CRISPR/Cas9

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BRIEF REPORT

CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

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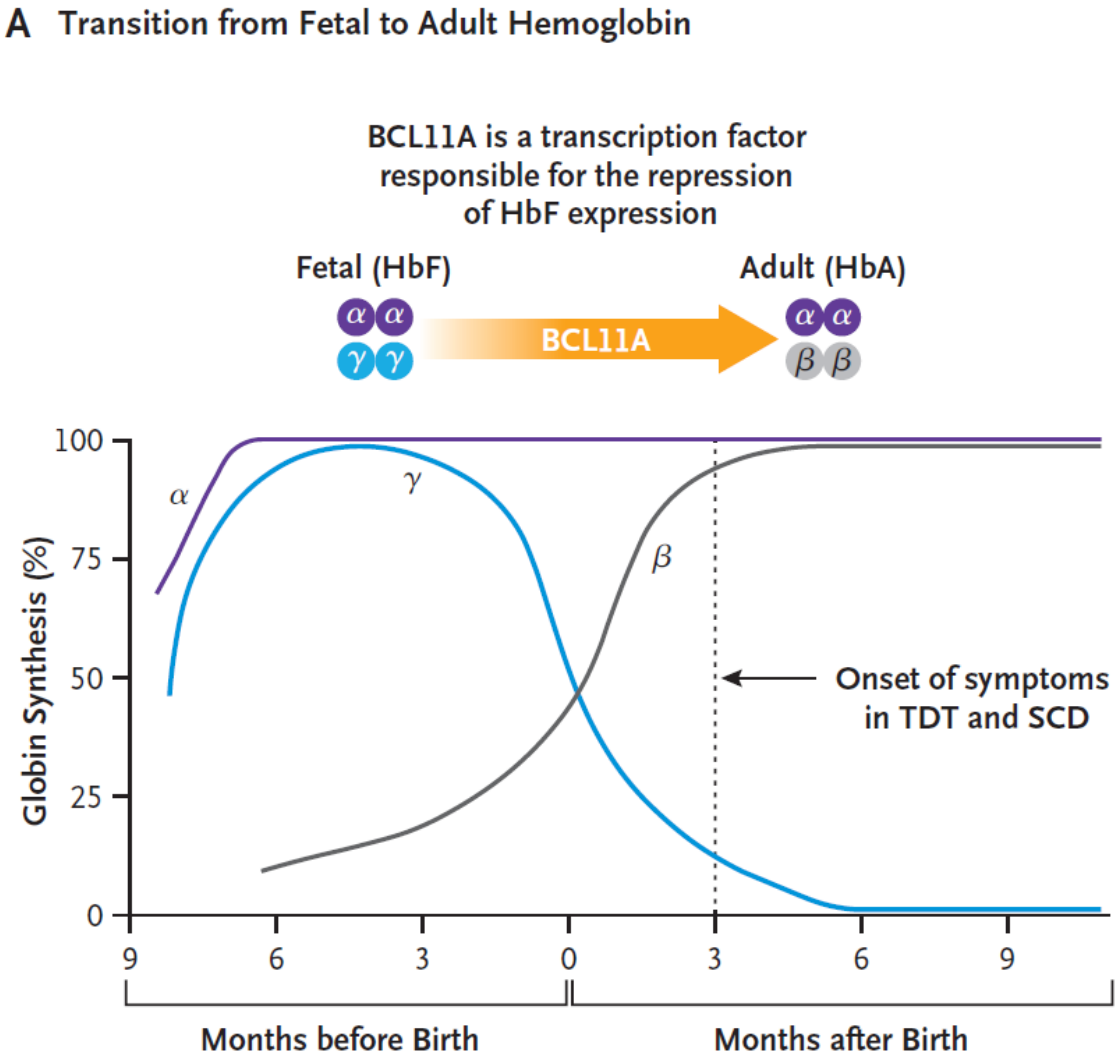
SUMMARY

Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are severe monogenic diseases with severe and potentially life-threatening manifestations. BCL11A is a transcription factor that represses γ-globin expression and fetal hemoglobin in erythroid cells. We performed electroporation of CD34+ hematopoietic stem and progenitor cells obtained from healthy donors, with CRISPR-Cas9 targeting the *BCL11A* erythroid-specific enhancer. Approximately 80% of the alleles at this locus were modified, with no evidence of off-target editing. After undergoing myeloablation, two patients — one with TDT and the other with SCD — received autologous CD34+ cells edited with CRISPR-Cas9 targeting the same *BCL11A* enhancer. More than a year later, both patients had high levels of allelic editing in bone marrow and blood, increases in fetal hemoglobin that were distributed pancellularly, transfusion independence, and (in the patient with SCD) elimination of vaso-occlusive episodes. (Funded by CRISPR Therapeutics and Vertex Pharmaceuticals; ClinicalTrials.gov numbers, NCT03655678 for CLIMB THAL-111 and NCT03745287 for CLIMB SCD-121.)

Relevant for exam: Figures 1 and 2, and Table 1

Figure 1a



- What is HbF and HbA?
- Why is HbF first produced? Is it different from HbA?
- What is BCL11A?

