SWISSTRANSFUSION
JAHRESKONGRESS 2024

UNIVERSITÄTS KLINIKUM FREIBURG

Gene Therapy for Hemoglobinopathies

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Disclosures

Reviewed by Board of Directors at UMC Freiburg

Sponsored Research Collaboration
- Cellectis

Scientific Consultancies
- AaviGen
- Cimeio Therapeutics
- Excision BioTherapeutics
- GenCC
- Novo Nordisk

Patents
- Several patents in the field of Gene Editing

Honorarium
- AstraZeneca
- CSL-Behring
- Pfizer

Hemoglobinopathies

genetic disorders of hemoglobin

- encompass all genetic disorders of hemoglobin
- originally found mainly in Mediterranean area and large parts of Asia and Africa
- among the most common inherited diseases worldwide (7% carriers)

- two main groups:
  - thalassemia syndromes: α- and **β-thalassemia**
  - structural hemoglobin variants: e.g. HbS (**sickle cell disease**)
- highly variable clinical manifestations:
  - from mild hypochromic anemia
  - to lifelong, transfusion-dependent anemia with multiorgan involvement

hemoglobinopathies

genetic disorders of hemoglobin

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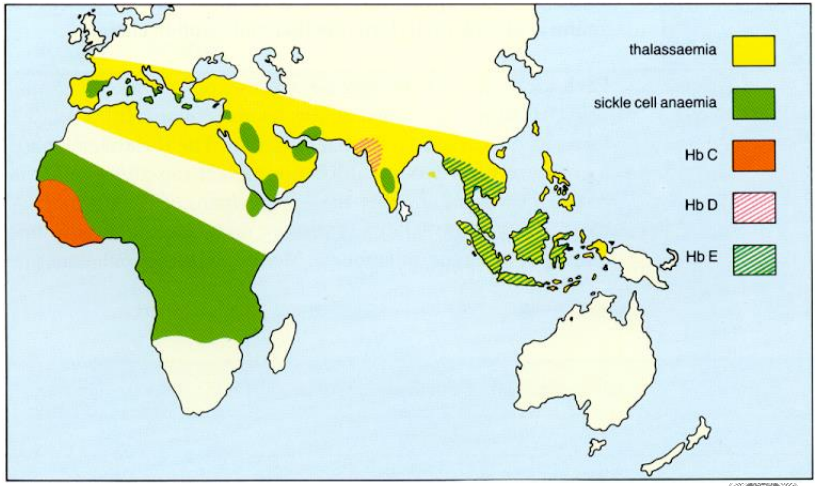
- among the most common inherited diseases worldwide (7% carriers)

Region	Gene carriers
Africa	5 to 30%
Arab nations	5 to 40% Up to 60% regionally
Central Asia and India	10 to 20%
South-East Asia	5 to 40% Up to 70% regionally
USA and Central America	5 to 20%
Italy	7 to 9%
Greece	6 to 7%
Turkey	7 to 10%



hemoglobinopathies

treatment

- **Supportive**:
  - periodic blood transfusions for life
  - combined with iron chelation

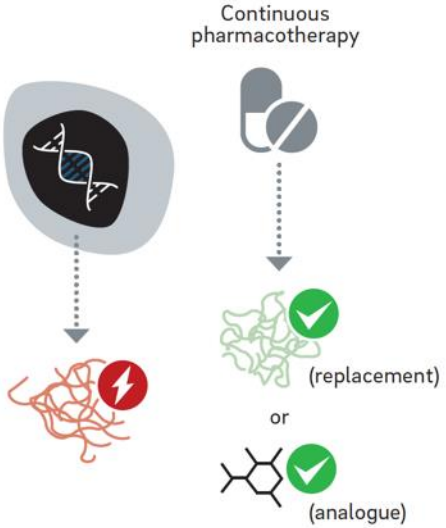
- **Curative**:
  - Allogeneic hematopoietic stem cell transplantation is the preferred treatment for severe forms of thalassemia

→ **Gene Therapy**

Gene Therapy

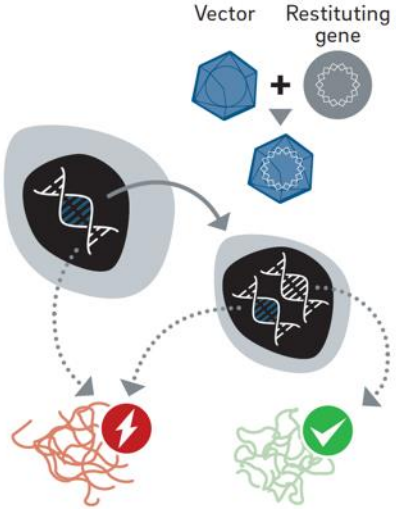
Main methods

Conventional



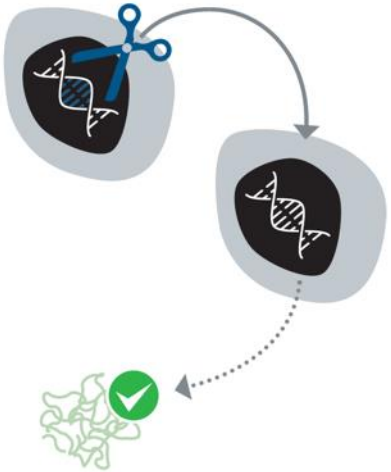
Often: symptomatic, chronic application

Gene Transfer



Often: curative, one-off application

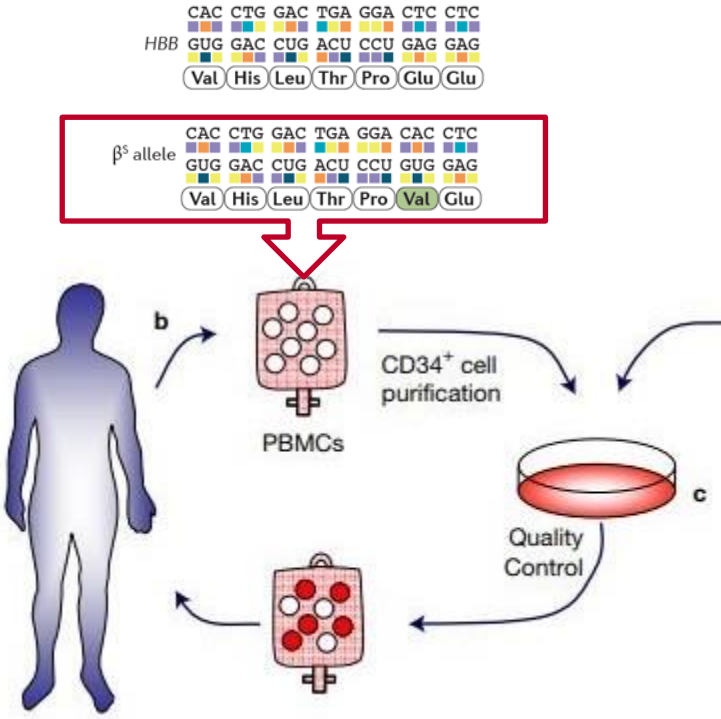
Gene Editing



Often: curative, one-off application

Gene Therapy Strategies

exemplified for sickle cell anemia



Gene Addition

- LV: overexpression of ß-Globin$_{T87Q}$

Genome Editing (correction)

- Correction of ß^{S} allele (GTG→GAG)

Genome Editing (knockout)

