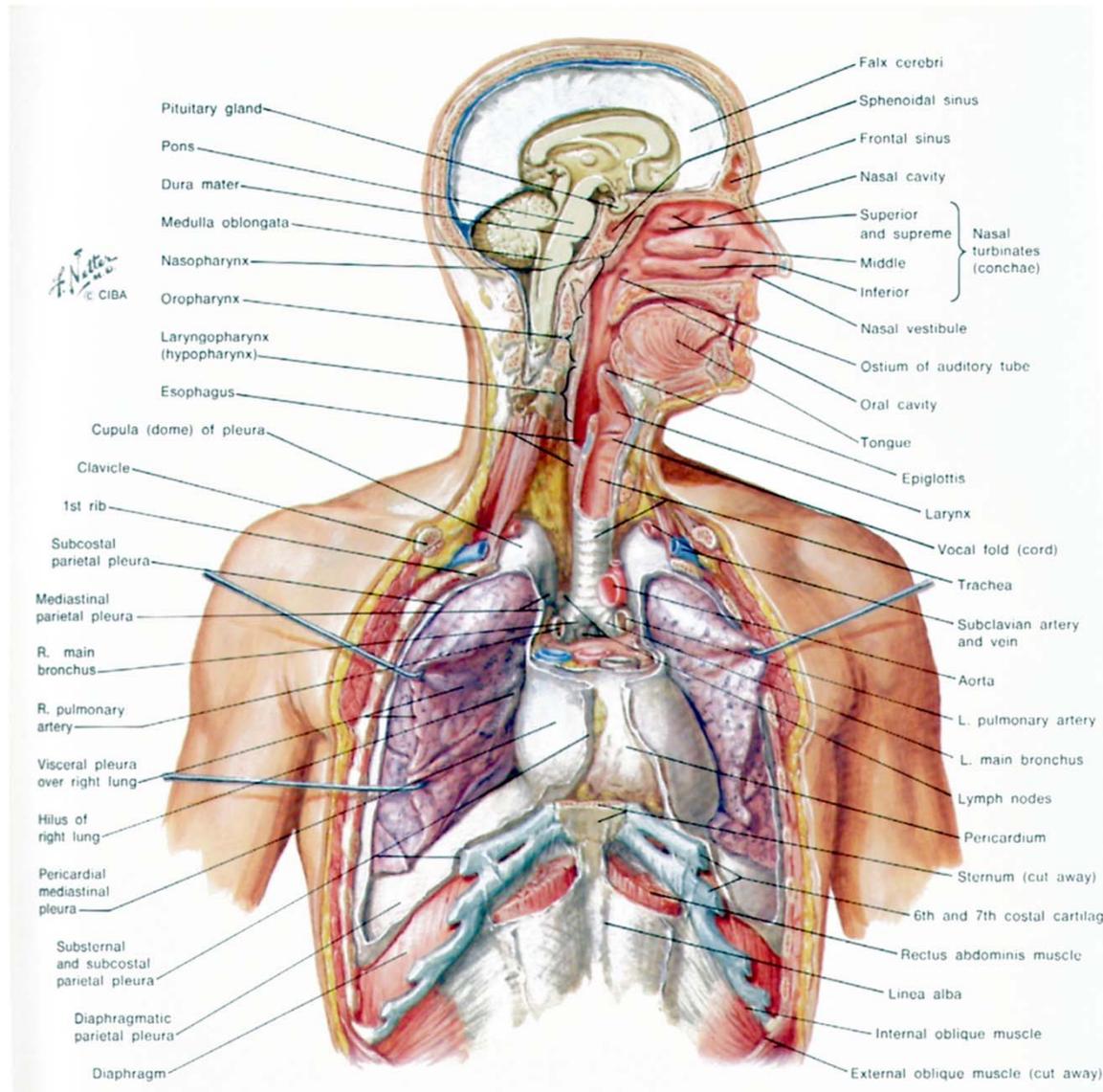


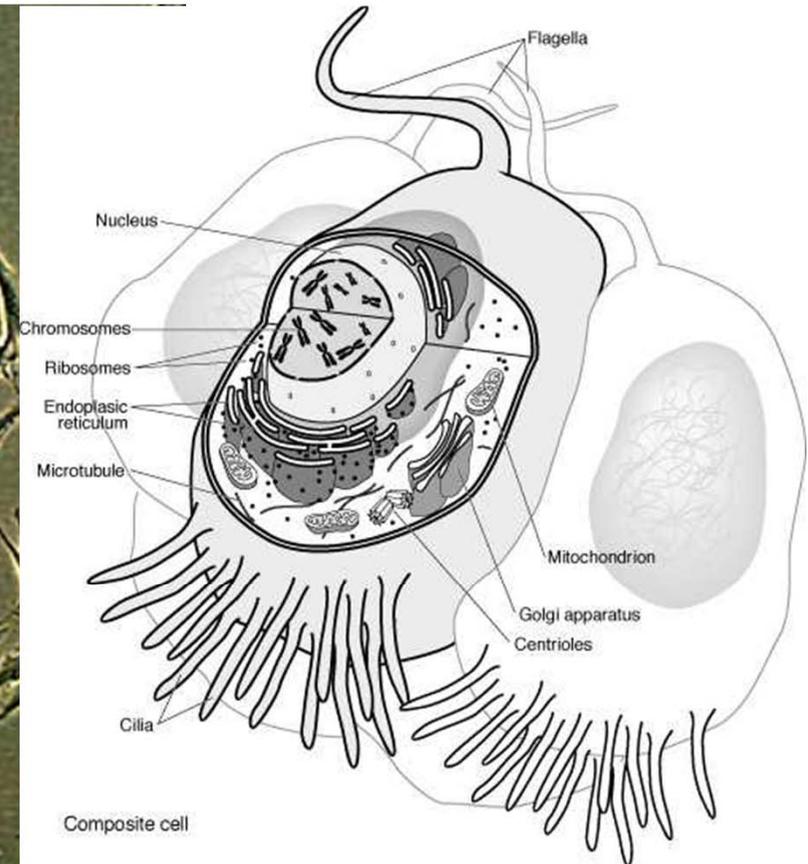
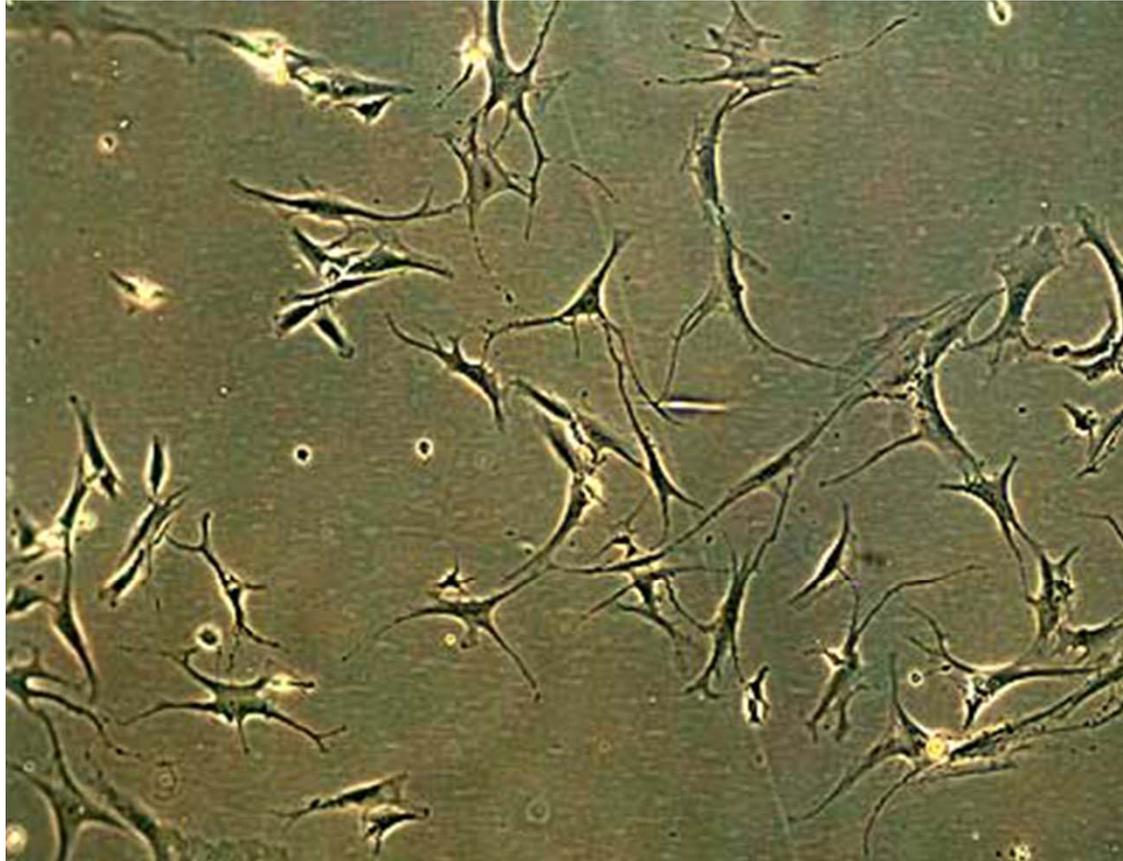
Individuum



