Nicht-maligne Erkrankungen mit genmodifizierten Stammzellen

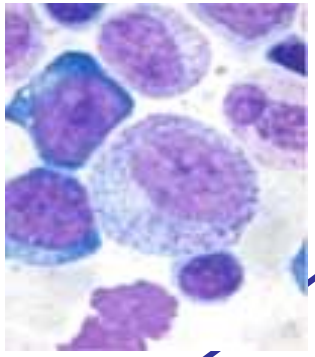
Rupert Handgretinger Abteilung für Hämatologie/Onkologie und Allgemeine Pädiatrie
Hoppe-Seyler-Strasse 1, 72076 Tübingen

Stammzellen

Selbsterneuerung

Ausdifferenzierung

Septische Granulomatose



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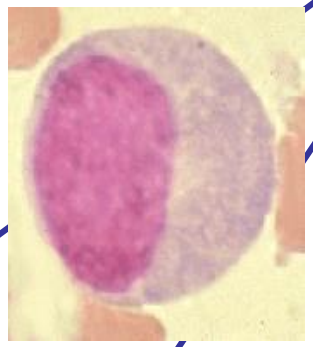
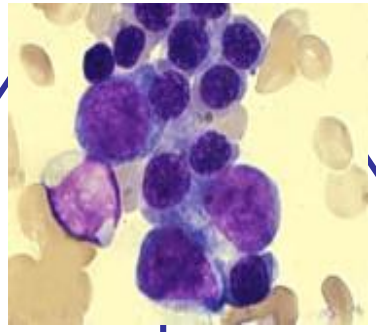
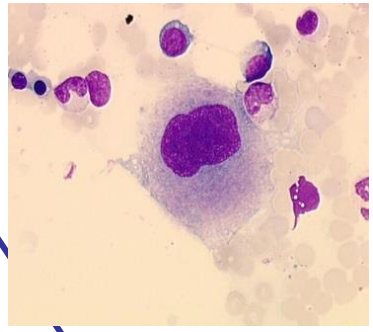


