Klinik für Kinder- und
Jugendmedizin

UNIVERSITÄTS
KLINIKUM
ulm

Gentherapie zur Behandlung angeborener Immundefekte

Manfred Hönig, Pädiatrische Stammzelltransplantation

Hämatologie heute, Köln, 21.03.2019

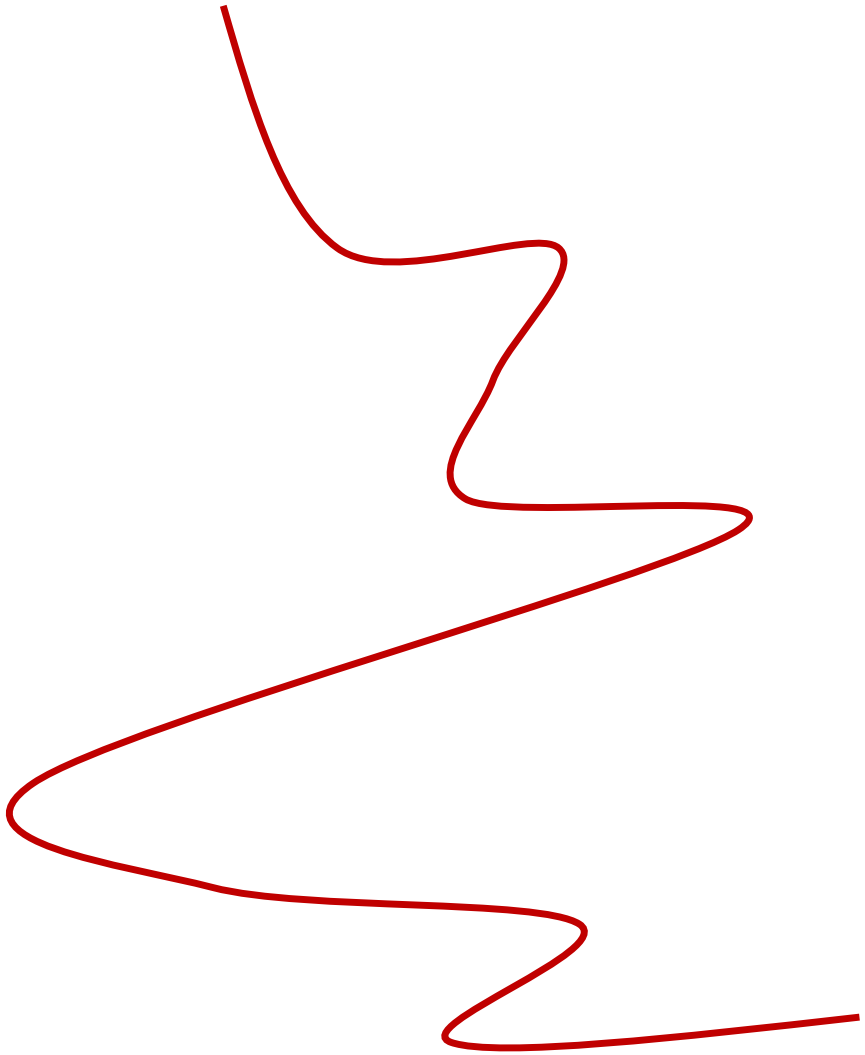
Offenlegung

CSL Behring
Jazz Pharmaceuticals
Medac
Octapharma

} Kongressbesuch/ Reisekosten
Vortragshonorar

Überblick

- Kasuistik
- abgeschlossene Studien
- rekrutierende Studien



Fall

- weibliches NG, 39 SSW, GG 3490g, pH 7,14, APGAR 9/10/10
- 7 Tage, klinisch unauffällig
- 2. Kind konsanguiner Eltern (Cousin/ Cousine I.°)
- Familienanamnese:
    - Schwester im Alter von 10/12 an CMV-Infektion verstorben

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Differentialblutbild:

Leuko	2500/µl
Granulo	2250/µl
Lympho	100/µl
Mono	150/µl

Fall

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- 7 Tage, klinisch unauffällig
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    - Schwester im Alter von 10/12 an CMV-Infektion verstorben

Differentialblutbild:		Immunphänotypisierung:	
Leukos	2500/µl	T-Ly	2/µl
Granulos	2250/µl	B-Ly	3/µl
Ly	**100/µl**	NK-Ly	20/µl
Mono	150/µl		

Fall

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- 7 Tage, klinisch unauffällig
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Leukos	2500/µl
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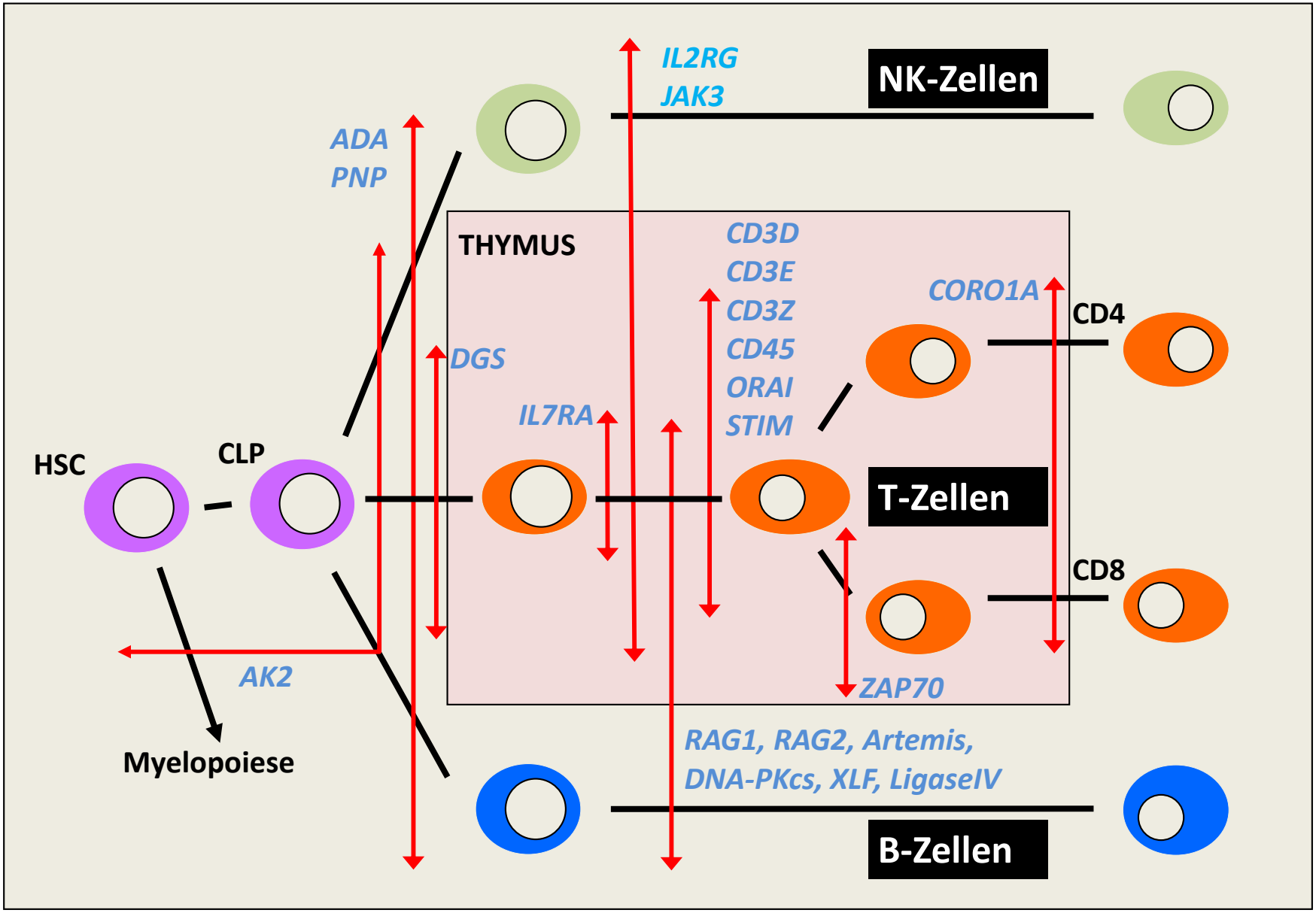
Ly	**100/µl**
Mono	150/µl

Immunphänotypisierung:	
T-Ly	2/µl
B-Ly	3/µl
NK-Ly	20/µl

Immunglobuline:	
IgG	6,9g/l
IgA	<0,06g/l
IgM	0,07g/l

Diagnose: $T^-B^-NK^-$ SCID


IL2RG
JAK3
NK-Zellen
ADA
PNP
THYMUS
CD3D
CD3E
CD3Z
CD45
ORAI
STIM
CORO1A
CD4
DGS
IL7RA
HSC
CLP
T-Zellen
CD8
AK2
ZAP70
Myelopoiese
RAG1, RAG2, Artemis,
DNA-PKcs, XLF, LigaseIV
B-Zellen

Fall

Adenosindesaminase: ↓ Enzymaktivität
Mutation in ADA: g.[864C>T][864C>T]; p.[Gln246*][Gln246*]
$T^-B^-NK^-$ SCID durch Defizienz der Adenosindesaminase

Indikation zur Stammzelltransplantation

Spender:	HLA-haploidentische Mutter
Konditionierung:	Bu12.8 Cy200
GvHD-Prophylaxe:	CD34-Selektion
Transplantat:	CD34+ $1x10^7$/kg, CD3+ $2x10^4$/kg
Engraftment:	zeitgerecht, Boost $2_{/12}$ nach KMT
Toxizität:	Mucositis 3-4°, Transaminasenanstieg
GvHD:	keine
Infektionen:	Rotaenteritis $7_{/12}$ nach KMT

Fall

Chimärismus: 100%

Fall

Chimärismus: 100%

Immunphänotyp:

Material: Vollblut

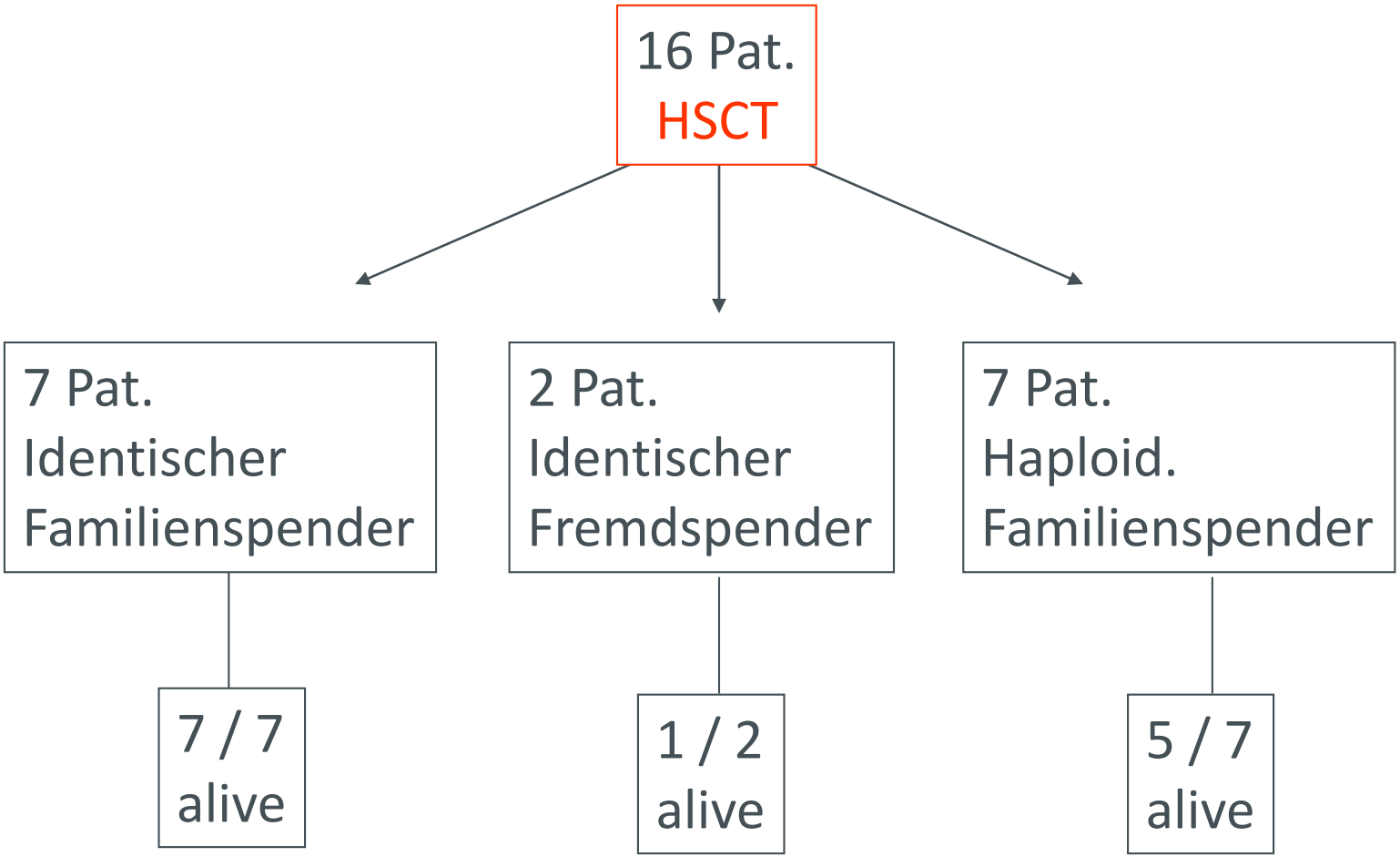
Oberflächenmarker (Lymphozyten)				
	Leukozyten:	**6600 /µl**	(4400-9500/µl) [1]	
	Lymphozyten:	**2706 /µl**	(1900-3700/µl) [1]	