BCL11A

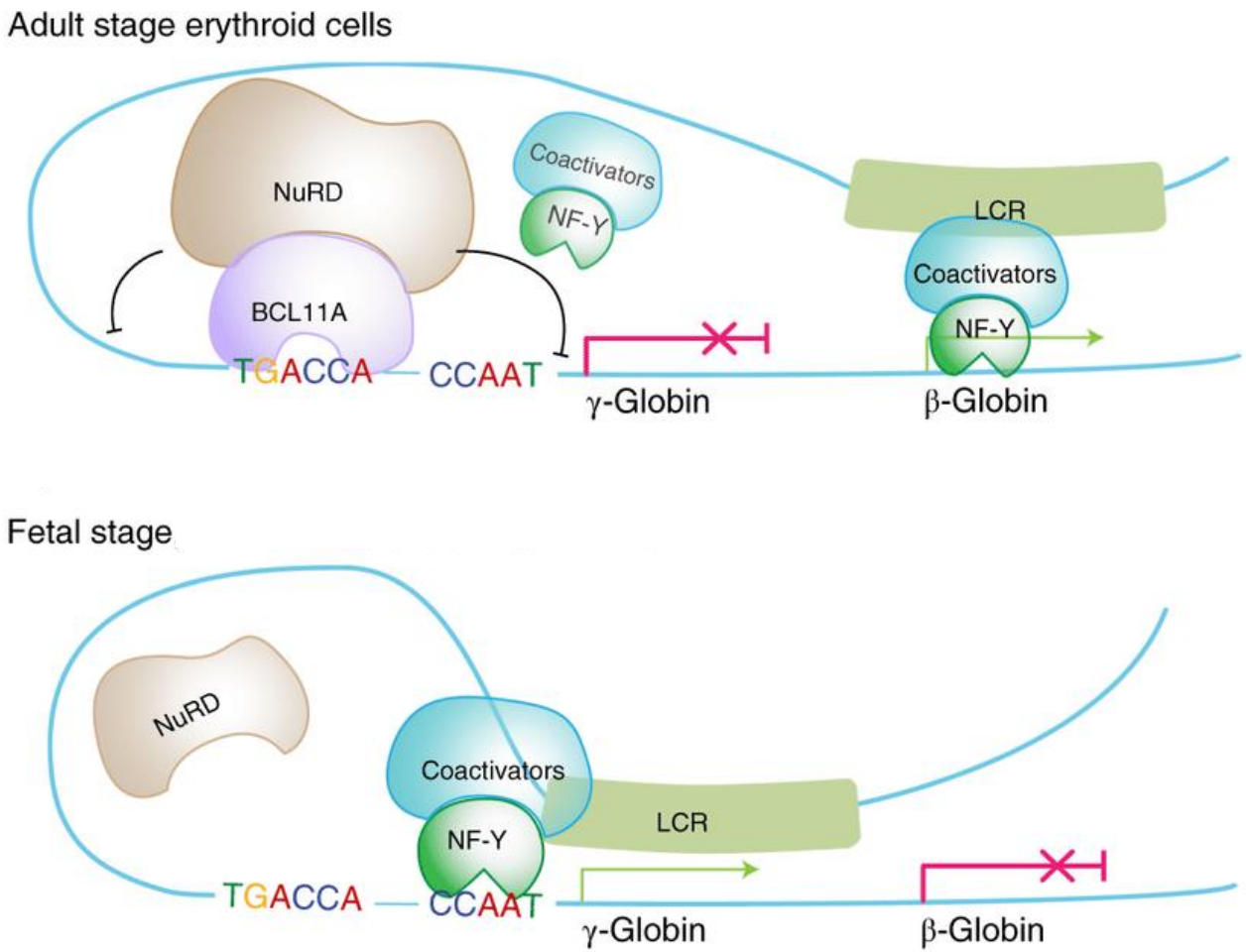
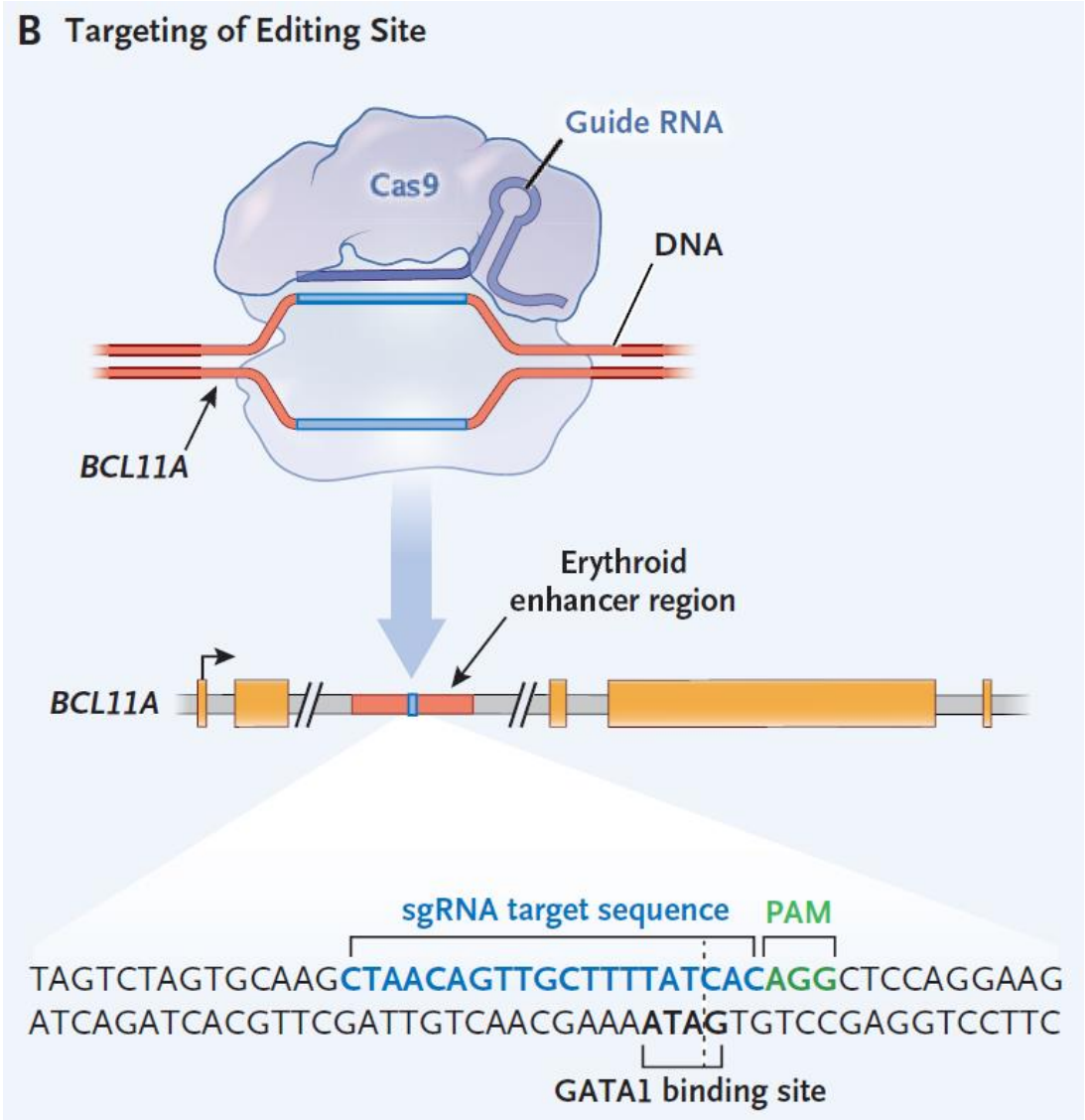

Adult stage erythroid cells
NuRD
Coactivators
NF-Y
BCL11A
TGACCA
CCAAT
γ-Globin
LCR
Coactivators
NF-Y
β-Globin
Fetal stage
NuRD
Coactivators
NF-Y
LCR
TGACCA
CCAAT
γ-Globin
β-Globin

Figure 1b



- What is Cas9/guide RNA doing with the DNA?
  Cut/repair/mutate/insert/delete?
- To which region is Cas9 directed? Why to this region?
- What is the effect of the Cas9-induced modification on BCL11A?