- Introduction of a compensatory mutation

Zynteglo®

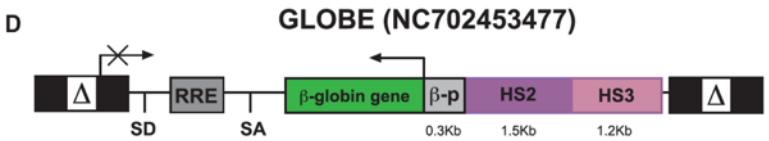
a.k.a. Lentiglobin

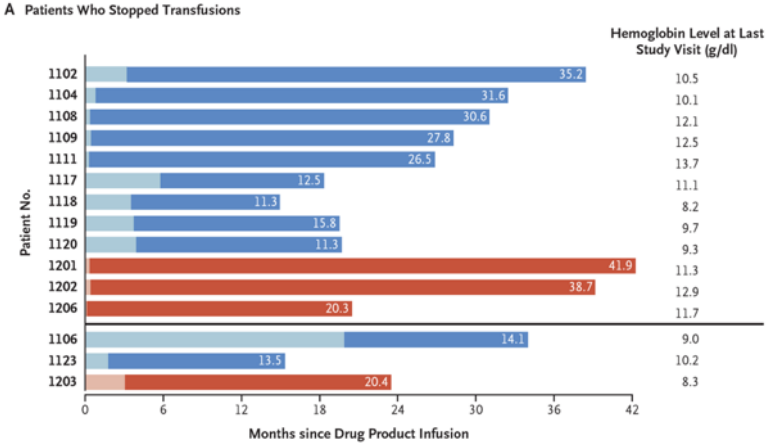
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 19, 2018 VOL. 378 NO. 16

Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia

A.A. Thompson, M.C. Walters, J. Kwiatkowski, J.E.J. Rasko, J.-A. Ribeil, S. Hongeng, E. Magrin, G.J. Schiller, E. Payen, M. Semeraro, D. Moshous, F. Lefrere, H. Puy, P. Bourget, A. Magnani, L. Caccavelli, J.-S. Diana, F. Suarez, F. Monpoux, V. Brousse, C. Poirot, C. Brouzes, J.-F. Meritet, C. Pondarré, Y. Beuzard, S. Chrétien, T. Lefebvre, D.T. Teachey, U. Anurathapan, P.J. Ho, C. von Kalle, M. Kletzel, E. Vichinsky, S. Soni, G. Veres, O. Negre, R.W. Ross, D. Davidson, A. Petrusich, L. Sandler, M. Asmal, O. Hermine, M. De Montalembert, S. Hacein-Bey-Abina, S. Blanche, P. Leboulch, and M. Cavazzana





Gene Therapy Strategies

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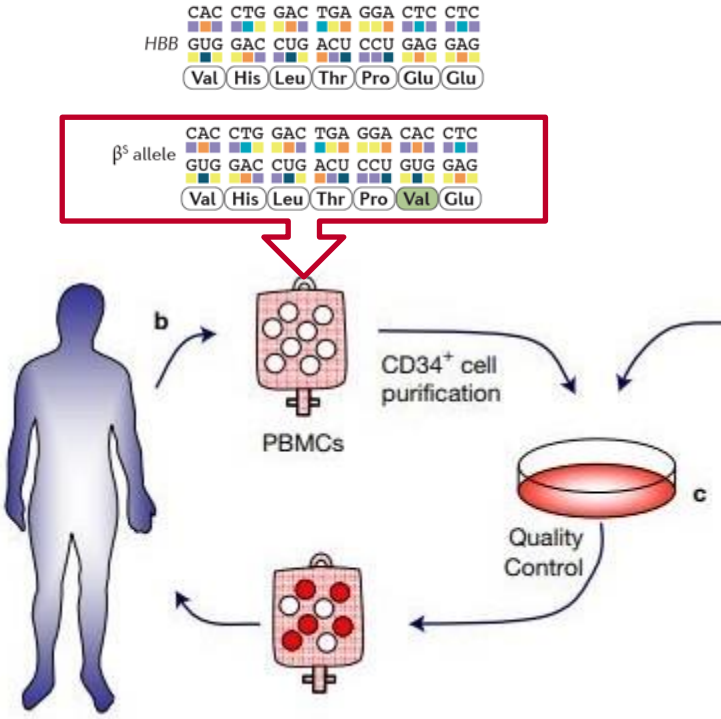

Gene Addition

- LV: overexpression of ß-Globin$_{T87Q}$

Genome Editing (correction)

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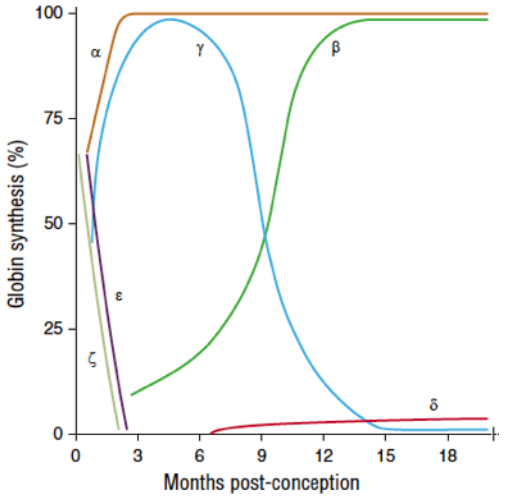
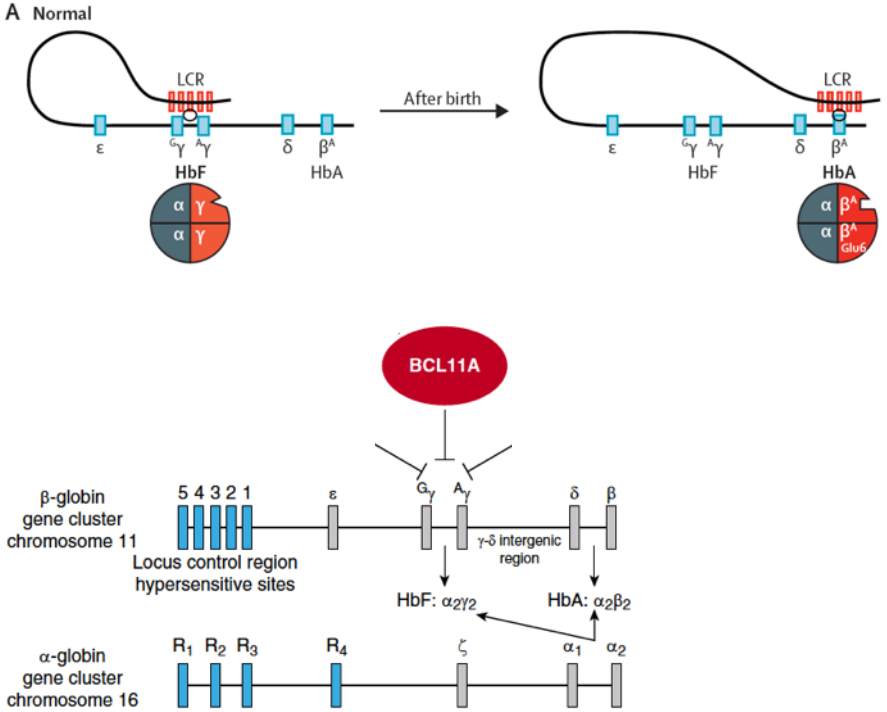
Genome Editing (knockout)