Organe/Gewebe



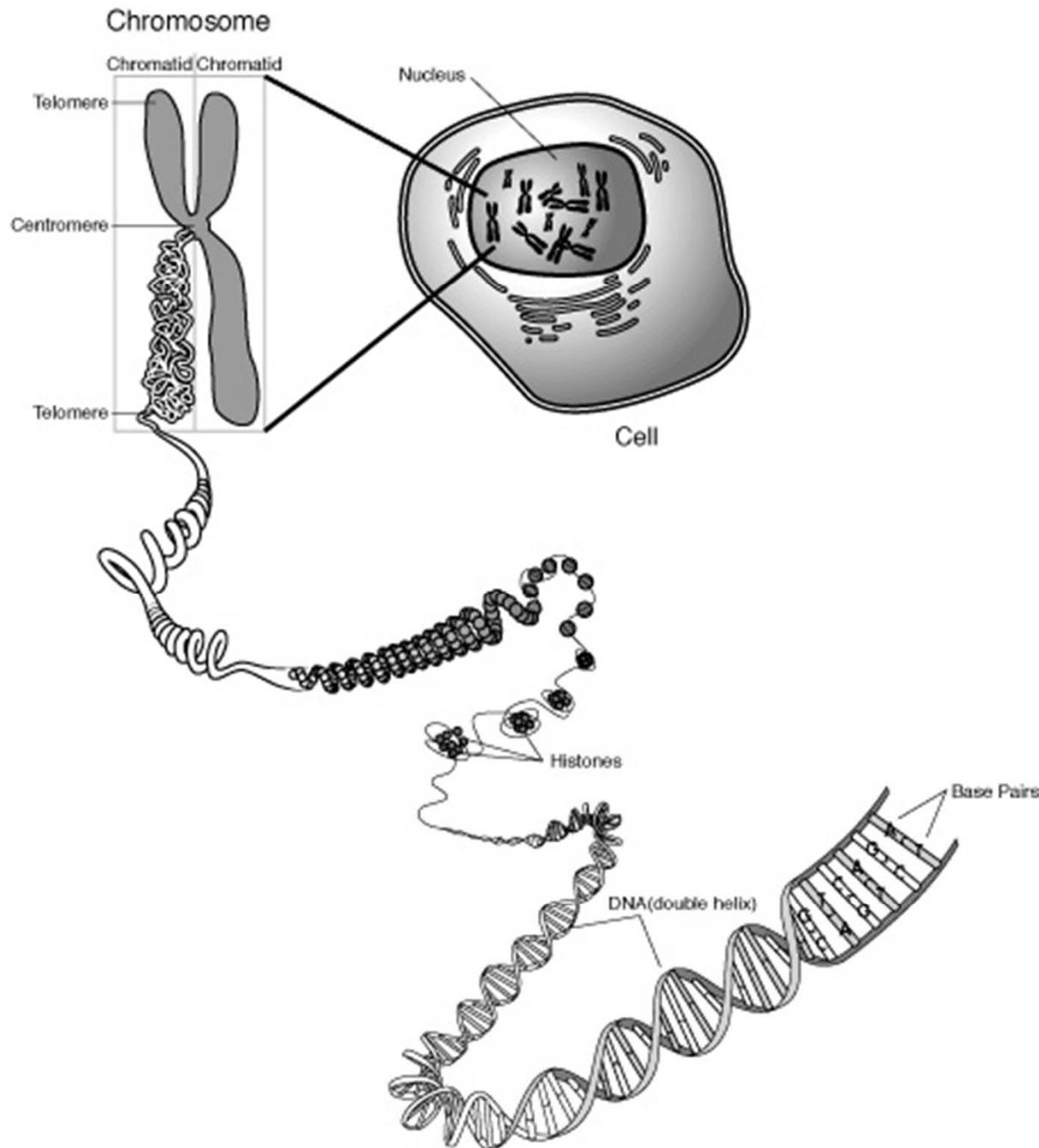
Zellen



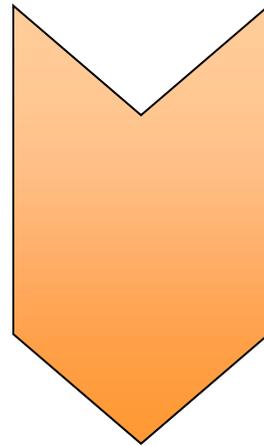
- 10.000.000.000.000 im Erwachsenen
 - > 1.000 Varianten
 - lebenslange Regeneration
 - Omnis cellula e cellula (Virchow)

Gene

~ 25.000 pro Zelle
Mehrere Editionen
Komplexe Interaktionen
Epigenetische Kontrolle
Gefahr von Mutationen



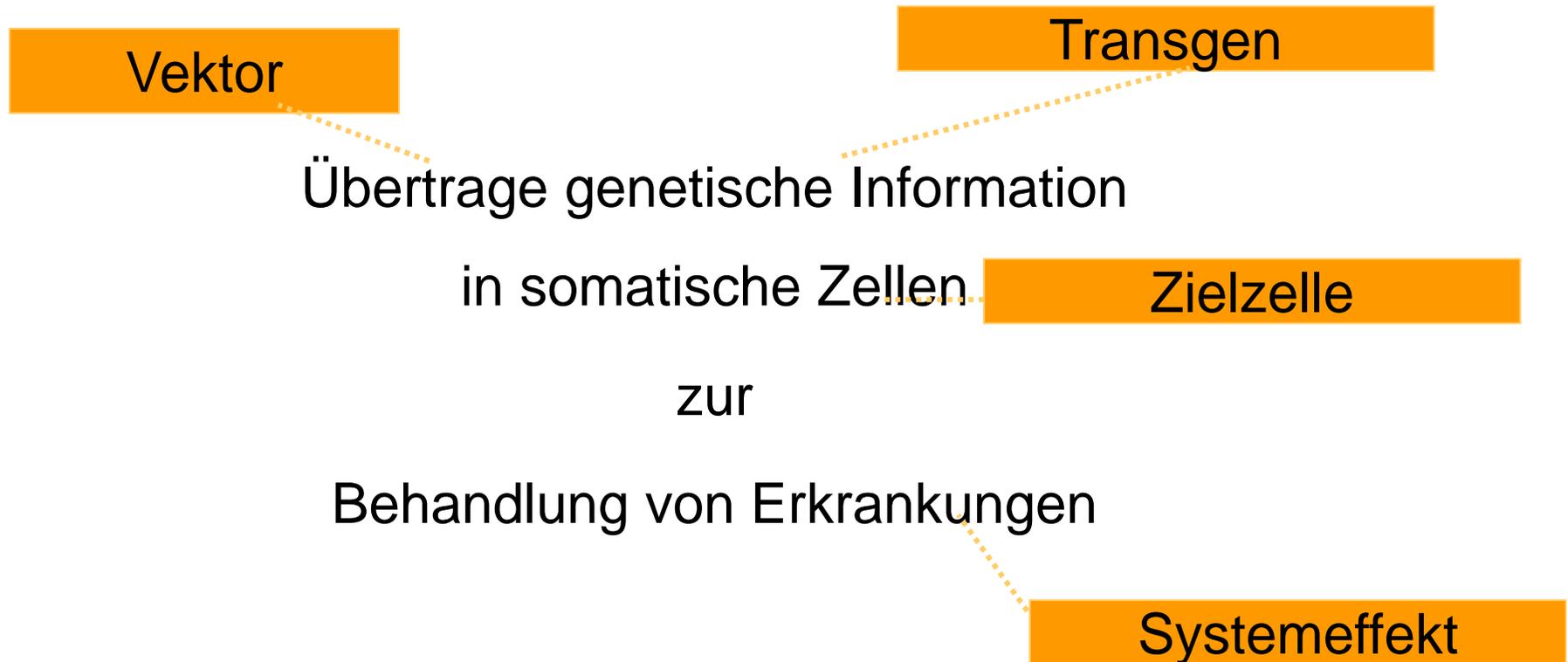
Erkrankungen infolge von Genfehlern



Therapie mit korrigierten Genen

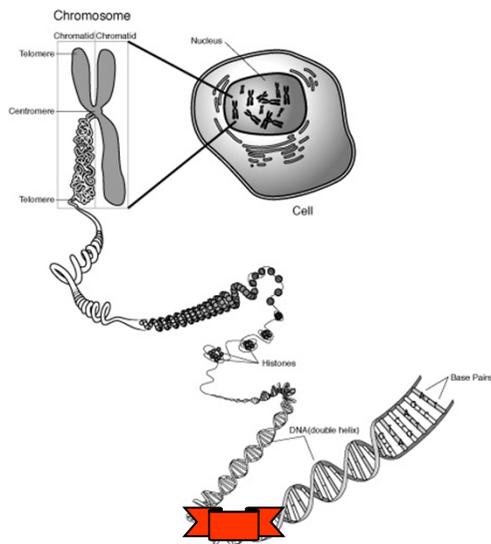
Information – Zelle - Quantität

Gentherapie



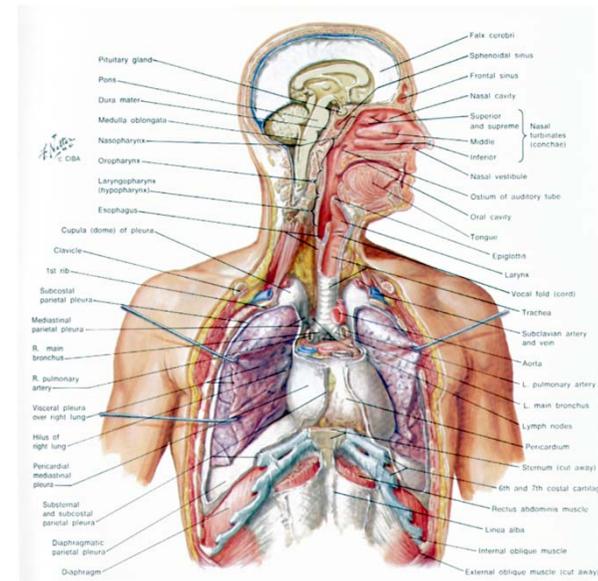
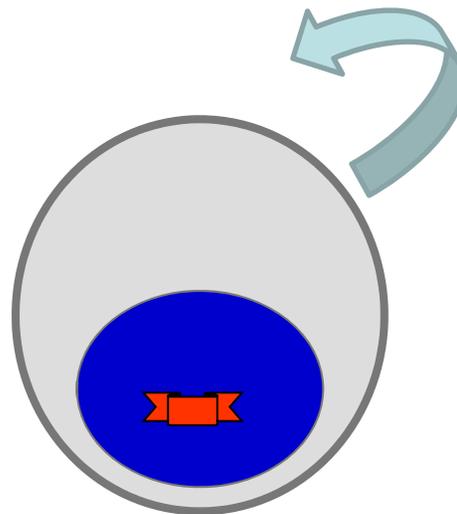
Vehikel? Dosis? Route? Therapeutische Breite?