B-Lymphozyten:			Referenzbereich
CD19+:	25 %	**676 /µl**	(270-860/µl) [1]
NK-Zellen:			
CD3-/CD56+/CD16+:	7 %	**189 /µl**	(80-600/µl) [4]
			(0-3%)
NK-like T-cells:			
CD3+/CD56+/CD16+:	1 %	**27 /µl**	(8-110/µl) [4]
Sonstige:			
DR+:	**27 %**	731 /µl	(13-30%) [4*]

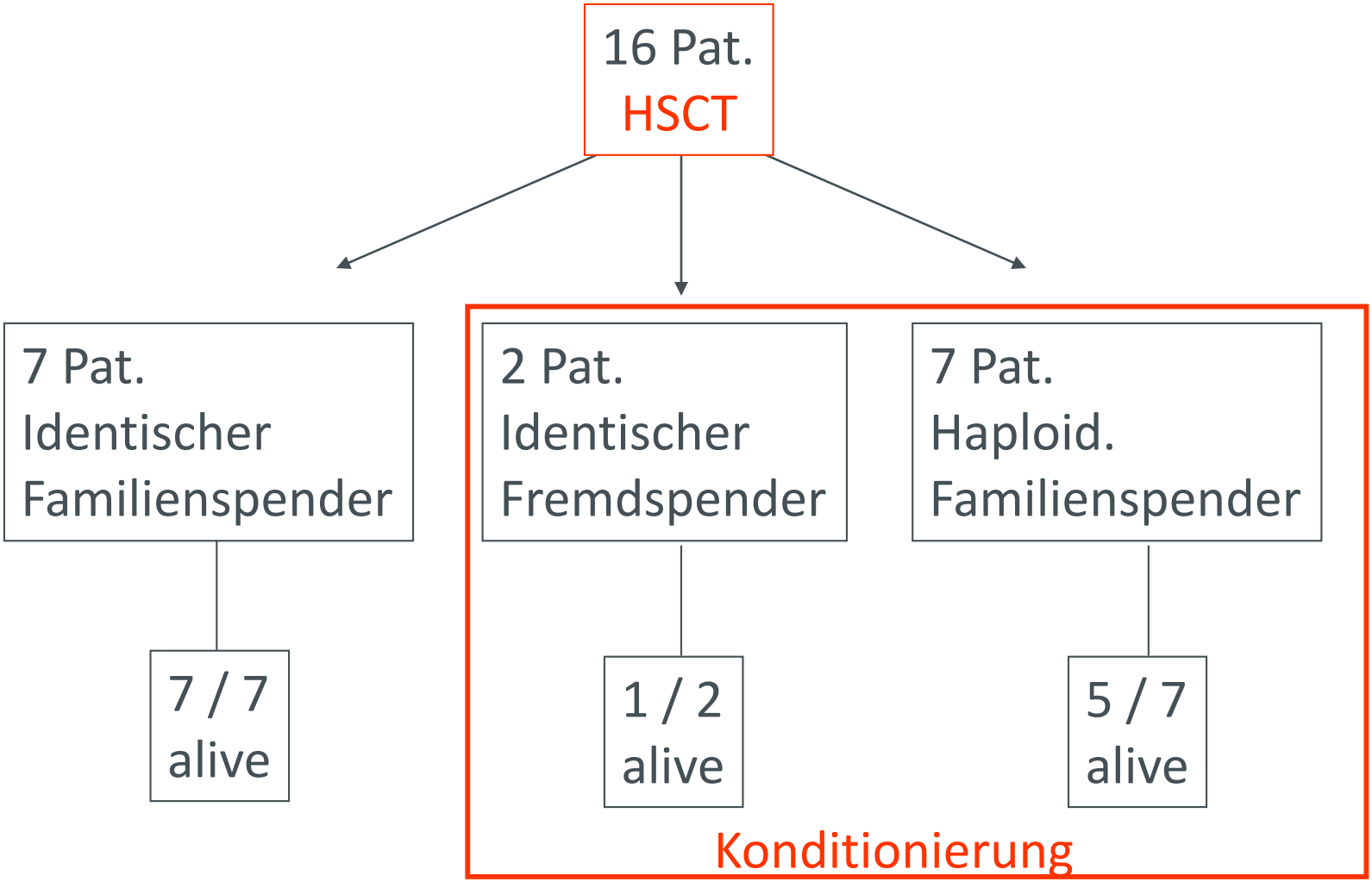
T-Lymphozyten:			Referenzbereich
CD3+:	62 %	**1678 /µl**	(1200-2600/µl) [1]
CD3+/DR+:	**1 %**	27 /µl	(0-4,1%) [4]
CD3+/CD4+:	32 %	**866 /µl**	(650-1500/µl) [1]
CD3+/CD8+:	20 %	**541 /µl**	(370-1100/µl) [1]
Ratio CD4/CD8:	**1,6**		(1-2,7) [4]
CD3+/CD4+/CD8+:	0 %		(5-30/µl)
CD3+/CD4-/CD8-:	7 %	**189 /µl**	(60-420/µl) [4]
TCRa,ß+:	54 %	**1461 /µl**	(760-3560/µl) [4]
TCRy,d+:	8 %	**216 /µl**	(60-420/µl) [4]
TCRa,ß+/CD4-/CD8-:	**1 %**	27 /µl	(0,6-2,8%) [4]
TCRy,d+/CD4-/CD8-:	**6 %**	162 /µl	(2,4-10%) [4]
von allen CD4+ Zellen:			
CD45RA+/CCR7+:			(34-79%)
CD45RA+/CCR7-:			(0,1-3,4%)
CD45 **RA+**/RO-:	**47 %**		(46-77%) [1]
CD45 **RO+**/RA-:	**47 %**		(16-57%) [4]
CD45RA+RO+:	**0 %**		(0-2,8%)
Ratio CD45RA/CD45RO:	**1**		
von allen CD8+ Zellen:			
CD45RA+CD62L+:	**63 %**		(23-74%)

Follow up: 18 Jahre

ADA-SCID Ulm


16 Pat.
HSCT
7 Pat.
Identischer
Familienspender
2 Pat.
Identischer
Fremdspender
7 Pat.
Haploid.
Familienspender
7 / 7
alive
1 / 2
alive
5 / 7
alive

ADA-SCID Ulm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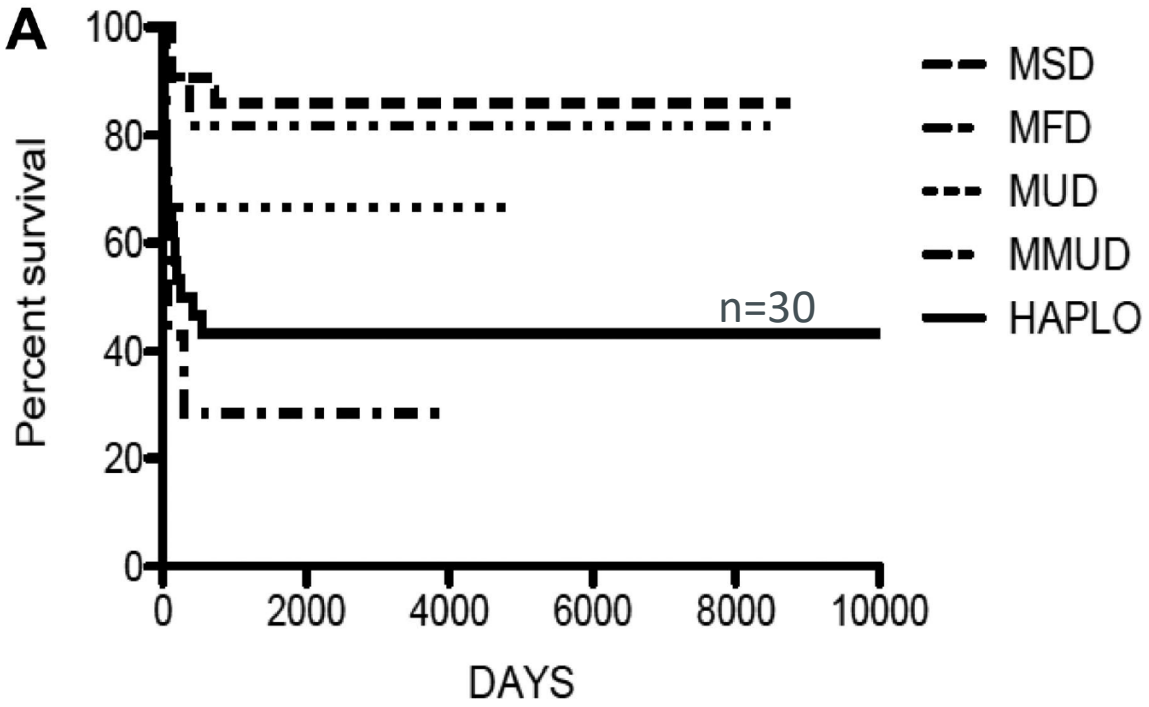
7 Pat.
Haploid.
Familienspender
7 / 7
alive
1 / 2
alive
5 / 7
alive
Konditionierung

Transplantation international ADA-Defizienz

Outcome of hematopoietic stem cell transplantation for adenosine deaminase-deficient severe combined immunodeficiency