- Introduction of a compensatory mutation

Regulation of β-globin locus

BCL11A as a master regulator





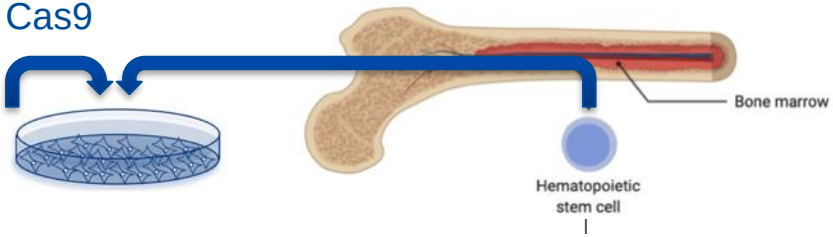
activation

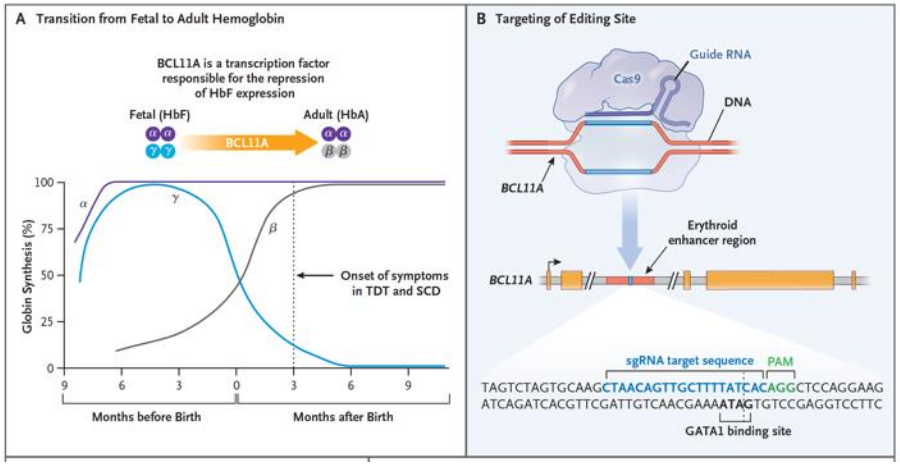
silencing

First approved CRISPR Medicine

Exa-Cel (Casgevy®) → hemoglobinopathies

CRISPR-Cas9





ORIGINAL ARTICLE

Exagamglogene Autotemcel for Transfusion-Dependent β-Thalassemia

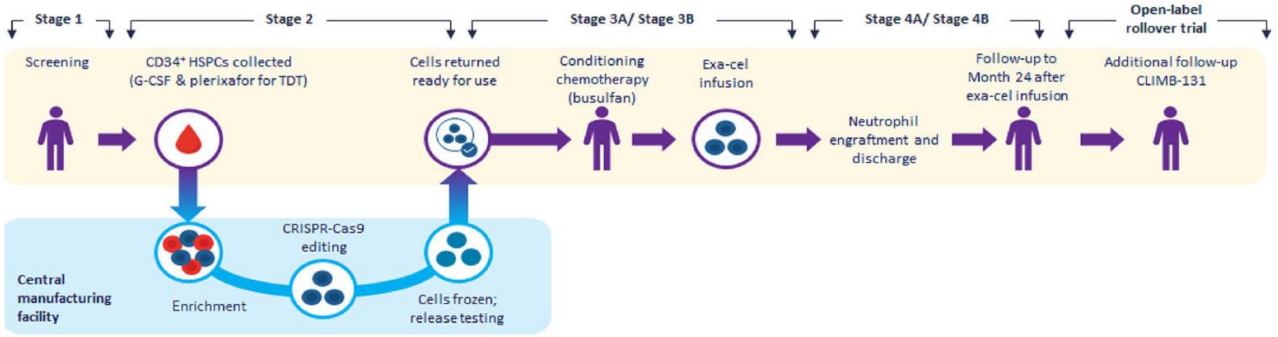
F. Locatelli, P. Lang, D. Wall, R. Meisel, S. Corbacioglu, A.M. Li, J. de la Fuente, A.J. Shah, B. Carpenter, J.L. Kwiatkowski, M. Mapara, R.I. Liem, M.D. Cappellini, M. Algeri, A. Kattamis, S. Sheth, S. Grupp, R. Handgretinger, P. Kohli, D. Shi, L. Ross, Y. Bobruff, C. Simard, L. Zhang, P.K. Morrow, W.E. Hobbs, and H. Frangoul, for the CLIMB THAL-111 Study Group*

ORIGINAL ARTICLE

Exagamglogene Autotemcel for Severe Sickle Cell Disease

H. Frangoul, F. Locatelli, A. Sharma, M. Bhatia, M. Mapara, L. Molinari, D. Wall, R.I. Liem, P. Telfer, A.J. Shah, M. Cavazzana, S. Corbacioglu, D. Rondelli, R. Meisel, L. Dedeken, S. Lobitz, M. de Montalembert, M.H. Steinberg, M.C. Walters, M.J. Eckrich, S. Imren, L. Bower, C. Simard, W. Zhou, F. Xuan, P.K. Morrow, W.E. Hobbs, and S.A. Grupp, for the CLIMB SCD-121 Study Group*

Pivotal Phase 3 Trial of Exa-cel in Participants With TDT



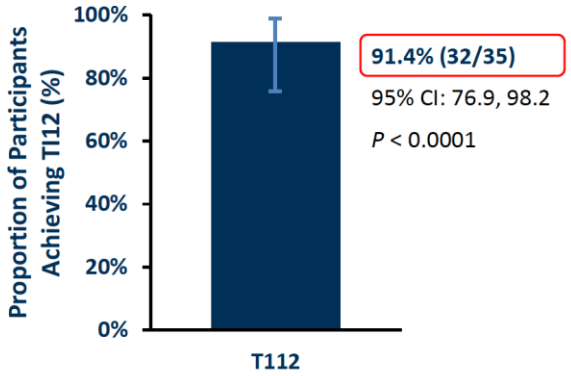
Study Design	Global, multicenter, open-label, single-arm, 2-year Phase 3 trial of a single infusion of exa-cel (NCT03655678)
Participants	52 (as of data cutoff: 16 January 2023); 12 to 35 years of age with TDT, including β^0/β^0 genotypes, defined as a history of ≥100 mL/kg/year or ≥10 units/year of packed RBC transfusions in the previous 2 years
Primary Efficacy Endpoint	Proportion of participants **transfusion independent for ≥12 consecutive months** while maintaining a weighted average hemoglobin ≥9 g/dL **(TI12)**
Key Secondary Efficacy Endpoint	Proportion of participants **transfusion independent for ≥6 consecutive months** while maintaining a weighted average hemoglobin ≥9 g/dL **(TI6)**
Analyses	• Full Analysis Set: participants who received exa-cel infusion • Primary Efficacy Set: participants followed for ≥16 months after exa-cel infusion (evaluable for primary & key secondary endpoints)

Participants who complete CLIMB THAL-111 can enroll in CLIMB-131 for 13 years of additional follow-up

	Full Analysis Set N = 52
Number of mobilization cycles, median (range)	1.0 (1, 4)
Exa-cel dose: 10^6 x $CD34^+$ cells/kg, mean (range)	8.4 (3.0, 19.7)
Duration (months) of follow-up after exa-cel infusion,[a] mean (range)	20.1 (2.1, 48.1)
Neutrophil Engraftment[b]	
Time to neutrophil engraftment (days), median (range)	29.0 (12, 56)
Platelet Engraftment[c]	
Time to platelet engraftment (days), median (range)	44.0 (20, 200)

Post-exa-cel AE Overview	Exa-cel N = 52
Participants with	
Any AEs, n (%)	52 (100.0)
AEs related to exa-cel, n (%)[a]	13 (25.0)
AEs related to busulfan, n (%)[a]	51 (98.1)
AEs Grade 3/4, n (%)	46 (88.5)
SAEs, n (%)	17 (32.7)
SAEs related to exa-cel, n (%)[a,b]	2 (3.8)
AEs leading to death, n (%)	0
Any malignancies, n (%)	0

