CRISPR Genome Editing 2

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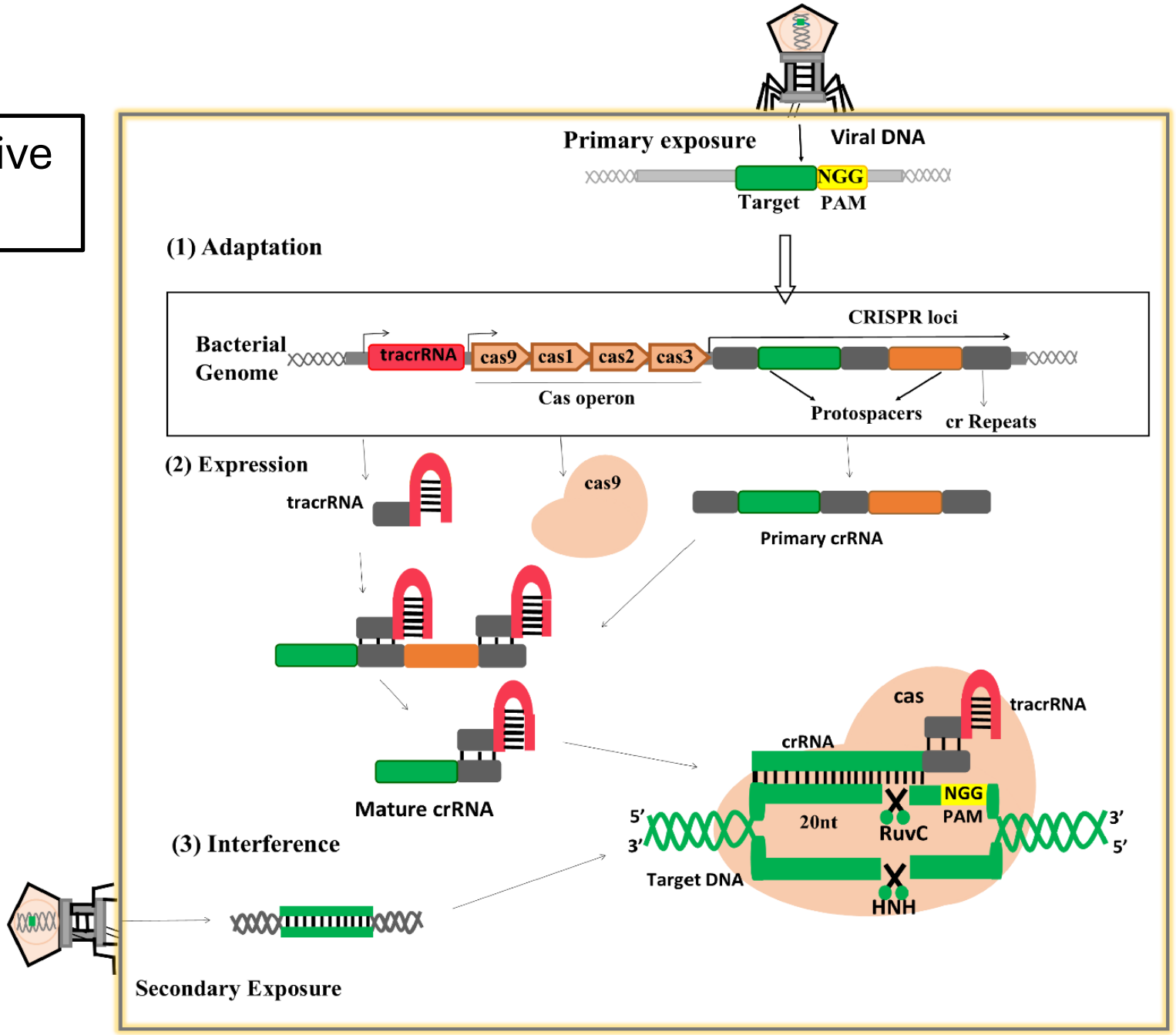
CRISPR/CAS 9 system of adaptive Immunity in bacteria



Farooq et al., 2018

Understanding the bacterial CRISPR locus

› J Mol Evol. 2005 Feb;60(2):174-82. doi: 10.1007/s00239-004-0046-3.

Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements

Francisco J M Mojica [1], César Díez-Villaseñor, Jesús García-Martínez, Elena Soria

Affiliations + expand

PMID: 15791728 DOI: 10.1007/s00239-004-0046-3

Abstract

Prokaryotes contain short DN repeats known as CRISPR, recognizable by the regular spacing existing between the recurring units. They represent the most widely distributed family of repeats among prokaryotic genomes suggesting a biological function. The origin of the intervening sequences, at present unknown, could provide clues about their biological activities. Here we show that CRISPR spacers derive from preexisting sequences, either chromosomal or within transmissible genetic elements such as bacteriophages and conjugative plasmids. Remarkably, these extrachromosomal elements fail to infect the specific spacer-carrier strain, implying a relationship between CRISPR and immunity against targeted DNA. Bacteriophages and conjugative plasmids are involved in prokaryotic population control, evolution, and pathogenicity. All these biological traits could be influenced by the presence of specific spacers. CRISPR loci can be visualized as mosaics of a repeated unit, separated by sequences at some time present elsewhere in the cell.

CRISPR for genome editing

Received the Nobel Prize in Chemistry in 2020

Science. 2012 Aug 17;337(6096):816-21. doi: 10.1126/science.1225829. Epub 2012 Jun 28.

A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.

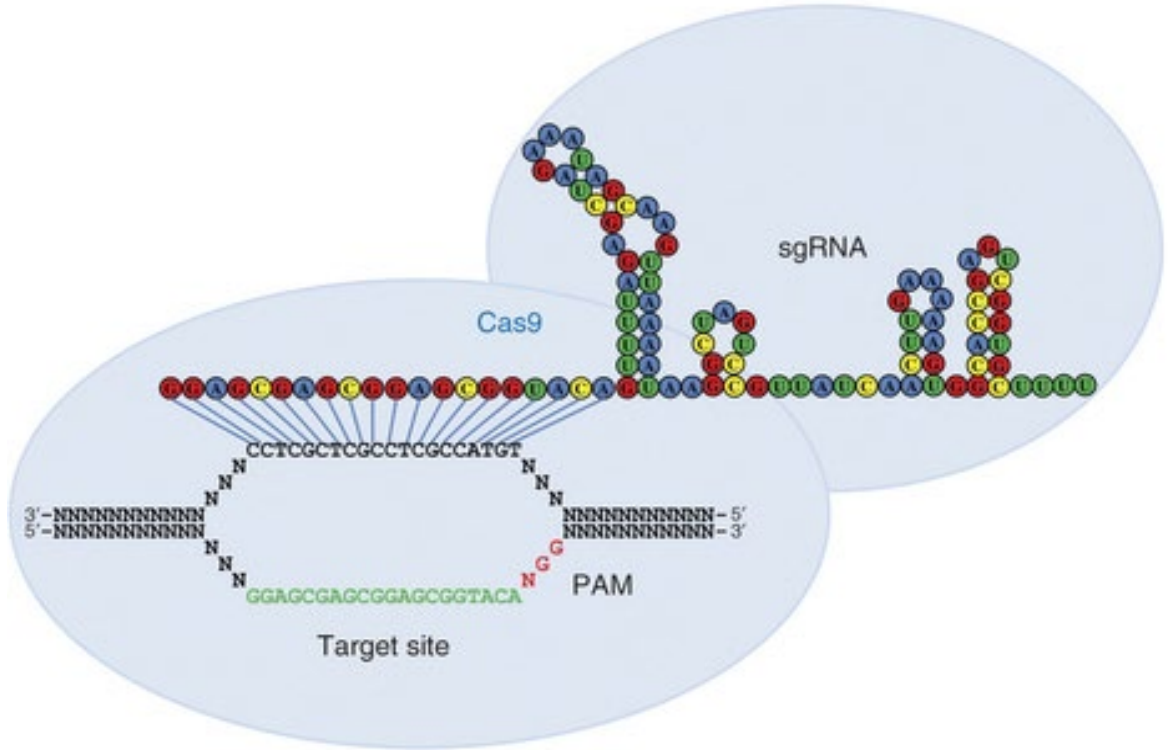
Jinek M[1], Chylinski K, Fonfara I, Hauer M, **Doudna** JA, **Charpentier** E.