Hassan et al., Blood 2012
n=106

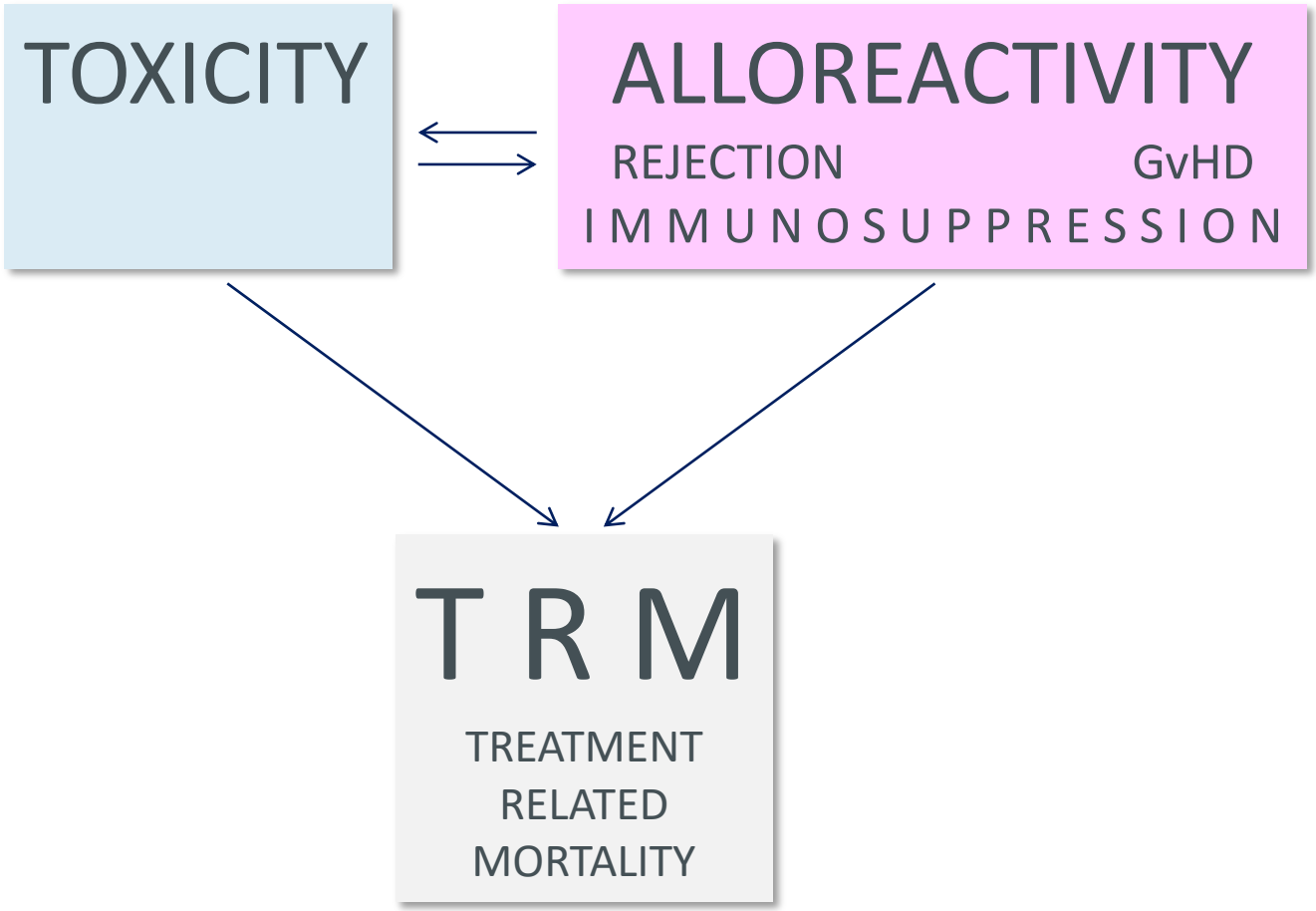


Therapieoptionen ADA-Defizienz

T R M

TREATMENT
RELATED
MORTALITY

Therapieoptionen ADA-Defizienz

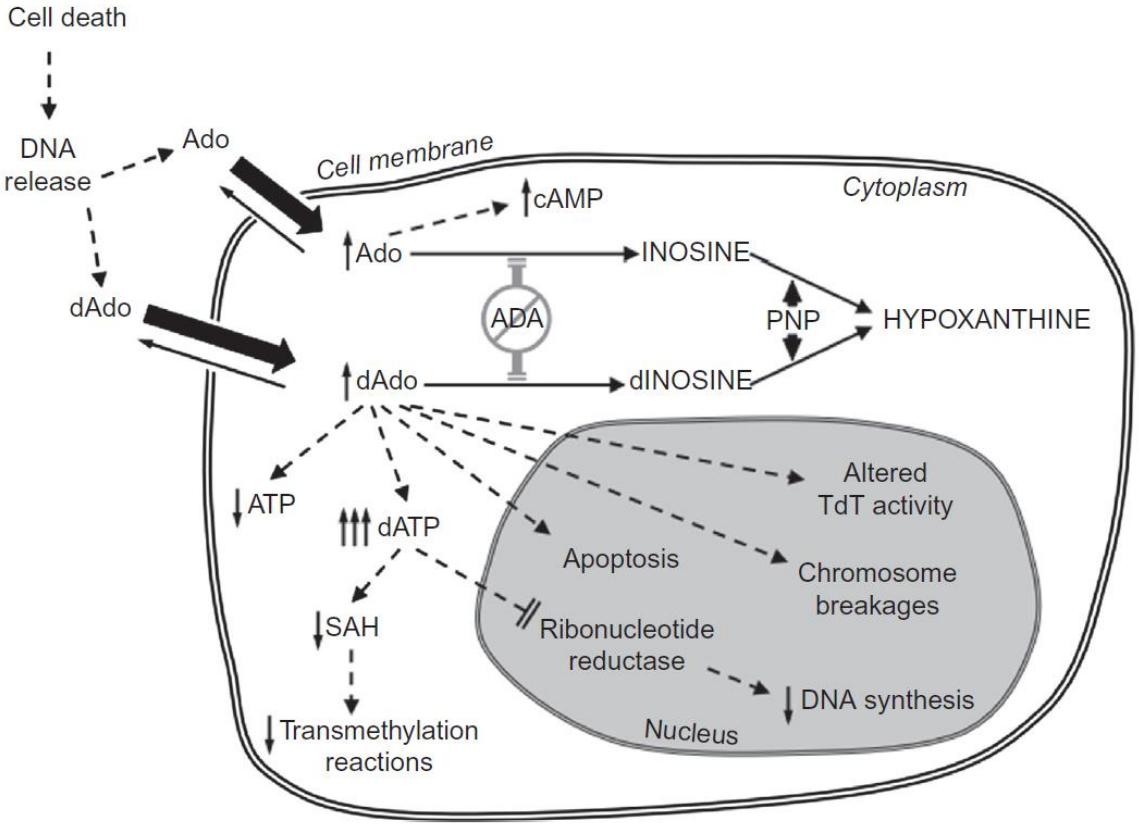


Therapieoptionen ADA-Defizienz

Transplantation

Enzymersatztherapie

ADA-Defizienz: Stoffwechselerkrankung



aus Stiehm's Immune Deficiencies

Edited by
Kathleen E. Sullivan
E. Richard Stiehm

Therapieoptionen ADA-Defizienz

Transplantation

Enzymersatztherapie

Gentherapie

Gentherapie ADA-Defizienz

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 April 2016
EMA/CHMP/230486/2016
Press office

Press release

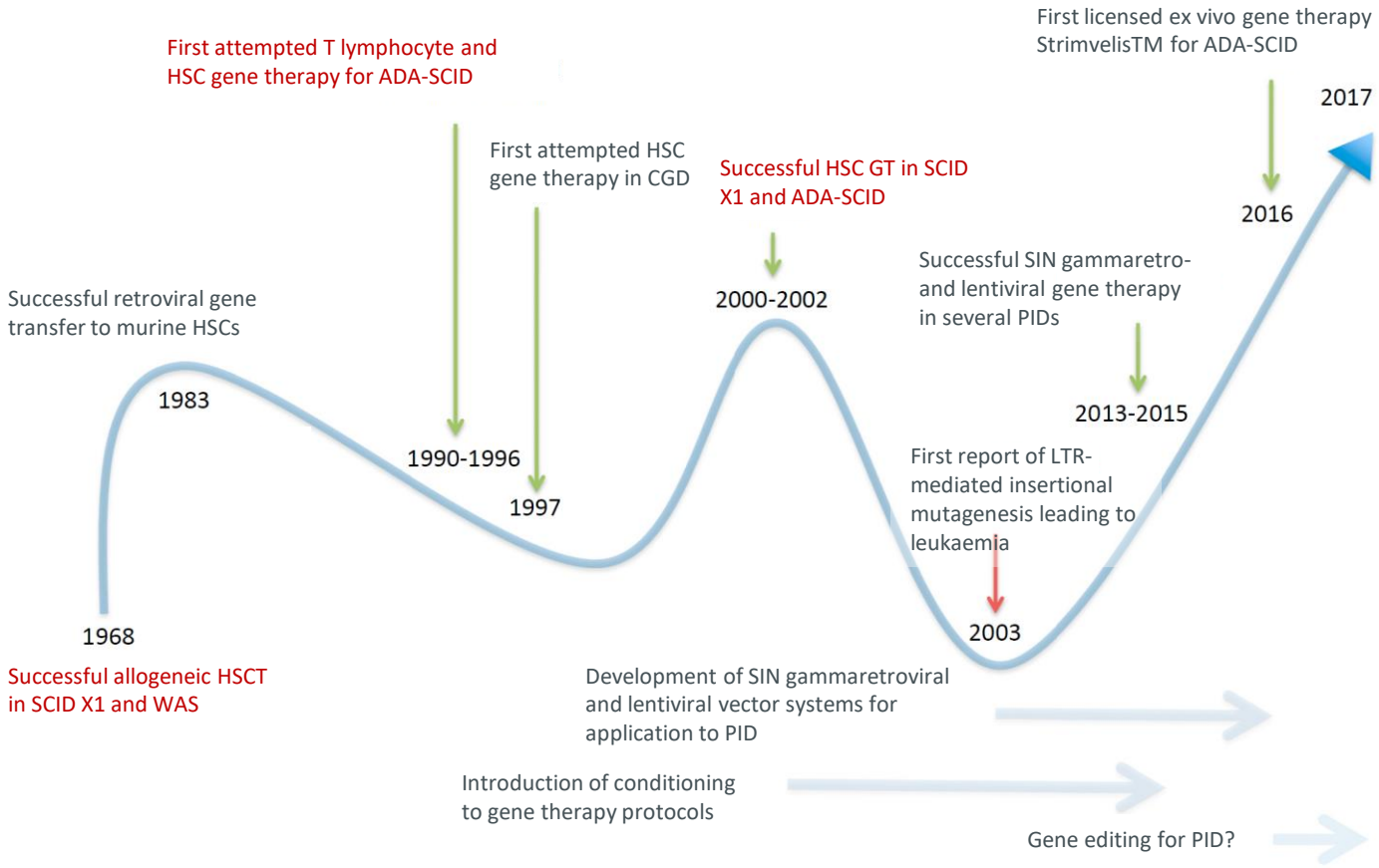
New gene therapy for the treatment of children with ultra-rare immune disorder recommended for approval

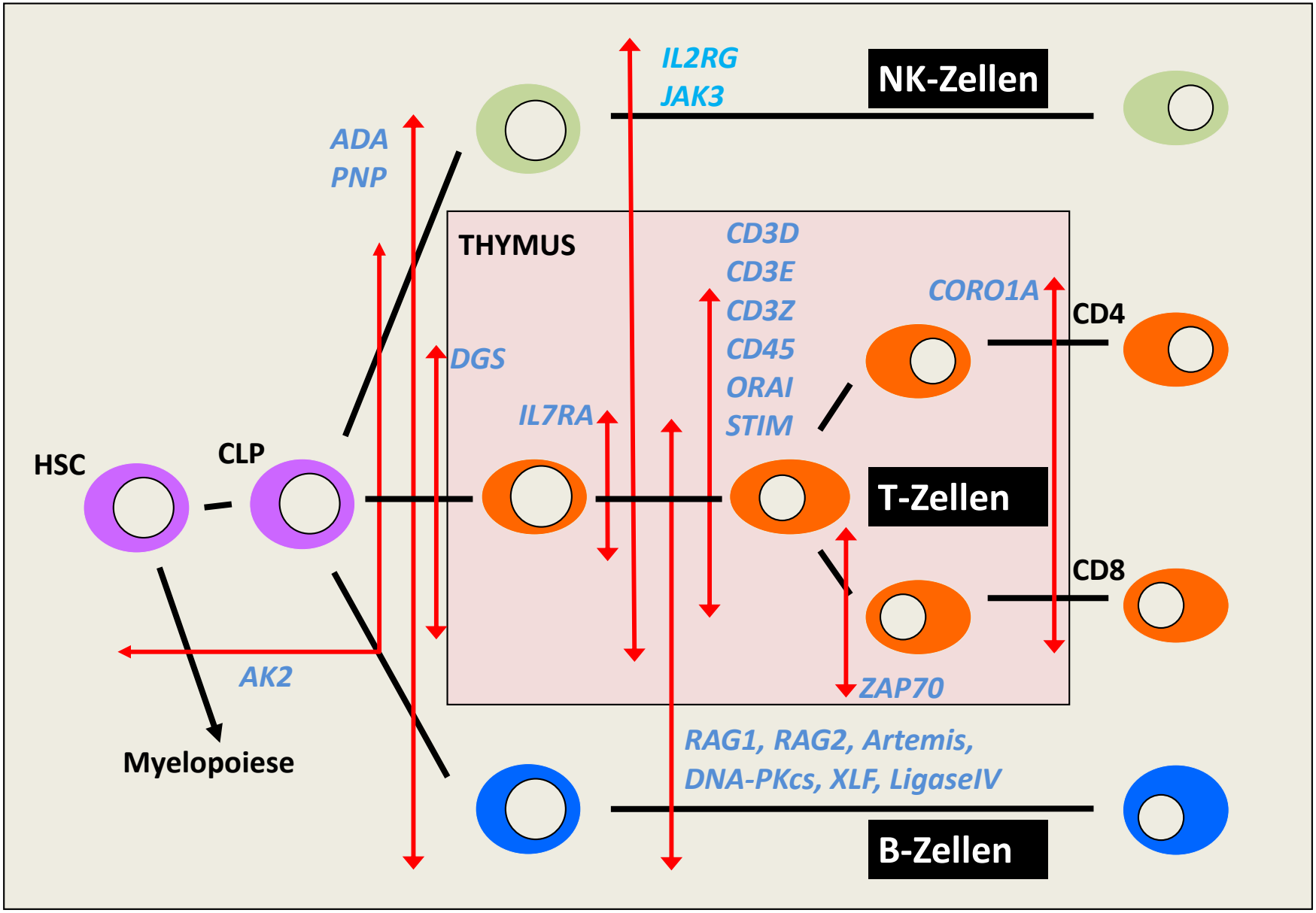
Orphan-designated Strimvelis to offer treatment option for patients with ADA-SCID who have no suitable stem cell donor