Primary Endpoint: TI12



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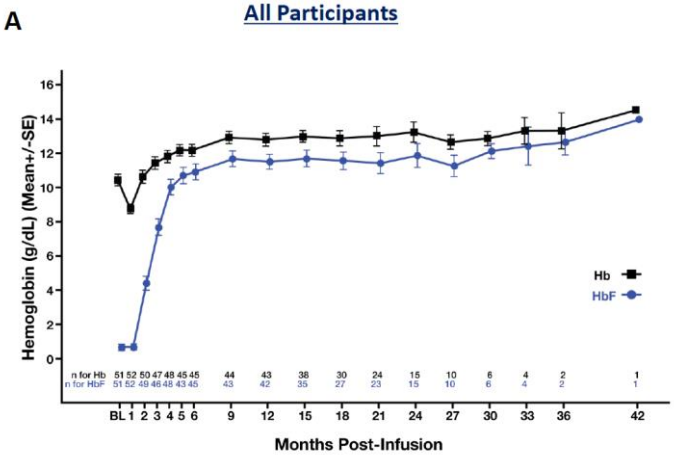
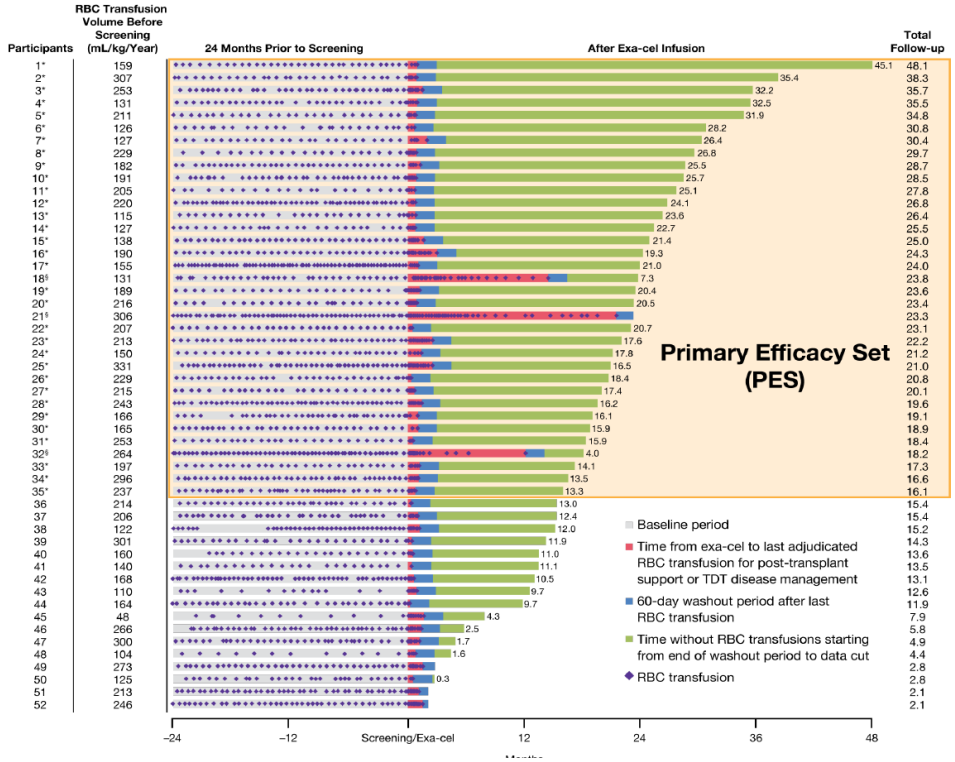


Participants Who Achieved Transfusion Independence (TI12) Maintained Transfusion Independence From 13.3 to 45.1 Months

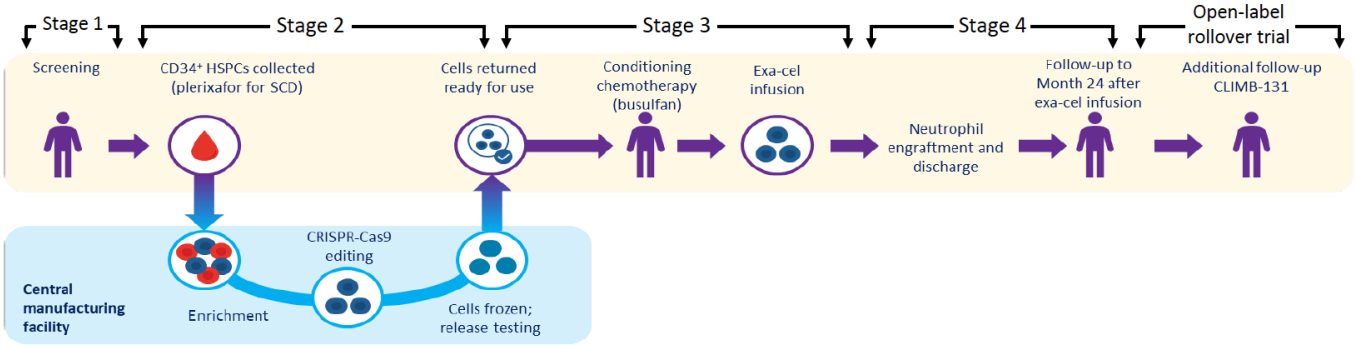


- Participants **stopped transfusions** after a mean of 35.2 days (PES); once TI12 achieved, all participants **remained transfusion independent**

- Three participants did not achieve TI12; however, had **substantial clinical benefit**
  - 1 participant had a relative reduction in annualized red blood cell transfusion volume of 83.9%
  - 2 participants stopped red blood cell transfusions 14.5 months and 12.2 months after exa-cel and have been transfusion-free for 7.3 months and 4.0 months, respectively

As of April 16, 2023, all 3 participants who did not achieve TI12 **stopped transfusions** 14.5, 12.2, and 21.6 months after exa-cel infusion, and were **transfusion-free** for 10.3, 7.0, 2.8 months

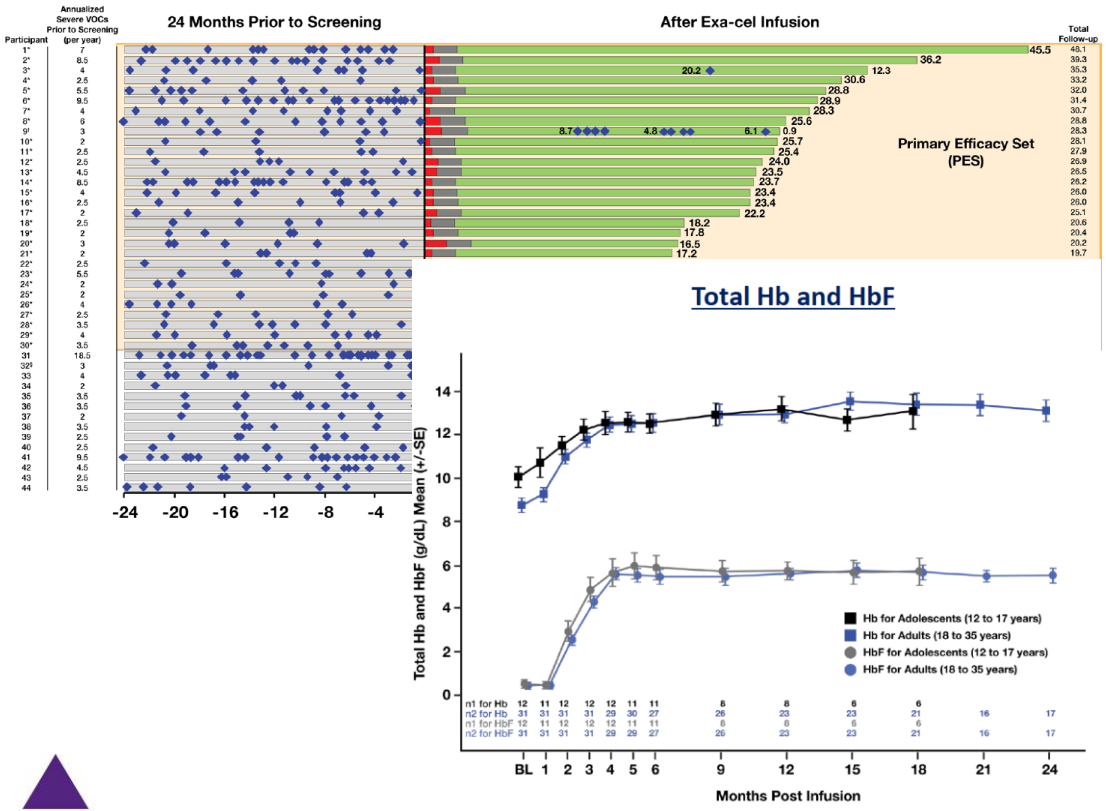
Pivotal Phase 3 Trial of Exa-cel in Participants With Severe SCD