Gentransfer: quantitative Fragen



1: 25,000

Transgen : Zelluläre Gene

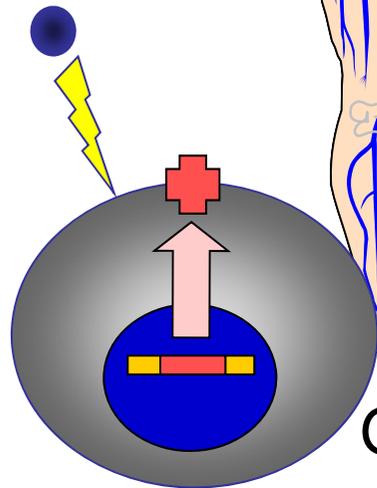


1:10¹³

Transgene Zelle : Körperzellen

Gentransfer: Prinzip

Milieu,
Pharmaka
= extrinsisch



Gene =
intrinsisch

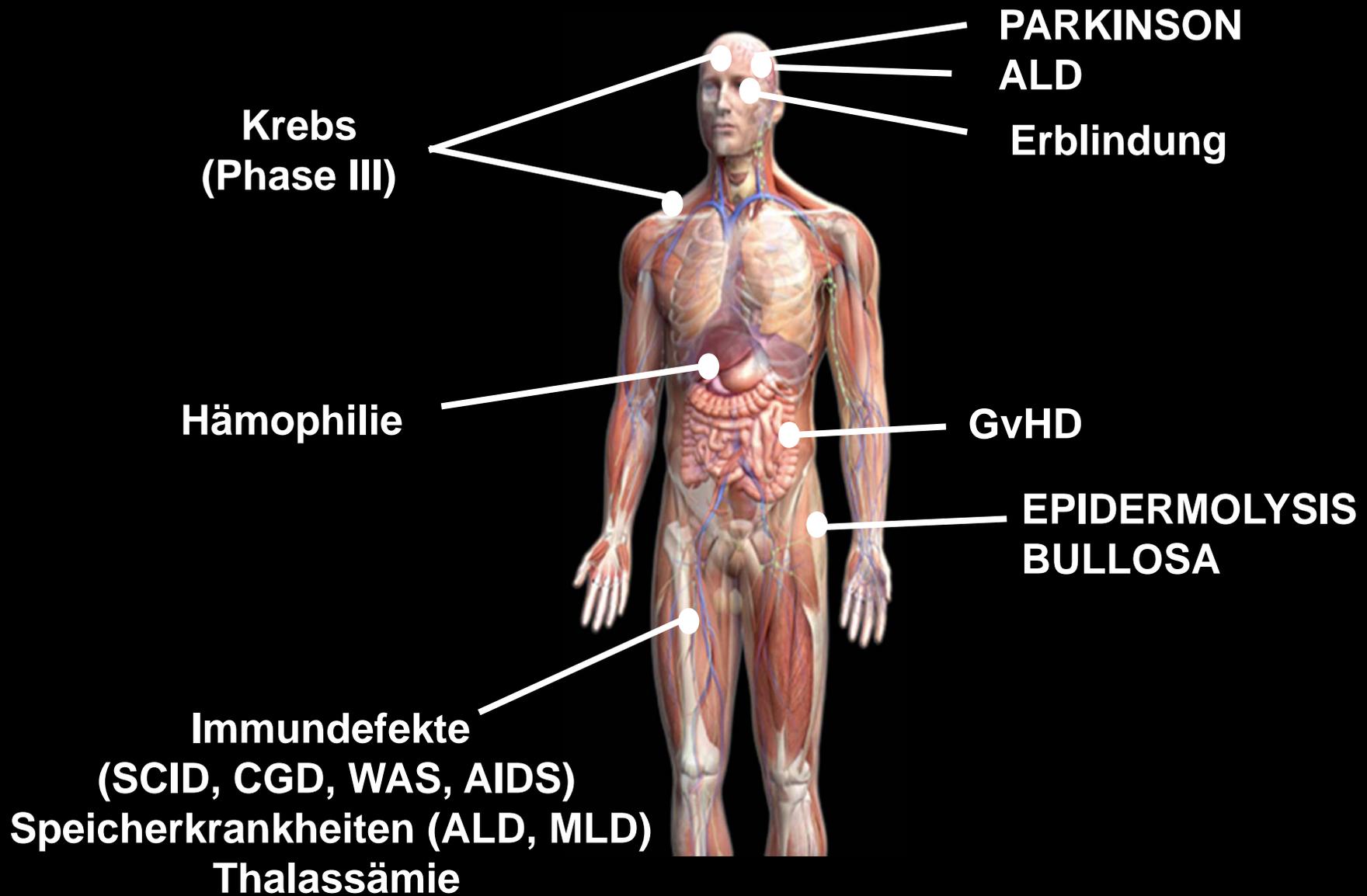
Funktionskontrolle

- Proliferation
 - Fitness
 - Migration
- Differenzierung
- Reprogrammierung
 - Eliminierung

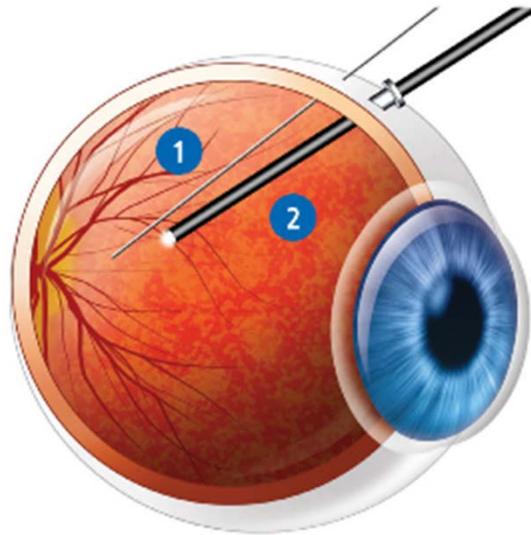
Korrektur

- Genetische Erkrankung
 - Infektion
 - Krebs
- Degenerative Erkrankung

GENTHERAPIE: Erste Effekte in klinischen Studien



Genherapie kuriert Farbsehen

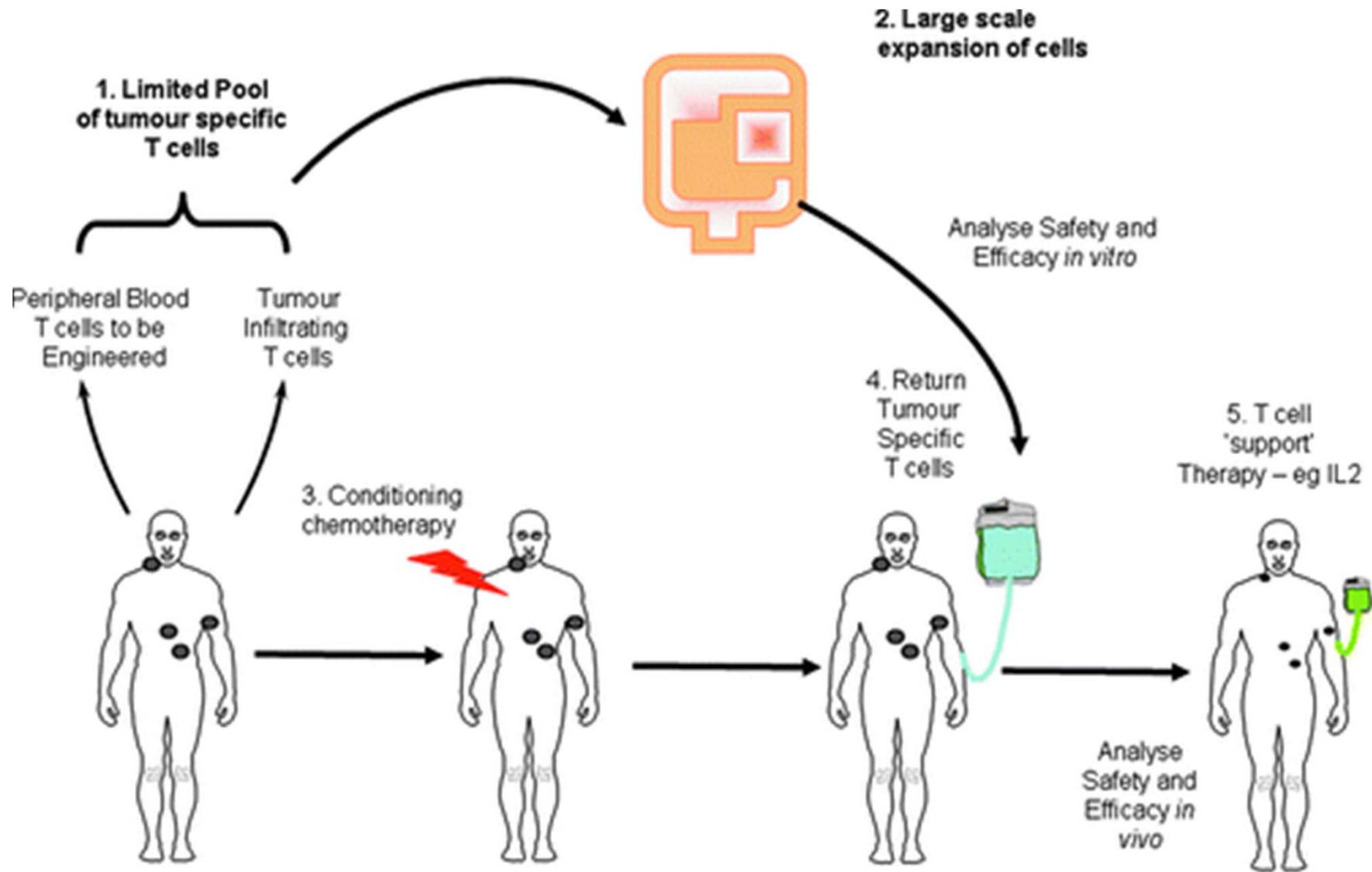


Perspektive:

- GT monogenetischer Netzhauterkrankungen
- GT der Makuladegeneration
- Diskussion: Enhancement?



