



Transfer of Autologous *ex vivo*-Modified T-Lymphocytes for Gene Therapy of HIV Infection

Jan van Lunzen

Retrovirus life cycle

Entry inhibitors

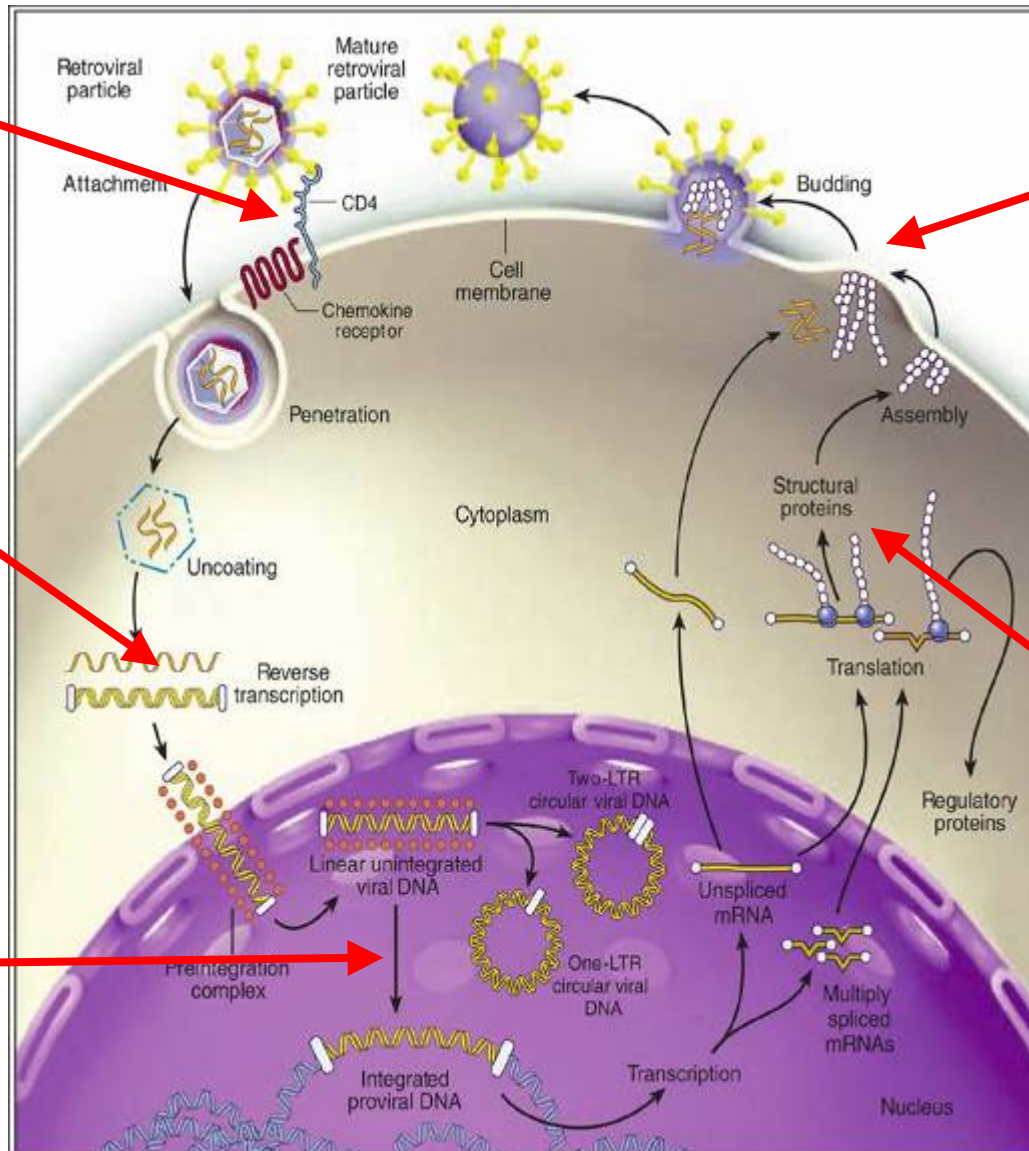
ENF MRV
VCV TNX355
AMD11070

Reverse transcriptase inhibitors

ZDV NVP
ddI DLV
ddC EFV
d4T 3TC
FTC ABC
TDF

Integrase inhibitors

GS9137
MK0518
others



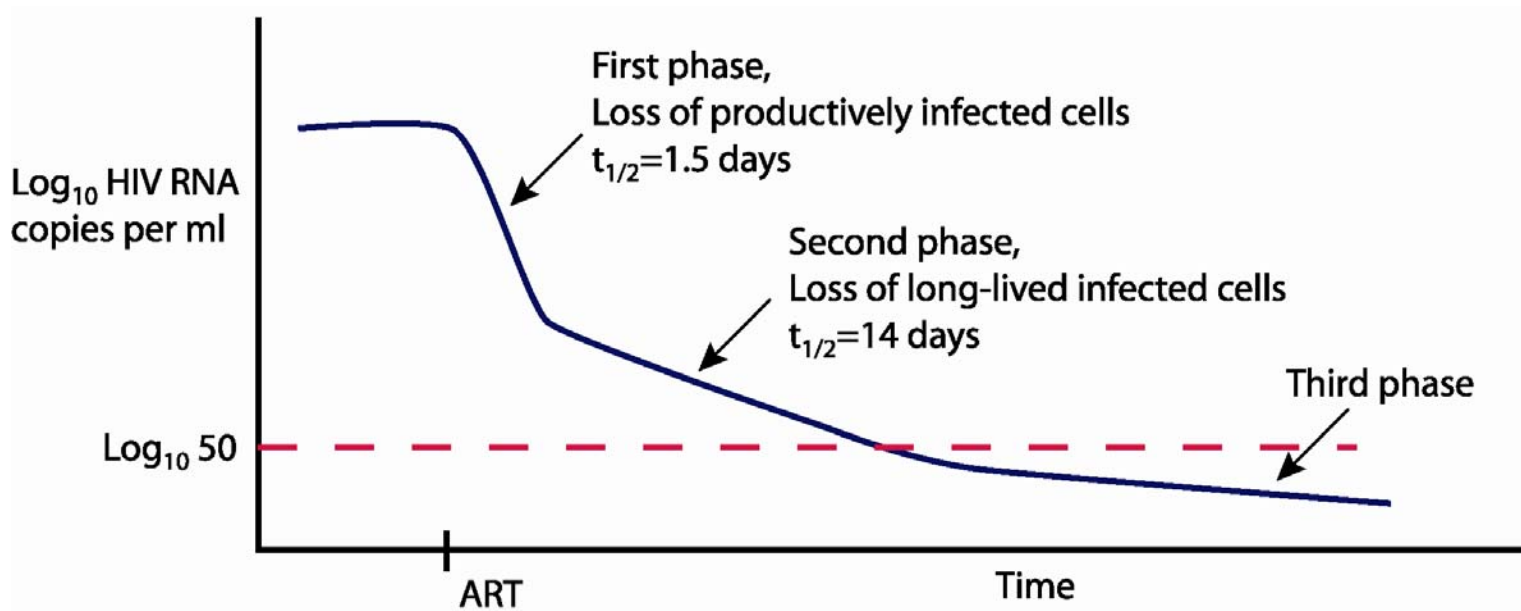