Thalassämie
Sichelzellanämie



Wiskott-Aldrich-Sy



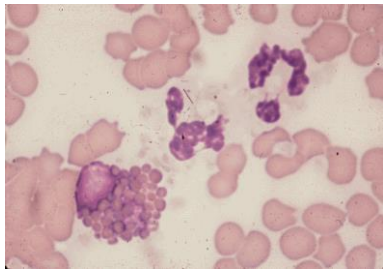
Myelopoese

Lymphopoese

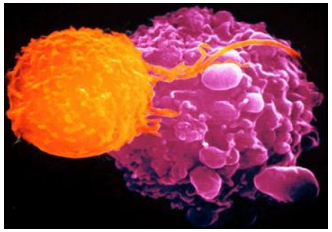
Erythropoese

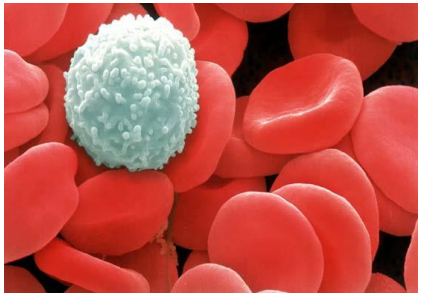
Thrombopoese

Granulozyten
Monozyten

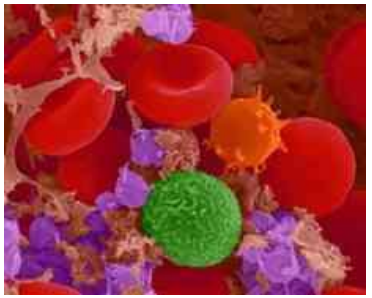


T-Zellen
B-Zellen
NK-Zellen





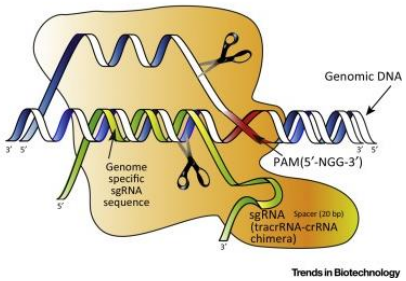
Erythrozyten



Thrombozyten

Genmodifizierung von Stammzelle

Crispr-Cas9



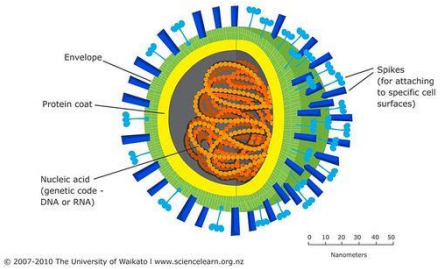
Kanchiswamy et al., 2016

Human CD34+ cells

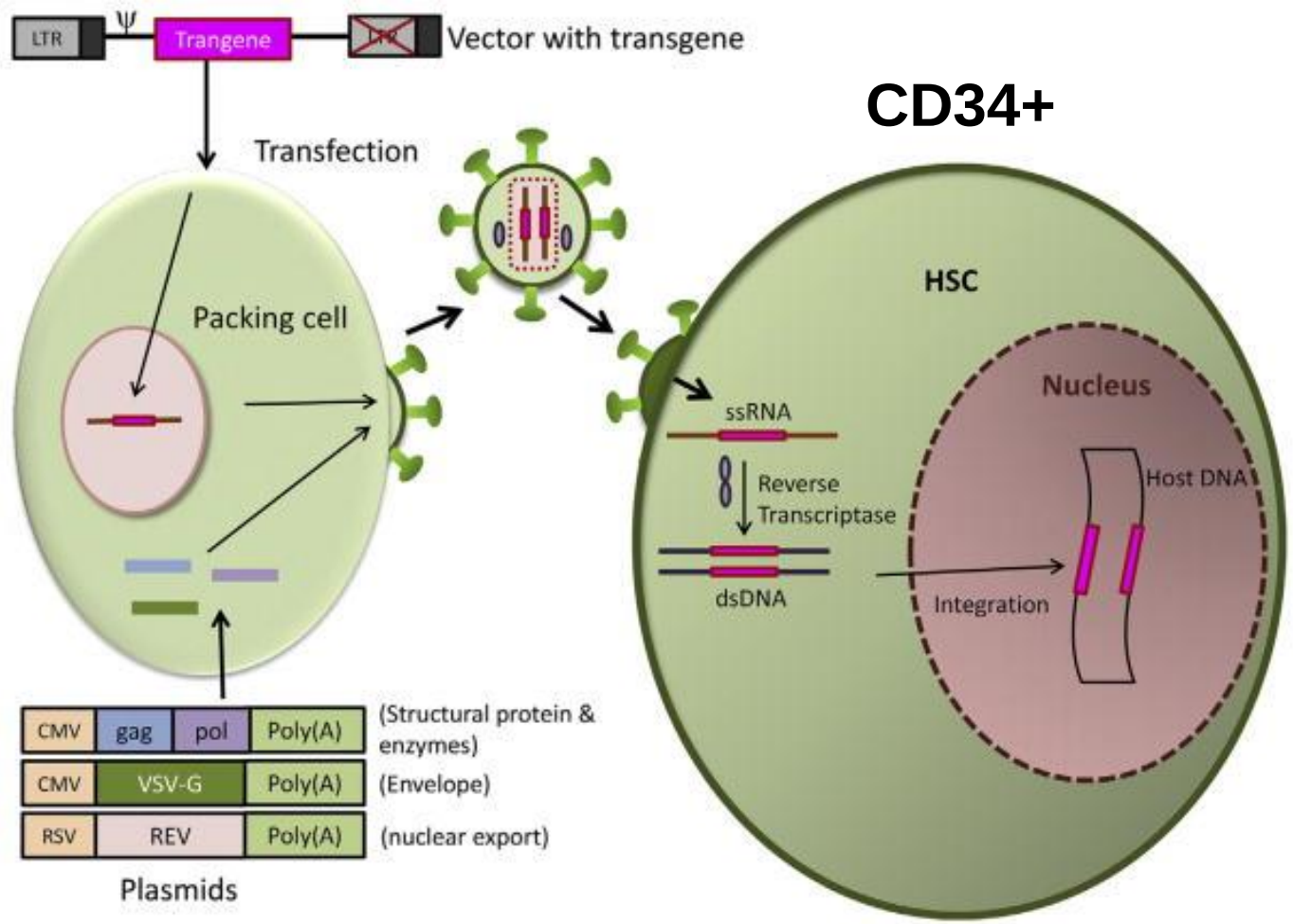
Gene editing | **Gene addition**

CD34+ modifizierte Stammzellen

Myeloablative Konditionierung plus
Autologe Stammzelltransplantation

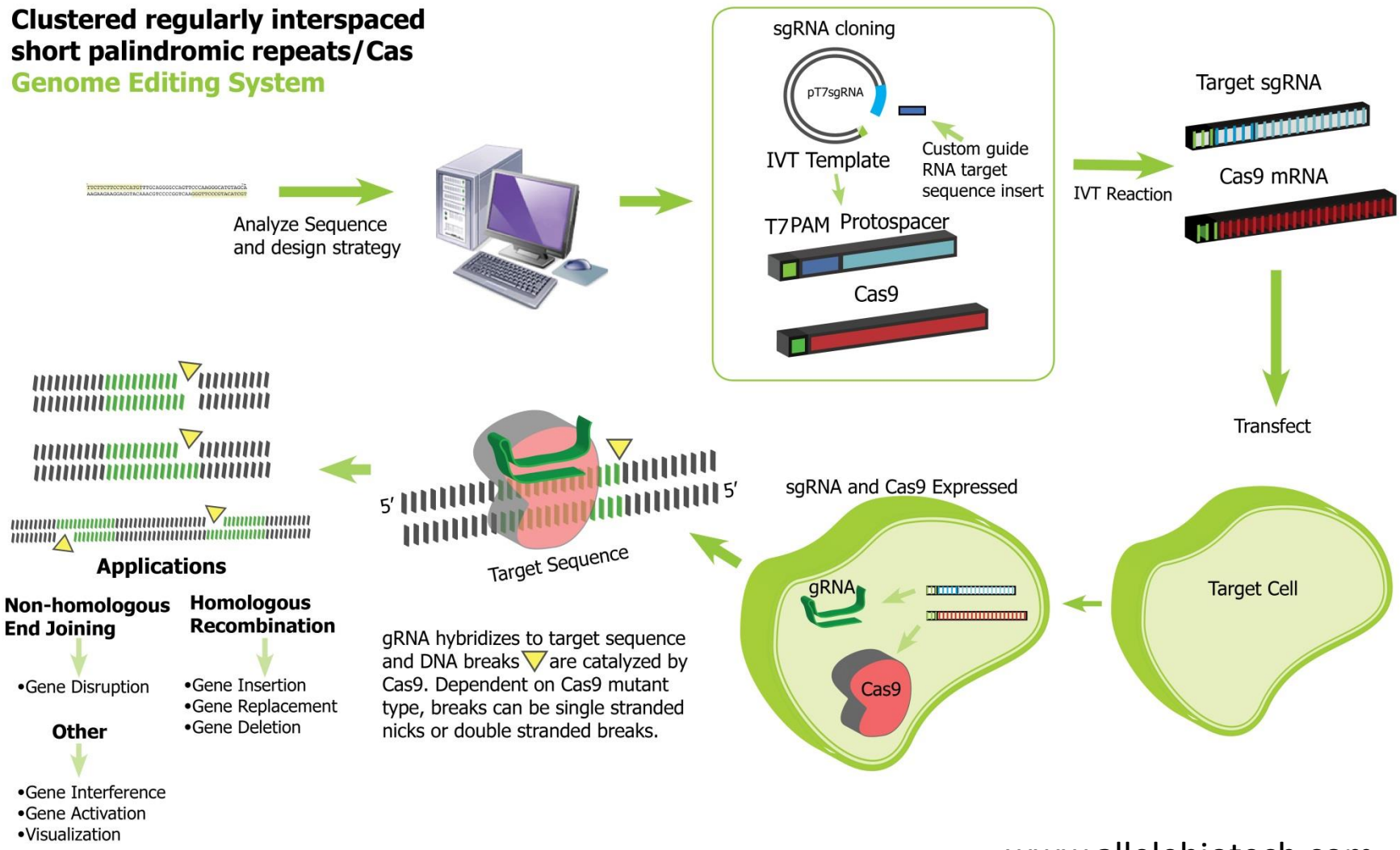


Lentiviral vectors for gene therapy



Chandrakasan & Malik, 2014

Gene Editing:CRISPR/Cas9



www.allelebiotech.com

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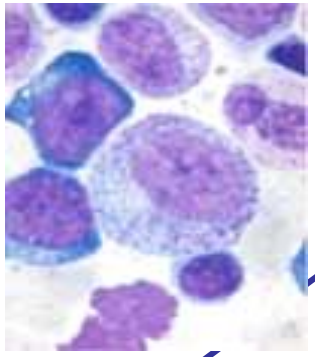

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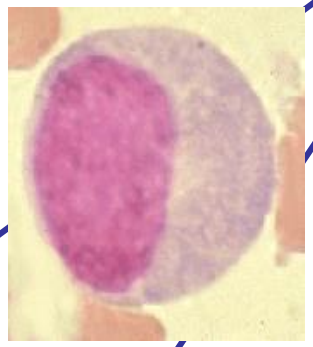
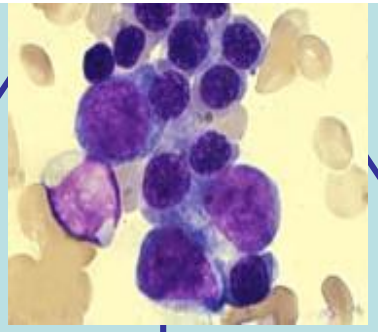


Thalassämie
Sichelzellanämie



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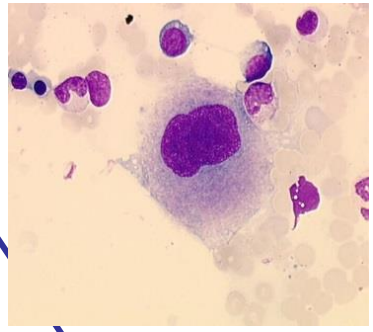

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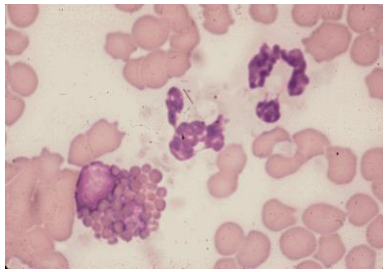

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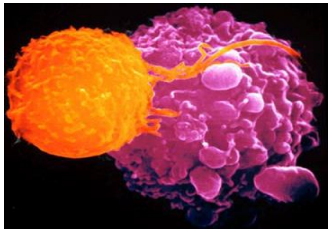
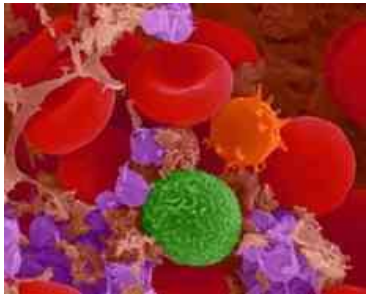