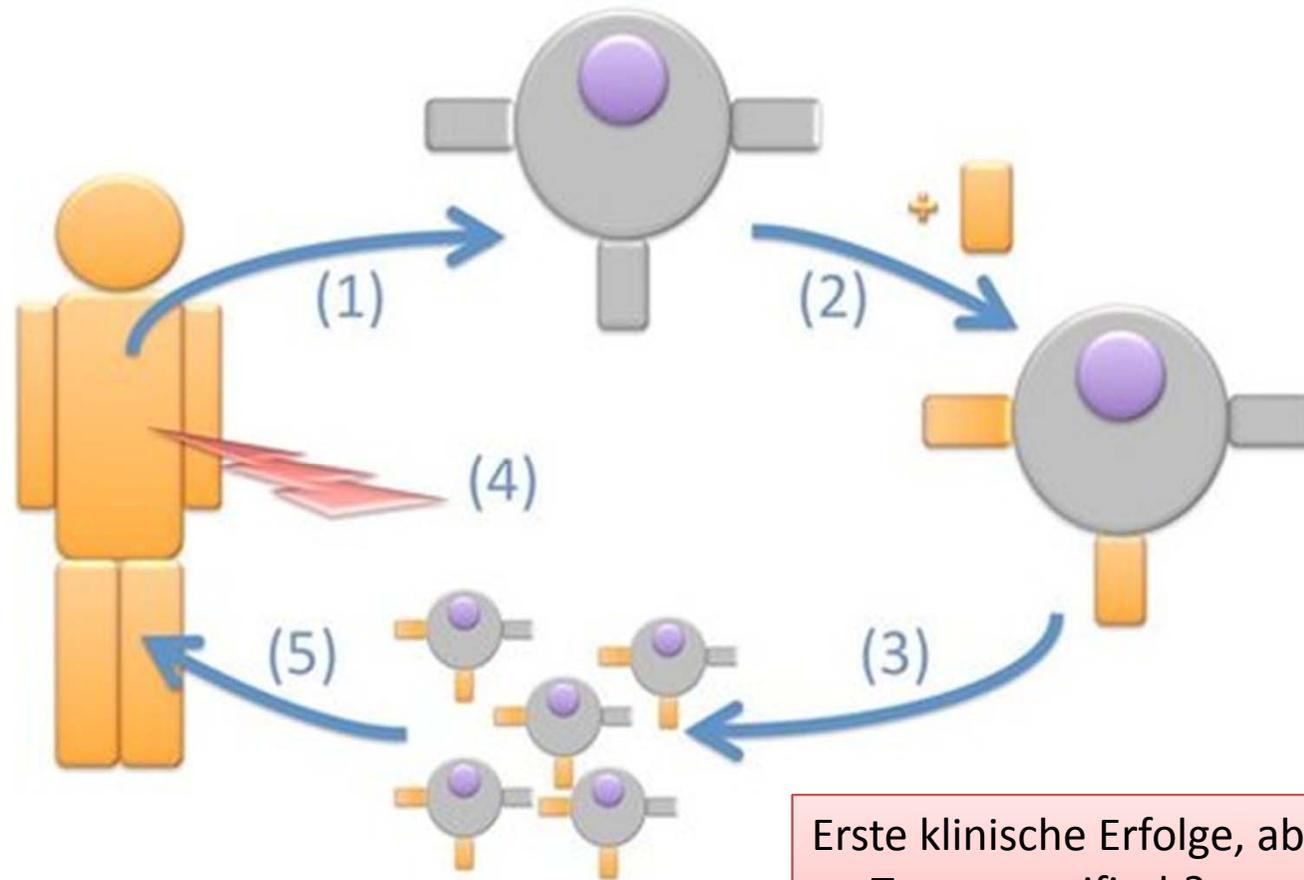
Adoptive Immunotherapy



Patient with cancer

Adoptive Immuntherapie:

Tumorspezifität durch Gentransfer



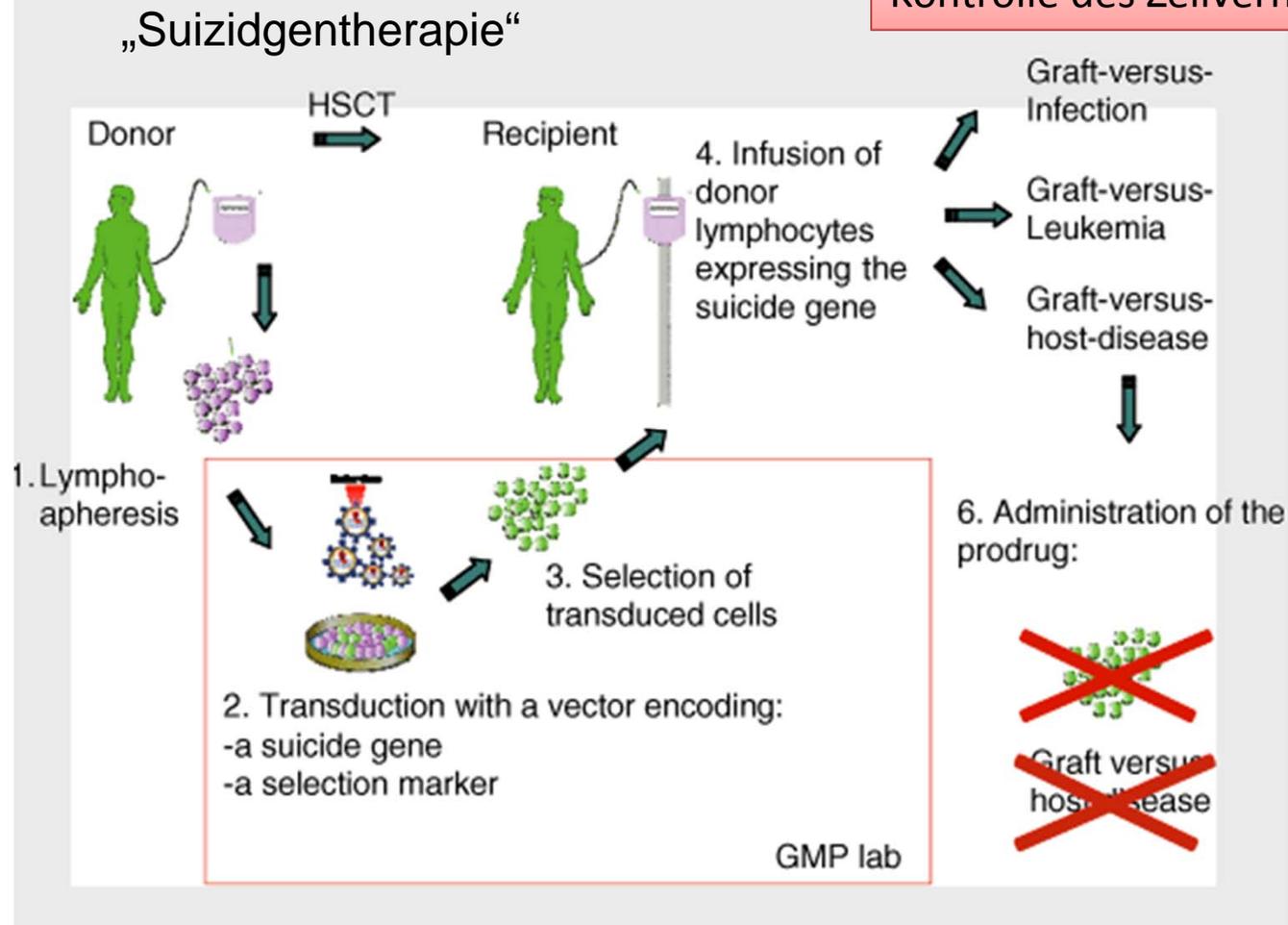
Erste klinische Erfolge, aber:

- Tumorspezifisch?
- Mischformen mit endogenem TZR?
- Revertierbar?

Adoptive Immunotherapy:

Konditionale Zellelimination durch Gentransfer

Wichtiges Prinzip:
Kontrolle des Zellverhaltens !



Adoptive Immuntherapie/Suizidgentransfer:

