Gregor Johann Mendel

Hugo de Vries

1865: Vater der Genetik

1901: Gene mutations are drivers of evolution

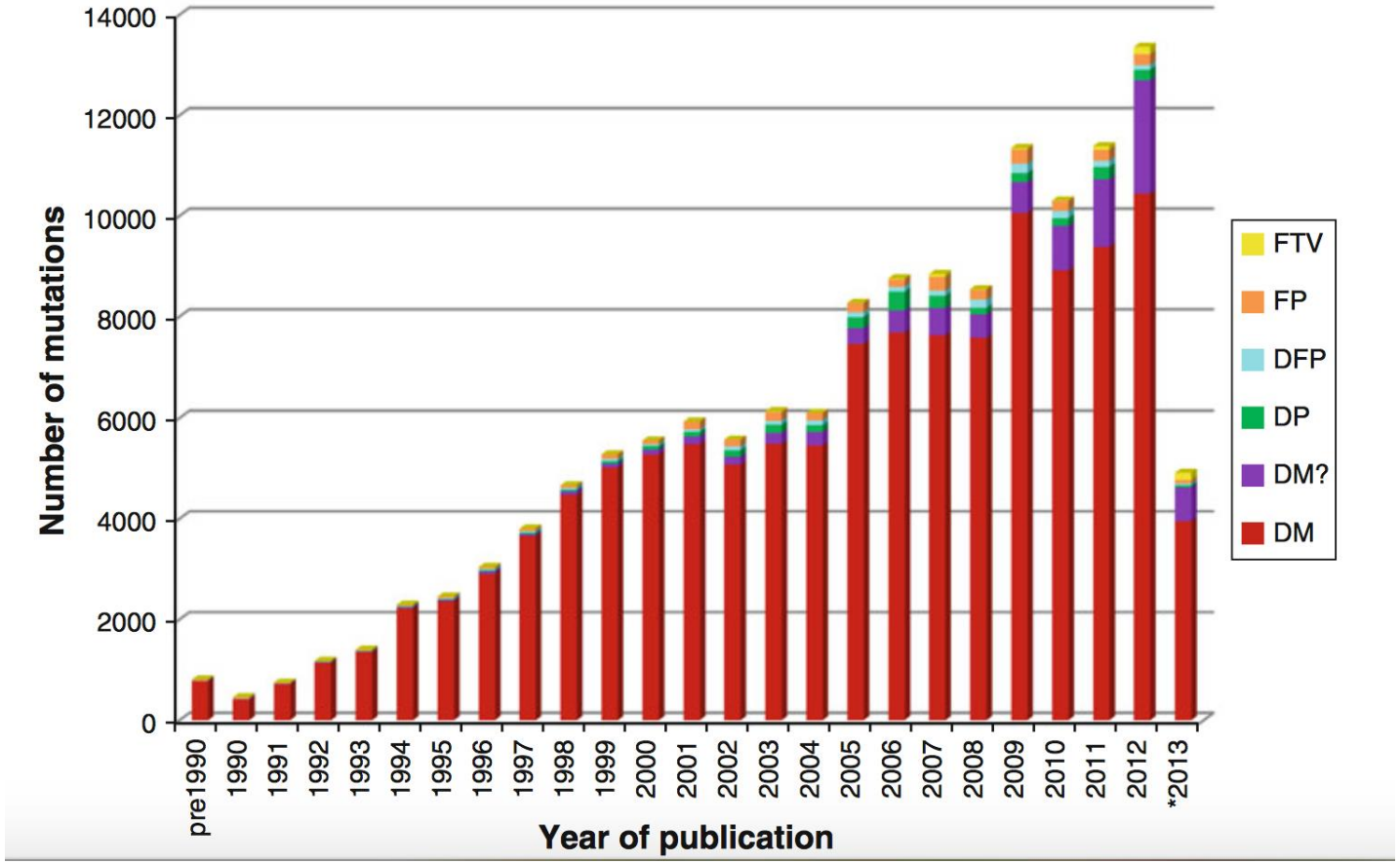
Masatoshi Nei expands on his evolution-busting theory in his 2013 book *Mutation-Driven Evolution*.

Humanes Genom: 25.000-30.000 Gene

Human Gene Mutation Database:

Es sind heute über 140.000 Genmutationen

in über 5.000 Krankheits-assoziierten Genen bekannt



Number of different disease associated mutations (DM) detected pro year

Cooper DN et al. Hum Genet. 2014; 133:1-9.