PID-Pioniere

„ups and downs“

Thrasher et al., Molecular Therapy 2017

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CLP
T-Zellen
CD8
AK2
ZAP70
Myelopoiese
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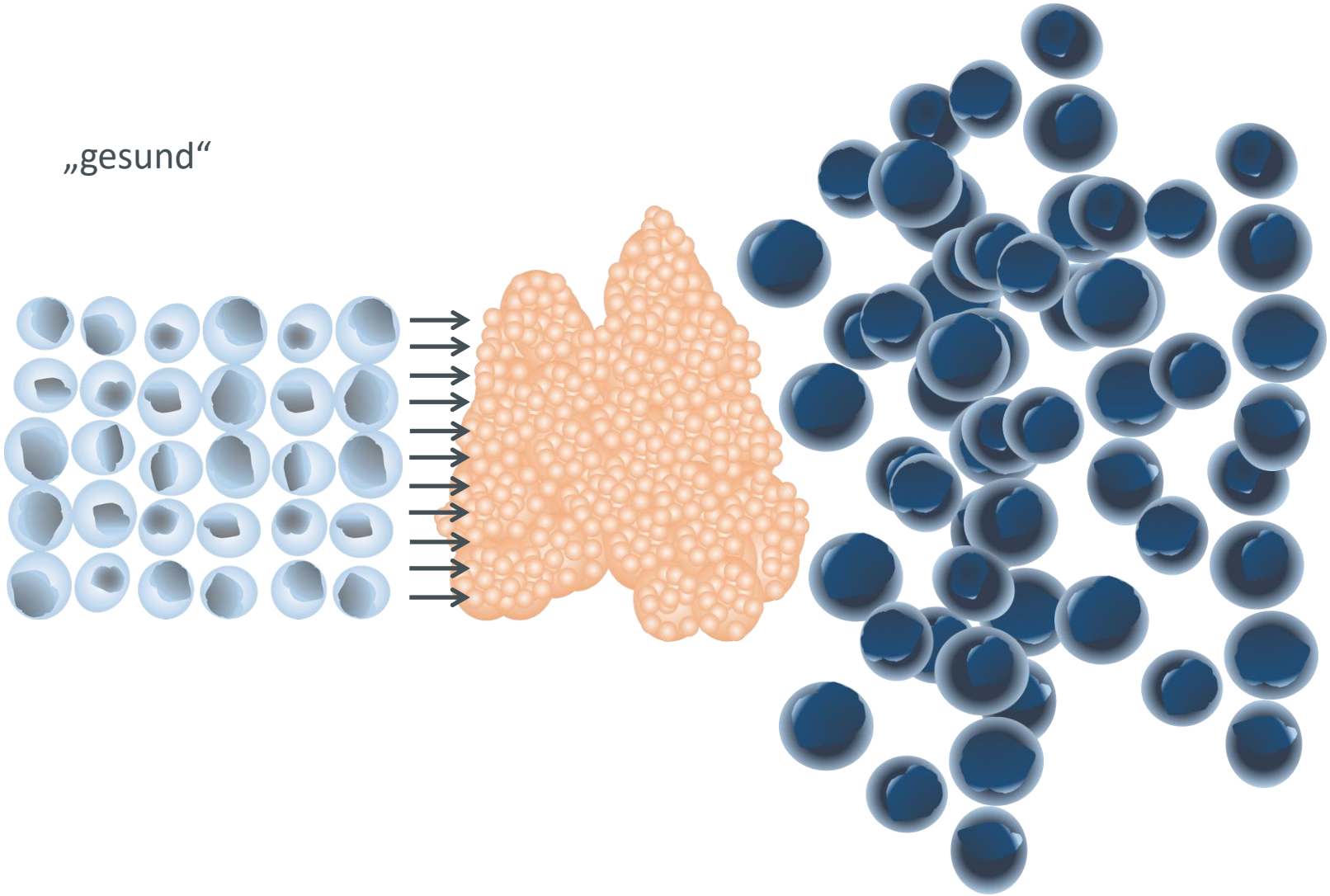
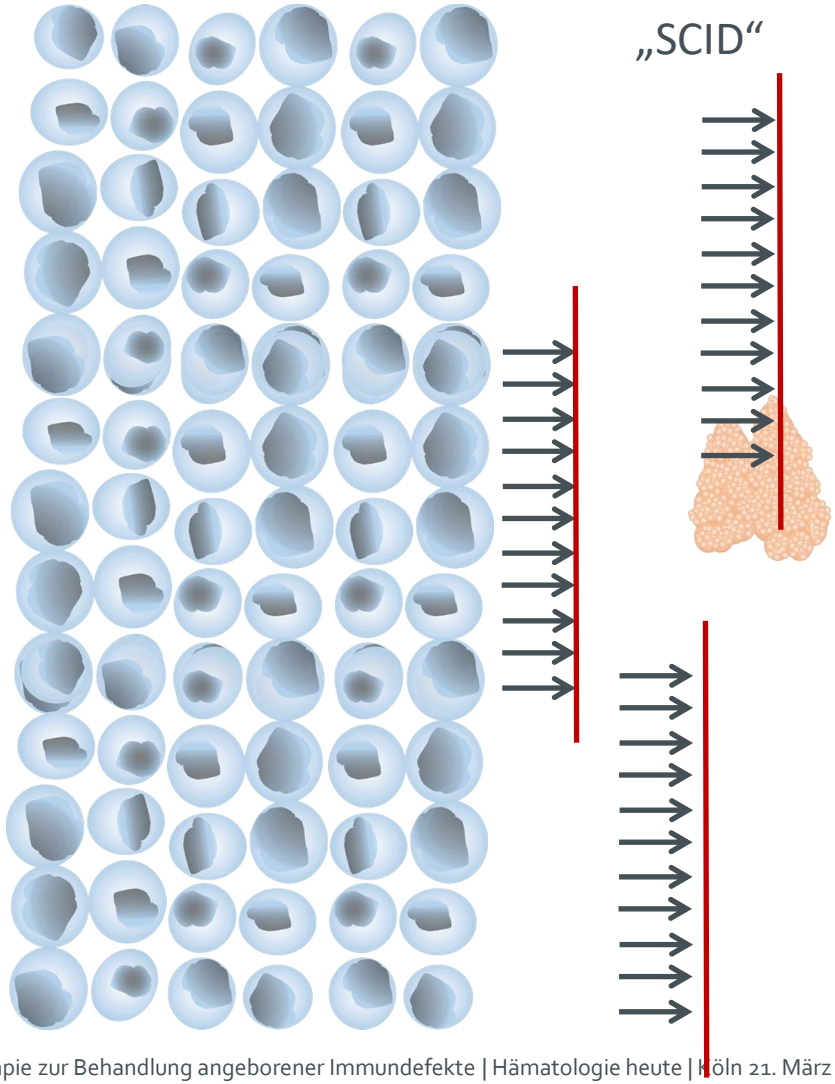
DNA-PKcs, XLF, LigaseIV
B-Zellen

Entwicklung T-Zellen



Entwicklungsarrest T-Zellen



Gentherapie

The New England Journal of Medicine
Volume 335 Number 21 November 21, 1996

Brief Report

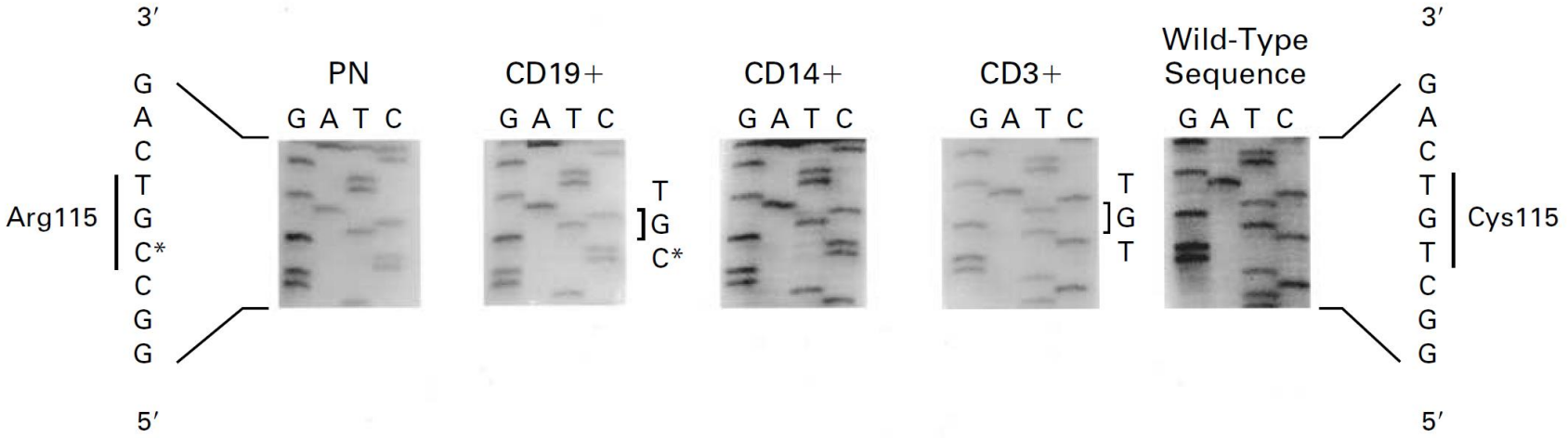
Atypical X-Linked Severe Combined Immunodeficiency Due to Possible Spontaneous Reversion of the Genetic Defect in T Cells

Volker Stephan, M.D., Volker Wahn, M.D., Françoise Le Deist, M.D., Uta Dirksen, M.D., Barbara Bröker, Ph.D., Ingrid Müller-Fleckenstein, Gerd Horneff, M.D., Horst Schroten, M.D., Alain Fischer, M.D., Ph.D., and Geneviève de Saint Basile, M.D., Ph.D.

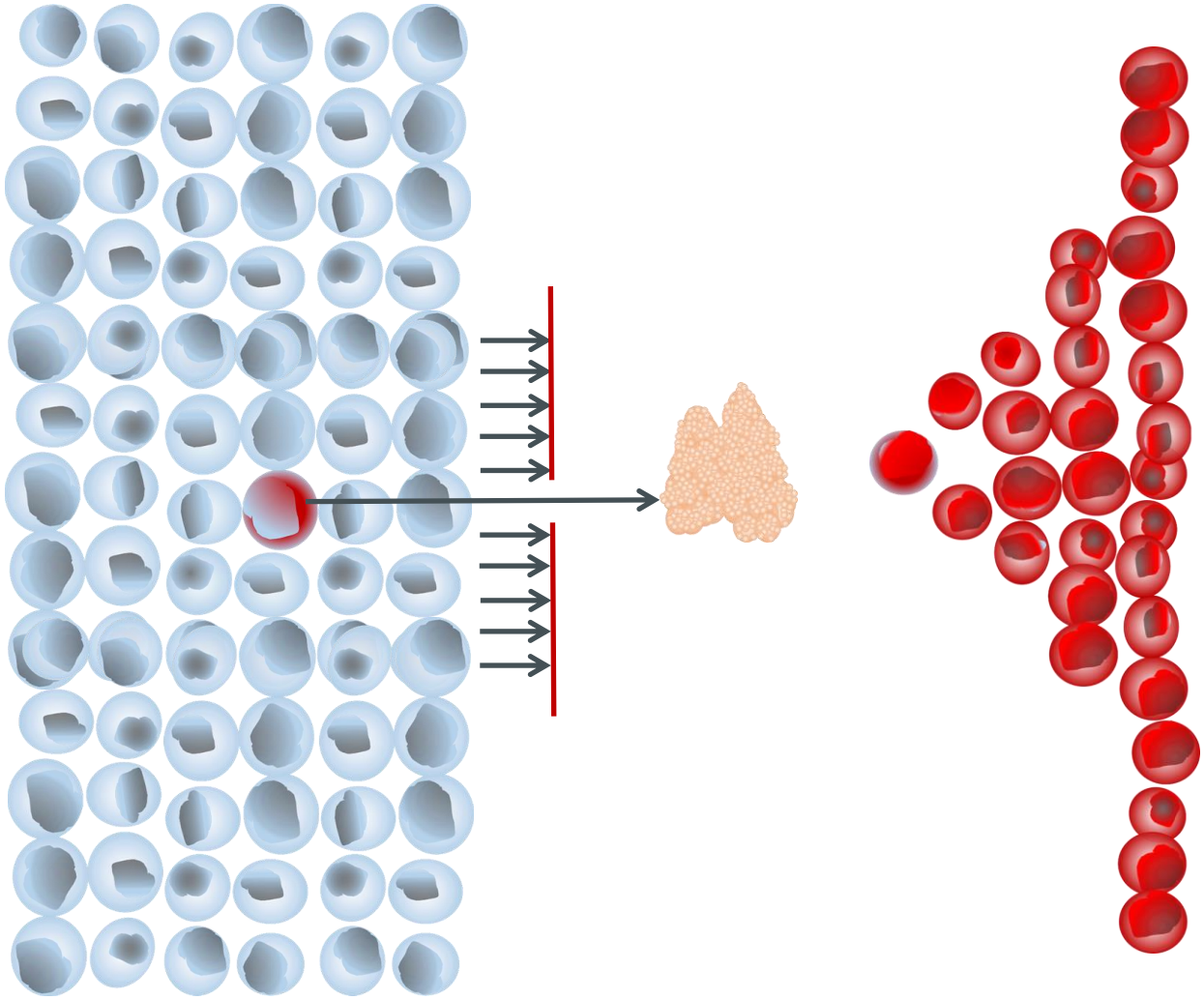
Type of Cell	Patient	Normal Range or Control Value
	cells/mm³	
T cells		
CD3+	815–2050	1200–2500
CD4+	250–828	720–2000
CD8+	360–1860	240–1000
B cells		
CD20+	930–2400	100–600
Natural killer cells		
CD56+	Undetectable	100–500
	T-cell proliferation (cpm ×10⁻³)	
Stimulus*		
None, day 4†	0.5±0.1	0.4±0.2
Phytohemagglutinin	3.3±1.3	34.0±15.0
OKT3	5.7±3.0	31.5±13.2
None, day 6‡	1.0±0.2	0.3±0.1
Tetanus toxoid	4.6±1.2	23.2±9.7
Purified protein derivative	7.1±2.3	24.0±8.1
Allogeneic cells	12.3±3.9	34.9±11.1