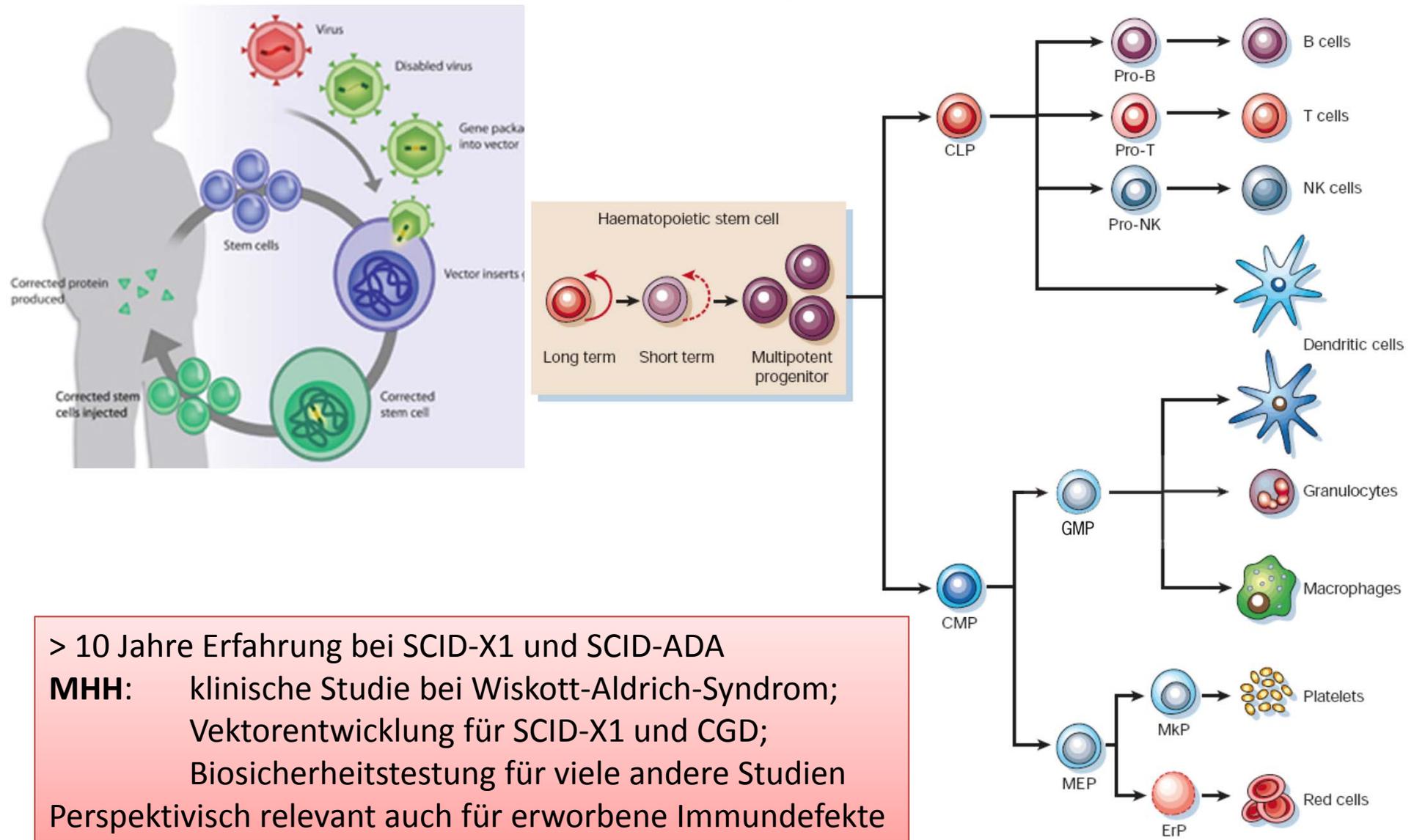
Umfangreiche klinische Studien

TABLE 2. CLINICAL TRIALS OF SUICIDE GENE THERAPY IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

<i>Clinical application</i>	<i>Vector (suicide gene/marker gene)</i>	<i>T cell manipulation (days)</i>	<i>No. of TK cells infused/kg per patient</i>	<i>No. of treated patients</i>	<i>Clinical response (no. of patients)</i>	<i>Incidence of GvHD (no. of patients)</i>	<i>Complete response of GvHD to GCV</i>	<i>Reference</i>
To treat disease relapse occurring after HLA-identical allo-SCT	RV (HSV-TK/ Δ LNGFr)	14	10^5 – 10^8	23	11	4	3/3 ^a	(Bonini <i>et al.</i> , 1997); (Ciceri <i>et al.</i> , 2007)
	RV (HSV-TK/NeoR)	NE	10^6 – 10^8	23	6	0	NE	(Champlin <i>et al.</i> , 1999)
	RV (HSV-TK/NeoR)	NE	10^6	3	1	1	NE	(Munshi <i>et al.</i> , 1997)
	RV (HSV-TK/NeoR)	24–48	10^6 – 10^8	9	2	1	1/1	(Burt <i>et al.</i> , 2003)
	RV (HSV-TK/ Δ LNGFr)	9–11	10^8	5	4	2	2/2	(Onodera, 2008)
Day 0 in TCD allo-SCT	RV (HSV-TK/NeoR)	12	10^5 – 10^6	12	4	5	5/5 ^b	(Tiberghien <i>et al.</i> , 2001)
	RV (HSV-TK/NeoR)	13	5×10^6	3	1	2	1/1	(Fehse <i>et al.</i> , 2004)
Day 60 in TCD allo-SCT	RV (HSV-TK/ Δ LNGFr)	10	10^7	9	7	1	NE	(Weissinger <i>et al.</i> , 2008)
Day 42 in TCD haplo-SCT	RV (HSV-TK/ Δ LNGFr)	14	10^5 – 10^7	8	3	1	1/1	(Bonini <i>et al.</i> , 2007)
	RV (HSV-TK/ Δ LNGFr)	10	10^7	28	22	11	10/10 ^c	(Ciceri <i>et al.</i> , 2009)
Total				123	61	28	23/23	

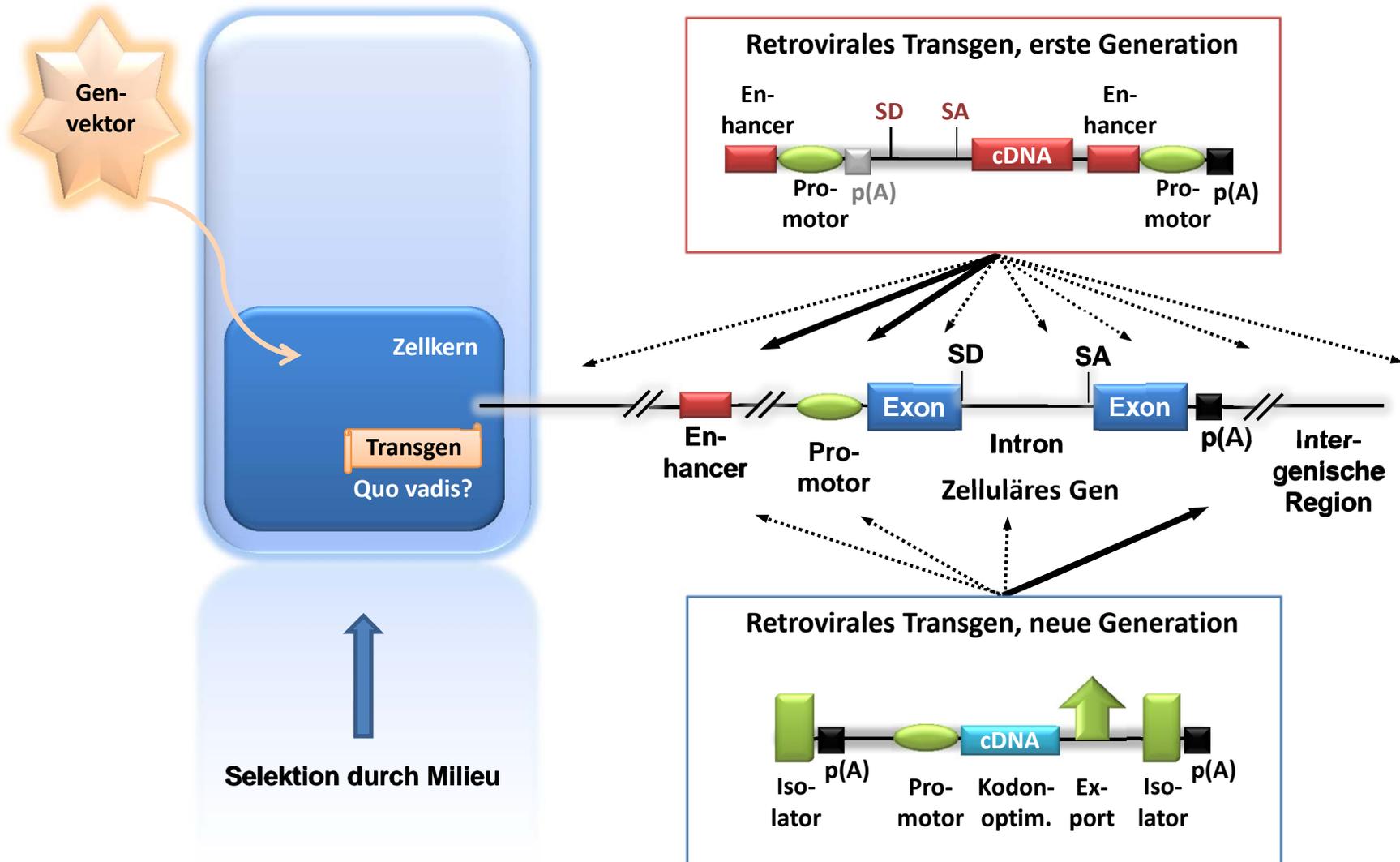
Abbreviations: GCV ganciclovir; GvHD, graft-versus-host disease; LNGF, low-affinity nerve growth factor; NE, not evaluable; RV, retroviral vector; SCT, stem cell transplantation; TCD, T cell depleted; TK, thymidine kinase.

Stammzellgentherapie zur Regeneration des Immunsystems



> 10 Jahre Erfahrung bei SCID-X1 und SCID-ADA
MHH: klinische Studie bei Wiskott-Aldrich-Syndrom;
 Vektorentwicklung für SCID-X1 und CGD;
 Biosicherheitstestung für viele andere Studien
 Perspektivisch relevant auch für erworbene Immundefekte

Cave! Insertionsmutagenese

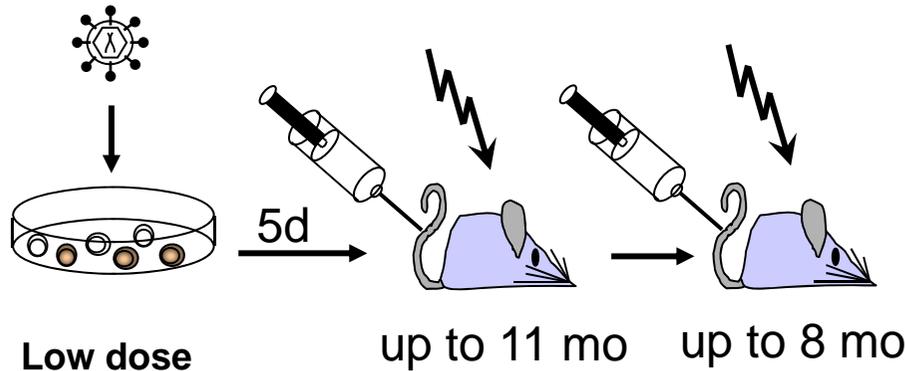


Gentransfer-Toxikologie

C57BL6 Maus, serielle KMT

PEI
EMA
FDA
(NTP)

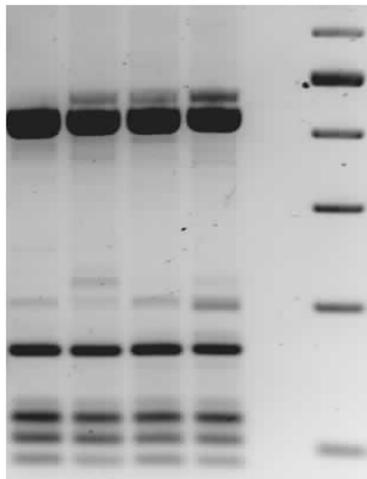
Vectors encoding
cell surface markers,
fluorescent proteins
or therapeutic genes



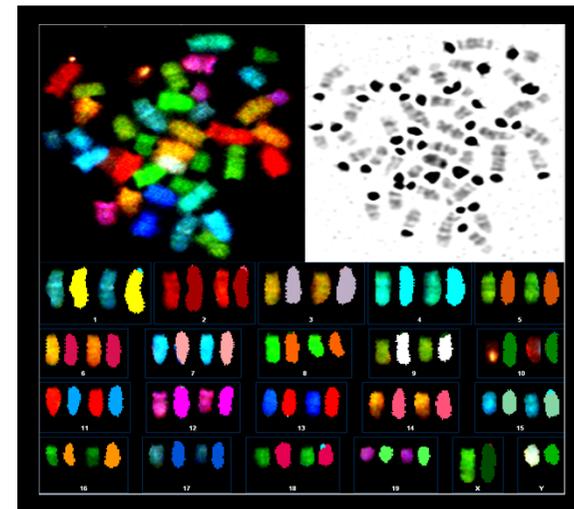
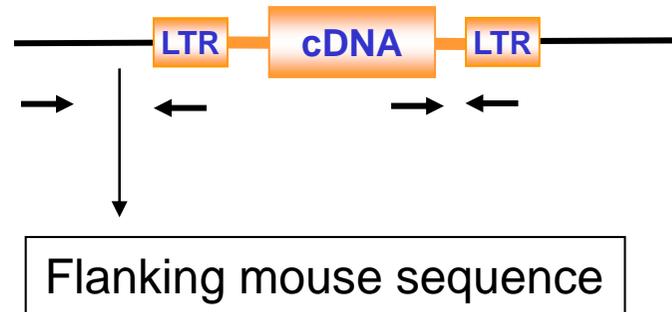
Low dose
versus
High dose

Leukemia
Clonal dominance
Integromics (IDDb)
“Phenotoxicity”