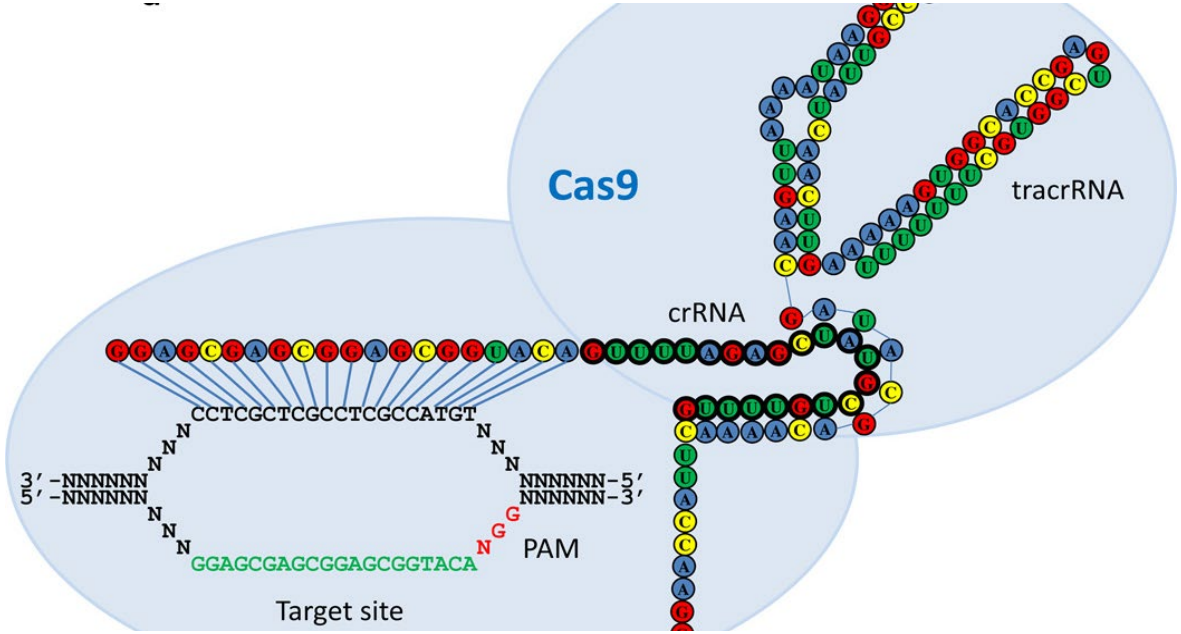
Author information

Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

CRISPR Genome editing





A cure for Sickle Cell Disease and β-Thalassemia

- **Sickle cell disease** affects about 100,000 Americans
  - About 5,000 Canadians - most common inherited disease
  - Higher frequency in **persons of African Decent**
- Mutation in the **Beta Globin Gene** (**β-globin**)
  - **S** allele: 2nd position **transversion** in 6th triplet

- **Sickle cell disease** (**SS** homozygous) & **Sickle Cell Trait** (**AS** heterozygous) at high frequency in **West Africa**
  - **Sickle Cell Trait** provides resistance to **Malaria**
  - **1 in 12 Africans** are heterozygous **carriers** (**AS**, Sickle Cell Trait) for the mutant hemoglobin allele