Methods

Supplementary Appendix to:

CRISPR-Cas9 Gene Editing for SCD and Transfusion-Dependent β-Thalassemia

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- How is Cas9 and gRNA brought into cells?
- Which cells? How were the cells obtained?
- How is Cas9 and gRNA produced?

CRISPR delivery strategies

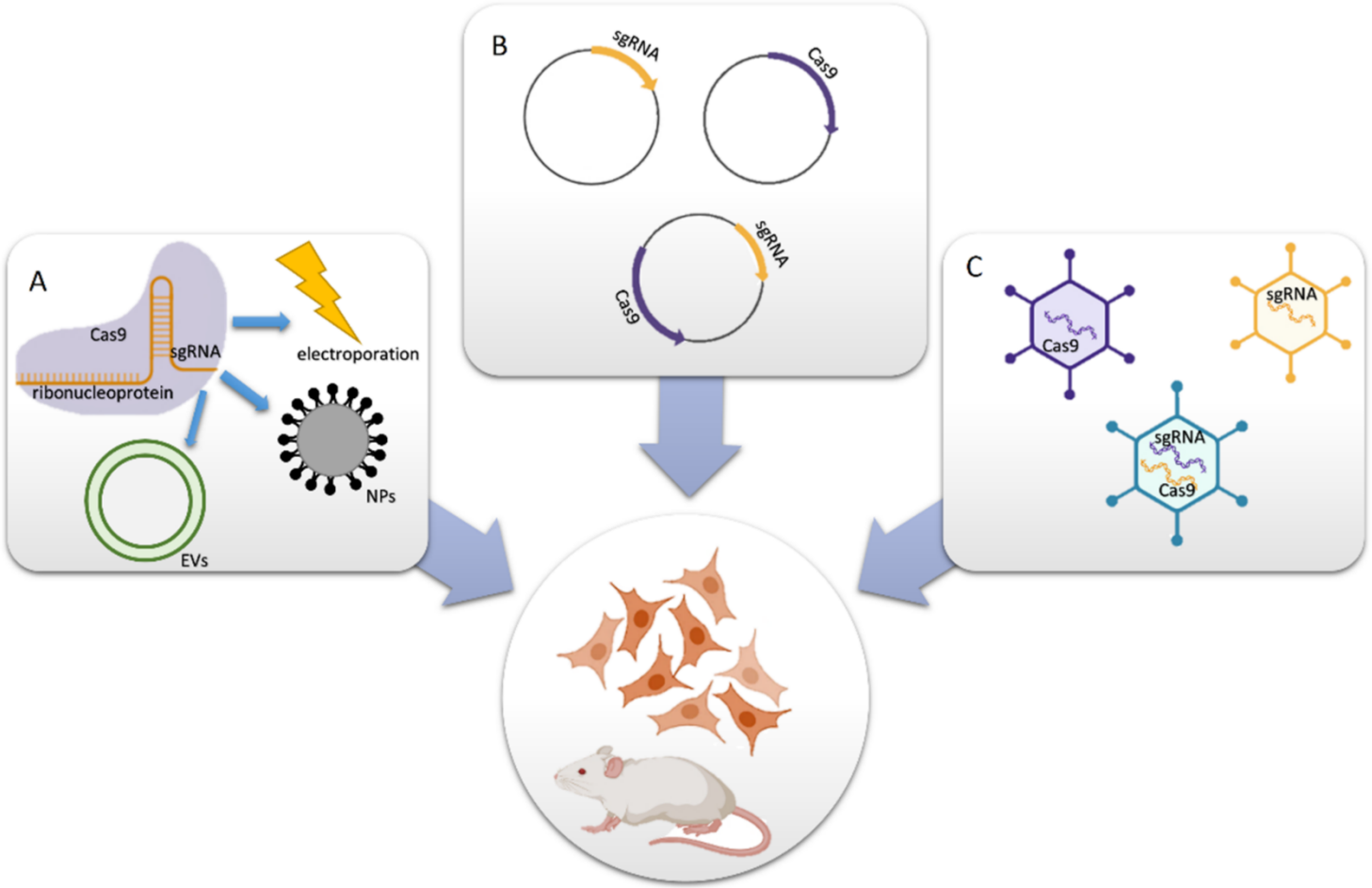
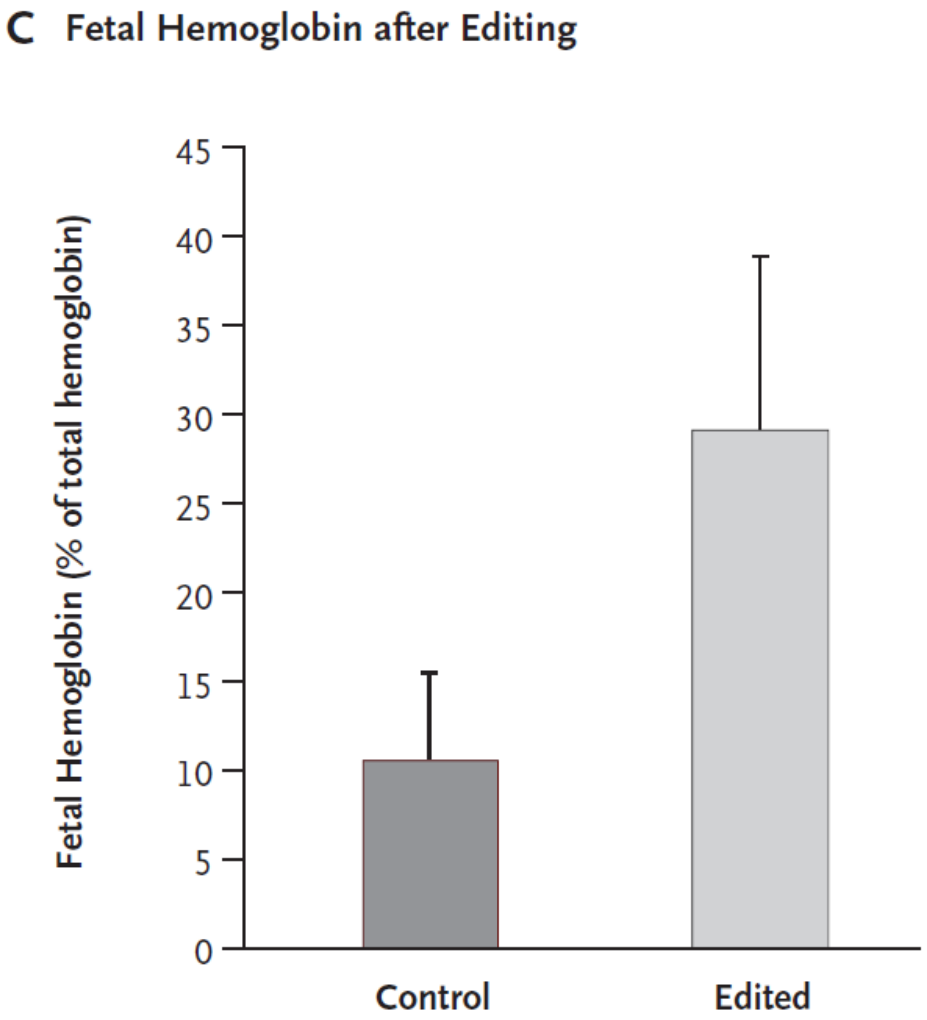