Protease inhibitors

SQV IDV
RTV NFV
FPV LPV
ATV TPV
DRV

Maturation inhibitor

bevirimat

Viral Dynamics Infer Viral Production



Limits of HAART

- Toxicity
- Resistance
- Costs
- Latency/Persistence

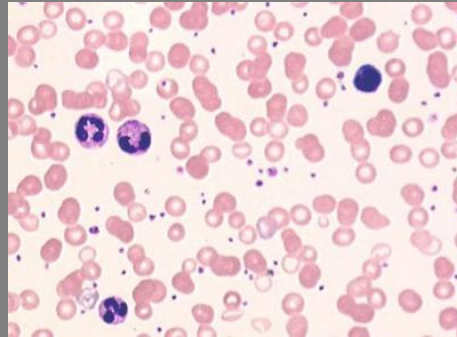


HAART: side effects

Reversible



Gastro-intestinal



Hematopoiesis



CNS

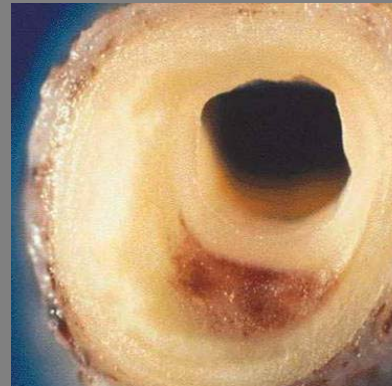
Not reversible ?