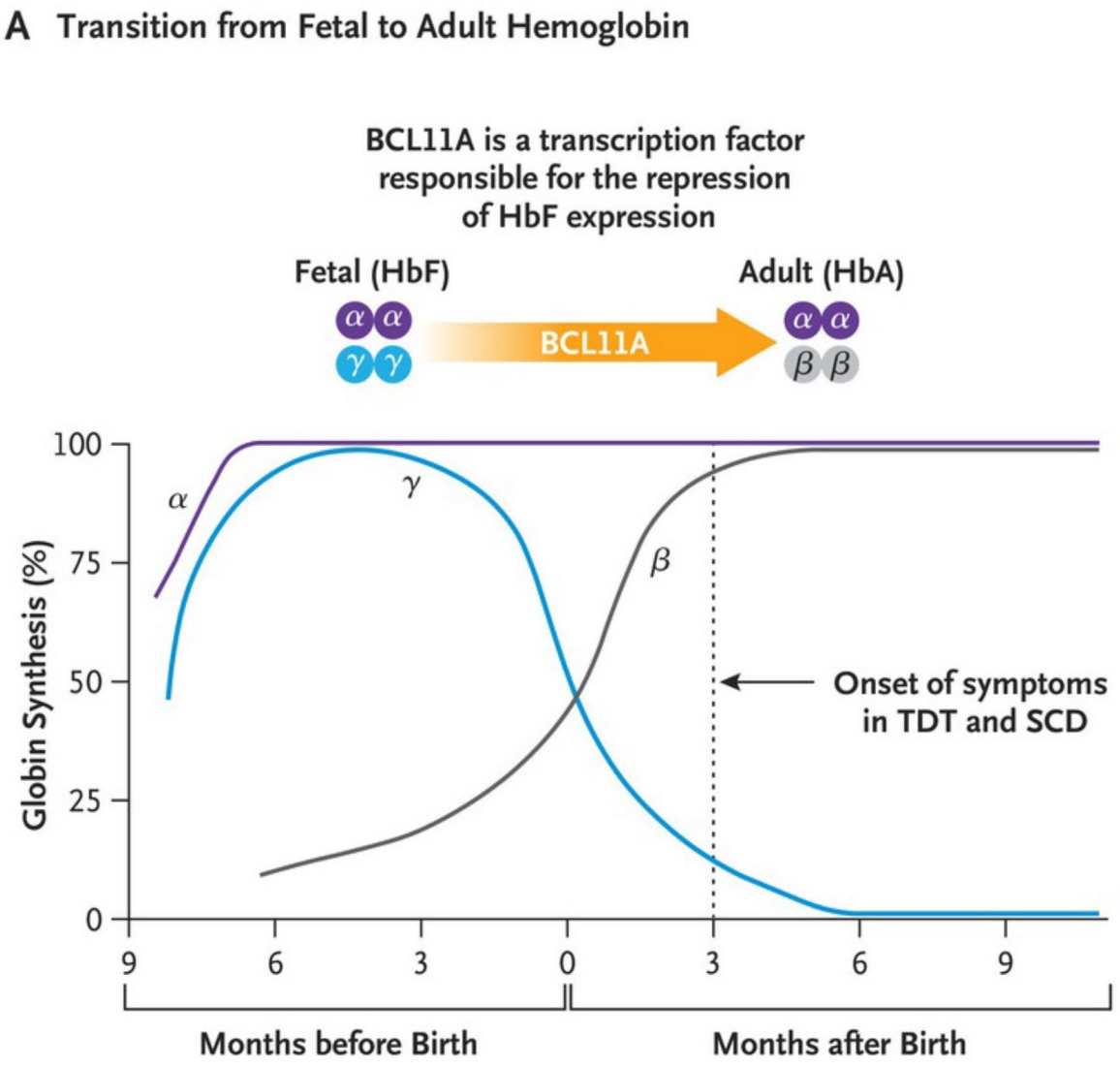
- **Hemolytic anemia, vaso-occlusive events that result in end organ damage, and reduced lifespan**.
  - Regular packed-RBC blood transfusions required.

- **β-Thalassemia**: Mutation in β-globin results in **severe anemia** but no sickling of RBCs
  - Weekly/monthly blood transfusions

A cure for Sickle Cell disease- a tale of two hemoglobins

Patients with both sickle cell disease and HPFH mutations, have few symptoms, if any (Jacob and Raper, 1958).