A Census of Human Cancer Genes

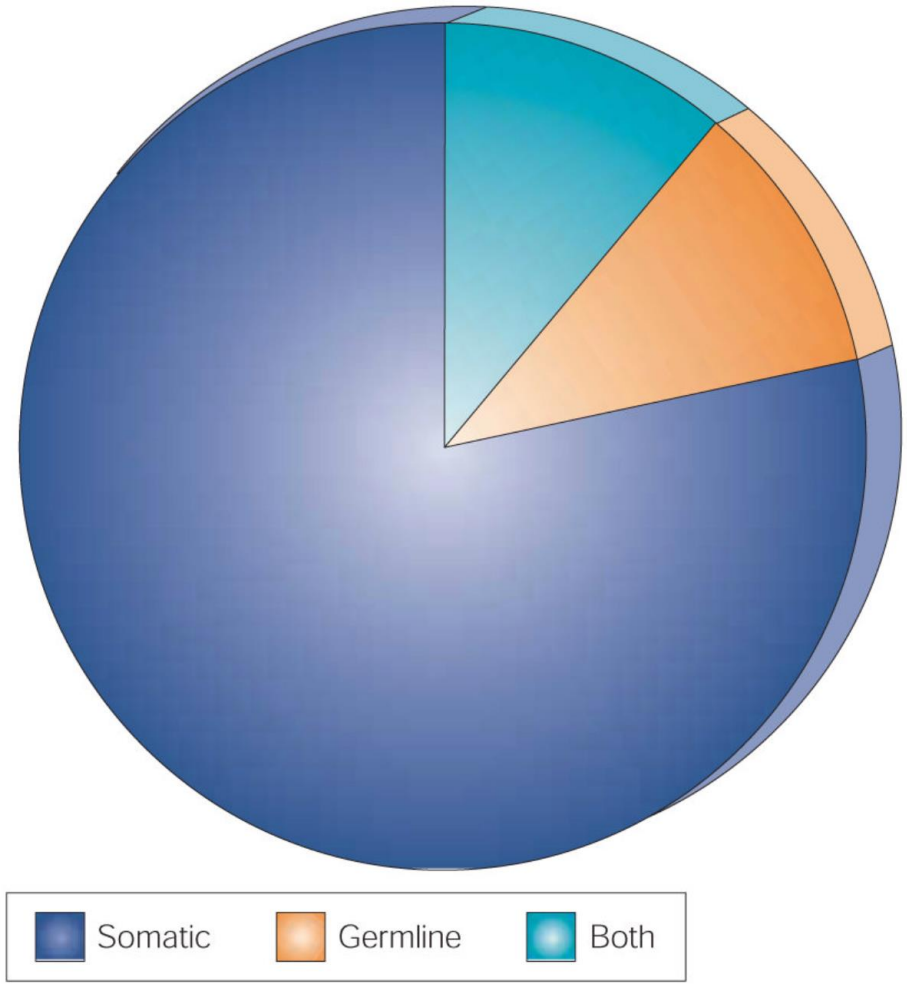
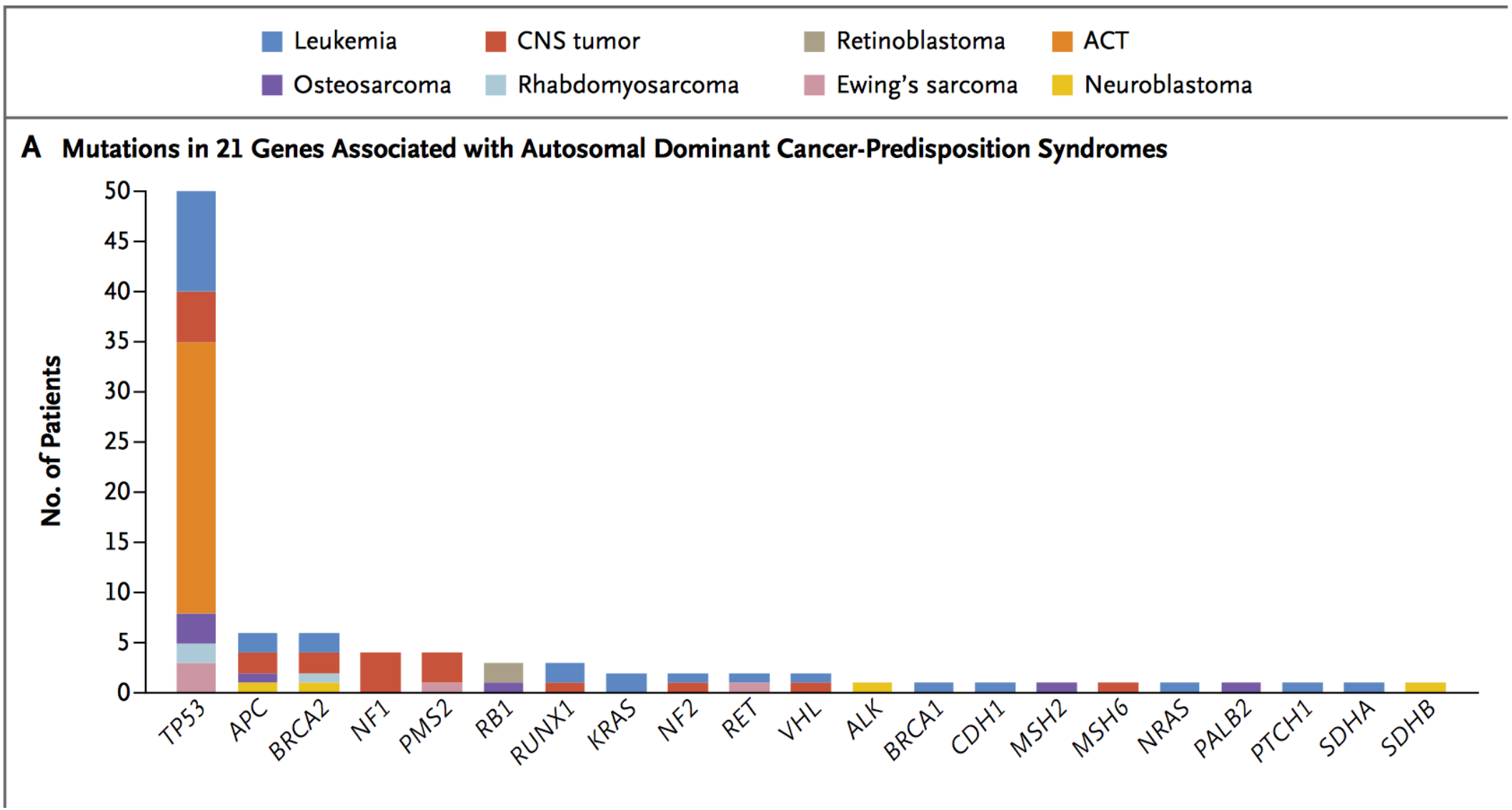