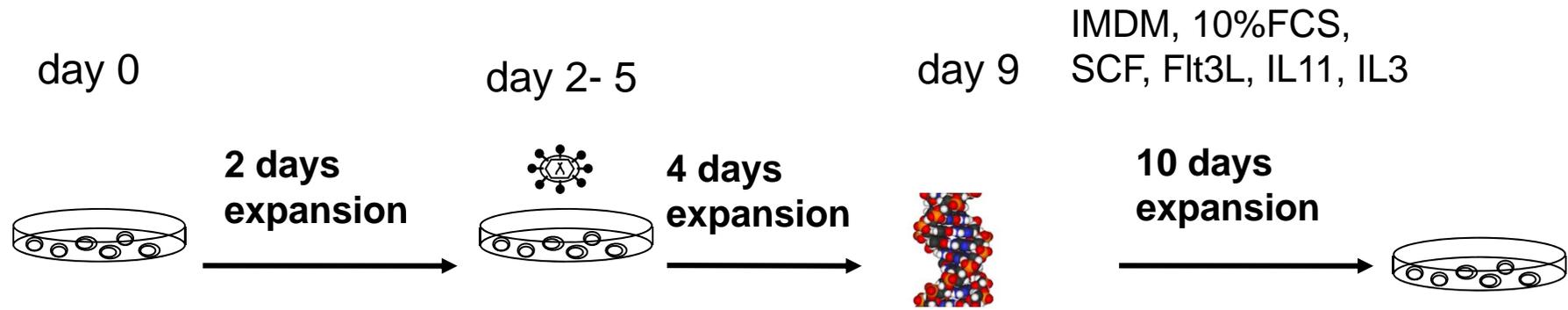
Li et al., Science 2002;
Kustikova et al., Science 2005;
Blood 2007; Mol Ther 2009
Modlich et al., Blood 2005;
Leukemia 2008
Wicke et al., Mol Ther 2010



Southern blot
LM-PCR



Quantifizierung des Risikos der Insertionsmutagenese im zellbasierten Verfahren



Primary
Lin.neg.
BM cells

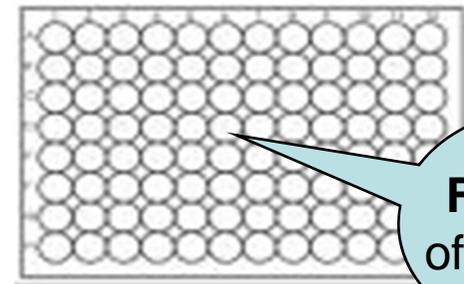
Transduction
100.000 cells
4x MOI 10

DNA Prep.
Mean copy no.
Facs analysis

14 days
replating

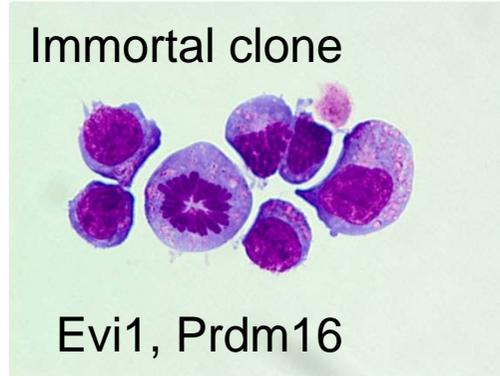
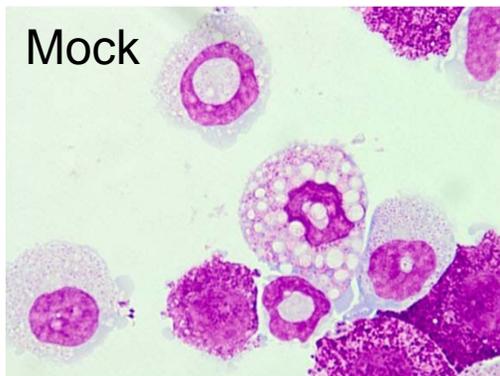
**Incidence
of mutants**

10 or 100 cells / well



**Fitness
of mutants**

Limiting Dilution

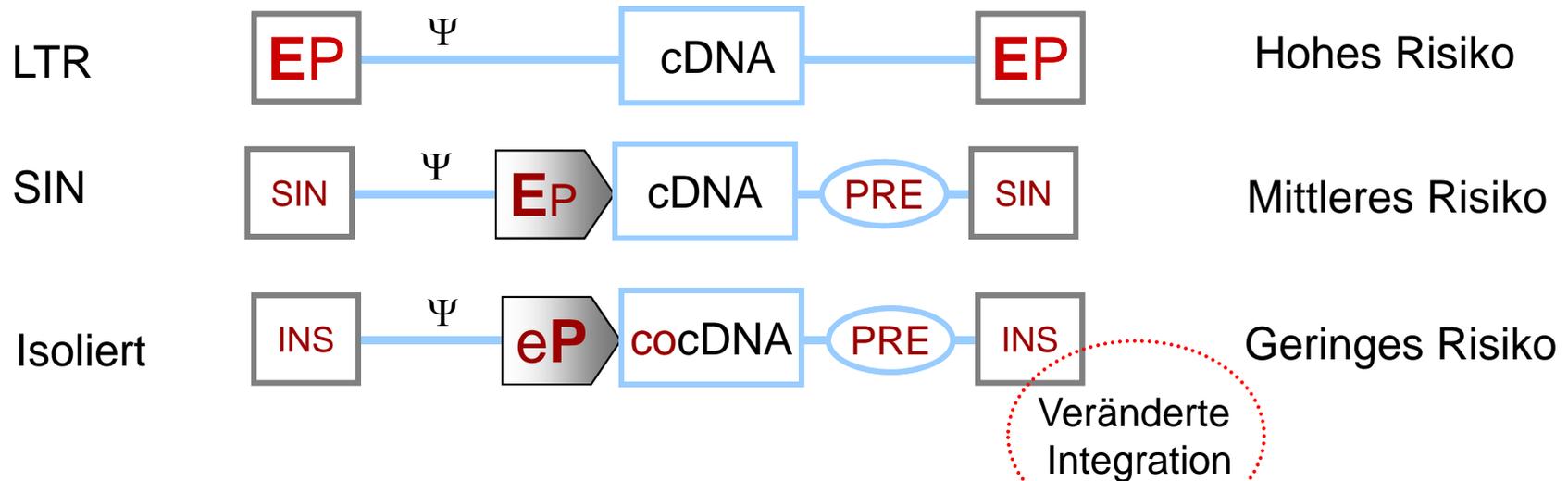


Vektormodifikationen reduzieren das Risiko

>10-facher Sicherheitsgewinn durch Umbau des Transgens

~ 9-facher Sicherheitsgewinn durch verändertes Integrationsmuster

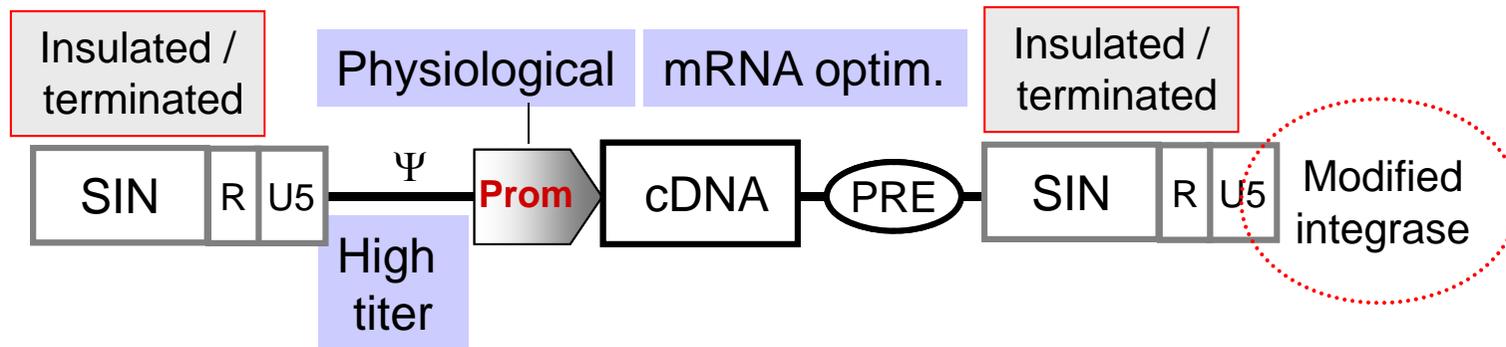
PEI
EMA
FDA
(NTP)



Modlich et al., 2006; 2009; Zychlinski et al., 2008; Arumugam et al., 2009; Suerth et al., 2010