bones



kidneys

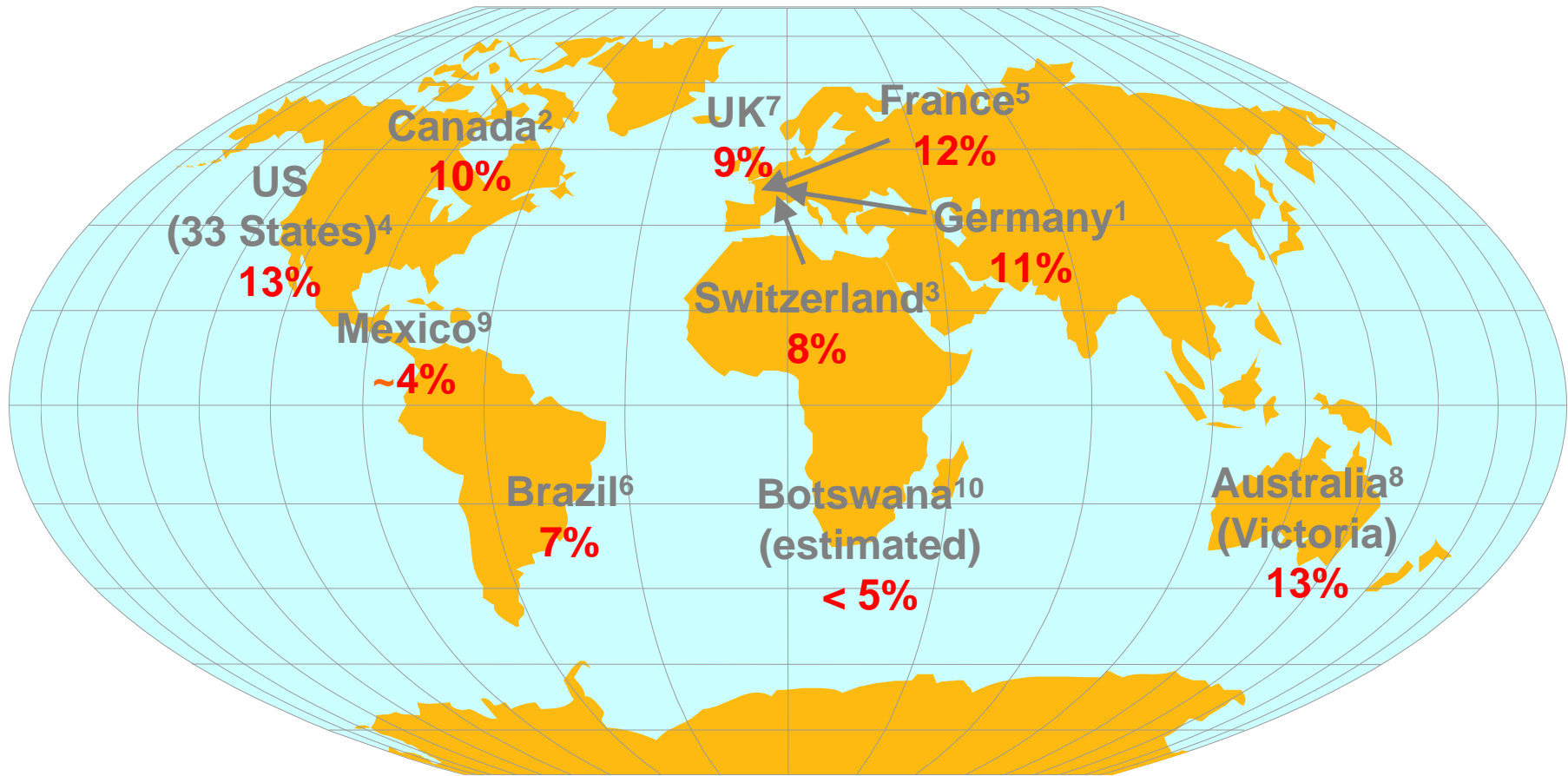


Dyslipidemia/CVD



Lipoatrophy

Prevalence of drug resistance in treatment-naïve patients



Oette M, et al. *J Acquir Immune Defic Syndr* 2006; **41**:573–581.