Figure 1. Mutation types in human cancer

Nat Rev Cancer. 2004; 4: 177–183

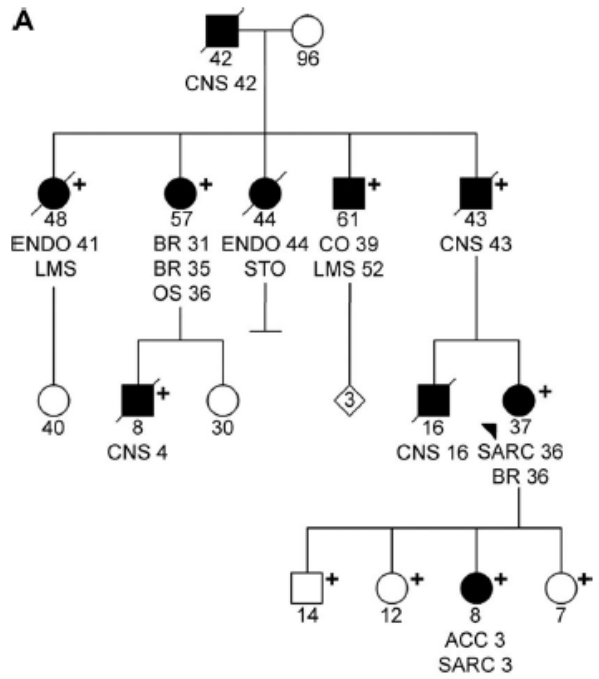
Germline Mutations in Predisposition Genes in Pediatric Cancer



Zhang, J., et al., N Engl J Med 2015; 373:2336-46

Germline TP53 Mutations and the Changing Landscape of Li–Fraumeni Syndrome

Kamihara J, et al., Human Mutation 35:654–662, 2014



OS=Osteosarcoma, ENDO=Endometrial Ca, LMS=Leiomyosarcoma, BR=Breast Ca, STO=Stomach Ca, CO=Colon Ca, CNS=Brain Tu, ACC=Adrenal Cortical Ca

Examples of Germline mutations in which CRISPR/Cas9 genome editing is evaluated

Disease	Gene mutation	Treatment
Cystic fibrosis	CFTR	No specific, special care
Tyrosinemia	FAH	Nitisinone (NTBC), Liver transplant.
Duchenne muscular dystrophy	Dystrophin	Symptomatic, Gene therapy
Hemophilia B	Factor IX	Factor IX substitution
Sickle cell disease	HbS	Transfusions, SZT
Huntington disease	HTT	Symptomatic
Achondroplasia	FGFR3	C-type natriuretic peptide

Korrektur der Keimbahnmutationen durch z.B. CRISPR/Cas9

- Probleme -

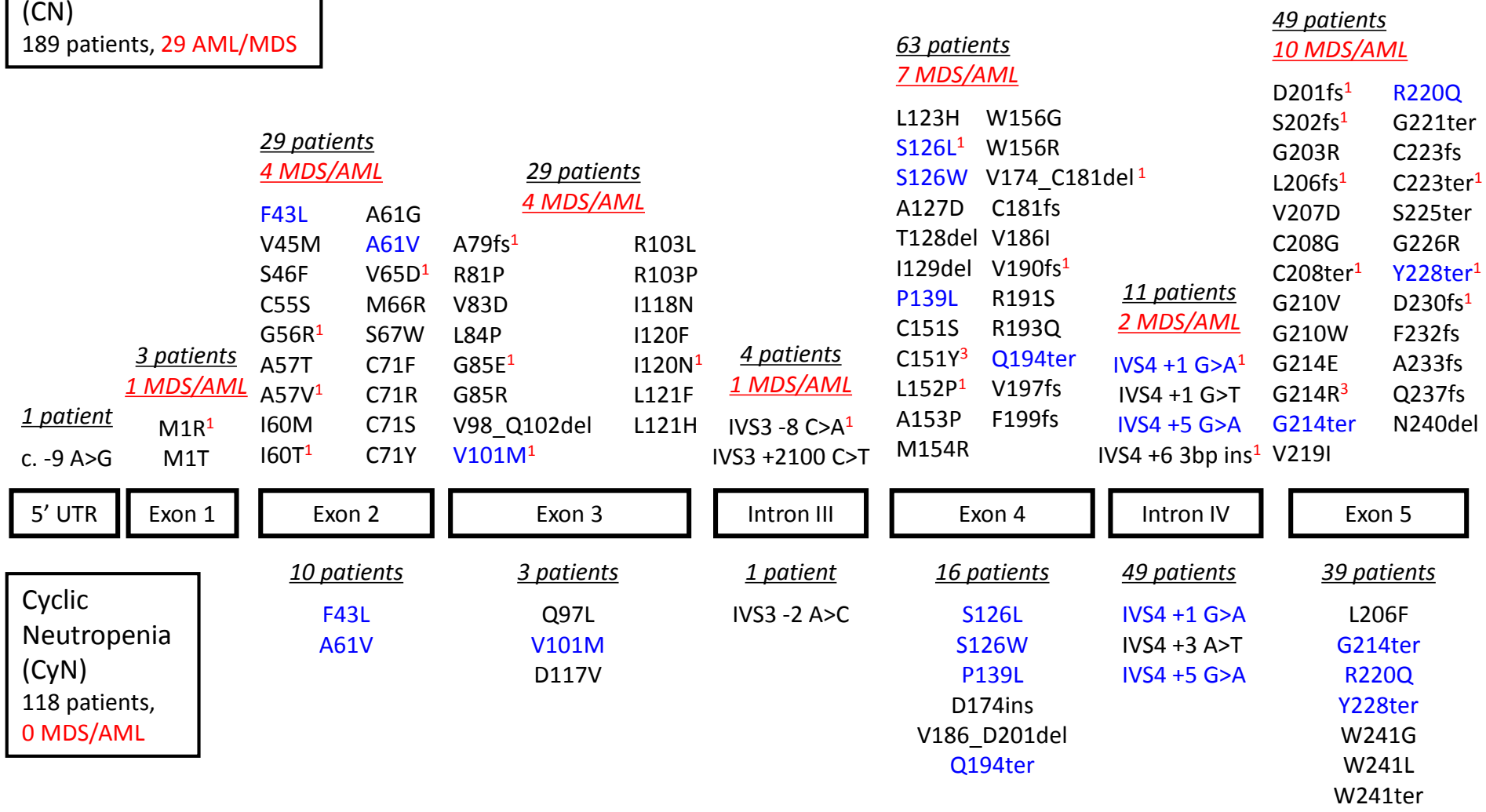
- „On-Target“- Effekte, z.B. bei Sichelzellerkrankung
- „Off-Target“-Effekte: unerwünschte, zusätzliche Schnitte im Genom
- Genetischer Chimerismus
- Epigenetische Veränderungen
- Fundamentale Konsequenzen für die Nachkommen
- Der Effekt der genetischen Modifikation eines Embryos wird erst nach der Geburt bekannt
- Polygene Erkrankungen (z.B. Alzheimer, Schizophrenie); Heterozygosität (50 % der Embryos sind gesund); recessive Erkrankungen (Krankheitsträger sind nicht „krank“)