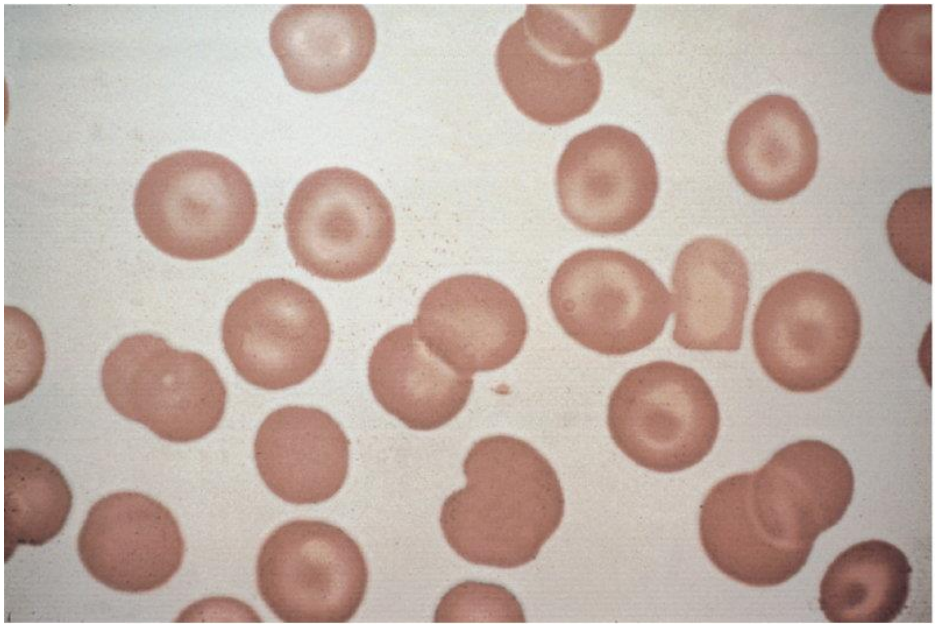
Erythrozyten

Thrombozyten

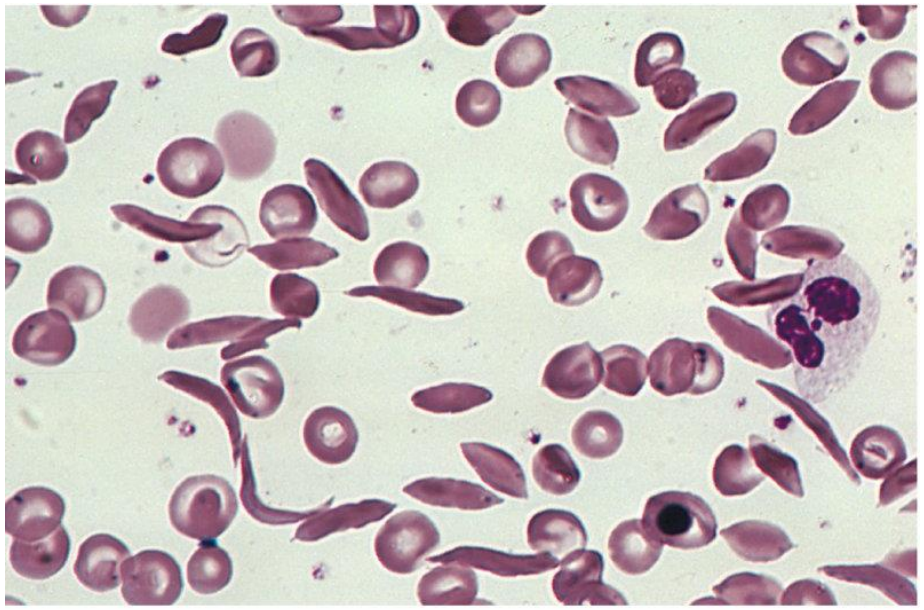


Hämoglobinopathien

Thalassämie



Sichelzellanämie



Erstbeschreibung des Hämoglobins durch Felix Hoppe-Seyler in Tübingen 1864

Felix Hoppe. Über die chemischen und optischen Eigenschaften des Blutfarbstoffs. Archiv für physiologische Anatomie und Physiologie. (1864), 29; 233-235.

β-Thalassemia

- most common autosomal recessive disorder worldwide
- 7% of world population carrier of β-thalassemia mutations

- 56.000 newborns/year with β-thalassemia major

Sickle Cell Disease (SCD)

- Mutation der beta Kette (Hb S)

- Homozygote und mildere heterozygote Formen

- 20-40% der Bevölkerung in Äquatorialafrika sind heterozygote Träger (1:250 erkrankt)

- 5-10 % der Afroamerikaner sind Träger

- 1:625 Geburten bei Afroamerikanern haben eine Sichelzellanämie
- 275.000 newborns/year with sickle cell disease

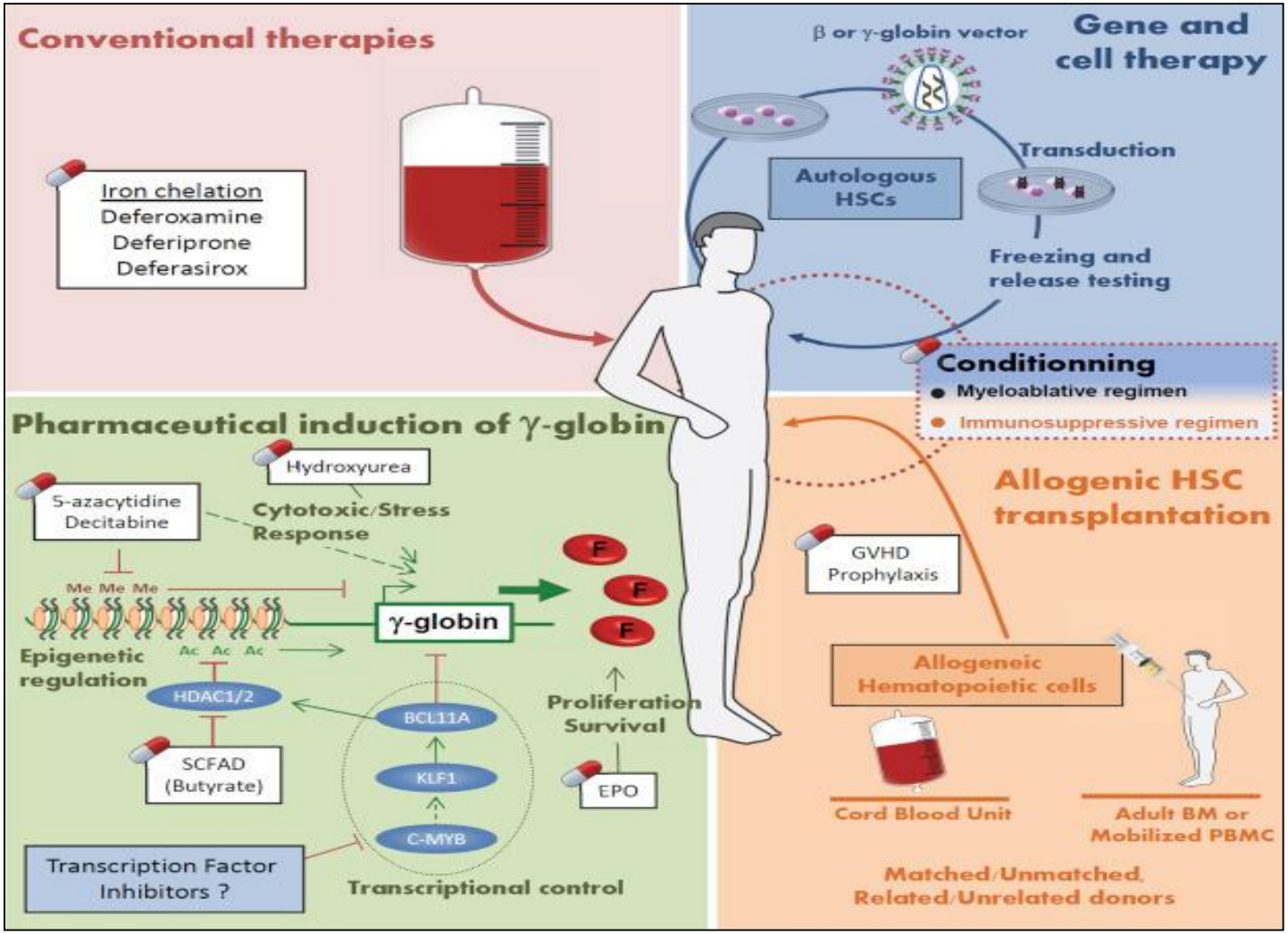
Beschreibung der Thalassämie durch Thomas Cooley