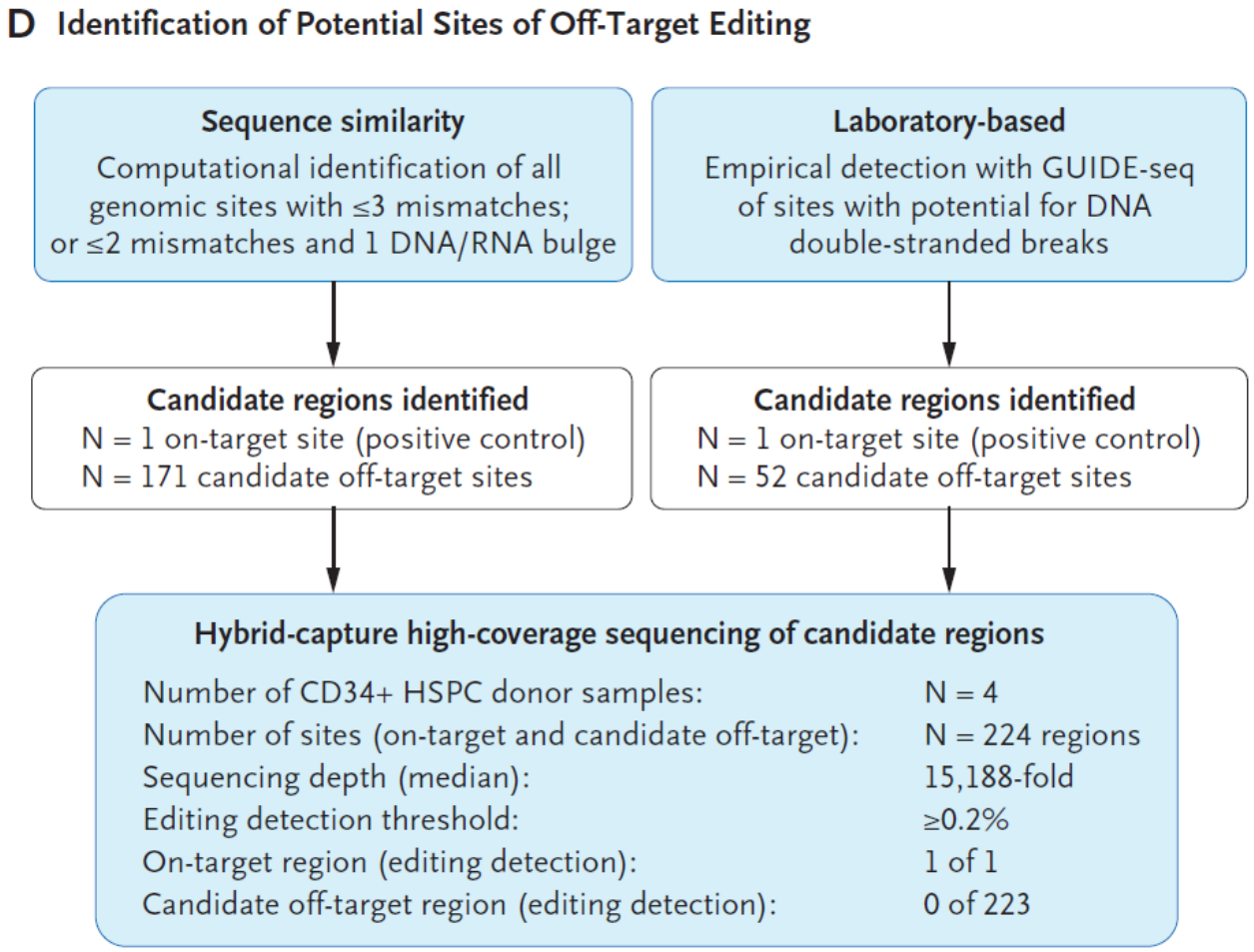
Figure 1c



Gene editing leads to HbF increase

- Which experiment was performed?
- With patients/persons? How many?
- At which time point after gene editing was the HbF level measured?
- Is the increase of HbF sufficient?

Figure 1d



- Why was off-target editing assessed?
- Off-target editing was assessed by DNA sequencing. From whom was the DNA taken?
- Two approaches were followed to identify regions that were then sequenced. Which ones? How do they work?
- How many regions were sequenced? Was off-target editing found?

Patients & treatment

- How many patients? Diseases?
- What are the main medical problems in TDT?
- What are the main medical problems in SCD?
- How were cells obtained from patients? What is «mobilization»?
- How were patients pre-treated before cell infusion (myoablative conditioning)?