Severe Chronic Neutropenia International Registry (SCNIR)

ELANE Mutations in Cyclic and Congenital Neutropenia

Linear Localization

Congenital Neutropenia (CN)
189 patients, 29 AML/MDS

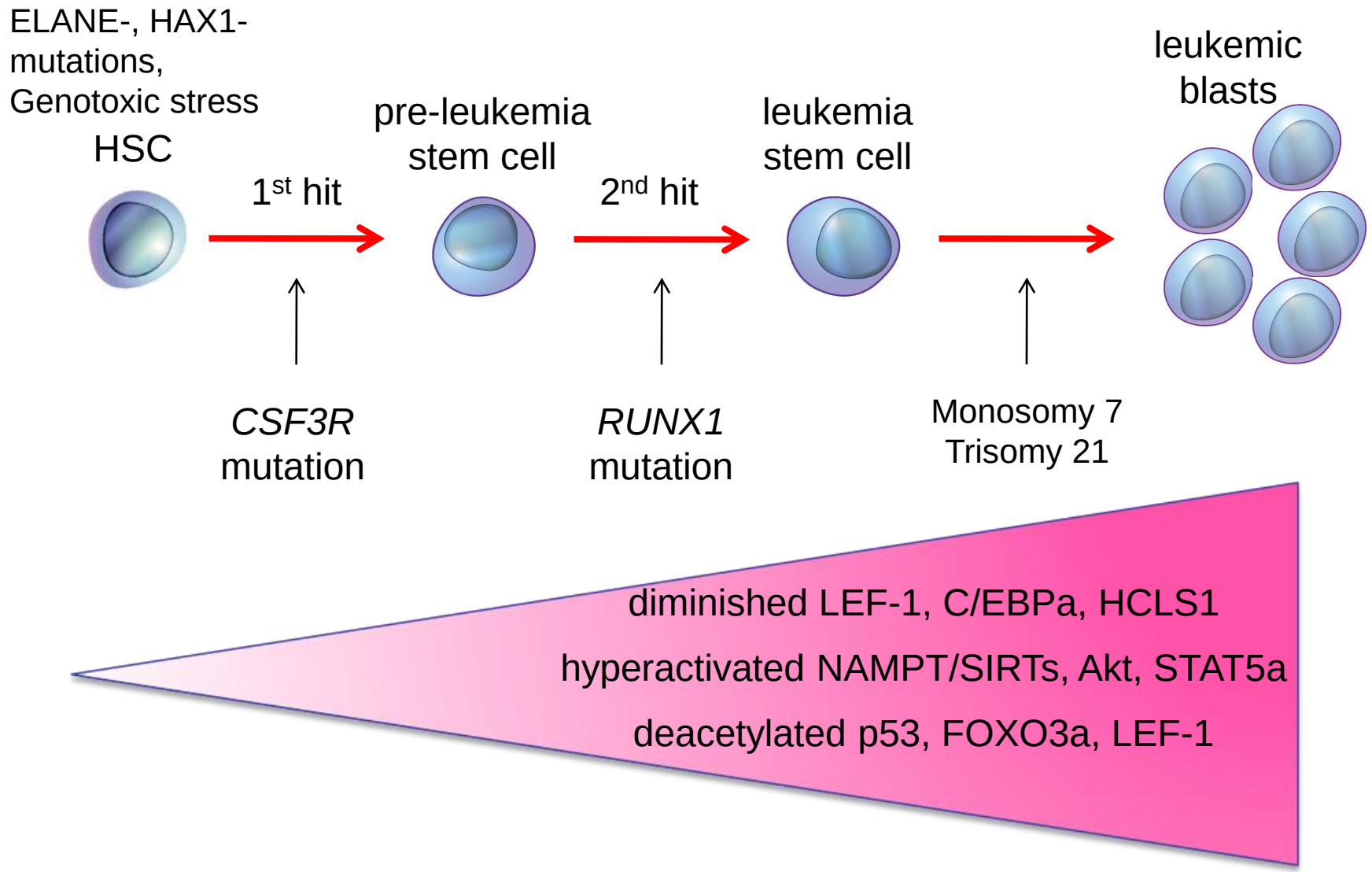


Cyclic Neutropenia (CyN)
118 patients,
0 MDS/AML

10 patients	3 patients	1 patient	16 patients	49 patients	39 patients
F43L	Q97L	IVS3 -2 A>C	S126L	IVS4 +1 G>A	L206F
A61V	V101M		S126W	IVS4 +3 A>T	G214ter
	D117V		P139L	IVS4 +5 G>A	R220Q
			D174ins		Y228ter
			V186_D201del		W241G
			Q194ter		W241L
					W241ter

Dale D, Welte K, et al.,Curr Opin Hematol. 2015;22:3-11

The two-hit hypothesis of leukemogenesis in CN



Genome editing divides scientists

Craig Mello, Univ. of Massachusetts :

In the distant future, I could imagine that altered germ lines
would protect humans against cancer, diabetes and other age-related problems

Craig Venter, La Jolla

Human germline engineering is inevitable, the question is when, not if

Eric S. Lander, Harvard and MIT, Boston:

If we want to eliminate all genetic diseases, we would have to do more than simply eliminate
the production of homozygous embryos. What about heterozygous state disease genes, recessive disease genes ?
If we really want to help parents avoid cases of genetic disease,
germline editing is not the first, second, third, or even fourth thing that we should thinking about.