Study Design	Global, multicenter, open-label, single-arm, 2-year Phase 3 trial of a single infusion of exa-cel (NCT03745287)
Participants	44 (as of data cutoff: 14 June 2023); 12 to 35 years of age with severe SCD and a history of ≥2 severe VOCs per year in the previous 2 years
Primary Efficacy Endpoint	Proportion of participants **free of severe VOCs for ≥12 consecutive months (VF12)**
Key Secondary Efficacy Endpoint	Proportion of participants **free from in-patient hospitalization for severe VOCs for ≥12 consecutive months (HF12)**
Analyses	• Full Analysis Set: participants who received exa-cel infusion • Primary Efficacy Set: participants followed for ≥16 months after exa-cel infusion (evaluable for primary & key secondary endpoints)

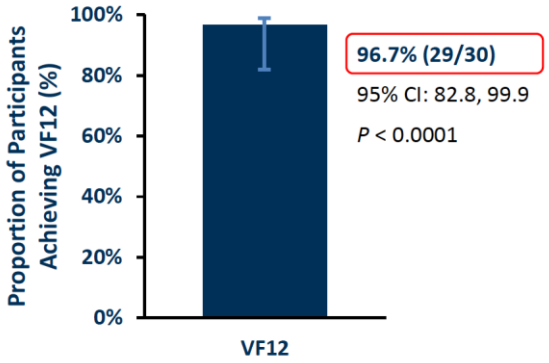
Participants who complete CLIMB SCD-121 can enroll in CLIMB-131 for 13 years of additional follow-up

Participants Treated With Exa-cel Achieved Clinically Meaningful and Durable Benefit - Free From VOCs



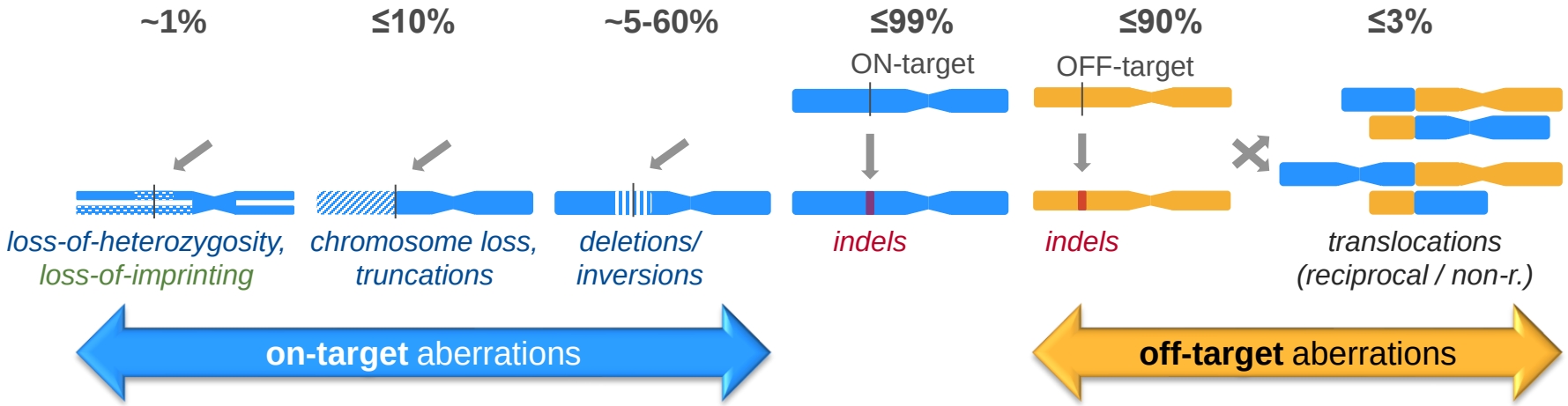
- Participants **stopped transfusions** after a median of **20 days**

Primary Endpoint: VF12



Genotoxicity

side effects of employing engineered genome editors



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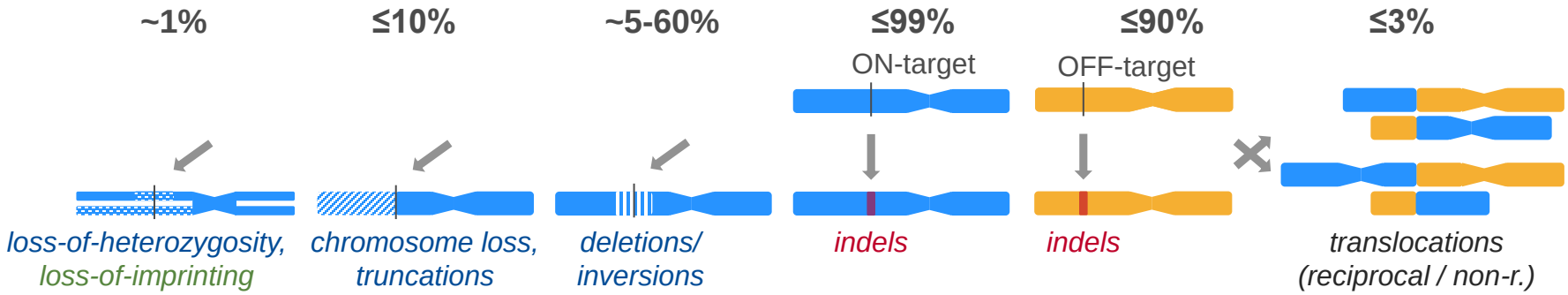


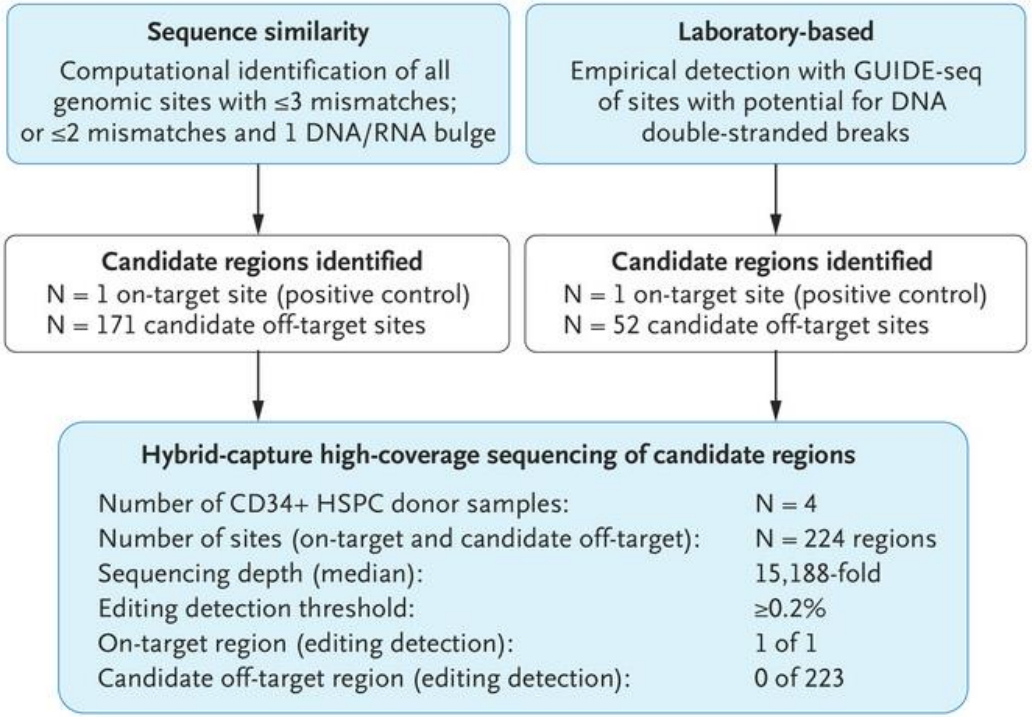
LOF: loss-of-function; GOF: gain-of-function; CNV: copy-number-variation