Reversion – Prinzip der Gentherapie

The New England Journal of Medicine
Volume 335 Number 21 November 21, 1996



„selective advantage“

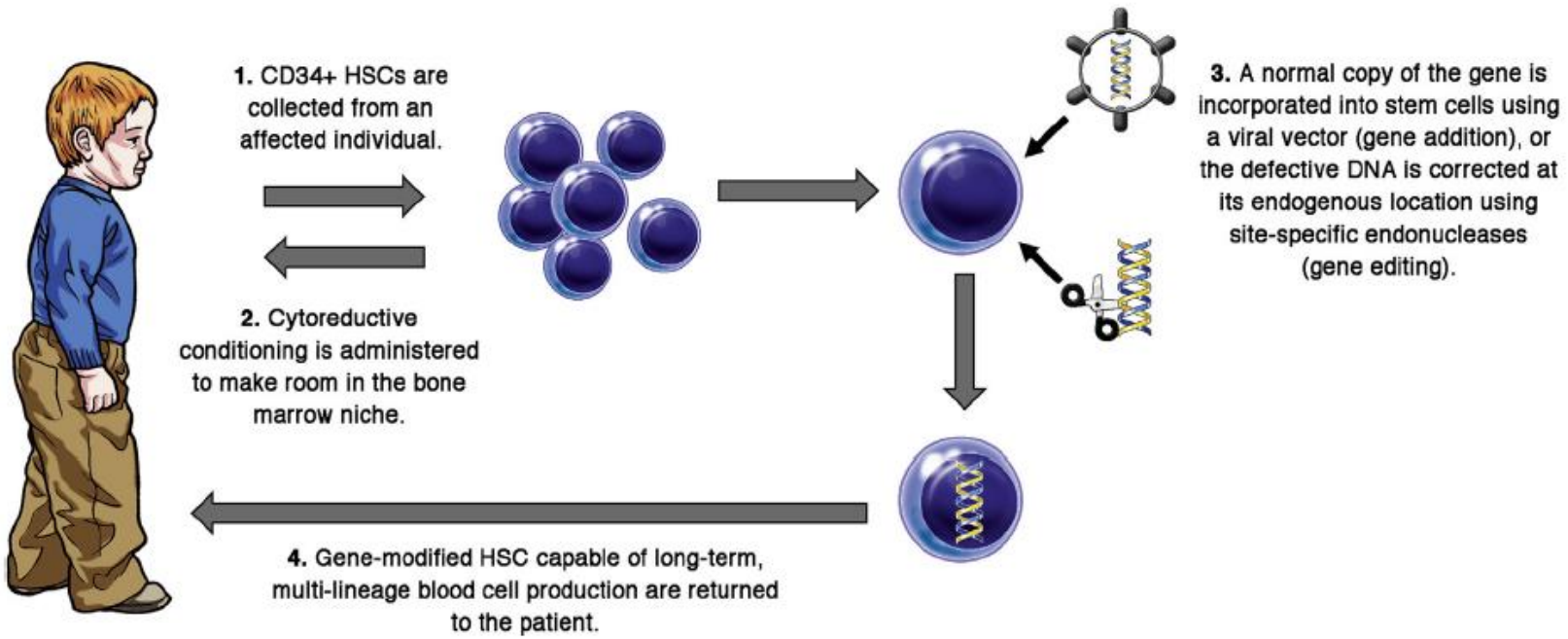


Gene therapy

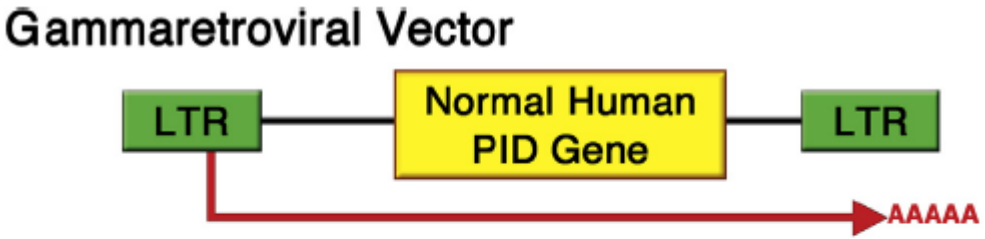
New frontiers in the therapy of primary immunodeficiency: From gene addition to gene editing

Donald B. Kohn, MD,[a,b,c,d] and Caroline Y. Kuo, MD[a] *Los Angeles, Calif*

J Allergy Clin Immunol 2017;139:726-32.



Gene therapy in PID

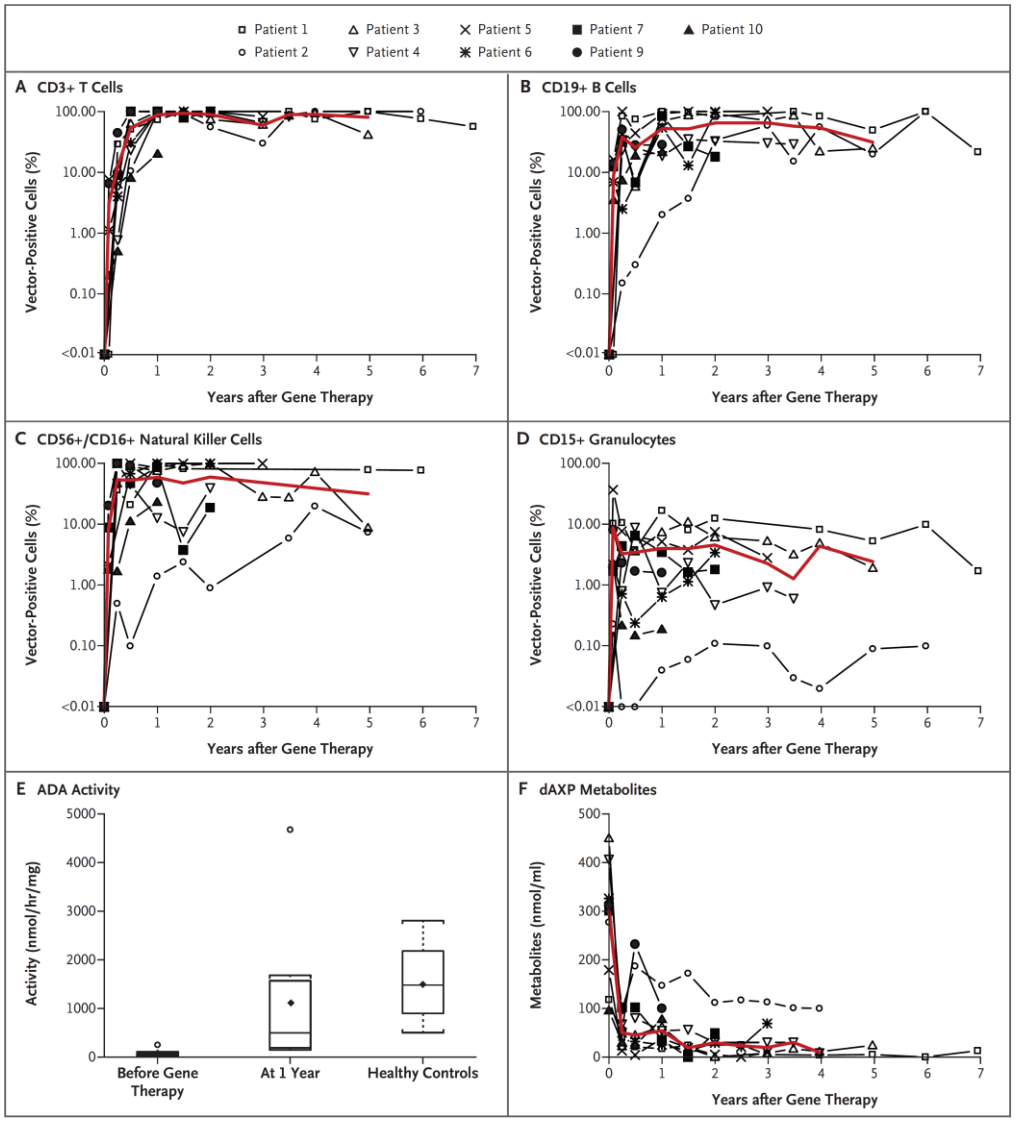


Kohn and Kuo, JACI, 2017

ADA	Milano/ London
X-SCID	Paris/ London
CGD	Frankfurt/ Zürich
WAS	Hannover/ Munich

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Aiuti et al., NEJM 2009



FDA halts gene therapy trials after leukaemia case in France

Charles Marwick *Washington, DC*

After the second occurrence of a leukaemia type illness in a patient in a gene therapy trial in France for X linked severe combined immune deficiency disorder (SCID), the US Food and Drug Administration has halted all trials that use retroviral vectors for inserting genes into bone marrow stem cells.

The move is described as a "precautionary measure" pending investigation. No evidence has been shown of leukaemia in any of the patients in the United States who have had this type of gene transfer, says the FDA.

Last September the French investigators, Dr Alain Fischer and Dr Marina Cavazzanna-Calvo at the Necker Hospital in Paris, reported that one of 11 patients with X linked SCID they were treating had developed T cell leukaemia about three years after receiving the gene transfer.

At that time the FDA suspended the three gene therapy trials of SCID patients in the United States that most closely resembled the French trial and stopped patient enrolment. The UK Department of Health's Gene Therapy Advisory Committee also recommended that additional measures be put in place to protect patients undergoing gene therapy trials (12 October, p 791).

The FDA's latest step puts on hold an additional estimated 27 trials that use retroviral vectors to insert the defective gene into haematopoietic stem cells.

The latest development is disappointing, as the early results of the French trial were so promising. Seven of the patients are in good health, with their immune system restored. One patient was regarded as too ill at the time of the procedure to benefit.

The development is regarded more as a temporary setback than the end of the road for gene therapy. Dr Philip Noguchi, acting director of the FDA office that regulates US gene therapy studies, says he regards gene therapy as a promising treatment for patients who have not benefited from current treatments, such as bone marrow transplantation.