Vektortechnologie: Neue Generationen

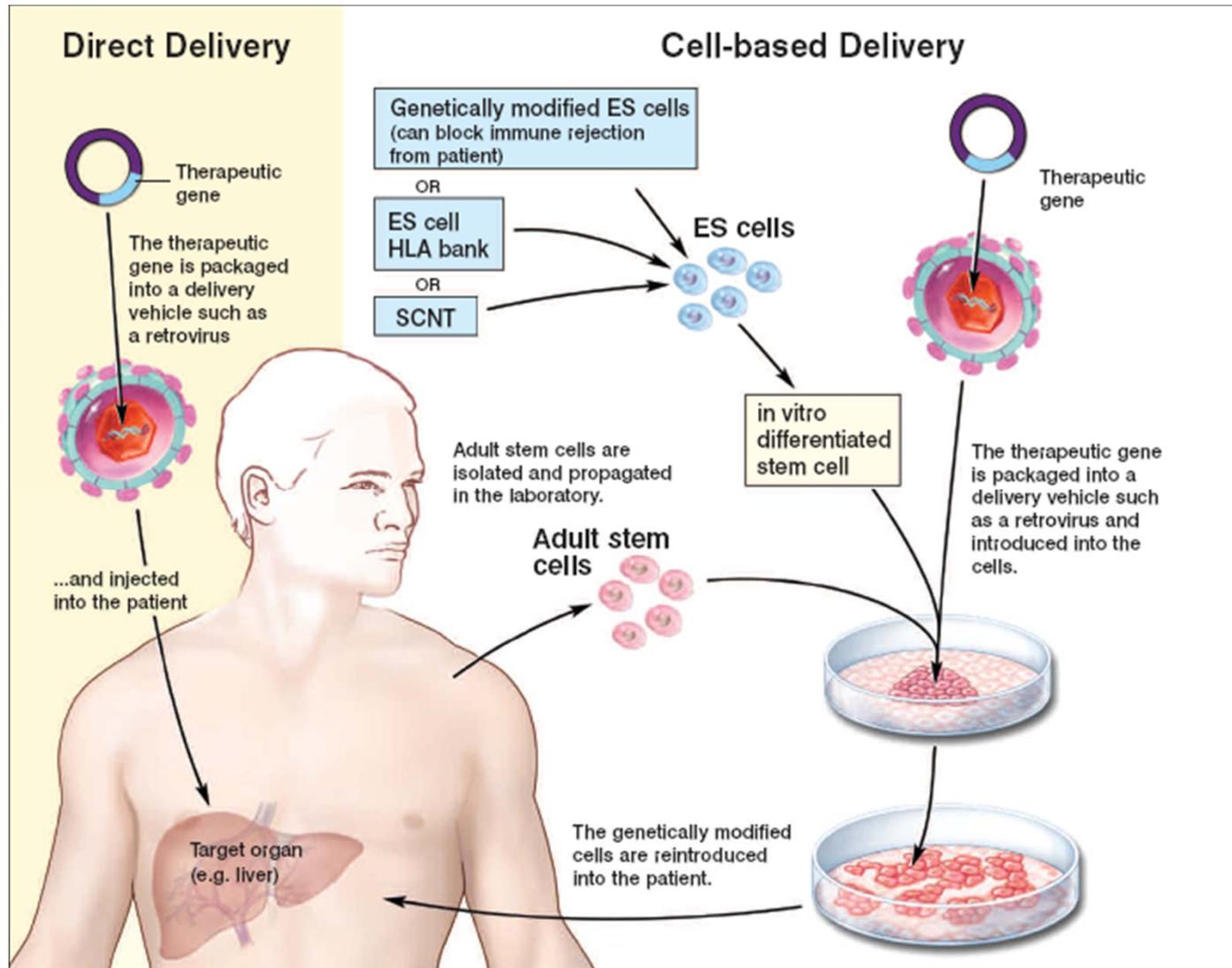
Neuer Vektor	klin. Prüfung
SCID-X1 (multicenter USA and EU)	2010
CGD (Grez et al., Frankfurt)	2011
WAS (Klein et al., Hannover)	2012
...	



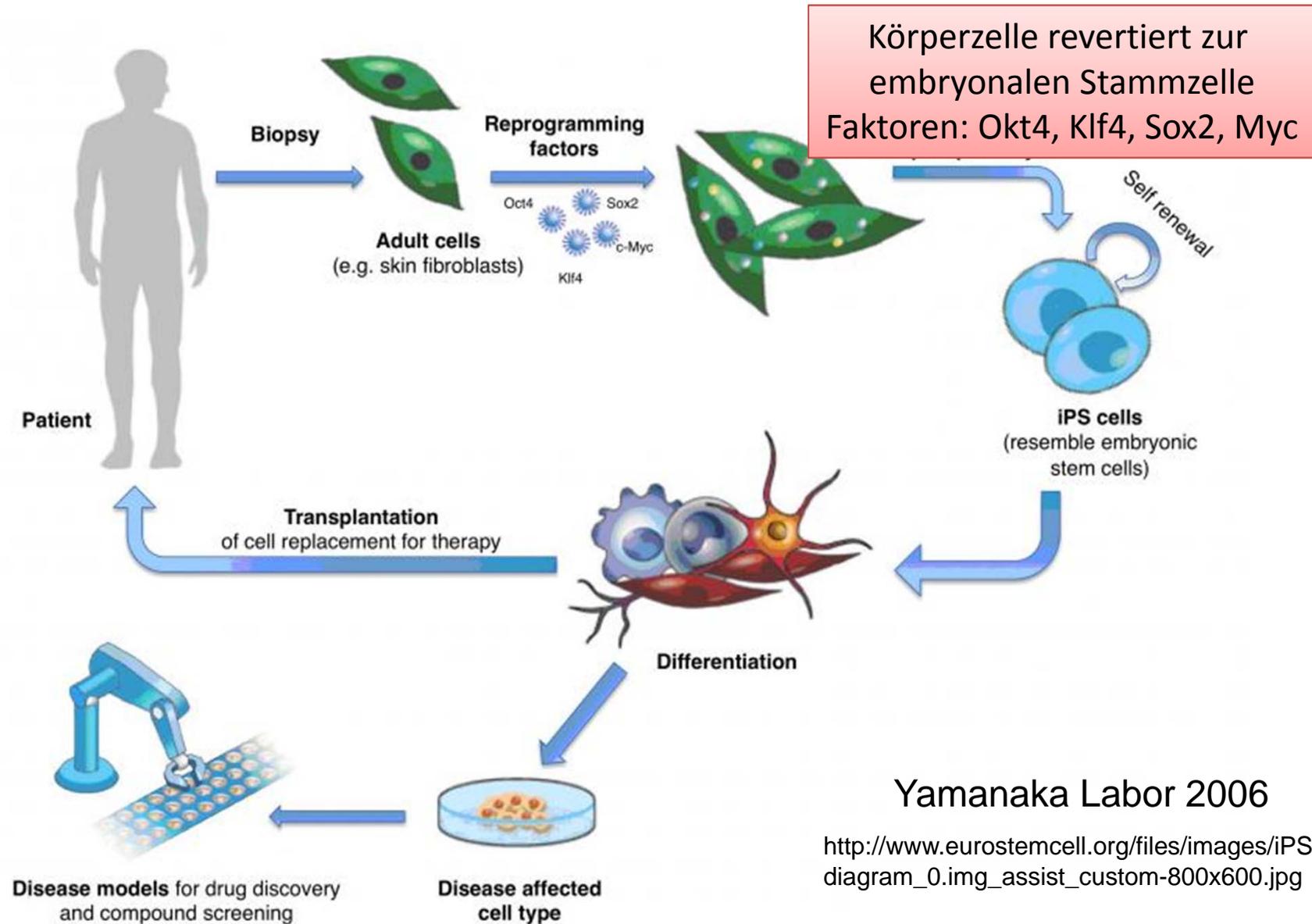
Verbesserte Gentransfereffizienz
Verbesserte Stammzellexpansion vor Tx

Gene transfer ex vivo oder in vivo

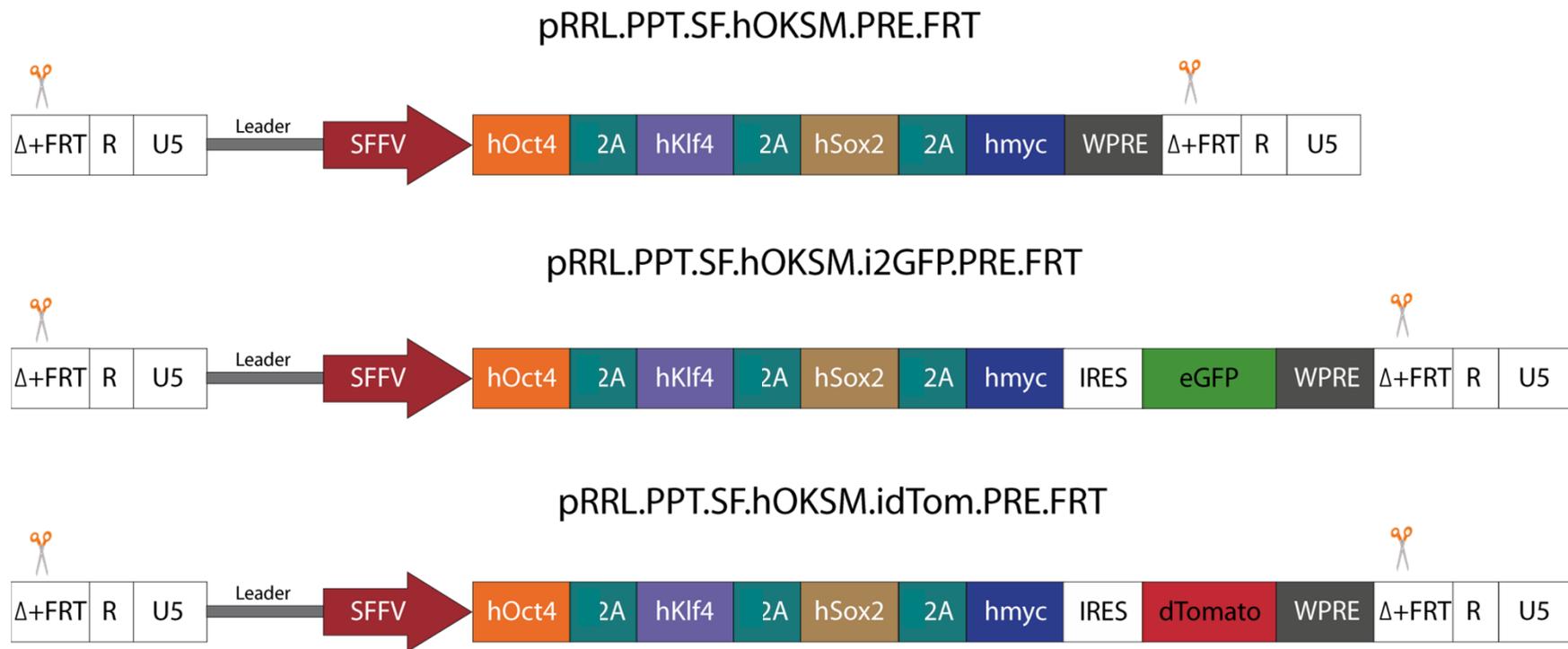
Polyklonal oder klonal?