Worst-case-scenario:

→ LOF in **tumor suppressor** gene(s)
→ GOF in **proto-oncogene**(s)
→ GOF **fusion gene** product

Off-Target Analysis

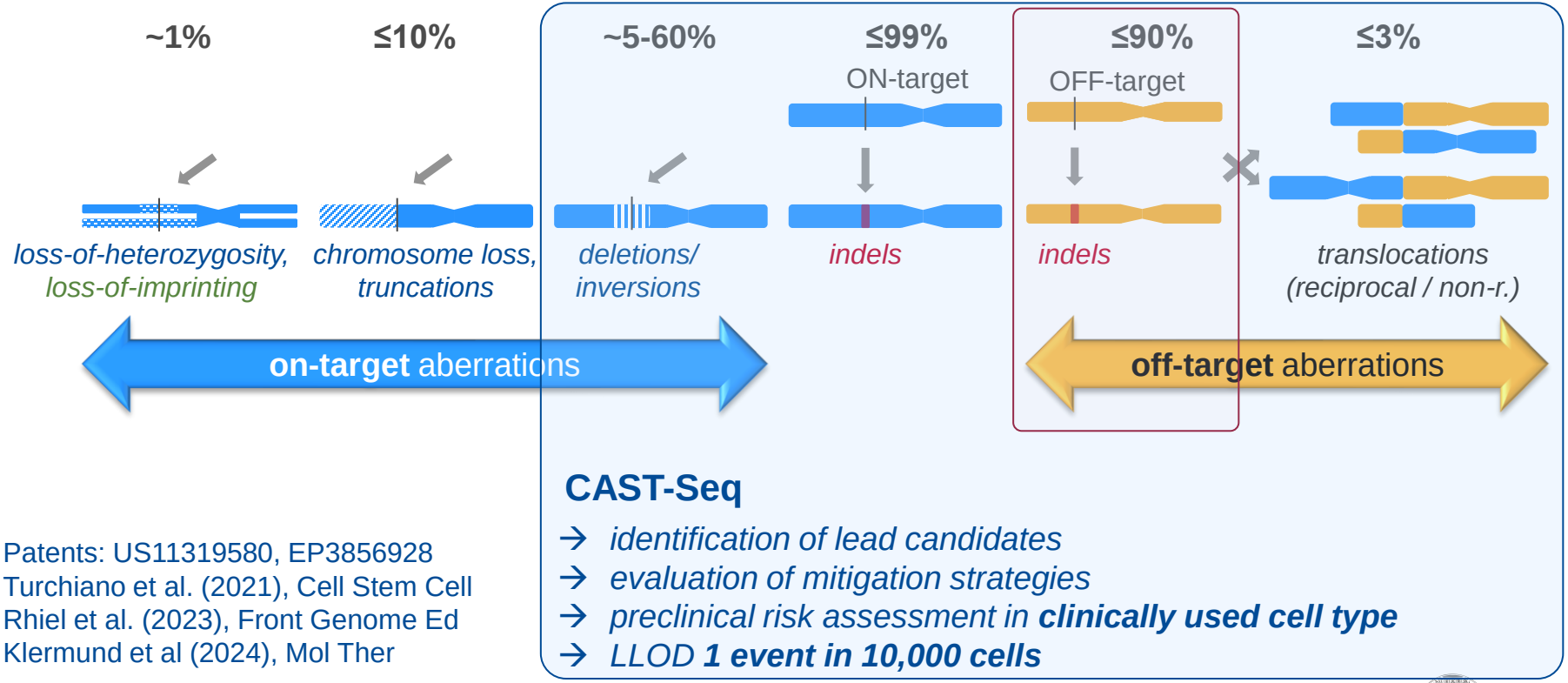
Casgevy®



Frangoul et al. (2021) N Engl J Med

Off-Target Assays

in silico – in vitro – in cellula



CAST-Seq

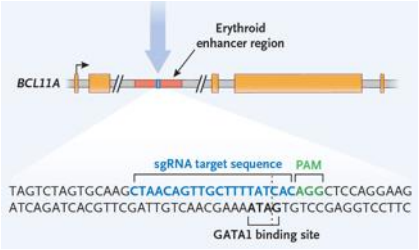
- → *identification of lead candidates*
- → *evaluation of mitigation strategies*
- → *preclinical risk assessment in **clinically used cell type***
- → *LLOD **1 event in 10,000 cells***

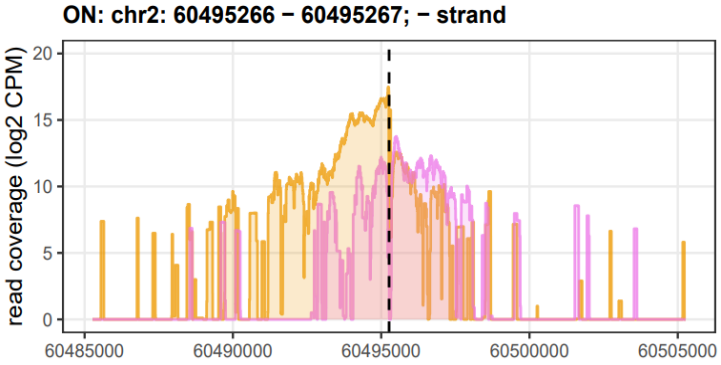
Patents: US11319580, EP3856928
Turchiano et al. (2021), Cell Stem Cell
Rhiel et al. (2023), Front Genome Ed
Klermund et al (2024), Mol Ther

