ready to start the trial and have the flexibility to fit with its schedule.

The third step is to start the trial and follow the protocol for the next 12 months. The pharmaceutical company responsible for the gene therapy provides the trial protocol and those conducting the clinical trial arrange for its delivery. The principal investigator of the study is accountable to the company for making sure the protocol is followed.

With the current AAV vector gene therapy trials, the protocol may involve:

- Multiple visits to the clinic in preparation
- A visit to the clinic for the gene therapy infusion, followed by a period of monitoring in the hospital to check for any negative (adverse) effects. You may need to stay overnight

- Then regular visits to the HTC over the next 12 months for testing and monitoring - at times weekly, at other times monthly or quarterly
- This may mean organising regular travel to the clinic and overnight accommodation
- Tests may include providing blood, faeces and other body fluid samples, such as semen and saliva
- Some trials may require you to abstain from alcohol or limit physical exercise
- Providing comprehensive and real-time electronic records of bleeds and treatments
- Committing to ensuring that a pregnancy is not possible, which may involve double contraception (eg, condom and contraceptive pill) for both you and your partner.

## What follow-up is involved?

The long-term effect of gene therapy is an important question and participants will be followed for some years. This may require visits to the HTC every 3 or 6 months for testing and monitoring for several years – on average 5 years.

## What support services are available on a clinical trial?

Support services such as psychological or social support would be provided through the clinic, and may be provided by a referral to an external service.

# Frequently asked questions

## Is gene therapy a cure for haemophilia?

**A.** In many cases successful gene therapy means that a person with severe haemophilia now has mild haemophilia, although a small number now have normal factor levels.

It is not yet known how long this effect will last or whether there are any long-term negative effects and trial participants will need to be followed up for many years. Currently it is not possible to deliver a second dose or booster for the same gene therapy, but this may be possible in the future.

Gene therapy cannot remove the joint and muscle damage a person with haemophilia has already developed over their lifetime. However, some people who have had successful gene therapy talk of no longer having the 'little niggles' that could be minor bleeds. Researchers are also investigating the long-term impact of stable factor levels – will they, for example, improve target joints?

If a person with haemophilia has a bleed after successful gene therapy, they may still need factor replacement therapy to treat their bleed.

## Who can have gene therapy for haemophilia?

**A.** Gene therapy for haemophilia is only available in clinical trials and trial participants are selected carefully.

Australian clinical trials currently require that you are an adult male with severe haemophilia

They may also have other requirements, for example, that you have had factor replacement therapy before, or that you have good liver and kidney health, or that you do not have 'active' blood borne virus infections.

Most clinical trials also require that you do not have inhibitors, but at least one new trial internationally is studying gene therapy in people with inhibitors.

Most clinical trials also exclude people with pre-existing immunity to AAV, but some new studies are now including people with low level AAV immunity to investigate whether new approaches to the treatment, such as altering the dose or changes to the vector, can manage the immune response.

Each clinical trial will also have its own specific requirements.

Clinical trials are not available at all hospitals. If you want to know about clinical trials at your hospital or others, speak to your treating doctor, and ask about the criteria to see if you might be a suitable candidate.

## Can children have gene therapy?

**A.** At present gene therapy clinical trials are only for adults. This is for several reasons but mainly because of safety. Researchers need to understand the long-term impact of gene therapy in adults before it is given to children, as the effect could last for their entire lives. Children are not old enough to give informed consent themselves and their parents would need to make the decision, and this may not be the same decision the child would make later when they are an adult. Children are also at earlier stage of development and some processes, like the immune response or how proteins are produced, may be accelerated or different in a child.

However, the question of gene therapy in children is high on the agenda for researchers. Childhood is a time when there is a lot of physical activity and growth and children have a lot to gain from



potentially normalising their factor levels with gene therapy. It is likely that at some stage in the future clinical trials involving children will be developed and become available.

### **Q** What is the difference between gene therapy for haemophilia A and for haemophilia B?

**A.** A challenge for AAV vector gene therapy for haemophilia A has been that the factor VIII gene mutation is large and it has been difficult to pack it inside the virus shell (capsid) used to deliver gene therapy. As a result, researchers have cut down the factor VIII gene sequence, only leaving the necessary parts, so that it can fit inside.

### **Q** Can my children still inherit haemophilia from me after I have gene therapy?

**A.** Yes. AAV vector gene therapy for haemophilia only affects the person receiving the gene therapy. It transfers the new functioning section of DNA into the nucleus of a liver cell. The DNA is not integrated into a chromosome and the liver cell is non-reproductive, ie, it does not produce eggs or sperm, so the effects of the gene therapy will not be passed on to your children. This is called **somatic gene therapy**.

**Germline gene therapy** can alter many types of cells, but it also transfers the new functioning

section of DNA to reproductive cells, like sperm and eggs. The effects of germline gene therapy are passed from parent to child and down the generations. Germline gene therapy is controversial because of the risks to future generations and safety concerns.

## What kinds of gene therapy are in the pipeline?

**A.** Gene therapy technologies are evolving rapidly, with many different clinical trials to test new technologies for health conditions. Some of these technologies are now also being tested in haemophilia.

This includes gene editing techniques, such as the zinc finger nucleases or the CRISPR-Cas9 system that work like molecular scissors to cut the DNA at a particular location so that a gene mutation can be repaired. These are experimental and need to be tested carefully in the laboratory first for safety - to make sure they only 'edit' the exact part of the DNA, can make the repair effectively and do not affect other parts of the DNA. Some of these techniques are now being tested in the laboratory for haemophilia. At least one has now commenced a clinical trial in humans.

## More information

For more information on gene therapy in haemophilia, speak to your treating haemophilia specialist doctor or a gene therapy expert who is undertaking clinical trials in haemophilia.

To find out how to get in touch with your local specialist Haemophilia Treatment Centre, contact:

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# Clinical trials

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