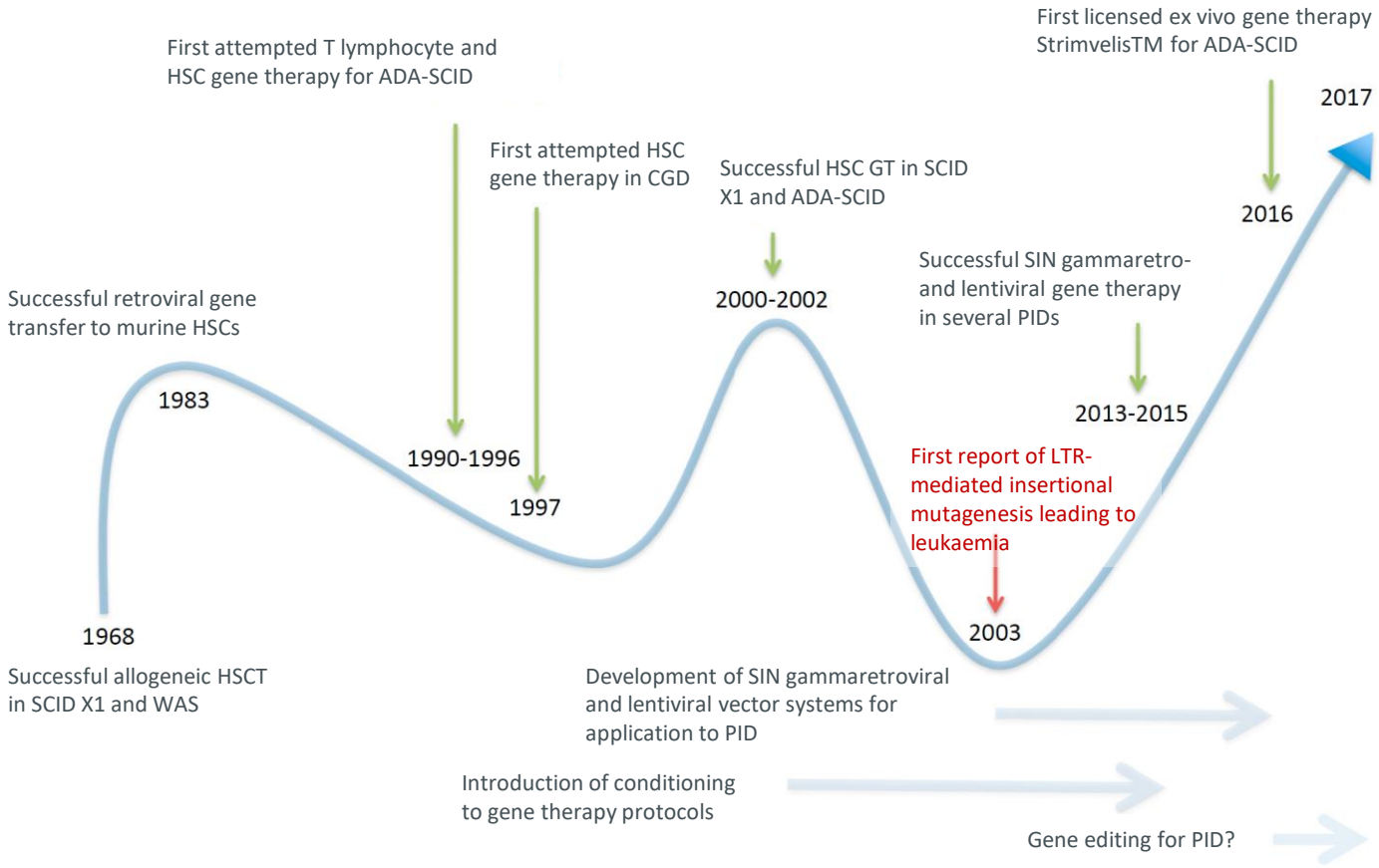
An FDA scientific advisory committee will hold a meeting late in February. Noguchi said the committee will be given the known data and asked to make recommendations.

Supporting the FDA's action, the American Society of Gene Therapy says that a key question is why this has occurred only in trials involving patients with SCID and not in the other clinical trials that use retroviral vectors targeted at haematopoietic stem cells.

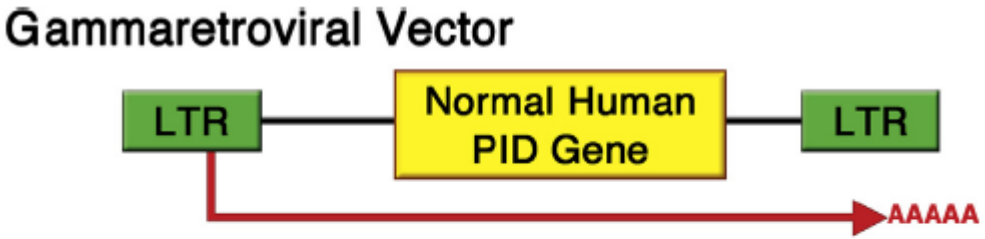
The society is conducting its own investigation and will report its findings at its annual meeting in June. It is possible, says the society, that the gene that encodes a T cell growth factor triggers the risk of leukaemia. Other possibilities are the fact that the patients are all infants, the nature of SCID itself, and the gene transfer technique. □

„ups and downs“

Thrasher et al., Molecular Therapy 2017



Gene therapy in PID



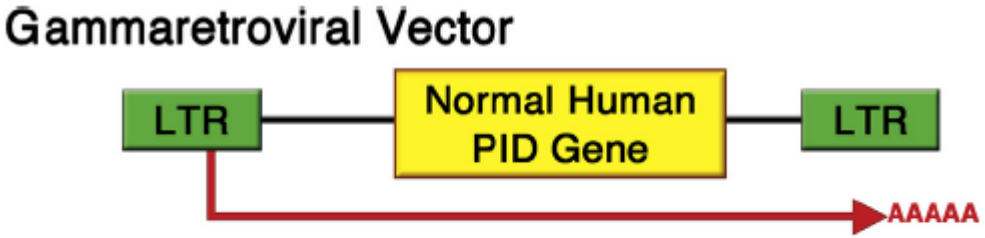
Kohn and Kuo, JACI, 2017

Starker viraler Promotor
-> beeinflusst die Transkription von Onkogenen
(LMO2, CCND2, MECOM- MDS/EVI1 complex locus, …)

Insertionale Mutagenese

X-SCID	Paris/ London	5/20	18/20 alive
CGD	Frankfurt/ Zürich	4/4	2/4 alive
WAS	Hannover/ Munich	7/10	8/10 alive

Gene therapy in PID



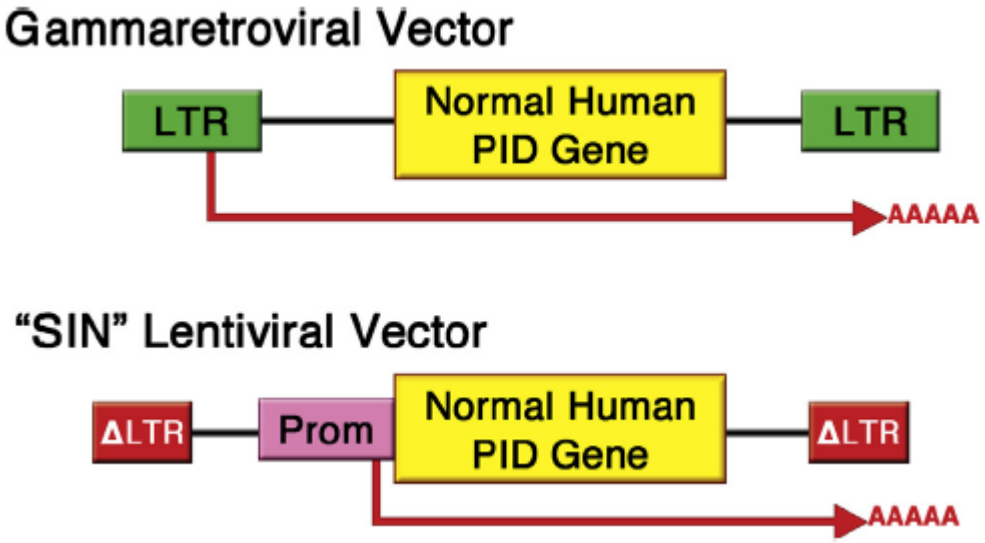
Kohn and Kuo, JACI, 2017

Starker viraler Promotor
-> beeinflusst die Transskription von Onkogenen
(LMO2, CCND2, MECOM- MDS/EVI1 complex locus)

Insertionale Mutagenese

ADA	**Milano/ London**	**0/>40**	100% alive, 75% DFS
X-SCID	Paris/ London	5/20	18/20 alive
CGD	Frankfurt/ Zürich	4/4	2/4 alive
WAS	Hannover/ Munich	7/10	8/10 alive

Gene therapy



Kohn and Kuo, JACI, 2017

- enthalten KEINE potentiell schädlichen viralen „long terminal repeat“ (LTR) Sequenzen, die Onkogene aktivieren könnten
- beinhalten endogene Promotoren, die die Expression des Transgens regulieren

Gentherapie vs. haplo IL2RG-Defekte

GENE THERAPY

BLOOD, 4 JUNE 2015 • VOLUME 125, NUMBER 23

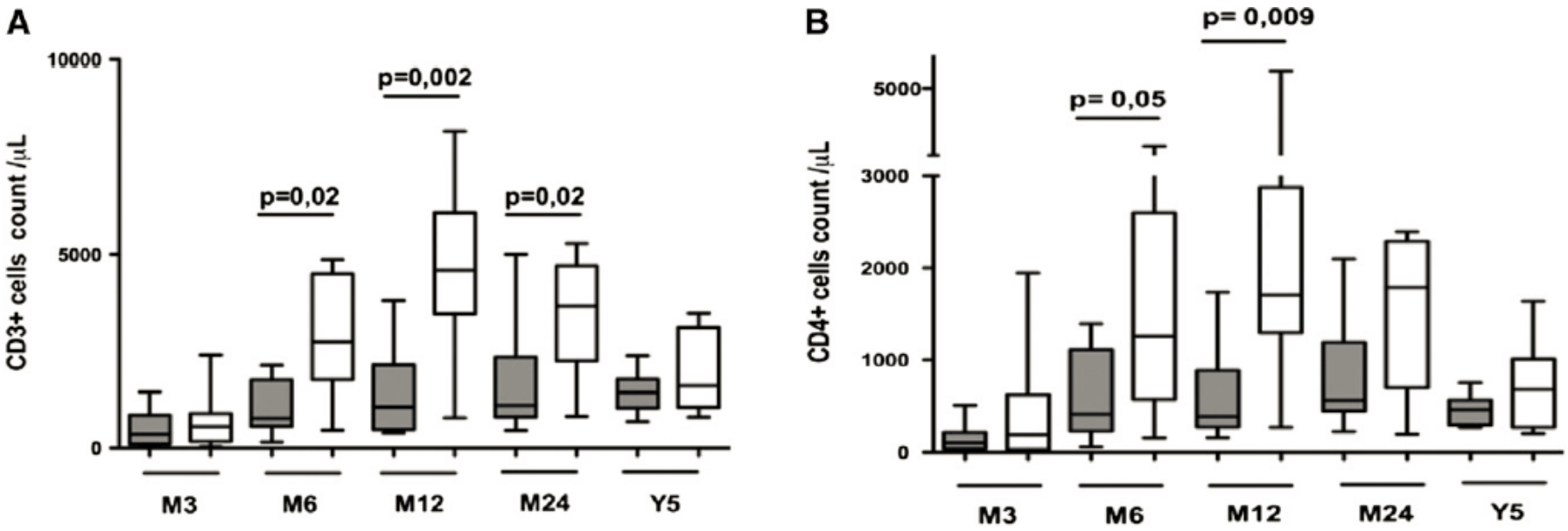
Faster T-cell development following gene therapy compared with haploidentical HSCT in the treatment of SCID-X1

Fabien Touzot,[1,2,3] Despina Moshous,[2,3,4] Rita Creidy,[5] Bénédicte Neven,[2,3,4] Pierre Frange,[6,7] Guilhem Cros,[2,3] Laure Caccavelli,[1] Johanna Blondeau,[1] Alessandra Magnani,[1,2,3] Jean-Marc Luby,[1] Brigitte Ternaux,[1] Capucine Picard,[2,8,9] Stéphane Blanche,[2,4] Alain Fischer,[2,3,4,10] Salima Hacein-Bey-Abina,[1,9,11] and Marina Cavazzana[1,2,3]

[1]Département de Biothérapie, Centre d'Investigation Clinique intégré en Biothérapies, Hôpital Necker–Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France; [2]Université Paris Descartes–Sorbonne Paris Cité, Institut Imagine, Paris, France; [3]INSERM UMR1163, Paris, France; [4]Unité d'Immunologie-Hématologie et Rhumatologie Pédiatrique, Hôpital Necker–Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France; [5]Laboratoire de Biochimie, Centre Hospitalier Intercommunal de Creteil, Paris, France; [6]Laboratoire de Microbiologie, Hôpital Necker–Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France; [7]EA 3620, Université Paris Descartes–Sorbonne Paris Cité, Paris, France; [8]Centre d'étude des Déficits Immunitaires, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France; [9]Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Paris, France; [10]College de France, Paris, France; and [11]Laboratoire d'Immunologie, Groupe Hospitalier–Universitaire Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin-Bicêtre, France