Patients & treatment

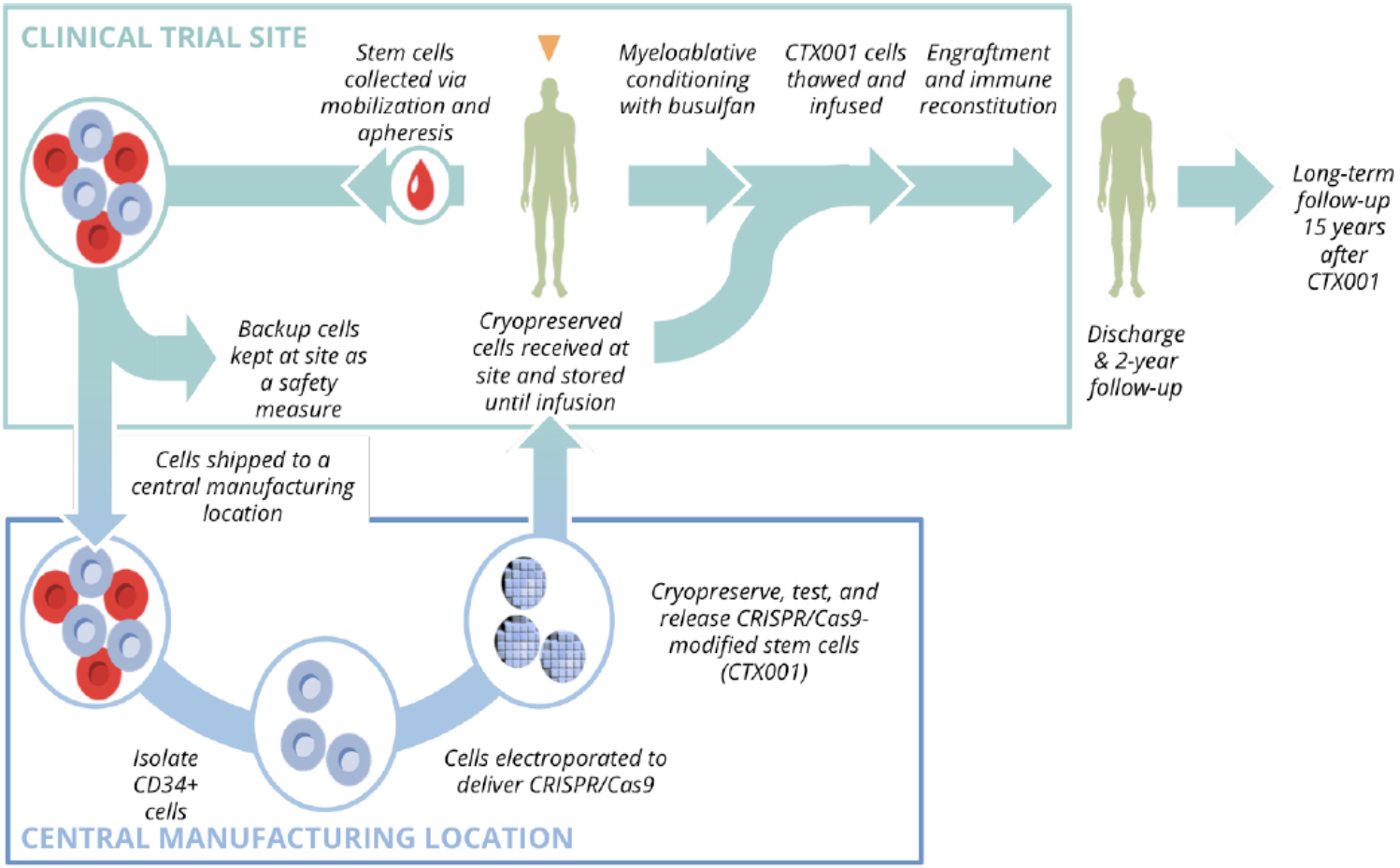


Table 1

Table 1. Frequency of Allelic Editing in CTX001 Cells, Nucleated Peripheral Blood, and Bone Marrow in the Study Patients.*

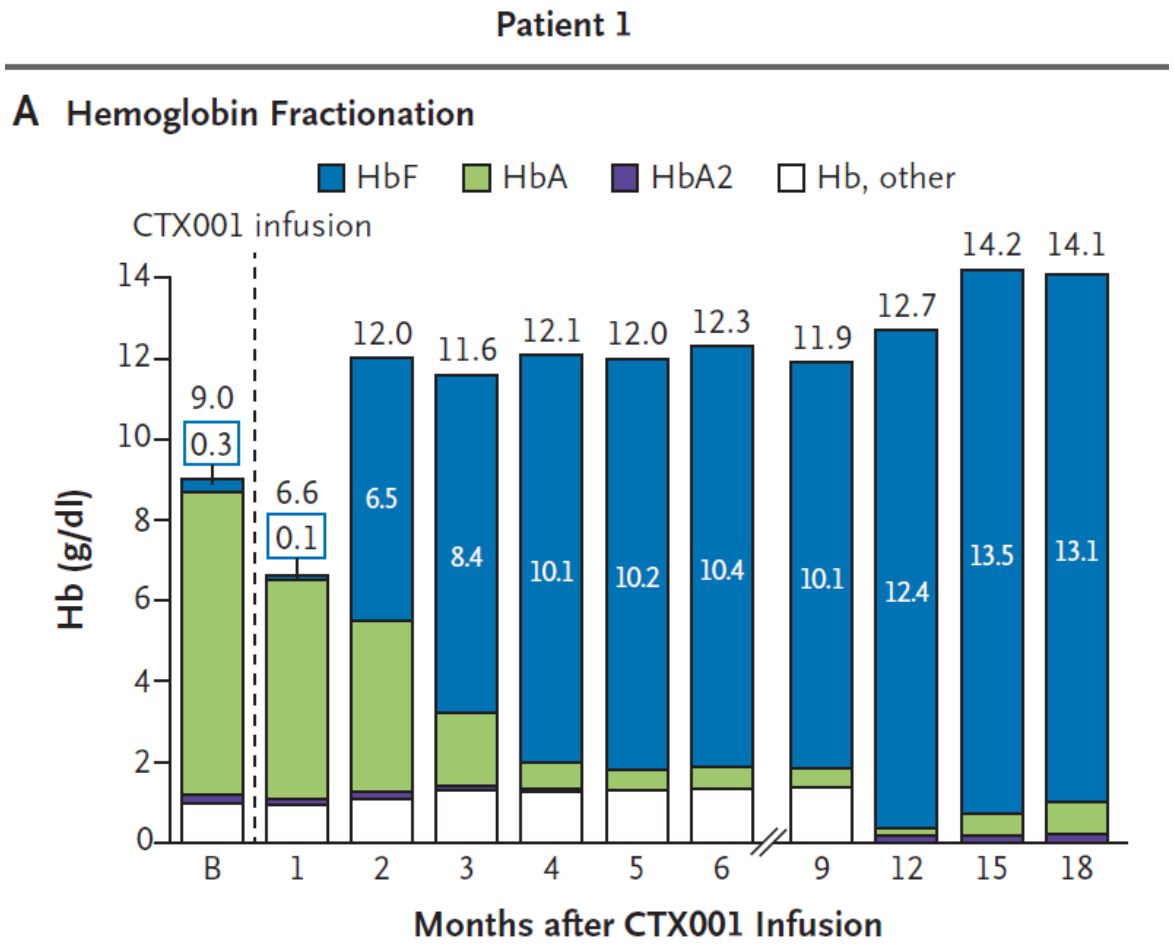
Allelic Editing	CTX001 Cells	Nucleated Peripheral-Blood Cells	Bone Marrow
		percentage of alleles	
Patient 1 with TDT			
Mobilization cycle 1†	68.9		
Mo 1		48.1	
Mo 2		69.7	
Mo 3		66.4	
Mo 4		62.3	
Mo 5		63.2	
Mo 6		60.0	78.1
Mo 9		62.8	
Mo 12		64.3	76.1
Mo 18		62.9	