- 1925
- Auffällige Veränderungen an Milz und Knochen bei Kindern mit Anämien
- Cooley's Anemia

http://en.wikipedia.org/wiki/Thomas_Benton_Cooley

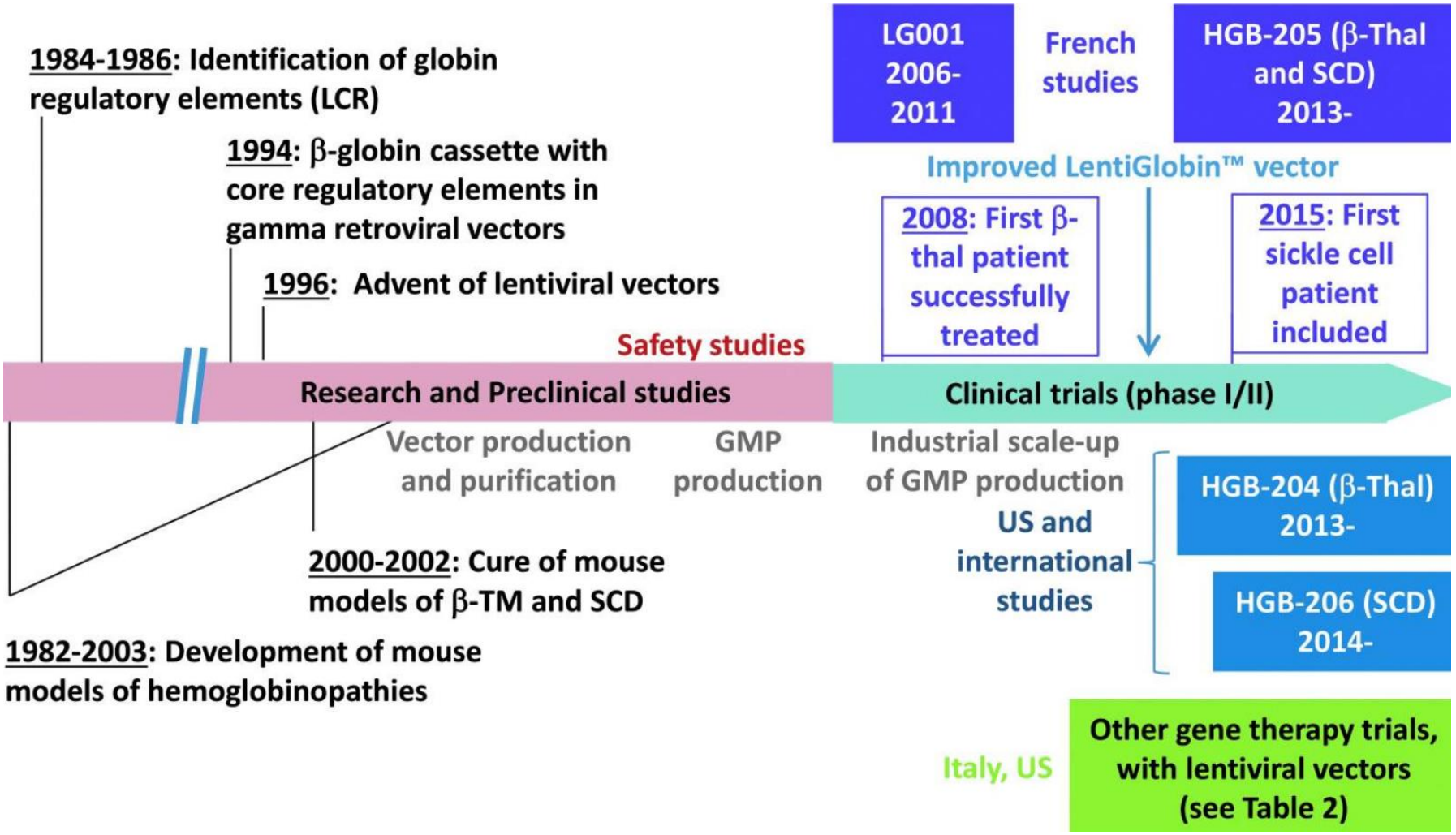
Am 8. Oktober 2014 wurde ein Asteroid nach ihm benannt: (4830) Thomascooley.

Treatments



Dreuzy et al. 2016. Current and future alternative therapies for B-thalassemia major.

Clinical trials



Negre et al., 2016

Clinical trials for gene therapy

Table 1. *Human clinical trials to date for gene therapy of β-TM and/or severe SCD in France and internationally with our lentiviral vectors (HPV569 and then BB305)*

Gene	Vector	Location	Protocol number	Sponsor	Condition	Conditioning	Intervention	Phase	Title	Start date	Results as of December 2015	Estimated primary completion
$\beta^{A\text{-}T87Q}$-globin	HPV569	France	LG001 study[159]	bluebird bio (formerly Genetix Pharmaceuticals)	β-thalassemia major and severe sickle cell disease	Myeloablative conditioning	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I/II	A Phase I/II Open Label Study with Anticipated Benefit Evaluating Genetic Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and β-Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Modified *ex-vivo* with a Lentiviral bA-T87Q Globin (Lentiglobin™) Vector	Sept 2006	First βE/β0-treated patient in the world, independent of transfusions for more than 7 years	Terminated
$\beta^{A\text{-}T87Q}$-globin	BB305	France	NCT02151526 (HGB-205 study)[159]	bluebird bio	β-thalassemia major and severe sickle cell disease	Myeloablative conditioning	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I/II	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and β-Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral βA-T87Q-Globin Vector (LentiGlobin® BB305 Drug Product)	July 2013	First βS/βS-treated patient in the world, with >50% βT87Q-globin2 βE/β0 patients independent of transfusions, 1 β0/β0 treated recently	December 2017
$\beta^{A\text{-}T87Q}$-globin	BB305	USA, Thailand, Australia	NCT01745120 (HGB-204 study)[163]	bluebird bio	β-Thalassemia major	Myeloablative conditioning	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I/II	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with β-Thalassemia Major by Transplantation of Autologous CD34+ Cells Transduced Ex Vivo with a Lentiviral β-A(T87Q)-Globin Vector (LentiGlobin® BB305 Drug Product)	August 2013	10 subjects infused: 5 β0/β0, 3 β0/βE, 1 β0/β+, and 1 with another genotype Transfusion independence for the majority	September 2017
$\beta^{A\text{-}T87Q}$-globin	BB305	USA	NCT02140554 (HGB-206 study)[164]	bluebird bio	Severe sickle cell disease	Myeloablative conditioning	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I	Phase 1 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease	August 2014	3 βS/βS subjects treated. No clinical results available yet	March 2019

Results were given at several international meetings.[159,163,164]

Negre et al., 2016

Clinical trials for gene therapy

Table 2. *Human clinical trials for gene therapy of β-TM or severe SCD with other lentiviral vectors*

Gene	Vector	Location	Protocol number	Sponsor	Condition	Conditioning	Intervention	Phase	Title	Start date	Results	Estimated primary completion
β-globin	TNS9.3.55	USA	NCT01639690[165]	Memorial Sloan Kettering Cancer Center	β-Thalassemia major	Partial cytoreduction (Bu 8 mg/kg) for 3 patients, myeloablative conditioning (Bu 14 mg/kg) for 1 patient	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I	A Phase I Clinical Trial for the Treatment of β-Thalassemia Major with Autologous CD34+ Hematopoietic Progenitor Cells Transduced with TNS9.3.55 a Lentiviral Vector Encoding the Normal Human β-Globin Gene	July 2012	Four patients treated. Three β0/β+ and one β0/β0. One patient had a significant decrease in transfusion requirements.	July 2016
γ-globin	sGbG	USA	NCT02186418[a]	Children's Hospital Medical Center, Cincinnati	Severe sickle cell disease	Unknown	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I/II	Gene Transfer for Patients with Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open Label Phase I/II Pilot Study	July 2014	No results available yet	July 2017
βAS3-globin (T87Q, G16D, E22A)	Lenti/βAS3-FB	USA	NCT02247843[a]	University of California, Children's Hospital, Los Angeles	Severe sickle cell disease	Unknown	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector	I	Clinical Research Study of Autologous Bone Marrow Transplantation for Sickle Cell Disease (SCD) Using Bone Marrow CD34+ Cells Modified with the Lenti/βAS3-FB Lentiviral Vector	August 2014	No results available yet	April 2017
β-globin	GLOBE	Italy	NCT02453477[166,a]	IRCCS San Raffaele	β-Thalassemia major	Myeloablative conditioning	Transplantation of HSCs transduced *ex vivo* with a lentiviral vector (intrabone injection)	I/II	A Phase I/II Study Evaluating Safety and Efficacy of Autologous Hematopoietic Stem Cells Genetically Modified with GLOBE Lentiviral Vector Encoding for the Human Beta Globin Gene for the Treatment of Patients Affected by Transfusion Dependent Beta-Thalassemia	May 2015	First patient recently treated	August 2019

[a]Clinicaltrials.gov
Results were provided at international meetings.[165,166]