Gentherapie vs. haplo IL2RG-Defekte

Touzot et al., Blood 2015



Explanation: „allogeneic reaction against the thymic tissue“ in haplo pts

Gentherapie vs. haplo IL2RG-Defekte

	haplo Tx	gene therapy
inclusion criteria	X-linked SCID (*IL2RG*)	
	single centre 2000-2013	
	lack of HLA-identical donor	
patients	13	14
cell product	CD34 positive selection	ex-vivo transduced CD34+ 9 pts y-retrovirus 5 pts SIN-y-retrovirus
conditioning	rabbit-ATG (Thymoglobuline) 2.5mg/kg on days -3 and -2	none *
graft failure	3	1
GvHD	4 (°2)	n.a.
autoimmunity	3	0
deaths	2 (viral infection, autoimmune enteropathy)	2 (ADV, T-ALL)

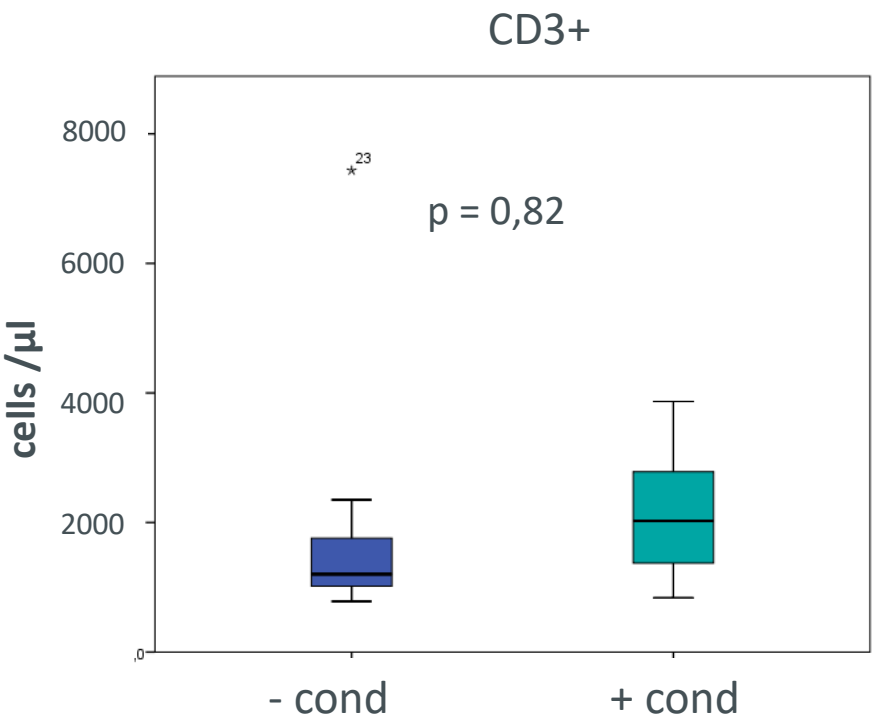
*single pt Fludarabine because of MFT

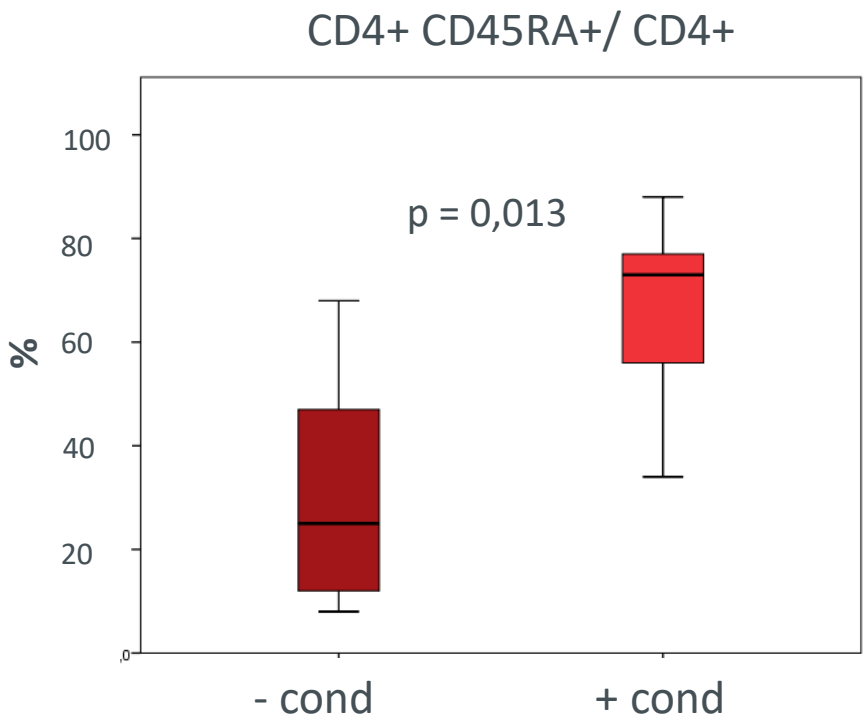
Touzot et al., Blood 2015

Gentherapie vs. haplo IL2RG-Defekte

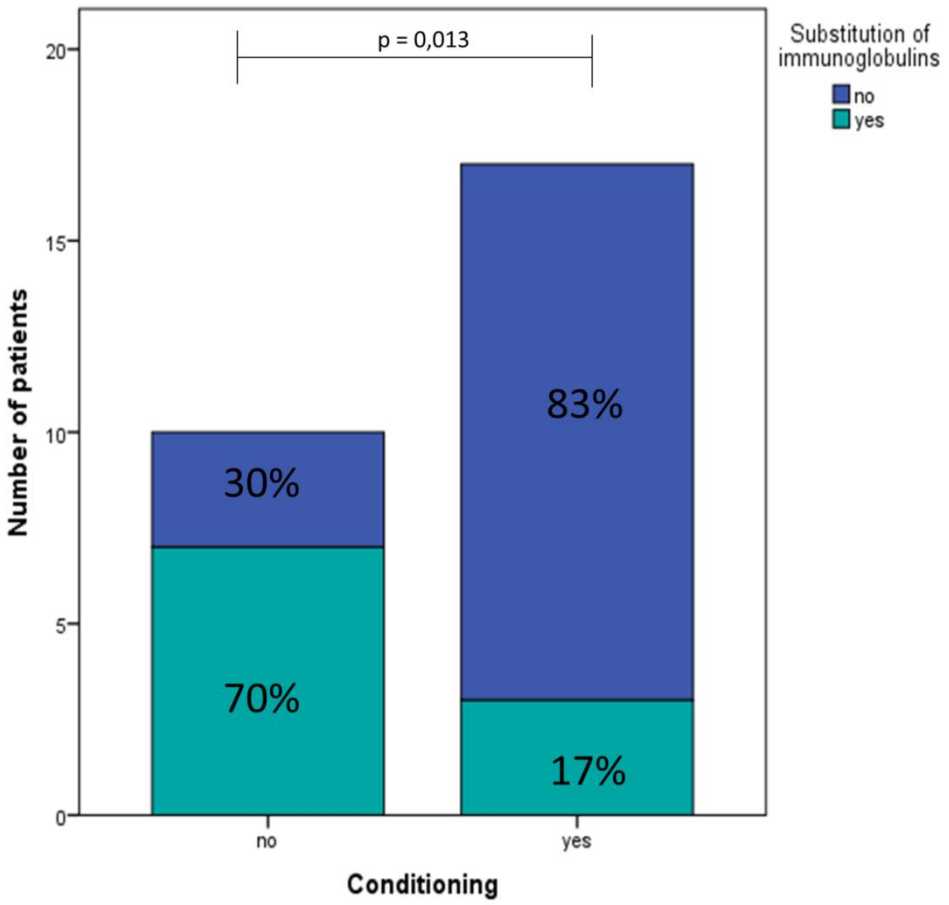
33 X-SCID patients after haplo Tx, 1982-2005

most recent follow up [10,3 years (1.4-24.6)]





Gentherapie vs. haplo IL2RG-Defekte



Touzot et al., Blood 2015

B-cell function: „…B-cell function remained absent in all patients.“

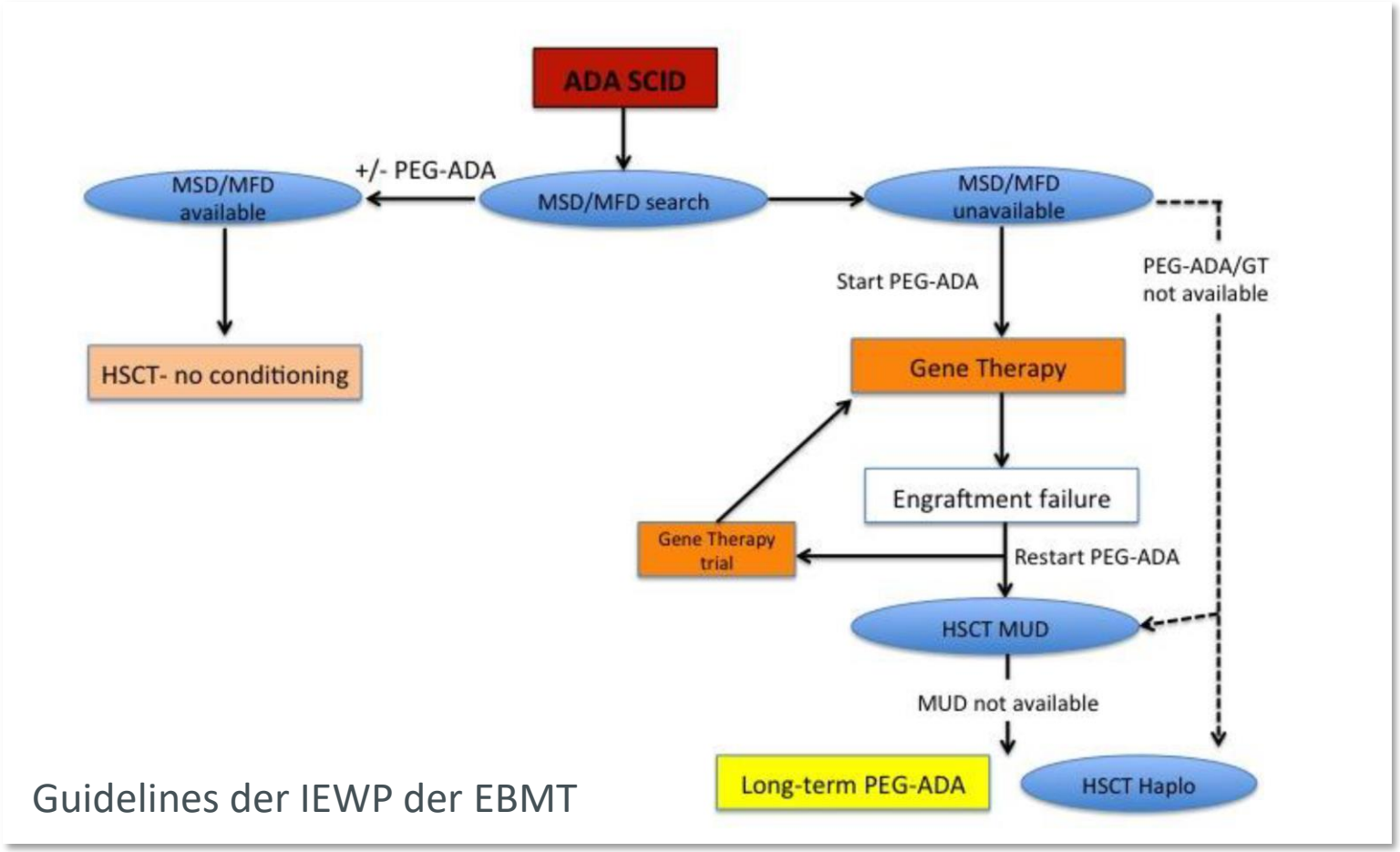
possible solution: conditioning to open niches for gene modified stem cells.