Medications that increase the production of gamma globin (Hydroxyurea) can improve quality of life for sickle cell patients



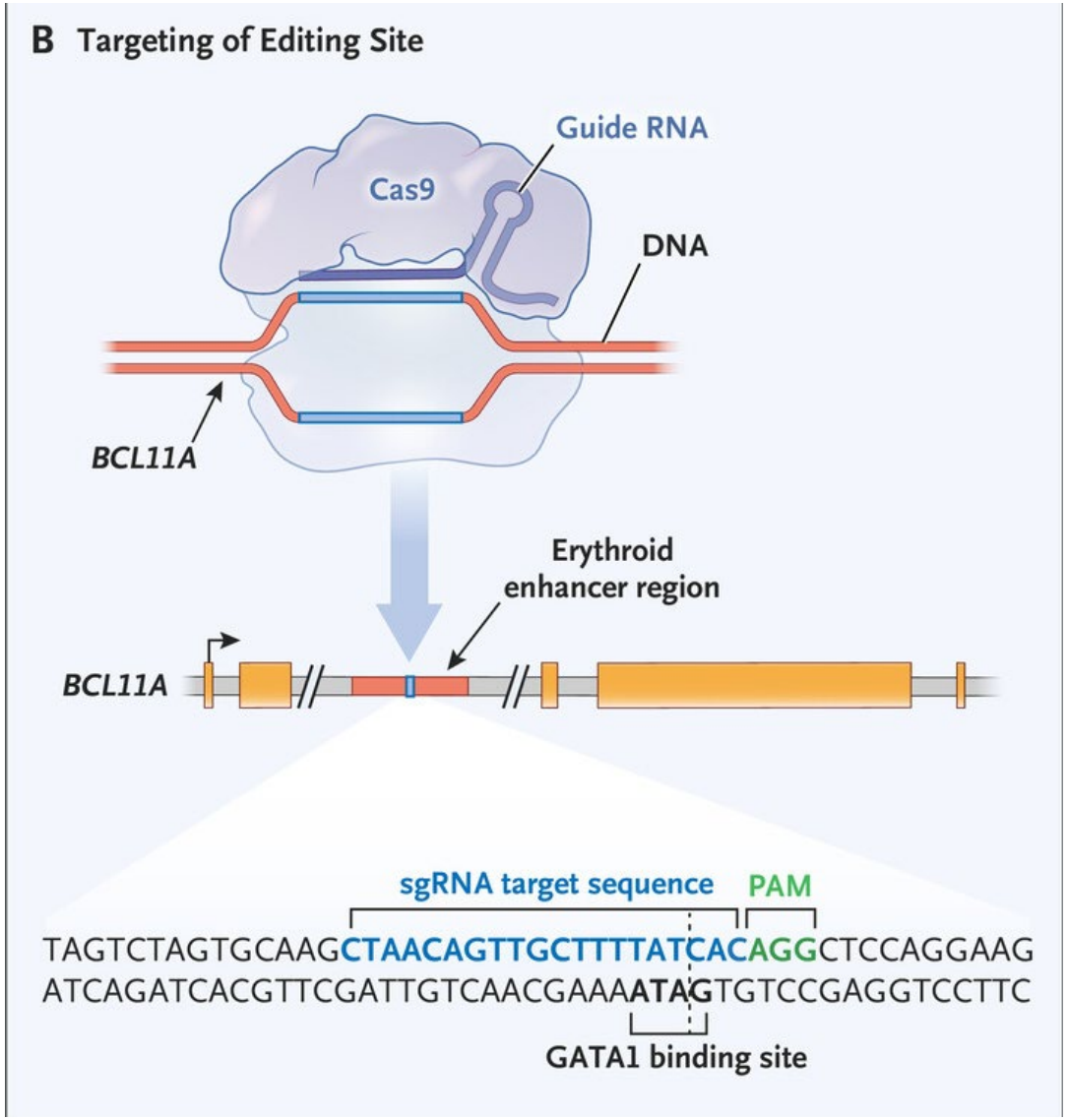
Frangoul et al., 2020

CTX001 (Casgevy)
Vertex Pharmaceuticals

Phase 1 clinical trials

CD34 +ve Cells from Patient 1 were edited at a frequency of 68.9%

CD34 +ve Cells from Patient 2 were edited at a frequency of 80.7%