2. Brooks JI, et al. *Antivir Ther* 2006; **11** (suppl 1):S119 (abstract 106)

3. Yerly S, et al. *Antivir Ther* 2006; **11** (suppl 1):S118 (abstract 105)

4. Ross LL, et al. *Antivir Ther* 2006; **11** (suppl 1):S120 (abstract 107)

5. Chaiz ML, et al. *Antivir Ther* 2006; **11** (suppl 1):S123 (abstract 110)

6. Brindeiro RM, et al. *AIDS* 2003; **17**:1063–1069

7. Health Protection Agency. *CDR Weekly* 2006; 16

8. Middleton T, et al. *Antivir Ther* 2004; **9**:S110

9. Escoto-Delgadillo M, et al. *XV International AIDS Conference* 2004; abst B11496

10. Vardavas R & Blower S. *Antivir Ther* 2005; **10** (suppl 1):S154 (abstract 141)

Kosten

- Medikation 10.000-15.000 US\$/Jahr
- Medikation senkt Gesamttherapiekosten
- Fortgeschrittener ID 2.5-fach höhere Kosten
- 71-84% der Gesamtkosten für Medikation
- 2% der Kosten für Arzt, etwa 360 US\$/Jahr
- Tatsächlicher Bedarf 1.500-2.000 US\$/Jahr
- Incremental cost per LYG 12.000-14.500 US\$
- Median progression time 3.8 y vs. 13.3 y

Wo persistiert HIV?

- Latent infizierte, ruhende CD4+ T-Zellen
- Monozyten/Makrophagen
- Astroglia/Mikroglia
- CD34+ Stammzellen?
- Dendritische Zellen?

Latenz versus persistierende Replikation!

Stadien der Latenz

Prä-Integrations Latenz

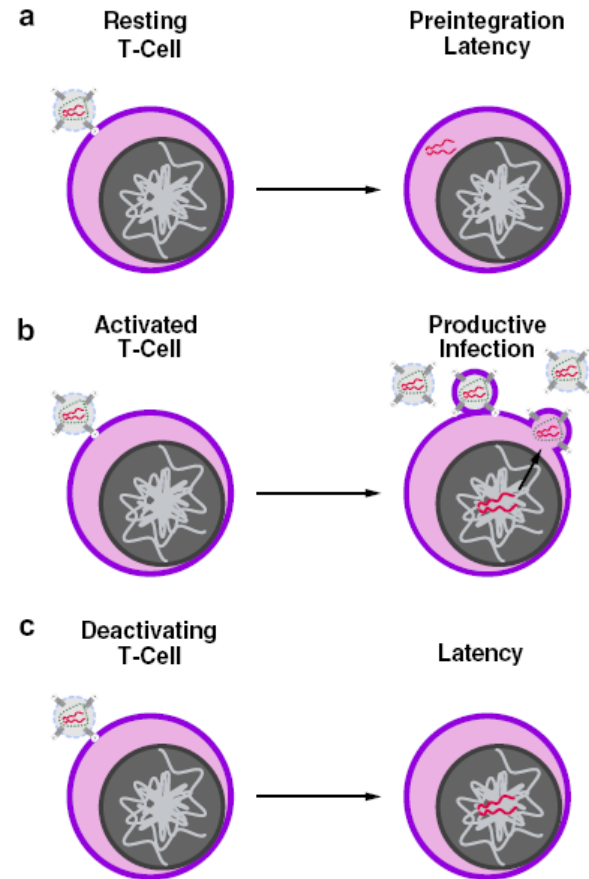
Infektion nicht-aktivierter ruhender T Zellen:
nicht-integrierte (zytoplasmatische) provirale DNA