Negre et al., 2016

Gene-editing mit Crispr/Cas9: In Planung

Program	Editing approach	Research	IND-enabling	Ph I/II	Partner	Structure
***Ex vivo:* Hematopoietic**						
CTX001: β-thalassemia	Disruption			*IND/CTA filing in late 2017*	VERTEX	Collaboration
CTX001: Sickle cell disease (SCD)	Disruption				VERTEX	Collaboration
Hurler syndrome (MPS-1)	Correction					Wholly-owned
Severe combined immunodeficiency (SCID)	Correction				CASEBIA	Joint venture
***Ex vivo:* Immuno-oncology**						
CTX101: CD19-positive malignancies	Various					Wholly-owned
Immuno-oncology – Other	Various					Wholly-owned
***In vivo:* Liver**						
Glycogen storage disease Ia (GSD Ia)	Correction					Wholly-owned
Hemophilia	Correction				CASEBIA	Joint venture
***In vivo:* Other organs**						
Duchenne muscular dystrophy (DMD)	Disruption					Wholly-owned
Cystic fibrosis (CF)	Correction				VERTEX	License option

http://www.crisprtx.com/our-programs/our-pipeline.php

Review article

Transcriptional regulation of fetal to adult hemoglobin switching: new therapeutic opportunities

Andrew Wilber,[1] Arthur W. Nienhuis,[2] and Derek A. Persons[2]

[1]Department of Surgery, Southern Illinois University School of Medicine, Springfield, IL; and [2]Department of Hematology, St Jude Children's Research Hospital, Memphis, TN

In humans, embryonic, fetal, and adult hemoglobins are sequentially expressed in developing erythroblasts during ontogeny. For the past 40 years, this process has been the subject of intensive study because of its value to enlighten the biology of developmental gene regulation and because fetal hemoglobin can significantly ameliorate the clinical manifestations of both sickle cell disease and β-thalassemia. Understanding the normal process of loss of fetal globin expression and activation of adult globin expression could potentially lead to new therapeutic approaches for these hemoglobin disorders. Herein, we briefly review the history of the study of hemoglobin switching and then focus on recent discoveries in the field that now make new therapeutic approaches seem feasible in the future. Erythroid-specific knockdown of fetal gene repressors or enforced expression of fetal gene activators may provide clinically applicable approaches for genetic treatment of hemoglobin disorders that would benefit from increased fetal hemoglobin levels. (*Blood*. 2011;117(15):3945-3953)

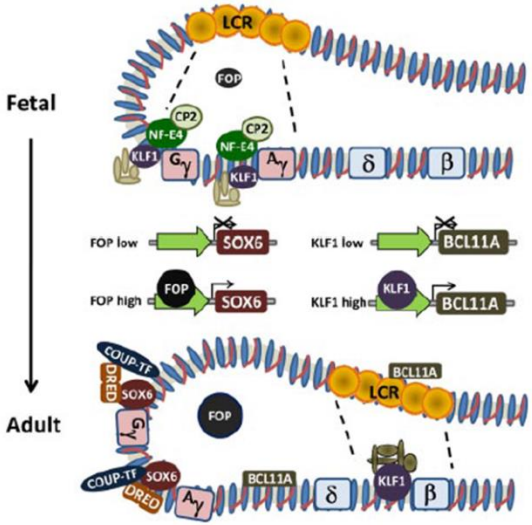
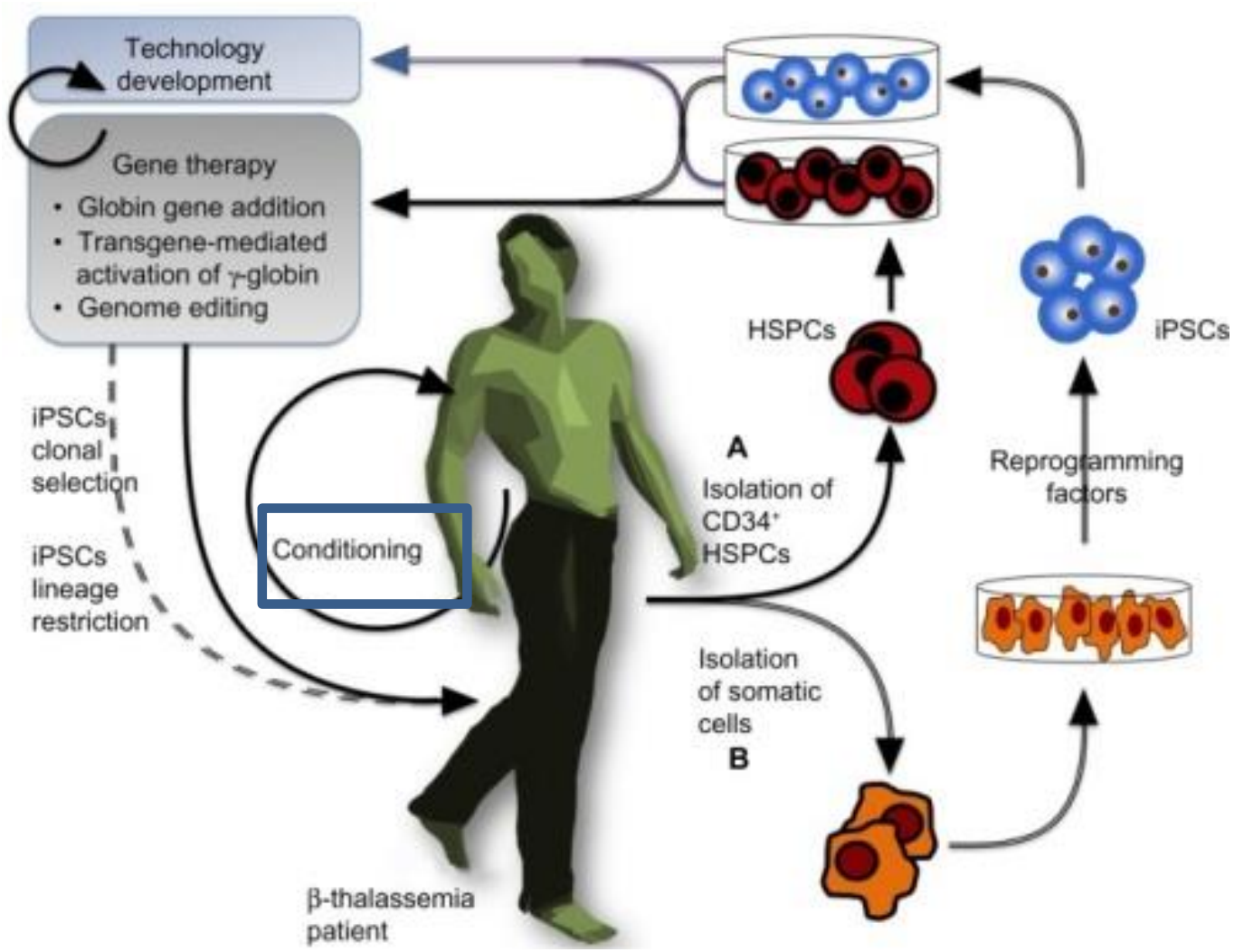


Figure 2. Schematic of hemoglobin switching model based on looping and interaction of the LCR with the individual globin gene promoters. The various proteins demonstrated experimentally to be involved in regulating the change in expression from γ-globin to β-globin and individual effects of FOP and KLF1 on transcriptional regulation of SOX6 and BCL11A, respectively.

Gene therapy for β-Thalassemia



Finotti et al., 2015

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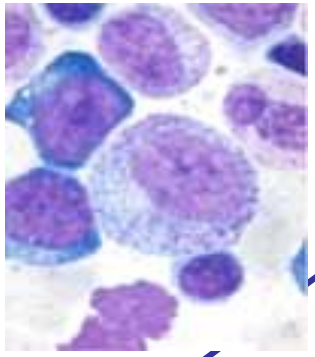

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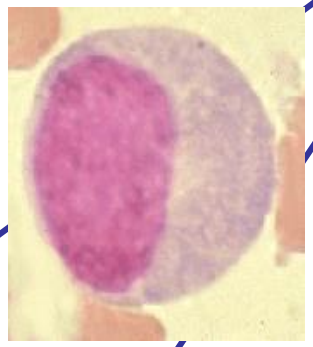

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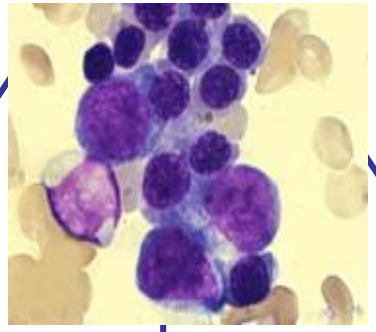