Frangoul et al., 2020

First test patients

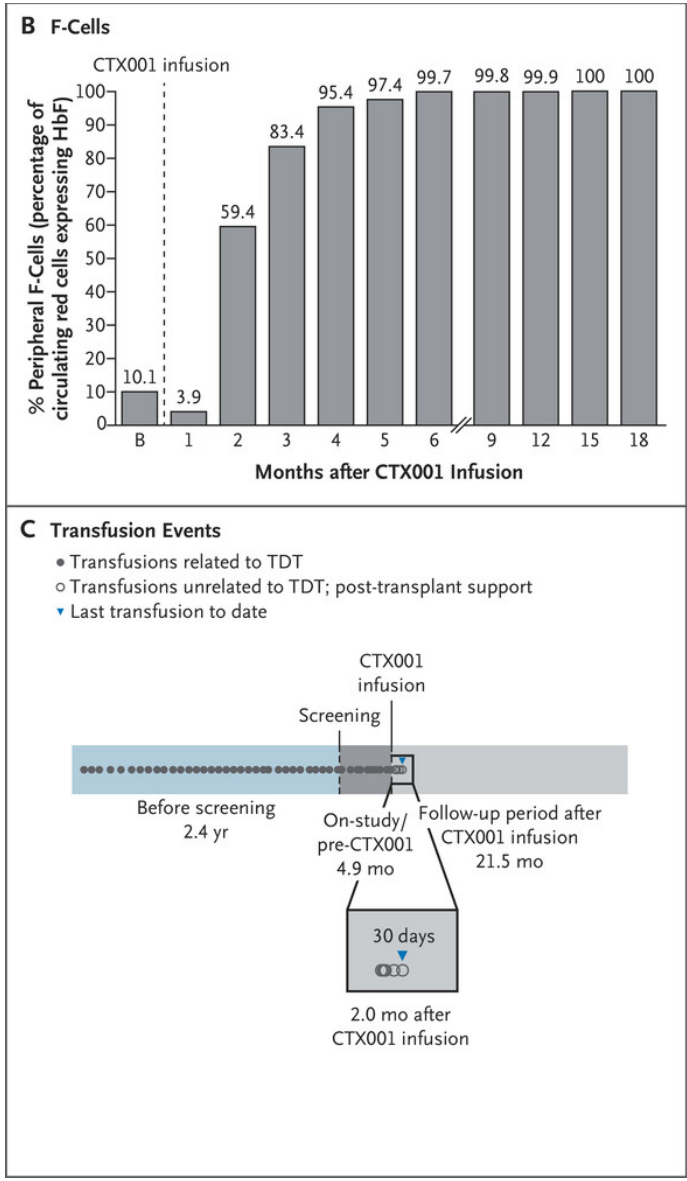
- Patient 1- 19-year-old woman
  - Transfusion-dependent **β-thalassemia**
  - Average of **34 units packed RBCs** per year

- Patient 2- 33-year-old woman
  - **Sickle Cell Disease**
  - Average of **seven vaso-occlusive events** per year
  - Average of 3.5 hospital stays per year
  - 5 RBC transfusions per year