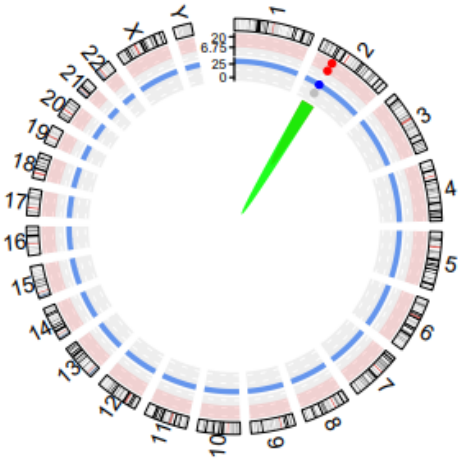
On- / Off-Target Effects

CAST-Seq Analysis of *BCL11A* locus

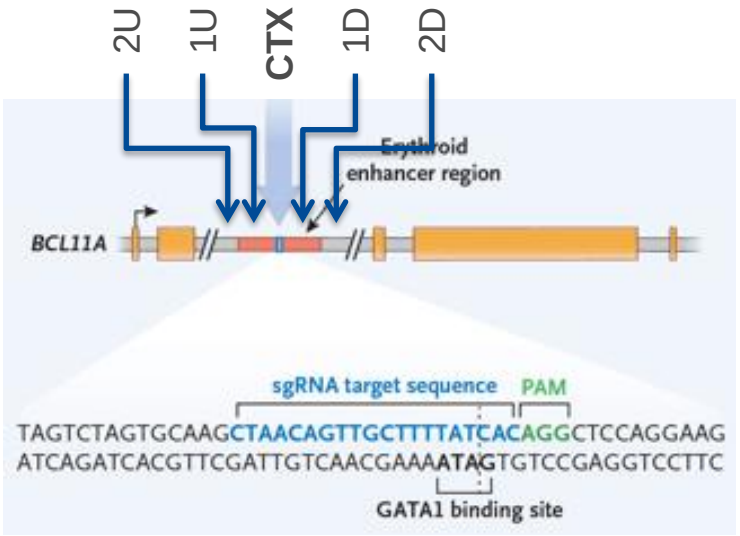
CTX

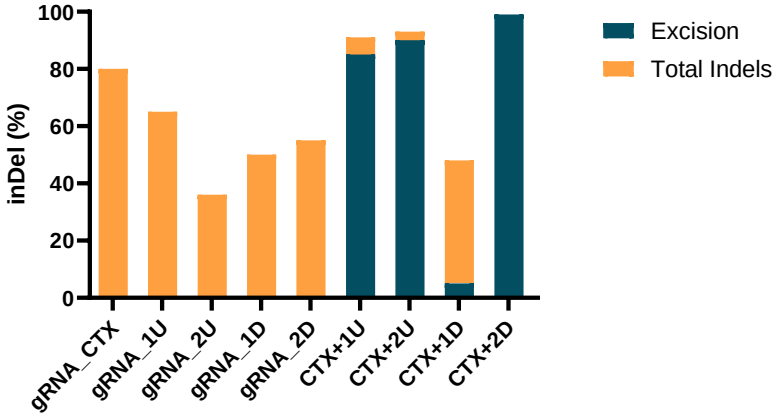






Double-Hit Strategy

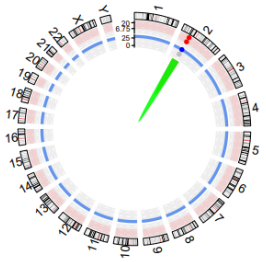




Chromosomal translocation analysis

CAST-Seq analysis of BCL11A edited HSCs

CTX



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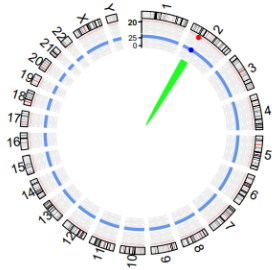

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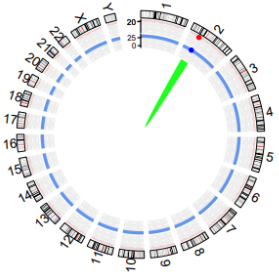
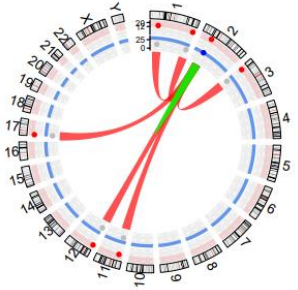
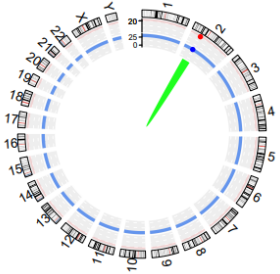


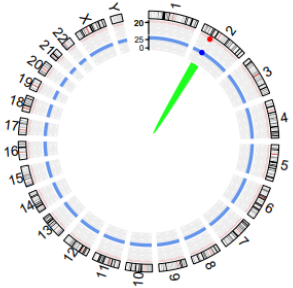
2D



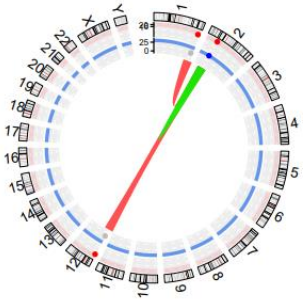
CTX+1U



CTX+2U

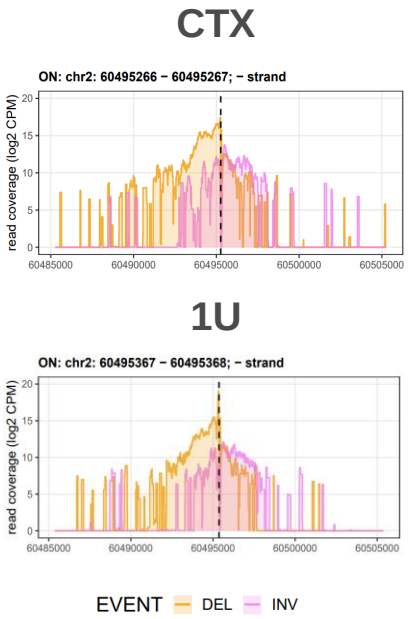


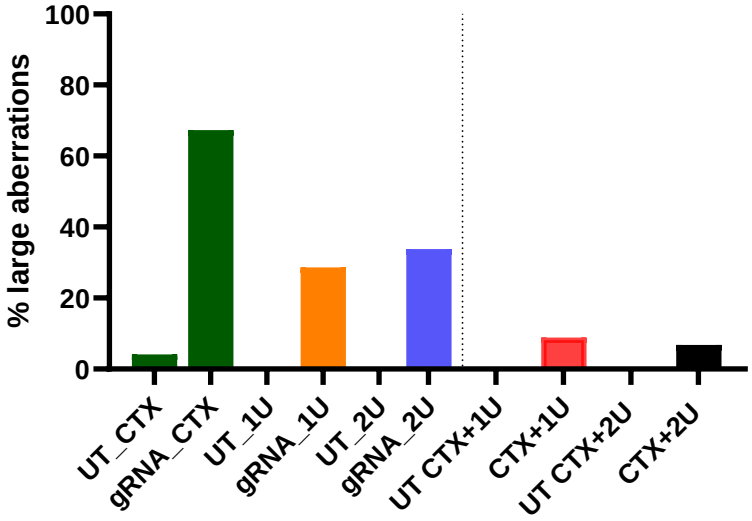
CTX+2D



On-Target Aberration Analysis

CAST-Seq coverage plots of BCL11A edited HSCs





Take Home Messages

- ✓ **Genome Editing** (nucleases, base editors) is a clinical reality
  with ~70 active interventional clinical trials worldwide
  with 1st CRISPR Medicine approved in the EU (Casgevy®, ~2 Mio.€ / treatment)

- ✓ **Genome Editing** (nucleases, base editors)
  can be highly specific = few to no **off-target effects**
  on-target effects still difficult to mitigate

- ✓ **Exa-Cel (Casgevy)**
  safe and efficacious in treatment of SCD and TDT
  therapeutic effect: rapid increase in HbF levels → persistent
  one-off medication to cure patients with SCD and TDT
  long-term effects ???