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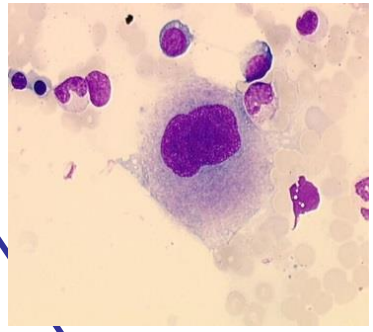

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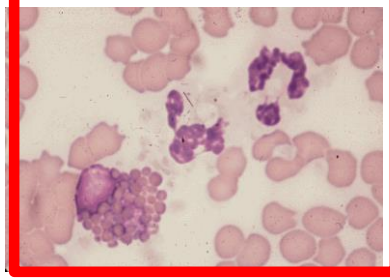

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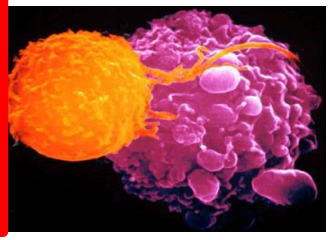

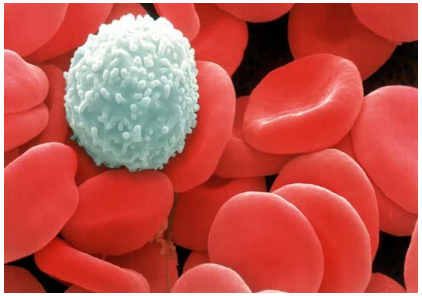

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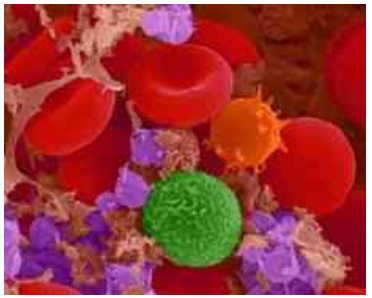
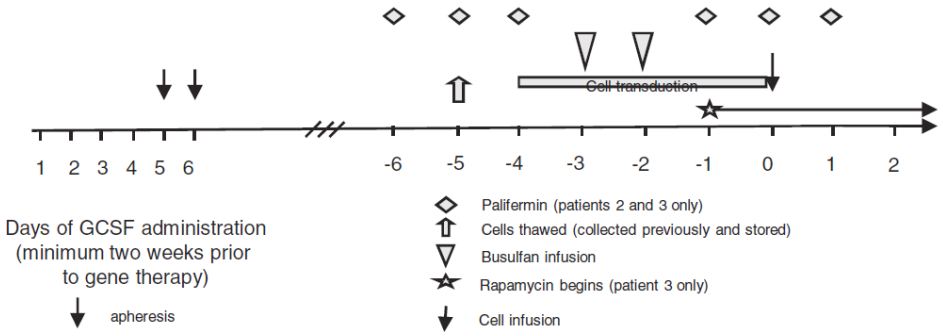


Septische Granulomatose (CGD)
(3 Patienten mit gp91^{phox})

Kang EM et al., Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. BLOOD 2010; 115: 783.

Conditioning



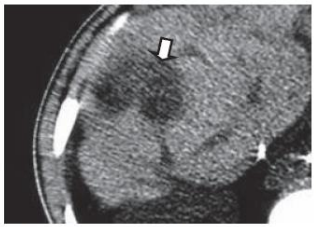


Image 1 pre gene therapy

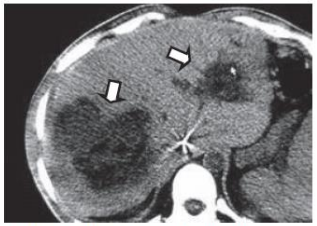


Image 2 pre gene therapy

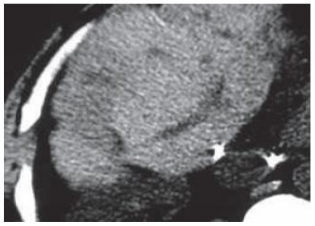


Image 1 post gene therapy

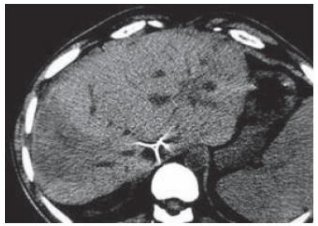
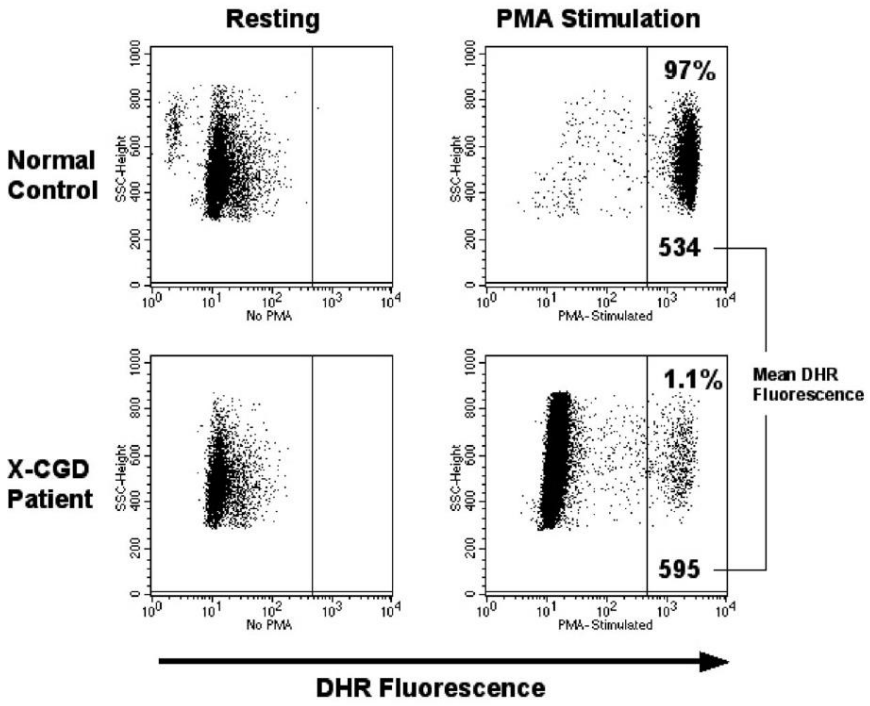
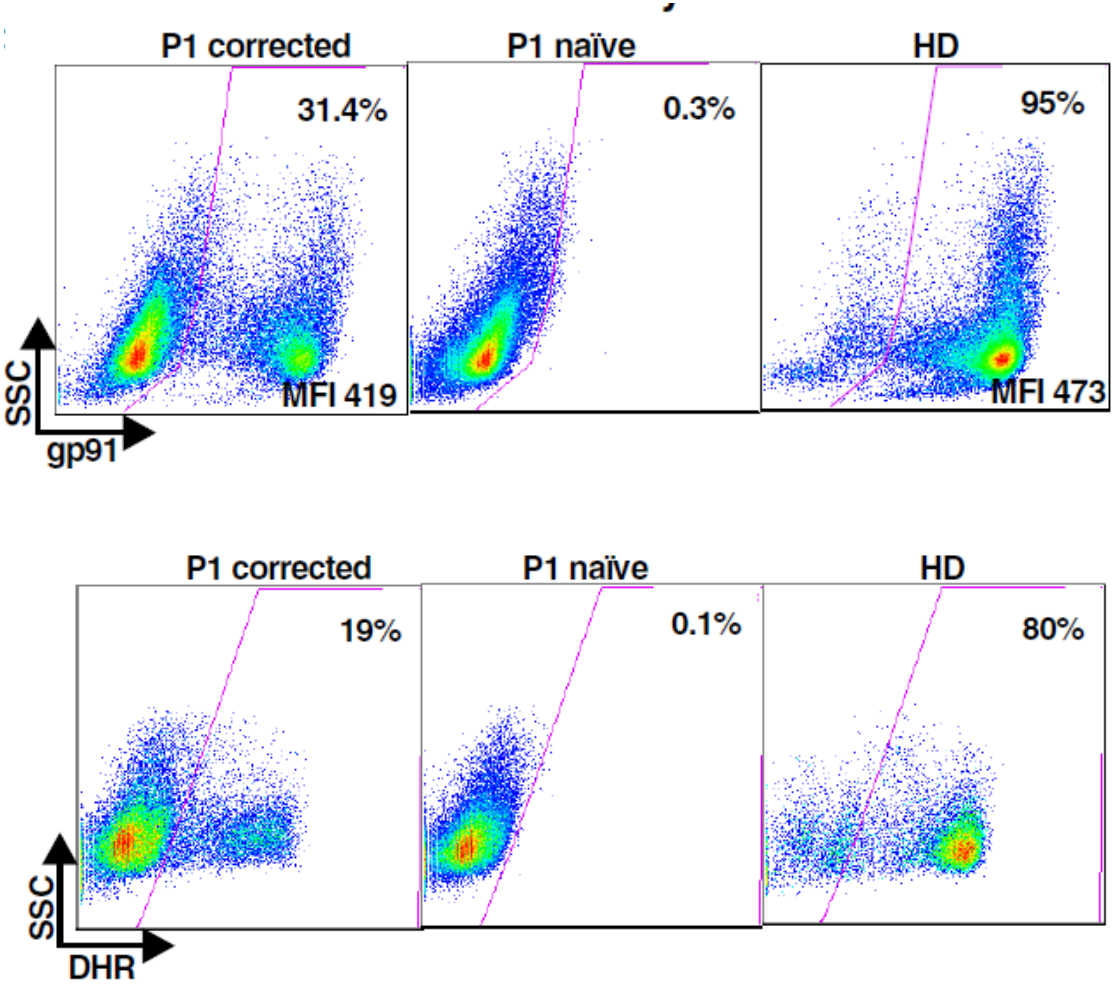