- What means «68.9» indicated for the CTX001 cells?
- What is «Mo 1», «Mo 2», etc.?
- What is «percentage of alleles»?
- Why is the number «percentage of alleles» increasing over time?

Table 1

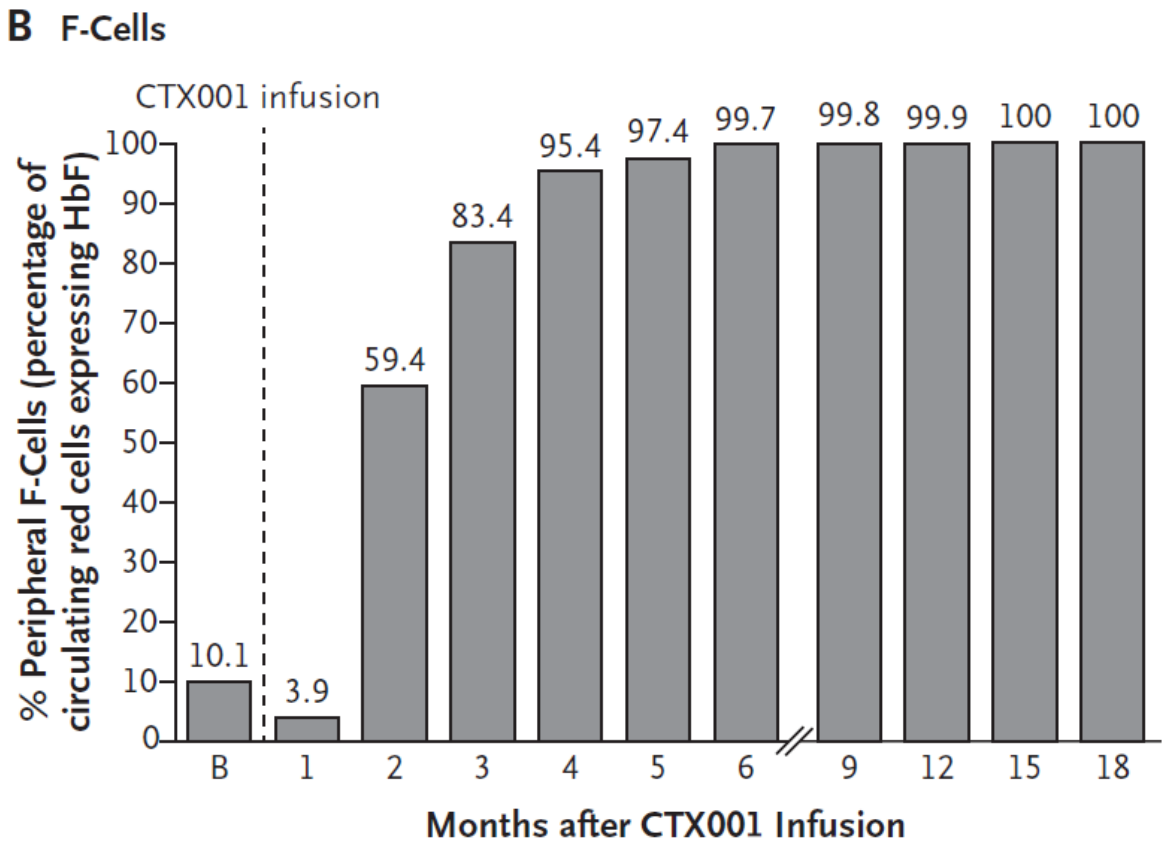
Patient 2 with SCD			
Mobilization cycle 1	82.6		
Mobilization cycle 2	78.7		
Mo 1		48.8	
Mo 2		72.0	
Mo 3		68.8	
Mo 4		72.6	
Mo 5		52.6	
Mo 6		69.4	81.4
Mo 9		64.3	
Mo 12		61.9	80.4