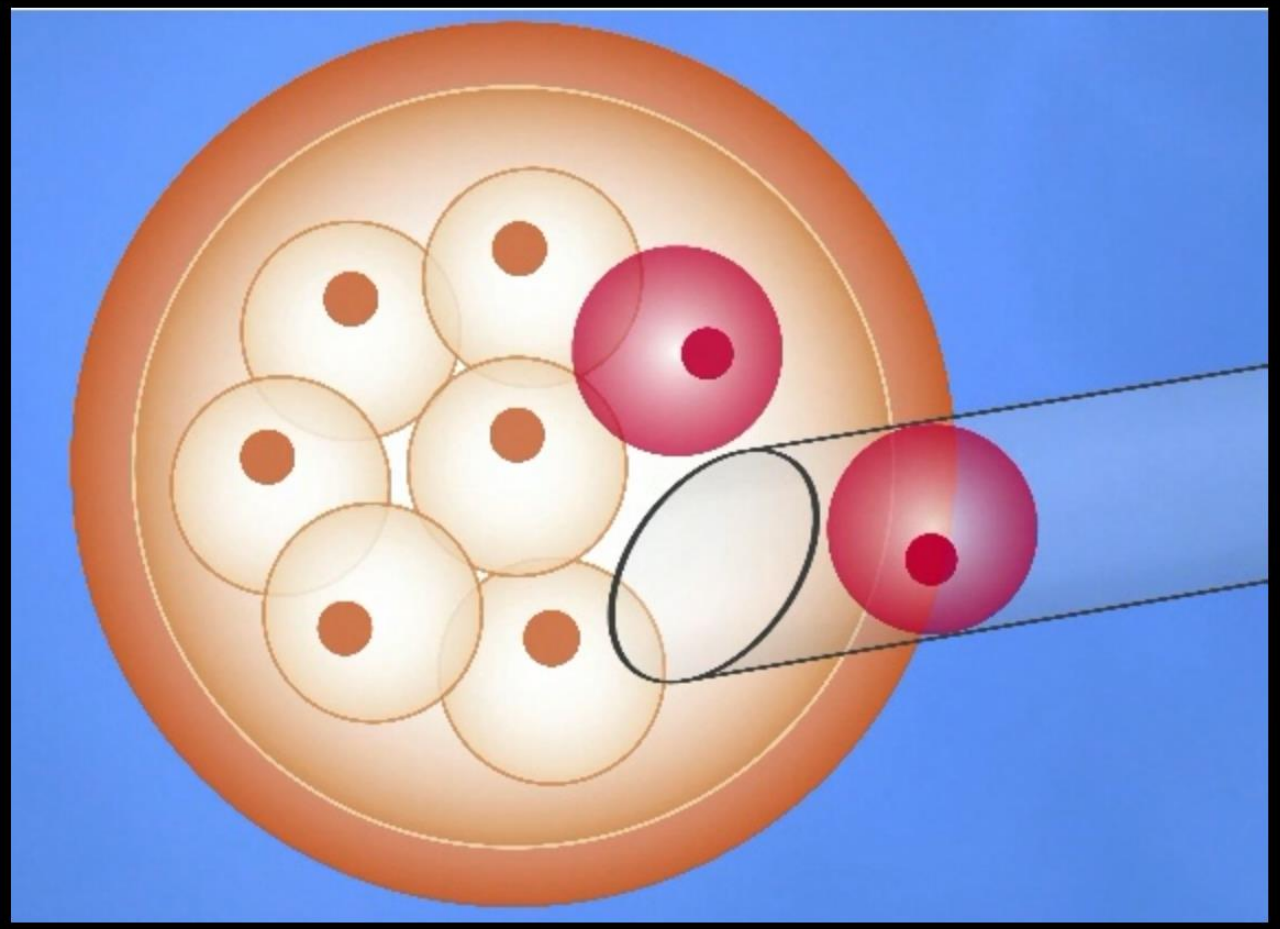
Edward Lanphier, Richmond

We believe there is a fundamental ethical issue in crossing the boundary to
modifying the human germ line.
Don‘t edit the human germline (Nature 2015)

Alternativen zu Eingriffen in die Keimbahn

- Präimplantationsdiagnostik
- Stammzelltransplantation
- Ex vivo somatische Gentherapie
- Ex vivo CRISPR/Cas9 Korrektur
- In vivo CRISPR/Cas9 Korrektur