Image 2 post gene therapy

Superoxide production



De Ravin SS. et al. CRISPR/Cas9 gene repair of hematopoetic stem cells from Patients with X-linked chronic granulomatous disease.
Science Translational Medicine 2017; 9: eaah 3480

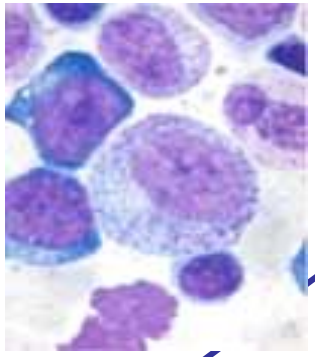


Stammzellen

Selbsterneuerung

Ausdifferenzierung

Septische Granulomatose



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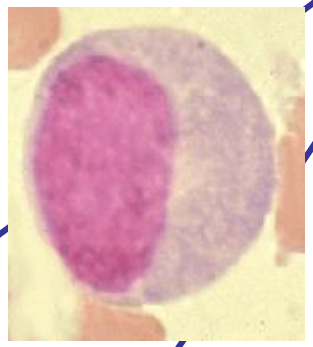
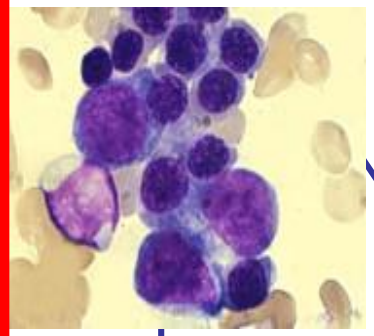
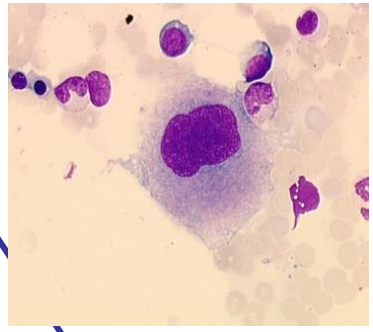


Thalassämie
Sichelzellanämie



Wiskott-Aldrich-Sy



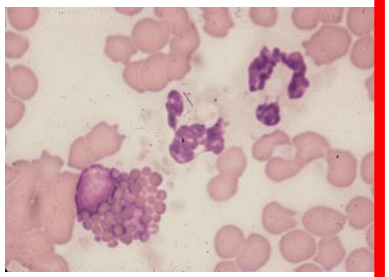
Myelopoese

Lymphopoese

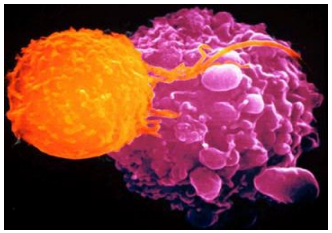
Erythropoese

Thrombopoese

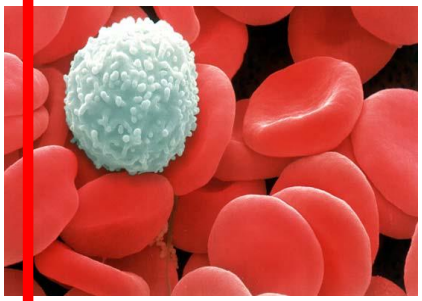
Granulozyten
Monozyten



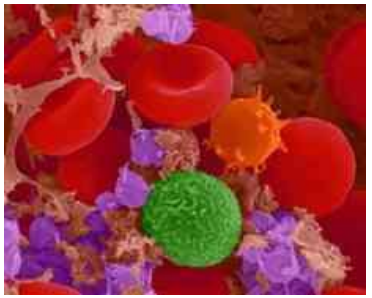
T-Zellen
B-Zellen
NK-Zellen



Erythrozyten



Thrombozyten



Kohn DB., Kuo CY. New frontiers in the therapy of primary immunodeficiency: From gene addition to gene editing.
J. Allergy Clin Immunol 2017; 139: 726.

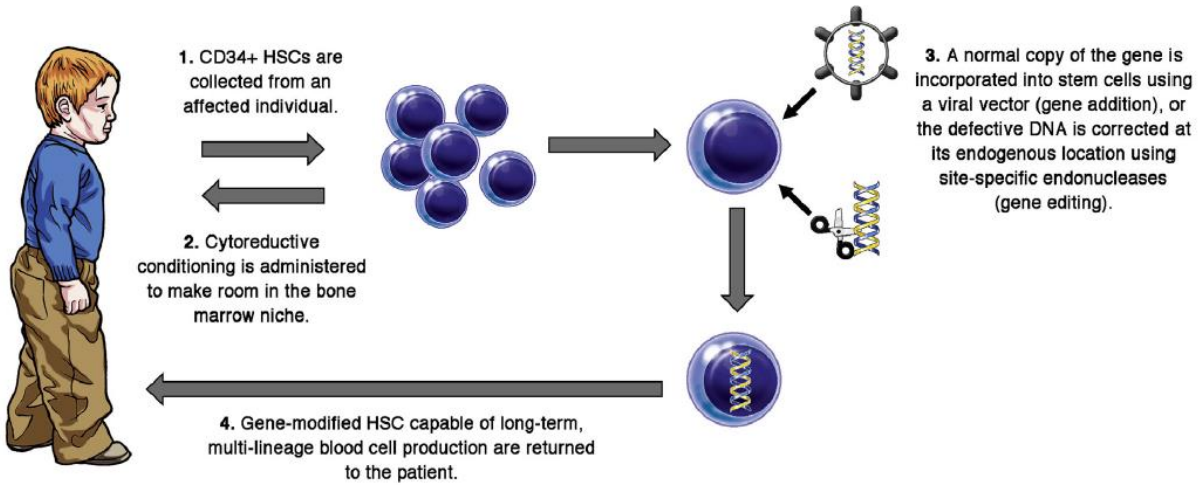


TABLE I. Diseases treated with gene therapy using HSCs in clinical trials

PIDs
ADA-SCID
X-SCID
WAS
X-CGD
Leukocyte adhesion deficiency (LAD)
Lysosomal storage and metabolic disorders
X-linked adrenoleukodystrophy (X-ALD)
Metachromatic leukodystrophy (MLD)
Hemoglobinopathies
β-Thalassemia
Sickle cell disease
Stem cell defects
Fanconi anemia

Table 1. Open Phase I/II Clinical Trials of HSC Gene Therapy for PIDs

Disease	Vector	Promoter	Conditioning	Stem Cell Source	Centre	Recruiting Since	No Patients	ClinicalTrials.gov Identifier
X-SCID	SIN-γRV	EFS	None	BM	Boston, Cincinnati, Los Angeles, London, Paris	2010	11	NCT01410019 NCT01129544 NCT01175239
	SIN-LV	EFS	Busulfan 6 mg/kg	PBSCs	Memphis, NIH Clinical Center Bethesda[a]	2010	5	NCT01306019
	SIN-LV	EFS	Busulfan 6 mg/kg	BM	Memphis, Seattle	2012	0	NCT01512888
ADA-SCID	SIN-LV	EFS	Busulfan 5 mg/kg	BM/PBSCs	London	2011	14	NCT01380990
	SIN-LV	EFS	Busulfan 4 mg/kg	BM/PBSCs	Los Angeles, Bethesda	2013	16	NCT01852071 NCT02022696
WAS	SIN-LV	WAS	RIC busulfan/ fludarabine[b]	BM/PBSCs	Milan	2010	8	NCT01515462
	SIN-LV	WAS	RIC busulfan/ fludarabine[b]	BM/PBSCs	Boston, London, Paris	2011	13	NCT01410825 NCT01347242 NCT01347346
CGD	SIN-γRV	Myeloid specific	Busulfan	PBSCs	Frankfurt	2013	0	NCT01906541
	SIN-LV	Chimeric	MAC busulfan[b]	PBSCs	London, Paris, Frankfurt, Zurich	2013	1	NCT01855685
	SIN-LV	Chimeric	MAC busulfan[b]	BM	Los Angeles, Boston, Bethesda	2015	1	NCT02234934

[a]This trial is recruiting patients aged 2–30 years.
[b]RIC, reduced intensity conditioning; MAC, myeloablative conditioning.