Figure 2a



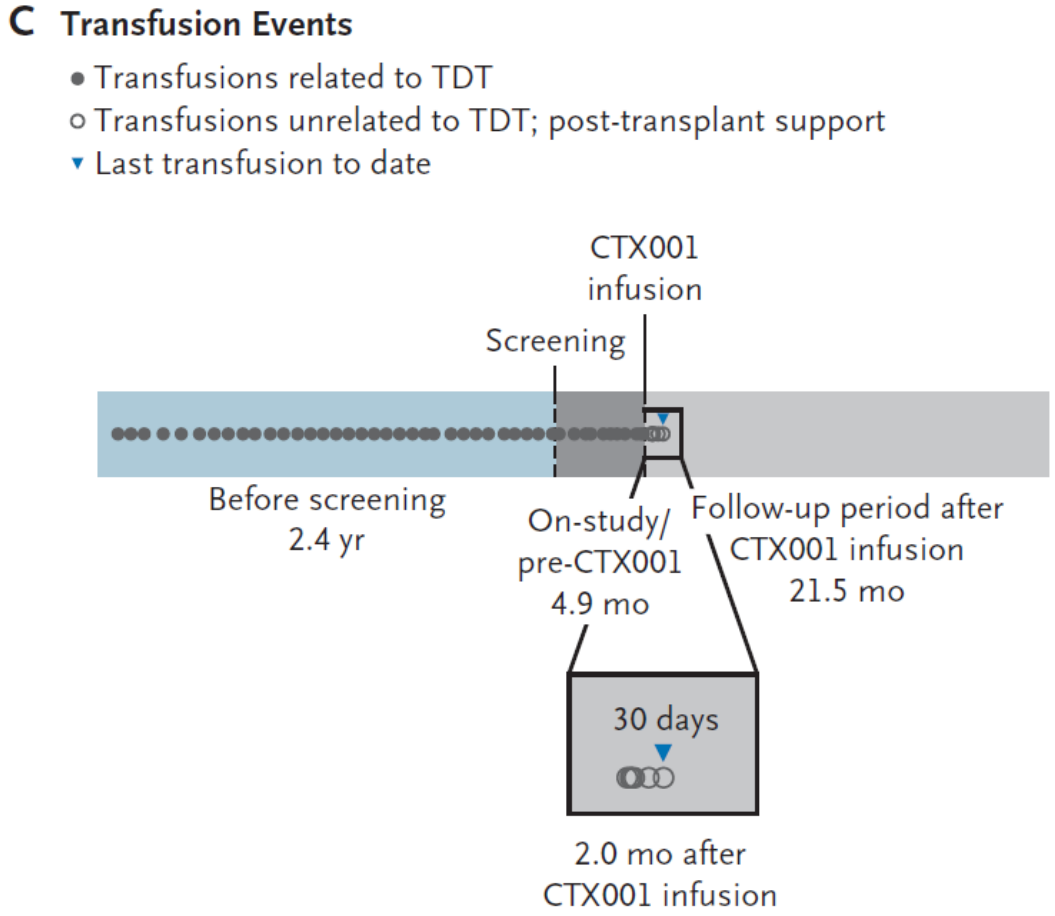
- What is Hb (g/dl)?
- What is «B»
- Why is HbF increasing over time (and is not high from the beginning)?

Figure 2b



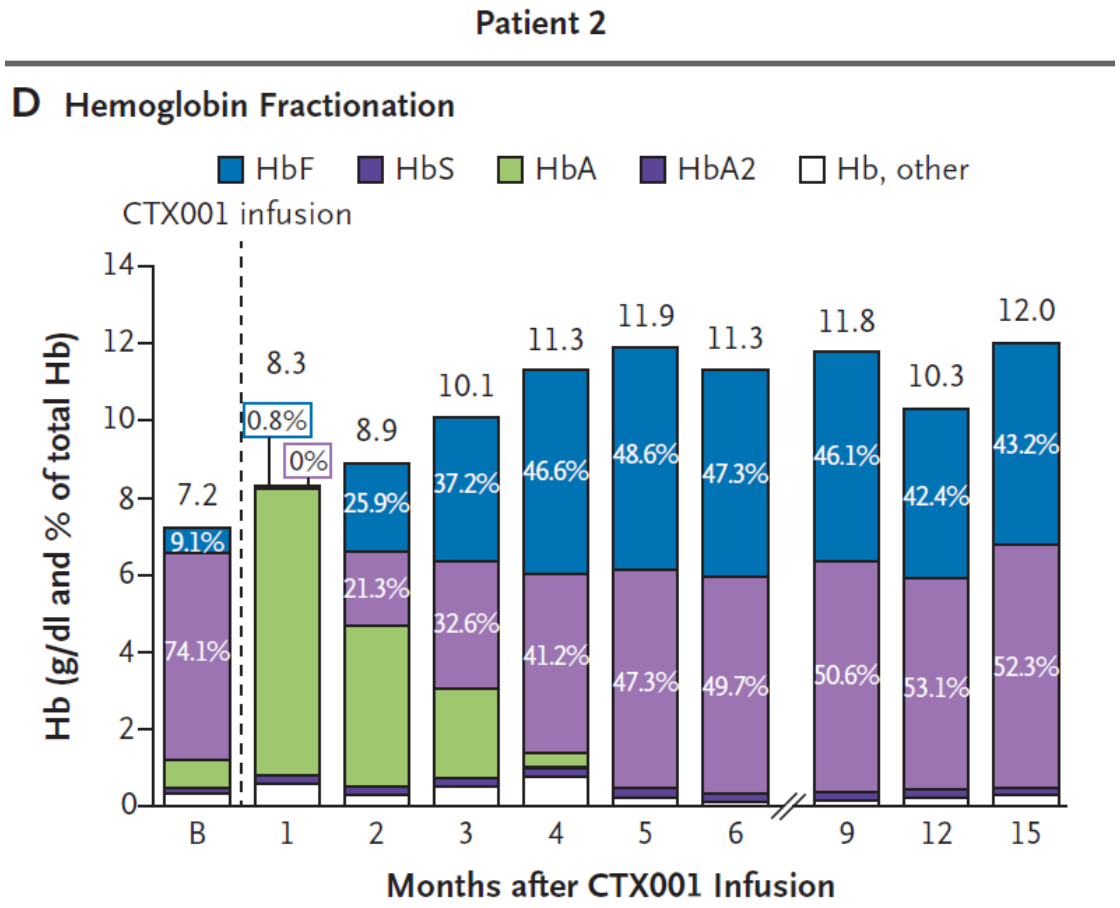
- What is «% peripheral F-cells»?

Figure 2c



- What does this graph show?
- Which is the main benefit for this patient?

Figure 2d



- Why is there much HbA (green) after CTX001 infusion?
- Which level of HbF is reached after 15 months?