Präimplantationsdiagnostik



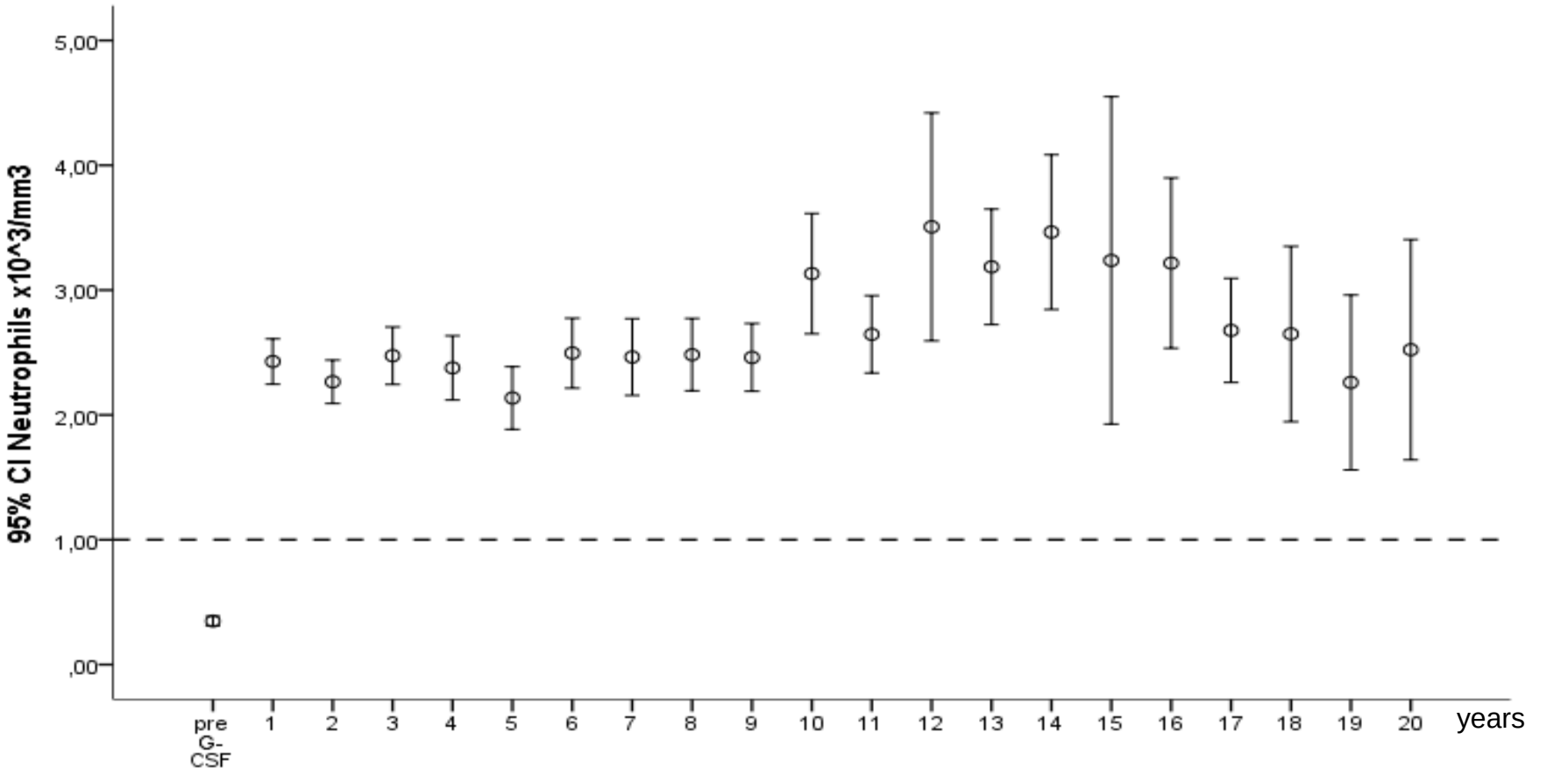
Etwa vier Tage nach der künstlichen Befruchtung besteht der Embryo aus acht Zellen. Ab diesem Zeitpunkt ist eine Erbgut-Analyse möglich - hierfür werden zwei Zellen entnommen.
Quelle: biotechnologie.tv

Bone marrow failure syndromes and Immunodeficiencies

Syndrome	Germline Mutations	Treatment
Fanconi Anemia	FANC-A, -B, -C, -D, BRCA1, etc.	SZT
Amegakaryocytic Thrombocytopenia	c-Mpl	SZT
Congenital Neutropenias	ELANE, HAX1, G6PC3, SDBS, etc.	G-CSF, SZT
SCID	IL2RG, ADA, etc.	SZT, Gene therapy
Wiskott-Aldrich-Syndrome	WASP	SZT, Gene therapy