Kohn DB., Kuo CY. New frontiers in the therapy of primary immunodeficiency: From gene addition to gene editing.
J. Allergy Clin Immunol 2017; 139: 726.

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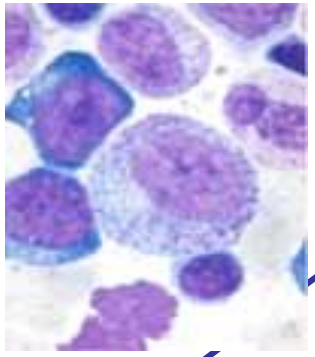
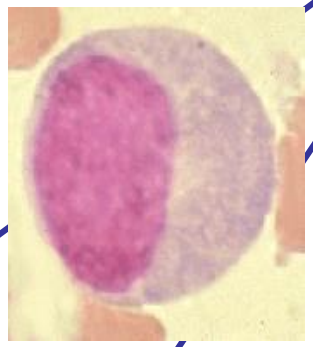
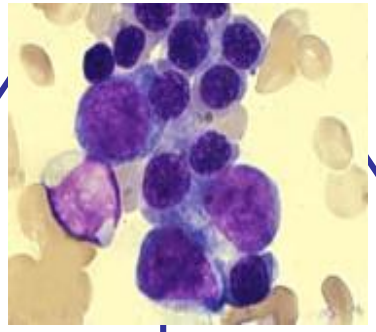


SCID



Thalassämie
Sichelzellanämie



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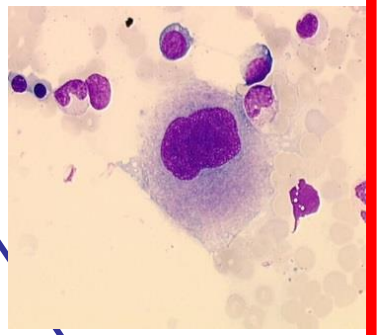


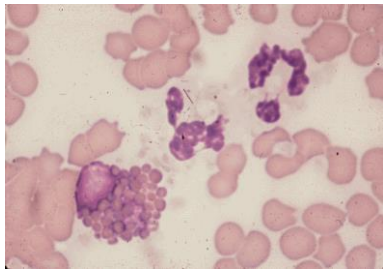
Myelopoese

Lymphopoese

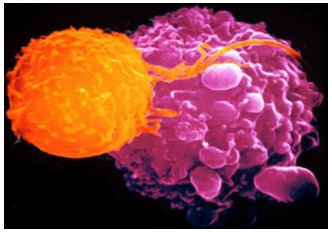
Erythropoese

Thrombopoese

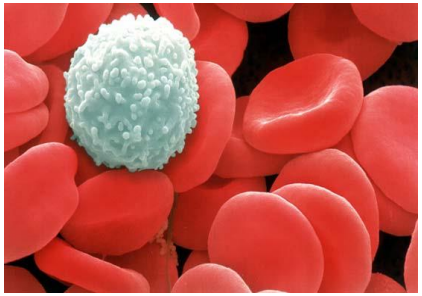
Granulozyten
Monozyten



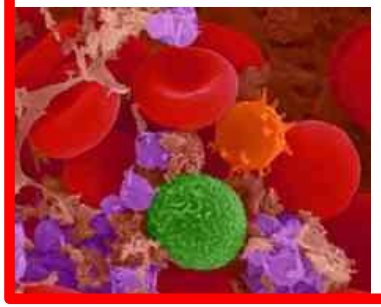
T-Zellen
B-Zellen
NK-Zellen



Erythrozyten



Thrombozyten



Triad of Wiskott Aldrich Syndrome

eczema-thrombocytopenia-immunodeficiency syndrome

Thrombozytopenie mit kleinen Thrombozyten

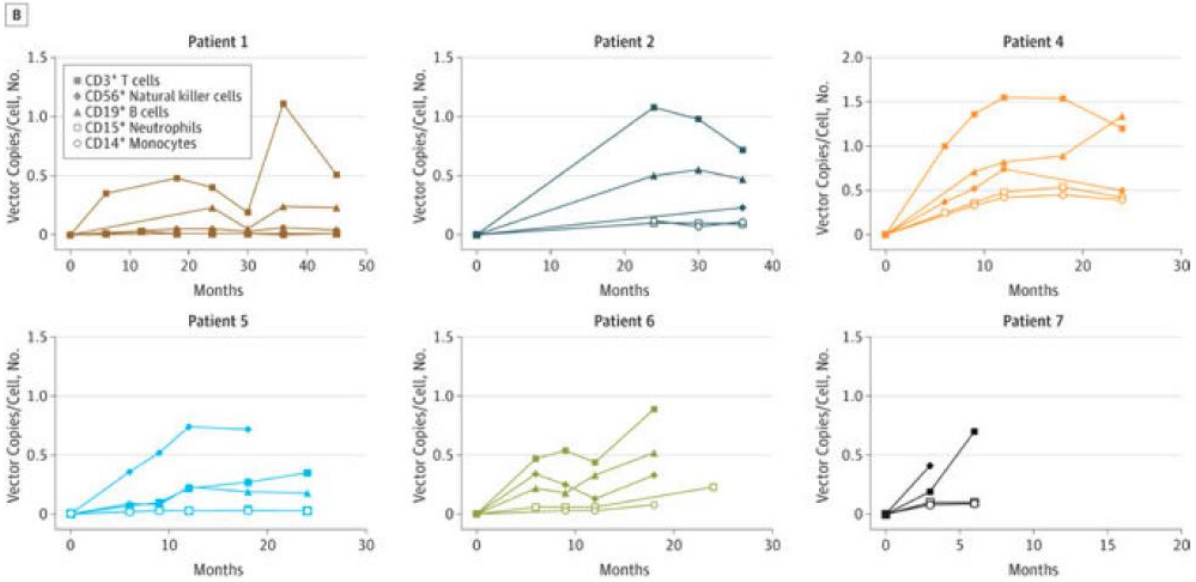
Ekzema (nicht immer)

Otitiden (Immunodefizienz))

-schlechte Antikörperbildung auf Carbohydrat-Antigene

-verminderte T-Zellfunktion

Abina SH. et al., Outcome following gene therapy in patients with severe Wiskott-Aldrich-Syndrome.
JAMA 2015; 313: 1550.



Results—Six out of the 7 patients were alive at the time of last follow-up (mean and median follow-up time: 28 and 27 months respectively) and showed sustained clinical benefit. One patient died 7 months after treatment from pre-existing drug- resistant herpes virus infections. Eczema and susceptibility to infections resolved in all 6 patients. Autoimmunity improved in 5/5 patients. No severe bleeding episodes were recorded after treatment, and at last follow up 6/6 patients were free from blood product support and thrombopoietic agonists. Hospitalization days were reduced

Fazit

Gentherapie kann eine Option sein/werden für angeborene monogene hämatologische Erkrankungen

Problem der Gentoxititität: sekundäre maligne Erkrankungen?

Transiente oder langlebige Genkorrektur?

Regulatorische Herausforderungen

Überlegenheit der Gentherapie im Vergleich zur allogenen Stammzelltransplantation?

Anurathapan U. et al., Hematopoietic stem cell transplantation for homozygous β thalassemia and B thalassemia/hemoglobin E patients from haploidentical donors . Bone Marrow Transplant 2016; 51: 813