Figure 2e

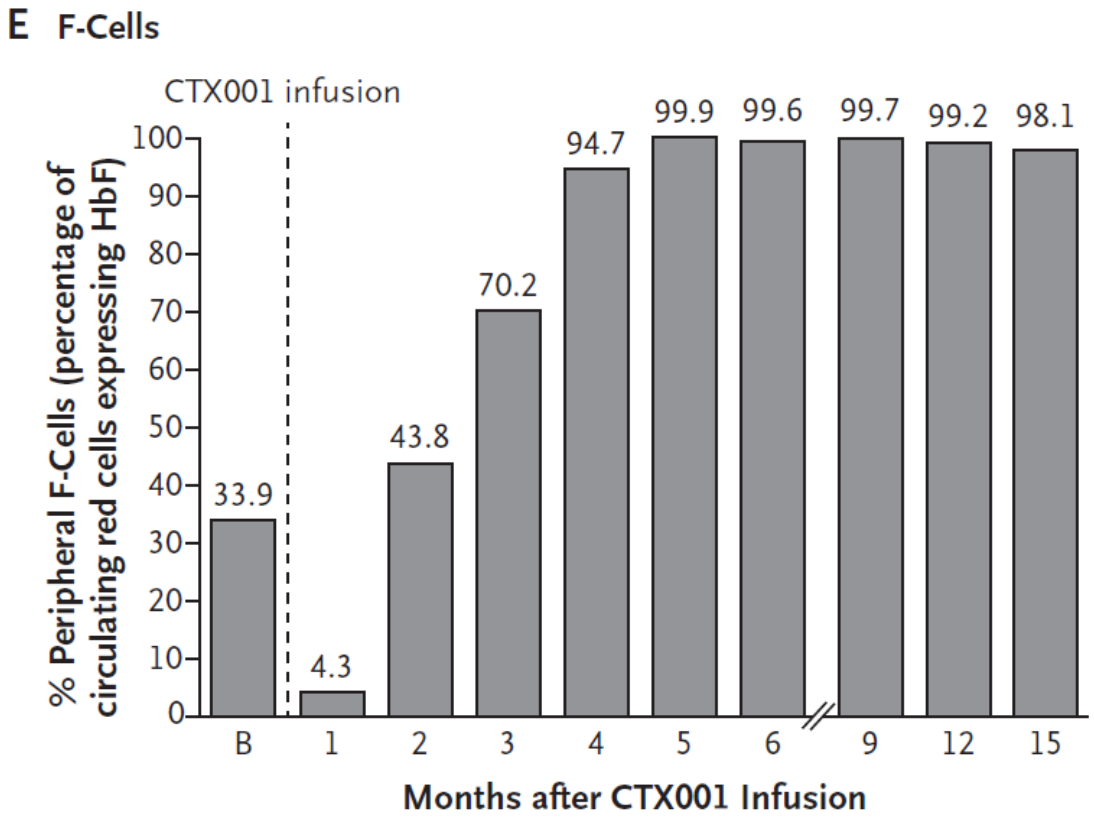
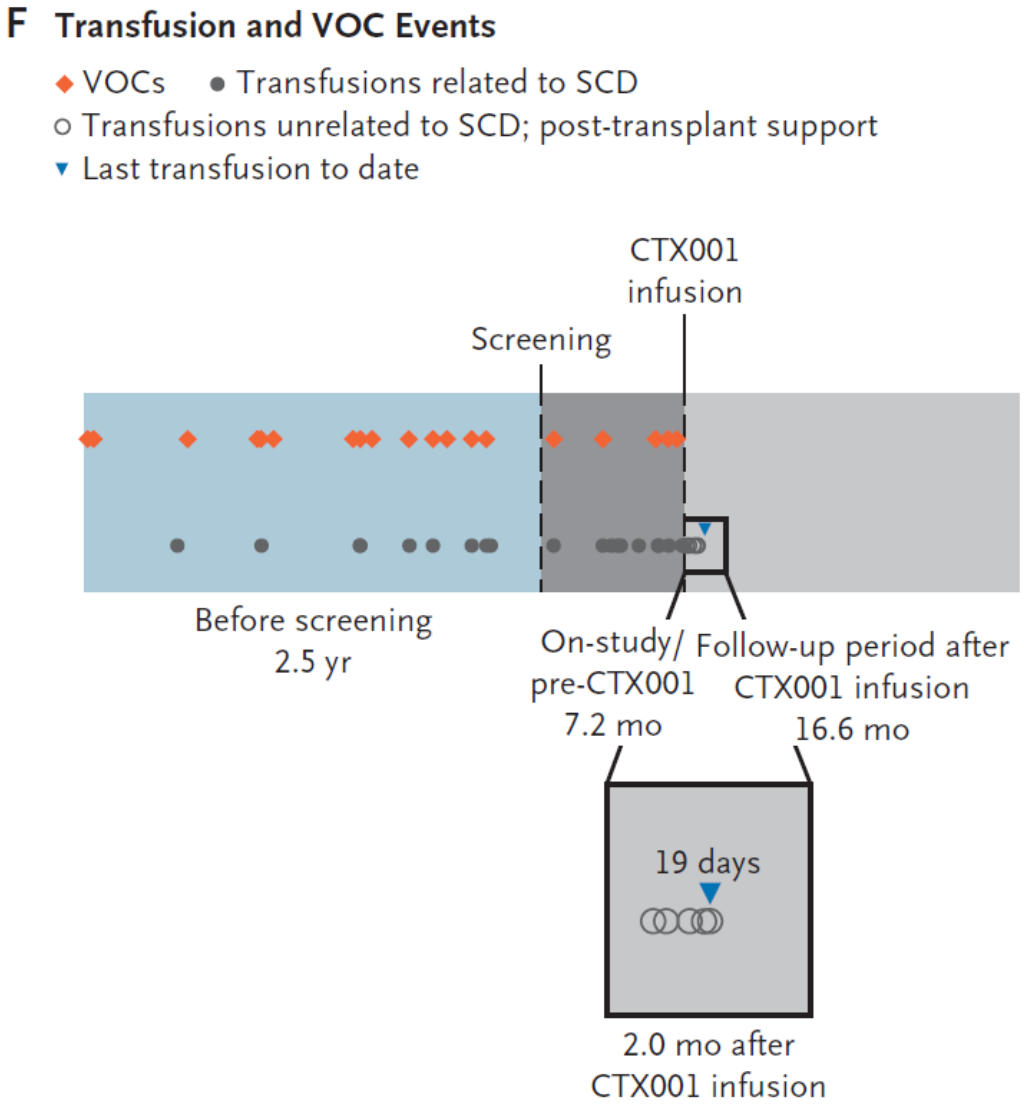

E F-Cells
CTX001 infusion
% Peripheral F-Cells (percentage of circulating red cells expressing HbF)
100
90
80
70
60
50
40
30
20
10
0
33.9
4.3
43.8
70.2
94.7
99.9
99.6
99.7
99.2
98.1
B
1
2
3
4
5
6
9
12
15
Months after CTX001 Infusion

Figure 2f



- What does this graph show?
- Which is the main benefit for this patient?