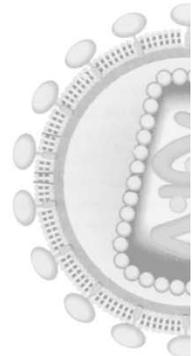
Induzierte pluripotente Stammzellen (iPS)



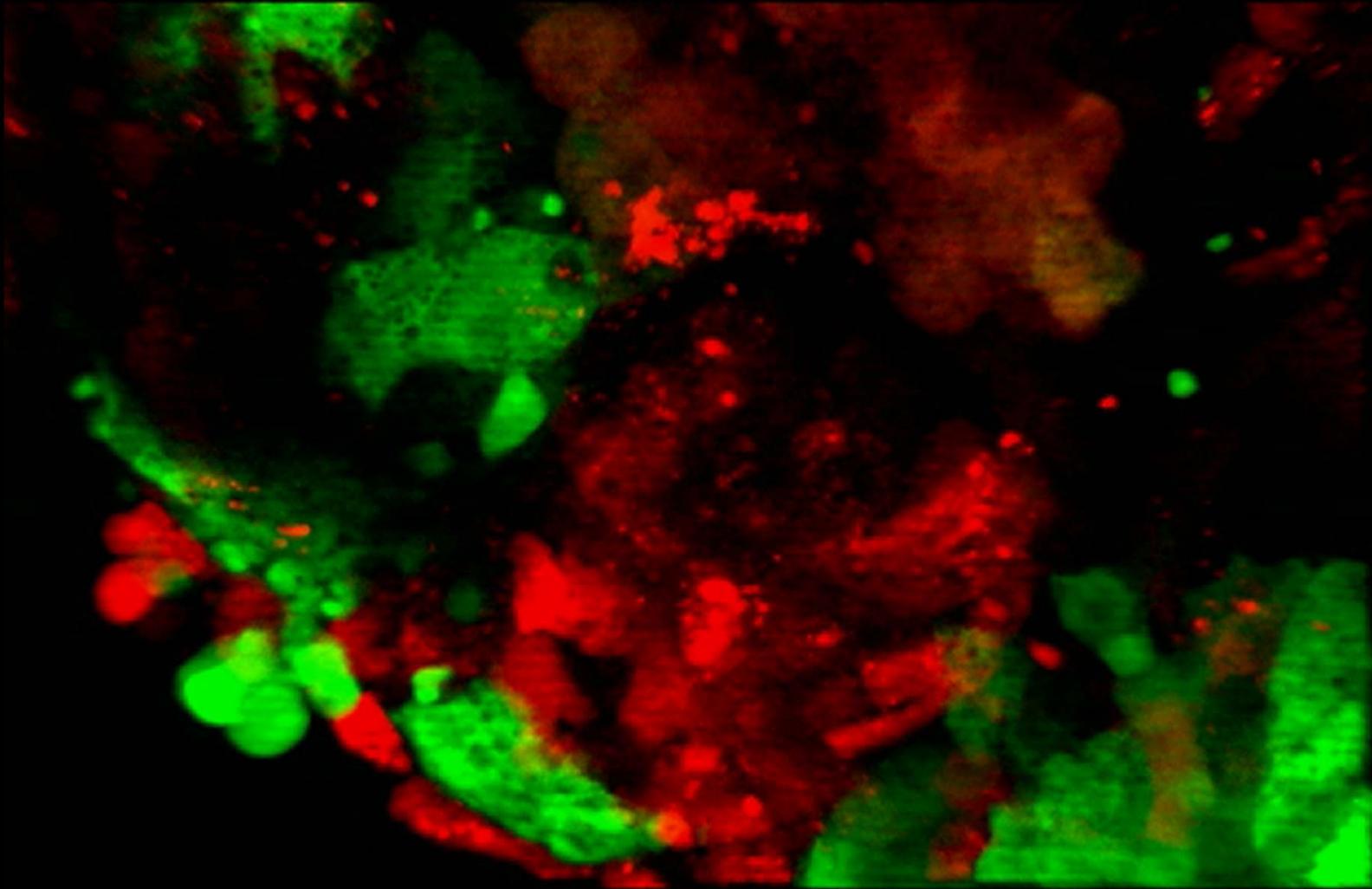
„Alles in 1“-Genvektor zur Reprogrammierung



Titer determined by qPCR $\approx 1 \times 10^7$ t.u./ml

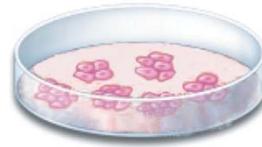


iPSC entstehen in Patchwork-Kolonien



Eva Warlich
Timm Schröder
Axel Schambach

Noch nicht am Ziel: Differenzierung der iPS Zellen

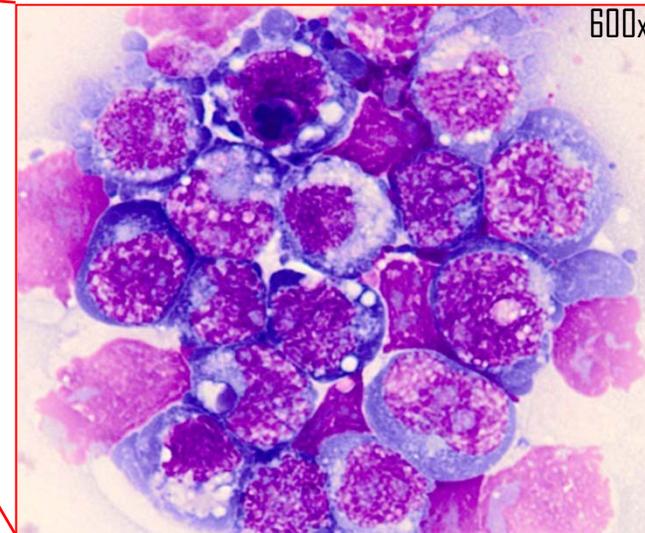
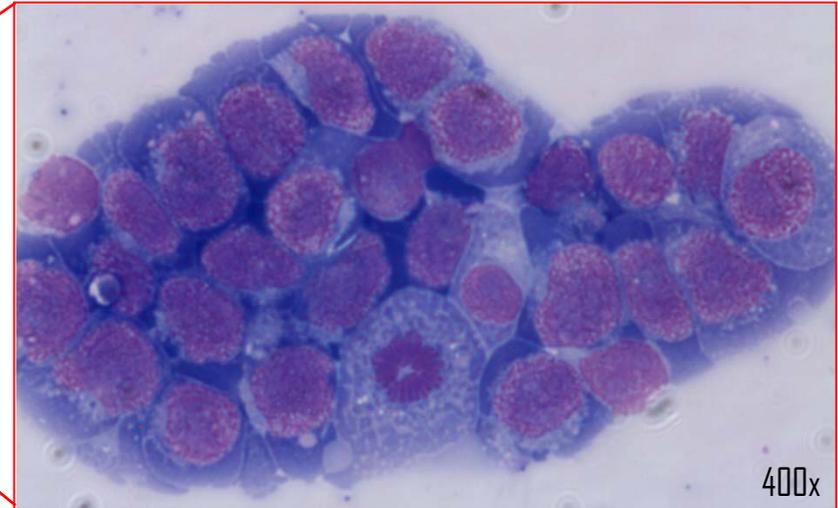
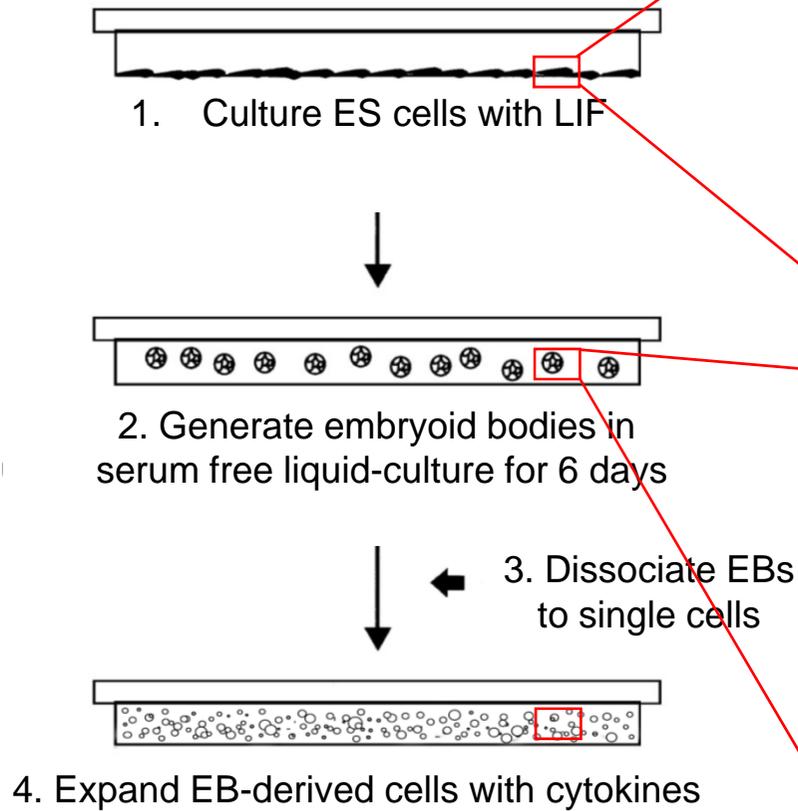


Pluripotente Zellen

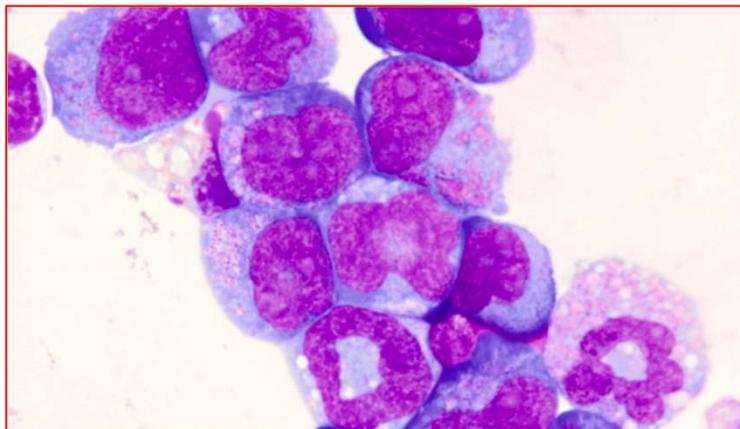


Blutzellen

Blut aus pluripotenten Zellen



May-Grünwald/GIEMSA-stain



Dietrich Lesinski, Niels Heinz,
Hannes Klump, Bernhard Schiedlmeier

Perspektiven

- Gentherapie öffnet neue Möglichkeiten für schwere Erkrankungen
- Aussichten maßgeblich abhängig von Vektorentwicklung, Krankheitsmodellen und Toxikologie
- Zunehmende therapeutische Breite der Gentherapie wahrscheinlich
- iPS Stammzelltechnologien interessant, aber noch viele offene Fragen (genetische Stabilität, funktionelle Integration, Langzeitverhalten)
- Großes offenes Feld: Gentransfer zur Kontrolle des Zellverhaltens



Vielen Dank

Collaborators

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Toni Cathomen
Punam Malik
Manuel Grez
Adrian Thrasher
Boris Fehse
Hannes Klump
Christof von Kalle
David Williams
Hans Schöler
Timm Schröder
Brigitte Schlegelberger

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Martijn Brugman, Zhixiong Li, Adrian Schwarzer, Johann Meyer,
Tamaryin Godinho, Ute Modlich

HSC expansion, ES-HC: Niels Heinz, Dietrich Lesinski,
Birgitta Ehrnström, Bernhard Schiedlmeier