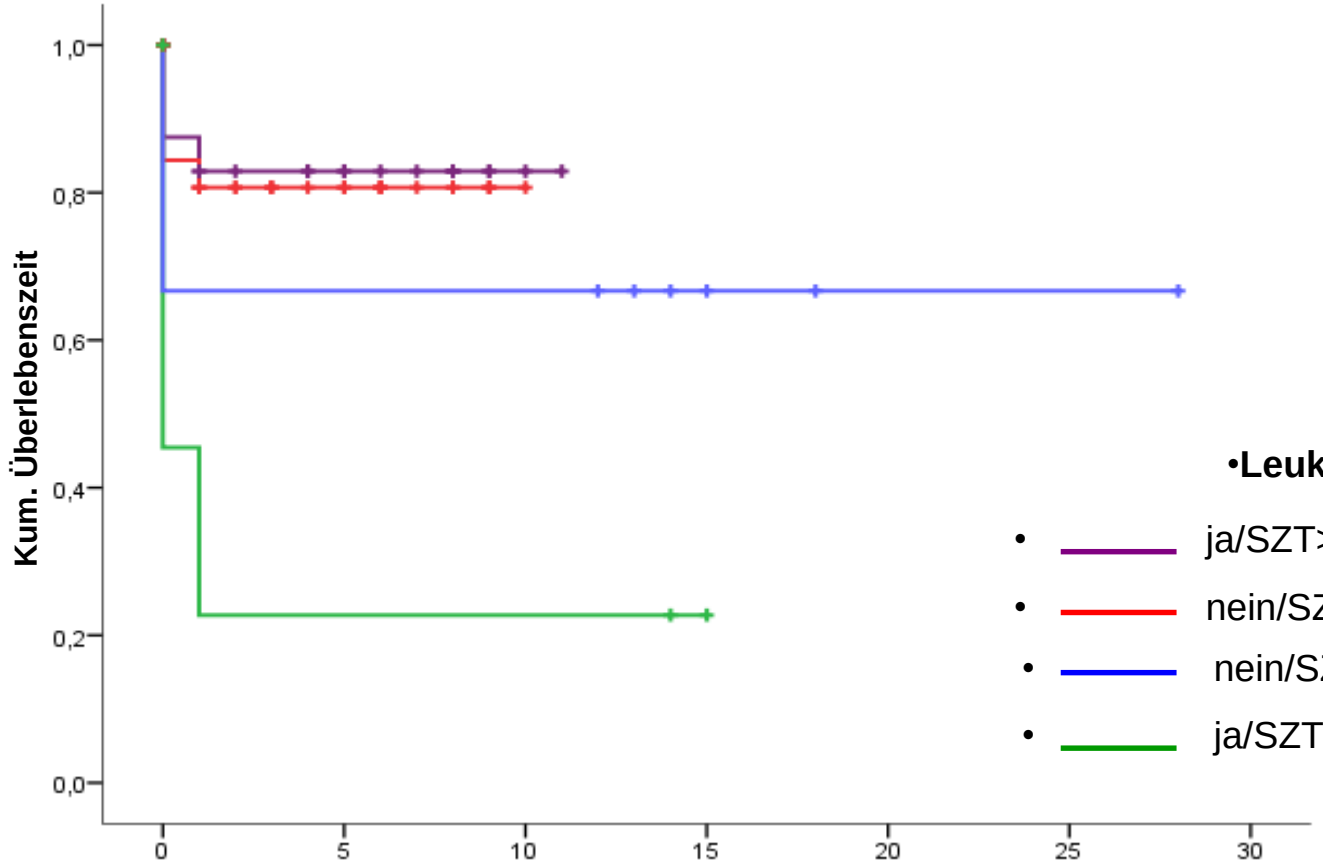
SZT=stem cell transplantation

Absolute neutrophil counts in patients with severe congenital neutropenia treated with G-CSF



SEVERE CHRONIC NEUTROPENIA International Registry

Hämatopoetische Stammzelltransplantation



Jahre nach SCT bis zum Tod oder bis zum letzten FU

P=0,002 (Log Rank)

•Leukämie und SZT-Datum

- ja/SZT>=2001 **Events/N=4/24**
- nein/SZT>=2001 **Events/N=6/32**
- nein/SZT<2001 **Events/N=3/9**
- ja/SZT<2001 **Events/N=8/11**