Produktive Infektion

Infektion aktivierter T Zellen:
integrierte transkriptions-aktive provirale DNA

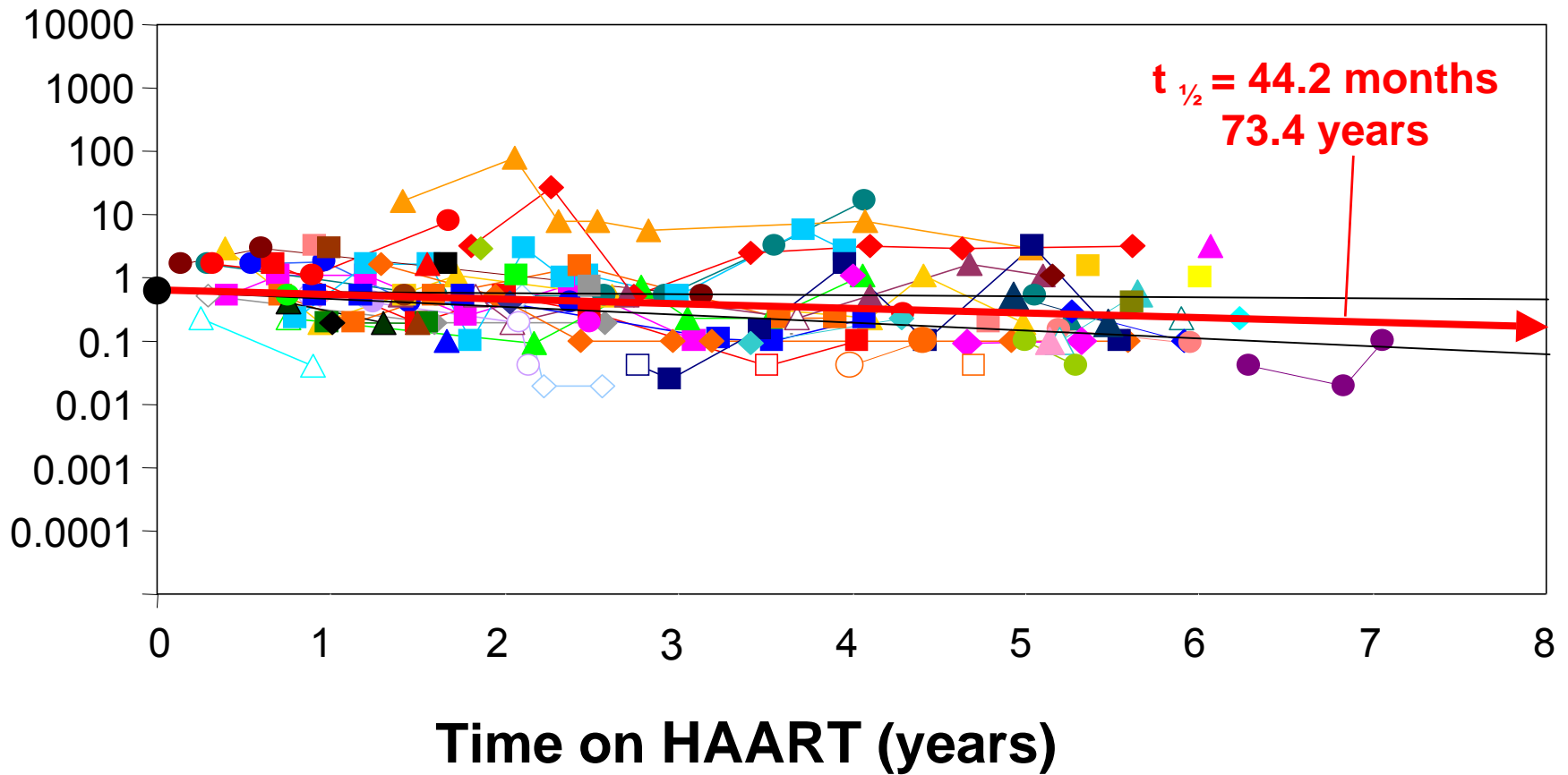
Post-Integrations Latenz

Infektion von Zellen, welche aus einem aktiviertem
in einen ruhenden Zustand zurückkehren:
integrierte transkriptions-inaktive provirale DNA



Frequency of Latently Infected CD4+ T Cells as a Function of Time on HAART

Frequency (per 10^6 resting CD4 cells)



Study Phase I/II

Immune Therapy of advanced HIV-1 Infection by Transduced Autologous T Helper Cells Expressing a Peptide which Inhibits Viral Entry - Results of a Phase I Pilot Study -

Jan van Lunzen

University Medical Center Hamburg-Eppendorf

Germany



**Universitätsklinikum
Hamburg-Eppendorf**

Success of gene therapy

The main reason for failure of gene therapy:

The number of gene-modified cells is too low.