Acknowledgments

Toni Cathomen
Jamal Alzubi
Sandra Ammann
Kay Ole Chmielewski
Alieh Fazeli
Carla Fuster Garcia
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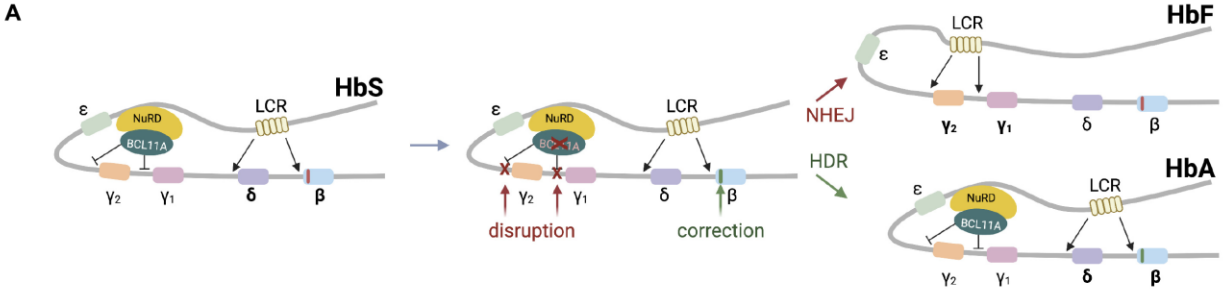
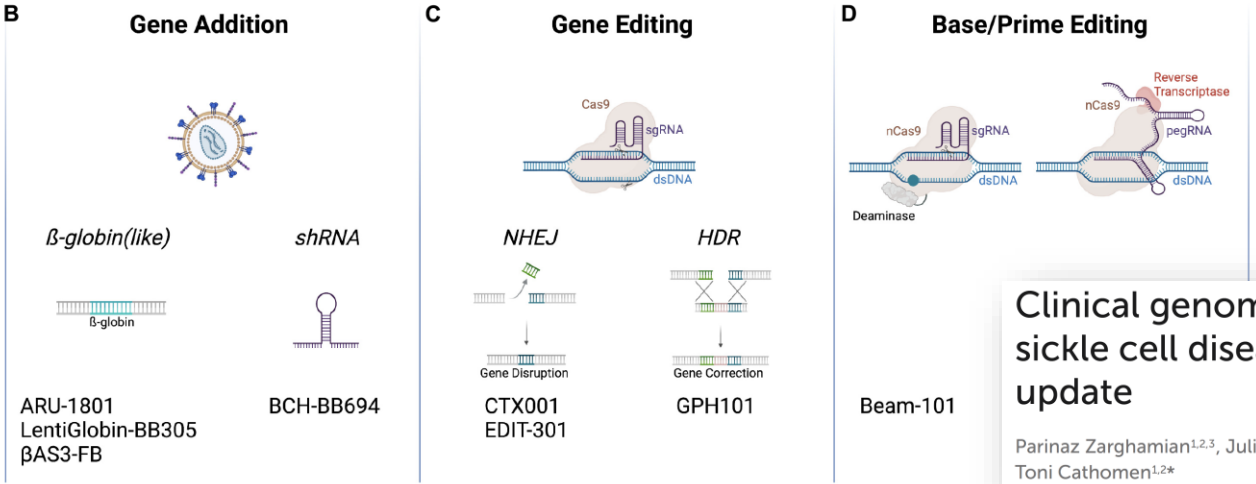
Extra Slides

Outlook





Clinical genome editing to treat sickle cell disease—A brief update

Parinaz Zarghamian[1,2,3], Julia Klermund [1,2] and Toni Cathomen[1,2*]

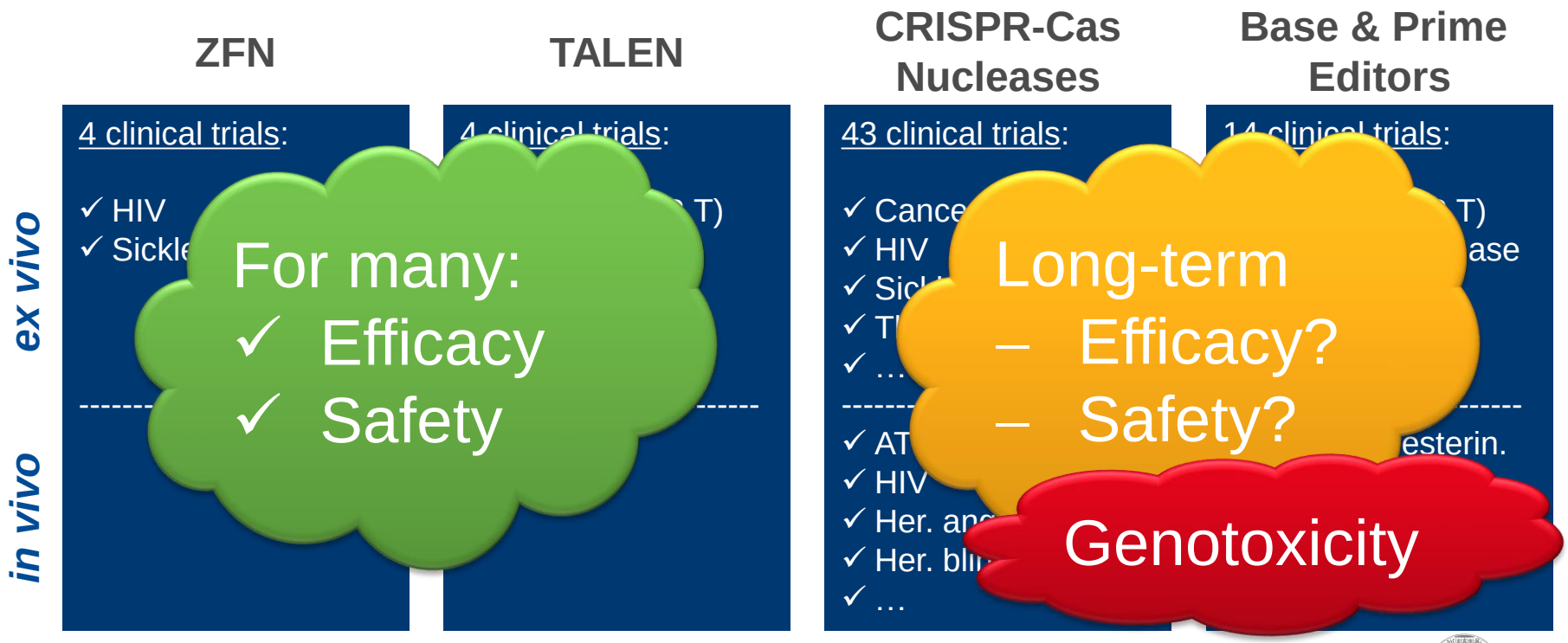
Approved Gene Therapies

15 approved gene therapy medicinal products in the EU (*as of Feb. 2024*)

	Indication	Appli-cation	Vector system	Target	Trade-name	Approv. by EMA	Manufacturer
Hereditary diseases	ß-Thalassemia, Sickle-cell anemia (HBB)	*Ex vivo*	CRISPR	CD34+ cell	Casgevy®	2024	Vertex / Crispr Therapeutics
	ADA - severe combined immunodeficiency (ADA)		RVV	CD34+ cell	Strimvelis®	2016	Fondazione Telethon Italy
	Metachromatic Leukodystrophy (ARSA)		LVV	CD34+ cell	Libmeldy®	2020	Orchard Therapeutics
	Inherited retinal dystrophy (RPE65)	*In vivo*	AAV2	retina	Luxturna®	2018	Spark Therapeutics / Novartis
	Spinal muscular atrophy (SMN1)		AAV9	nerves	Zolgensma®	2020	AveXis / Novartis
	L-amino acid decarboxylase deficiency (DDC)		AAV2	brain	Upstaza®	2022	PTC Therapeutics
	Hemophilia A (Factor VIII)		AAV5	liver	Roctavian®	2022	BioMarin Europe
	Hemophilia B (Factor IX)		AAV5	liver	Hemgenix®	2023	CSL Behring
Cancers	B-cell leukemia / B-cell lymphoma (CD19)	*Ex vivo*	LVV RVV LVV	T cell	Kymriah® Yescarta® Breyanzi®	2018 2018 2022	Novartis Kite Pharma / Gilead Celgene / Bristol-Myers Squibb
	Mantle cell lymphoma (CD19)		RVV	T cell	Tecartus®	2020	Kite Pharma / Gilead
	Multiple myeloma (BCMA)		LVV LVV	T cell	Abecma® Carvykti®	2021 2022	Celgene / Bristol-Myers Squibb Jansen
	Melanoma (GM-CSF)	*In vivo*	HSV1	tumor	Imlygic®	2015	Amgen

active interventional clinical trials

as of May 2024 (© ClinicalTrials.gov)



Delivering Genome Editing Tools

Editing the Genome

***in vivo* genome editing**

***ex vivo* genome editing**