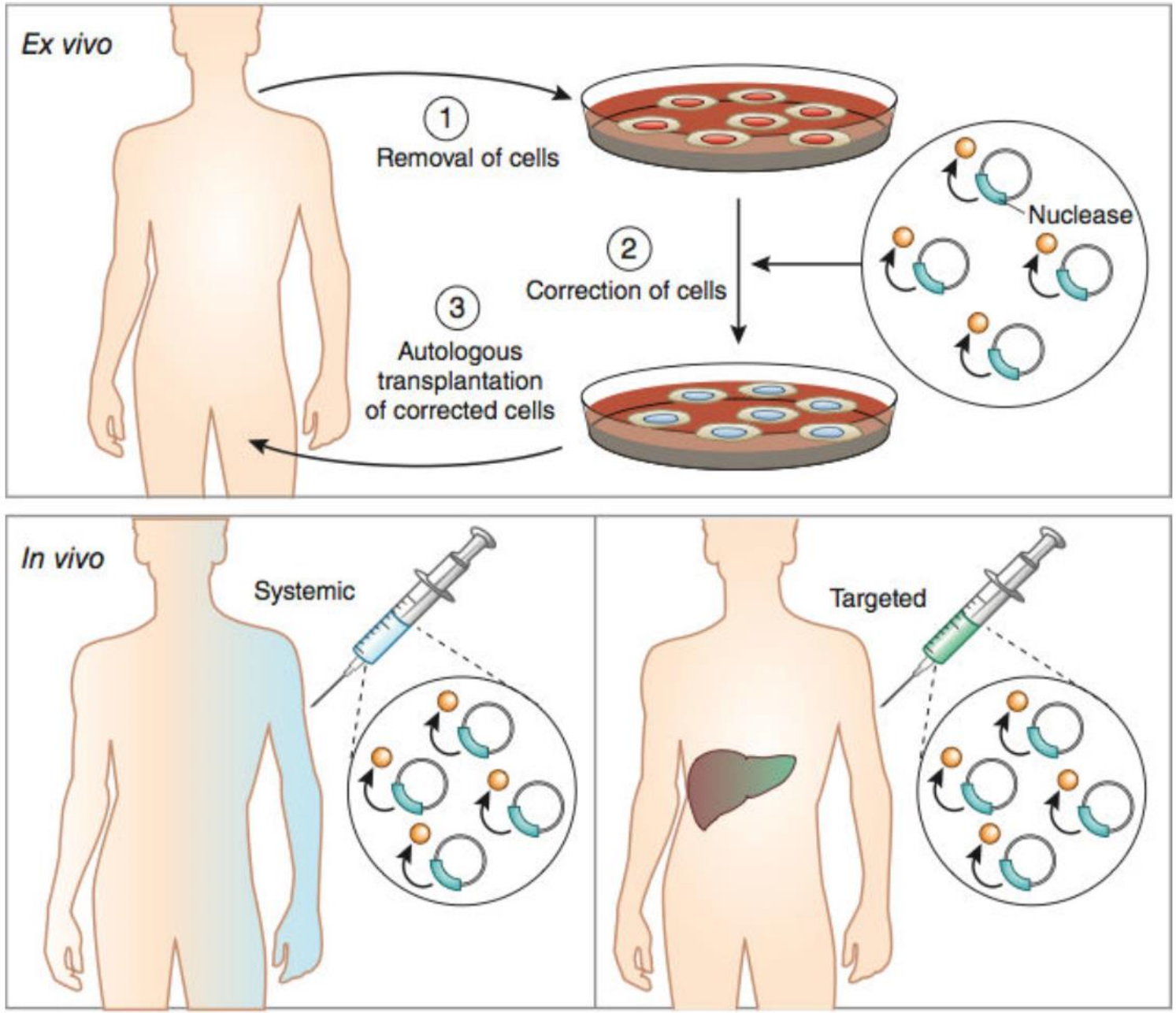
N=76

Gentherapie

Table 2 | ***Ex vivo* haematopoietic stem cell gene therapy trials for SCIDs**

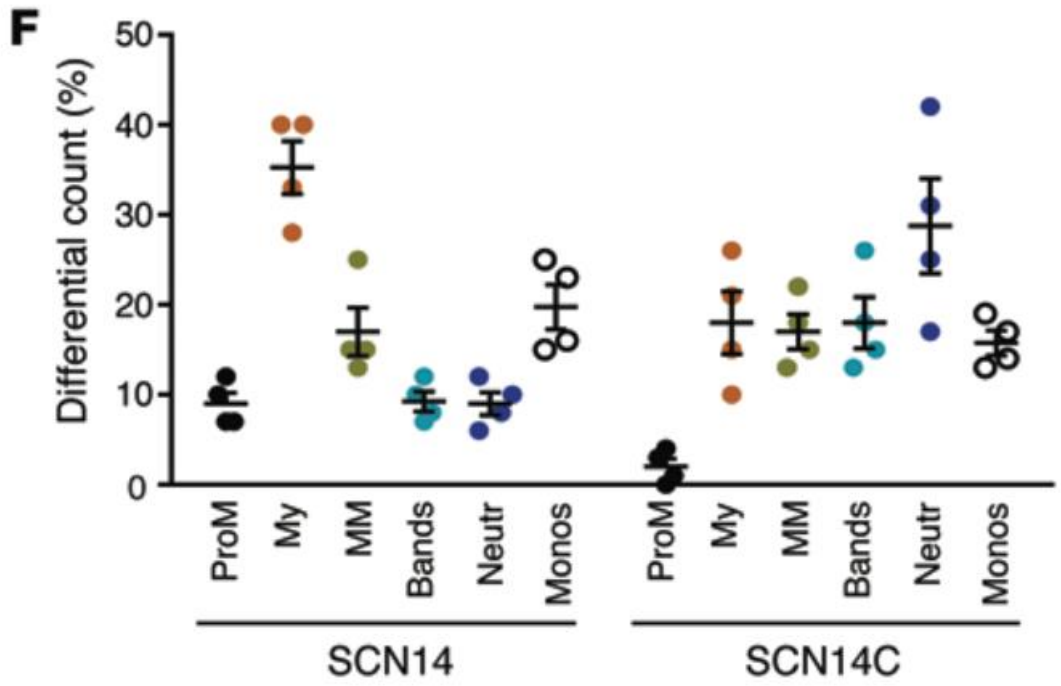
Disease	Targeted gene	Vector	*n*	Follow-up (years)	Outcome
SCIDX1	*IL2RG*	First-generation γ-retroviral vector	20	8–16	• Correction of T cell immunodeficiency in 18 patients • No long-standing correction of B cells, but half of the patients are immunoglobulin sufficient • NK cell reconstitution is limited • T cell leukaemia in 5 patients
	IL2RG	Second-generation γ-retroviral vector (SIN)	9	2.3–4.5	• Sustained immune function • No patients with genotoxicity • 8 patients are alive and well
ADA-SCID	*ADA*	First-generation γ-retroviral vector plus a mild conditioning regimen	42	1.5–14	• Successful correction of T cell immunodeficiency • 31 patients are alive and well • No patients with leukaemias
	ADA	Lentiviral vector (SIN) plus a mild conditioning regimen	16	0.5–3	• Sustained immune function • No patients with genotoxicity • 16 patients are alive and well

A. Fisher, et al., Nature Reviews Disease Primers 2015



Ex vivo versus in vivo editing therapy, Feng Zhang, et al., Nat. Med. 2015

Correction of ELANE mutations in iPSCs from a patient with congenital neutropenia by CRISPR/Cas9 technology

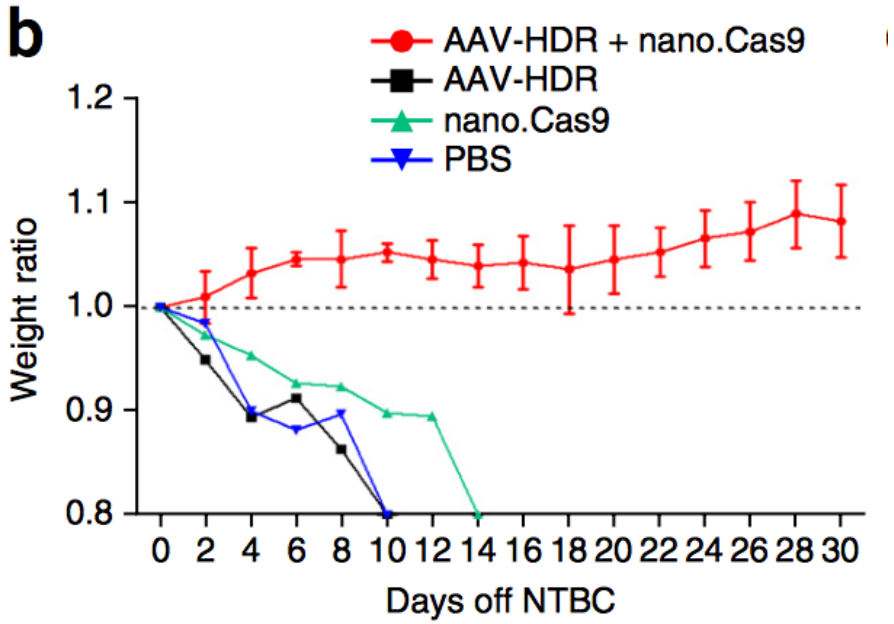


Nayak RC, et al. JCI 2015

Mouse model of human hereditary tyrosinemia

Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components *in vivo*

Yin H et al., Nature Biotechnology 2016



Schlussfolgerung

- Genetische Erkrankungen sind komplex, wir müssen noch viel darüber lernen. Eingriffe in die menschliche Keimbahn um dauerhaft Mutationen zu korrigieren sollten mit dem derzeitigen Stand der Forschung nicht durchgeführt werden.

- Die aufgezeigten Alternativen inklusive der CRISPR/Cas9 Technologie können jedoch erfolgreich zur Korrektur von Gendefekten in somatischen Zellen genutzt werden.

- Der Deutsche Ethikrat sollte sich intensiv mit diesem Thema befassen.