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

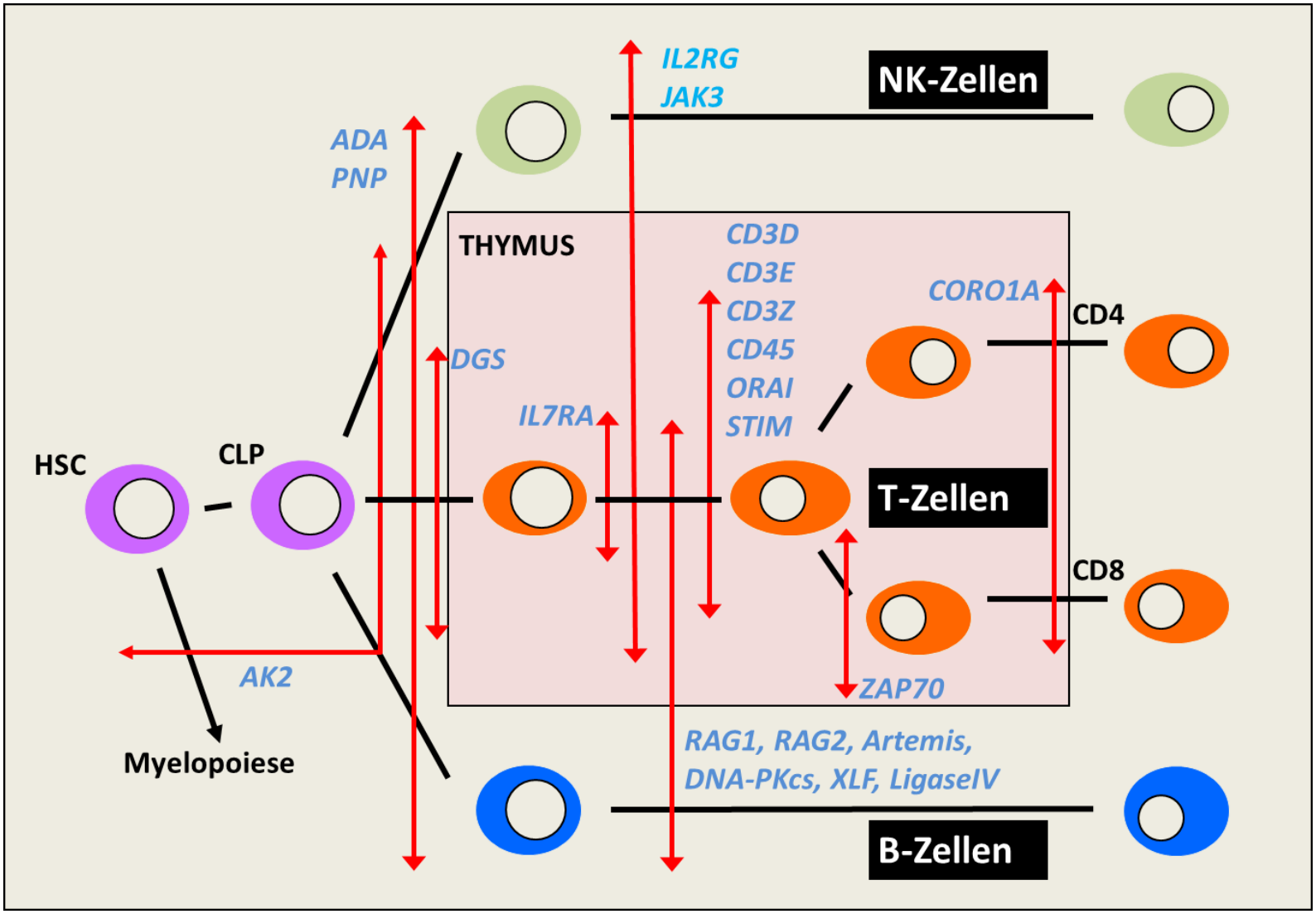
Booth et al., Trends in Molecular Medicine 2016

Therapieoptionen ADA-Defizienz



Guidelines der IEWP der EBMT

SCID-Entitäten

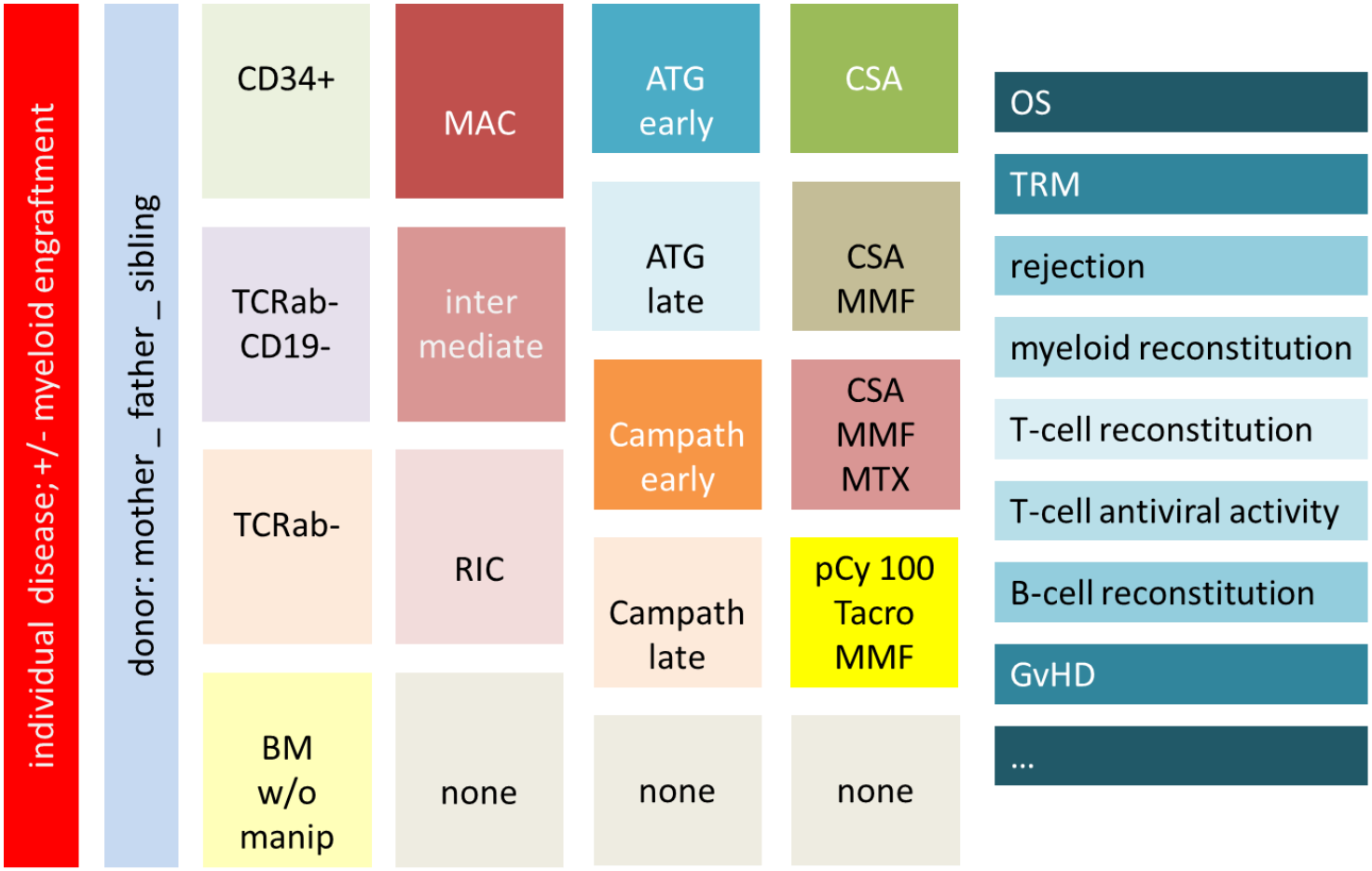


International Union of Immunological Societies, IUIS

WHO Klassifikation

1. kombinierte T- und B- Zell Defekte
2. Antikörpermangelsyndrome
3. andere definierte Immundefizienzsyndrome
4. Erkrankungen mit Immundysregulation
5. Angeborene Defekte der Phagozytenzahl oder –funktion oder Kombination
6. Defekte der angeborenen („innate“) Immunität
7. Autoinflammationssyndrome
8. Complementdefekte
9. „Phenocopies“ of PID

Zusammenfassung



Zusammenfassung

++ Gentherapie pro ++

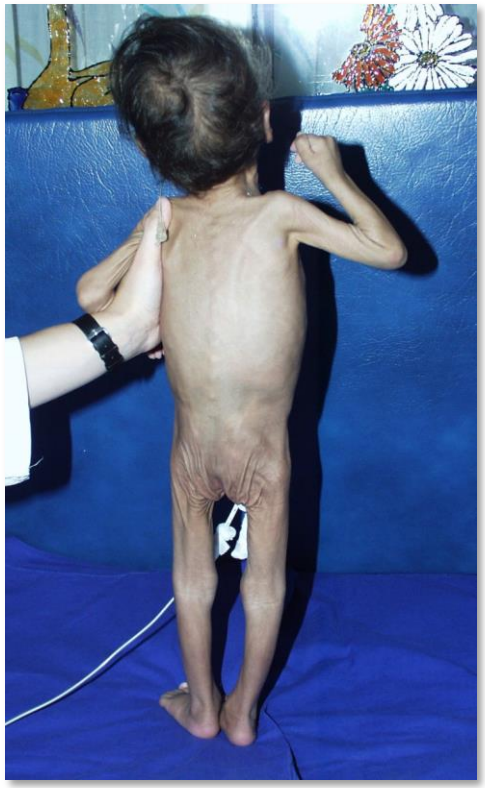
- **keine GvHD**
- Spender immer verfügbar
- Zentralisierte Zellmanipulation

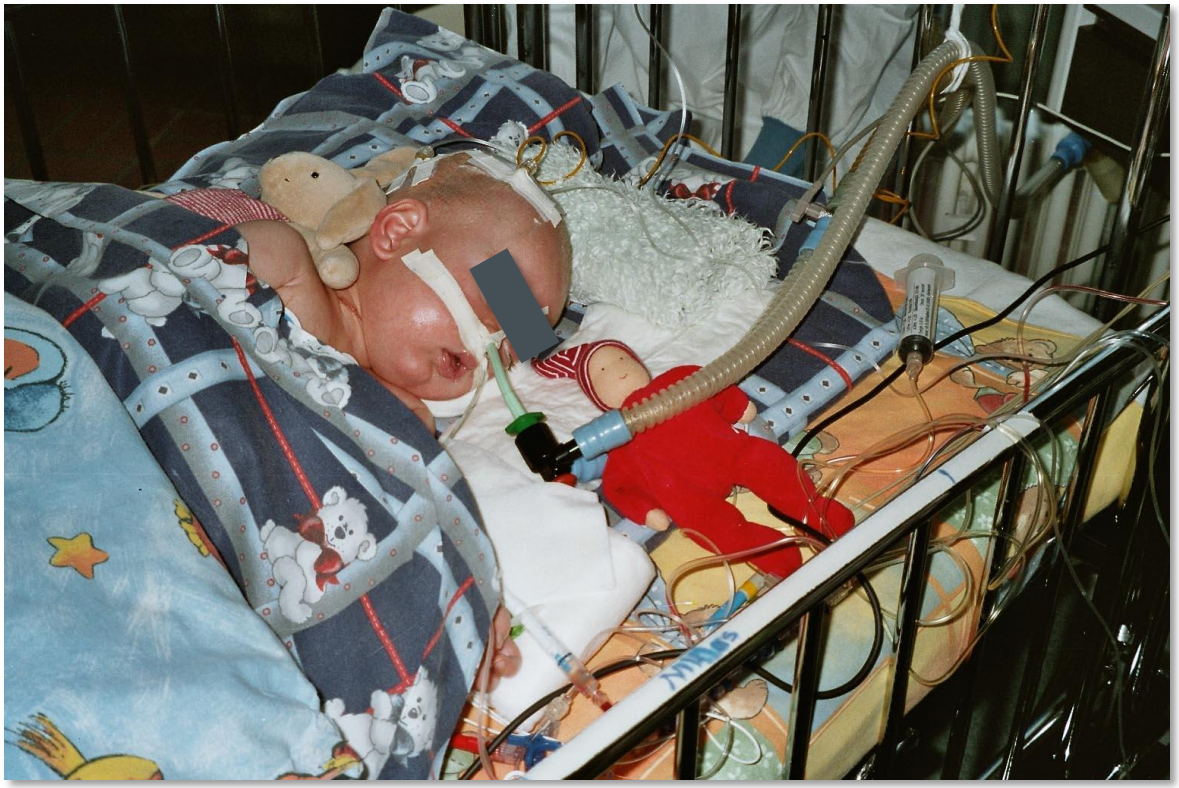
- Chemotherapie

-- Gentherapie contra –

- „Harvest“ Stammzellen
- Zentralisierte Zellmanipulation
- **„gemischter“ Chimärismus**
- „Survival“ ohne selective advantage?
- Chemotherapie (geringere Dosierung)
- **vergleichsweise „junge“ Therapie** **(Vergleich mit historischen SCT-Ergebnissen)**

Zusammenfassung





Vielen DANK!

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