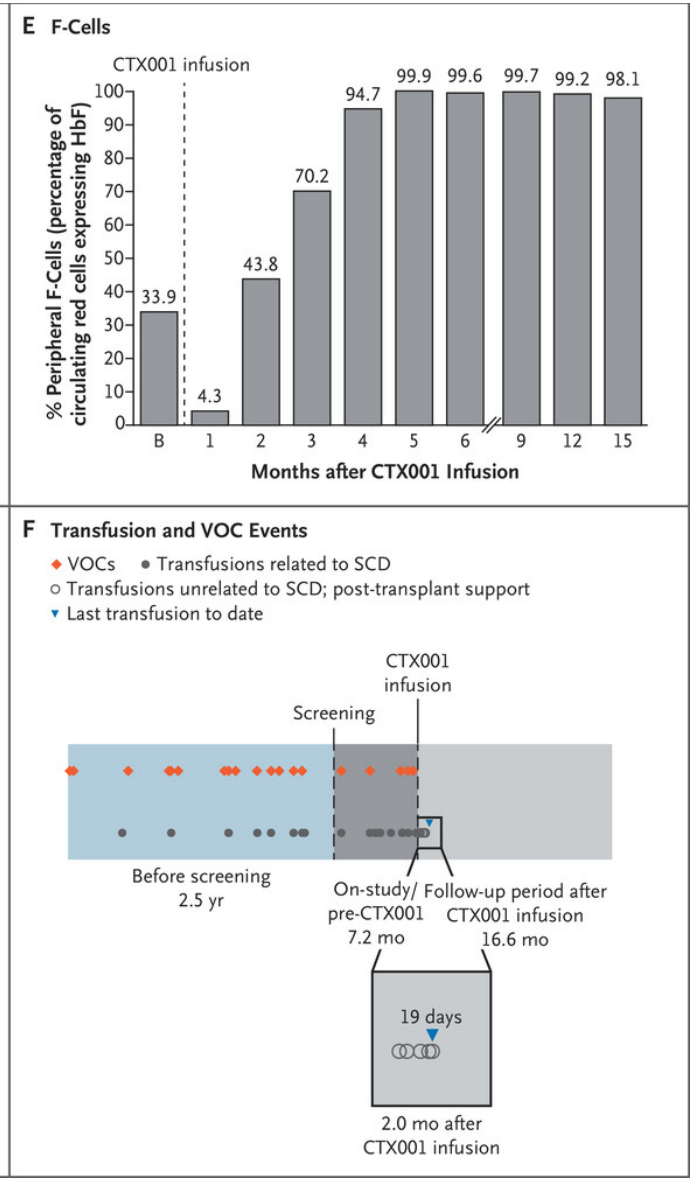
First test patients

Frangoul et al., 2020

Patient 1- TDT



Patient 2- SCD



Approval of CTX001 (Casgevy)

- **Approved in the UK** for patients 12 and over suffering from Transfusion dependent β-Thalassamia and Sickle Cell disease in November 2023.
- **Approved in the US** for patients 12 and over suffering from Transfusion dependent β-Thalassemia and Sickle Cell disease in January 2024.
  - Approval based on Phase Three study with 31 patients.
- Approved in European Union, Saudi Arabia, & Bahrain.
- Health Canada began priority review of Casgevy on April 1st 2024.
- Cost **US$2.2 million** per treatment

Casgevy treatment plan

- **STEP 1:** Mobilization medicine- moves hematopoietic stem cells from bone marrow to circulation. Stem cells collected.
  - Takes one week
  - May have to be repeated
- **STEP 2:** Stem cells sent to manufacturing site for gene editing.
  - **Casgevy** sent to healthcare provider.
  - Takes up to six months: editing efficiency and off target effects assessed.
- **STEP 3:** Conditioning medicine- clears stem cells form bone marrow.
  - 2 - 3 days, in hospital
- **STEP 4:** Intravenous infusion of Casgevy.
  - 4 - 6 weeks in hospital- ensure cells graft &
  - Blood cell counts return to normal.

Casgevy – equitable access

- **Cell and Gene Therapy Access Model**
- **Reduce cost of one-time gene therapies** to treat rare diseases.
- **US Federal Funding** for states for access to cell and gene therapies
- **Reduced price negotiated** between Federal Government and manufacturer
- States receive **rebates for Medicaid access** to treatments
  - Participation in the program is voluntary.
- **Ancillary costs covered -** travel, fertility preservation
- Rollout in **January 2025**.
- 75% of sickle cell patients live in Africa - approval, medical infrastructure, cost?

Comparable to other treatments

- **Casgevy (Vertex Pharmaceuticals)**
- CRISPR/CAS9 editing to prevent BCL11A expression in hematopoietic (CD34) stem cells.
- Reduced β-globin expression, increases γ-globin expression
- 29 (93.5%) of SCD patients had **no vaso-occlusive events** in 12 months following treatment.
- Patients will be followed for 15 years
- US$2.2 million cost of treatment.
- No serious adverse events reported.

- **Lyfgenia (bluebird Bio)** - **lentiviral vector** to insert **new copy of β-globin gene**.
- Approved in 2023/2024 for SCD (36 patients)
- 88 % of patients had **no vaso-occlusive events** in 12 months following treatment
- Patients will be followed 15 years
- US$3.1 million cost of treatment
- **Blood cancers occurred in some patients** (lifelong monitoring for blood malignancies required)