Success with a low number of transduced cells is possible if

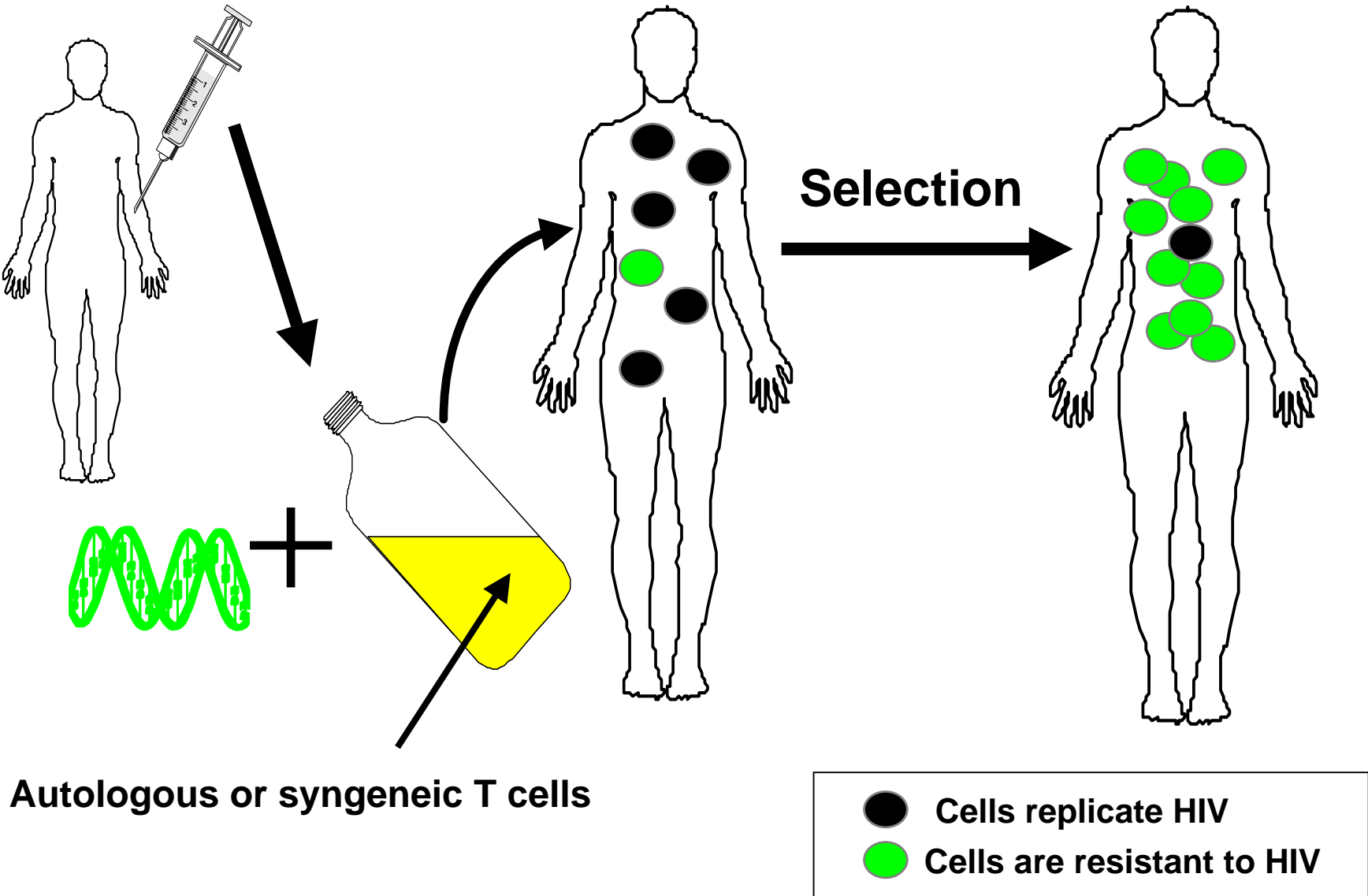
- the gene product has a therapeutic effect on non-modified cells also (e.g. secreted, immunizing gene product).

-> ***bystander effect***

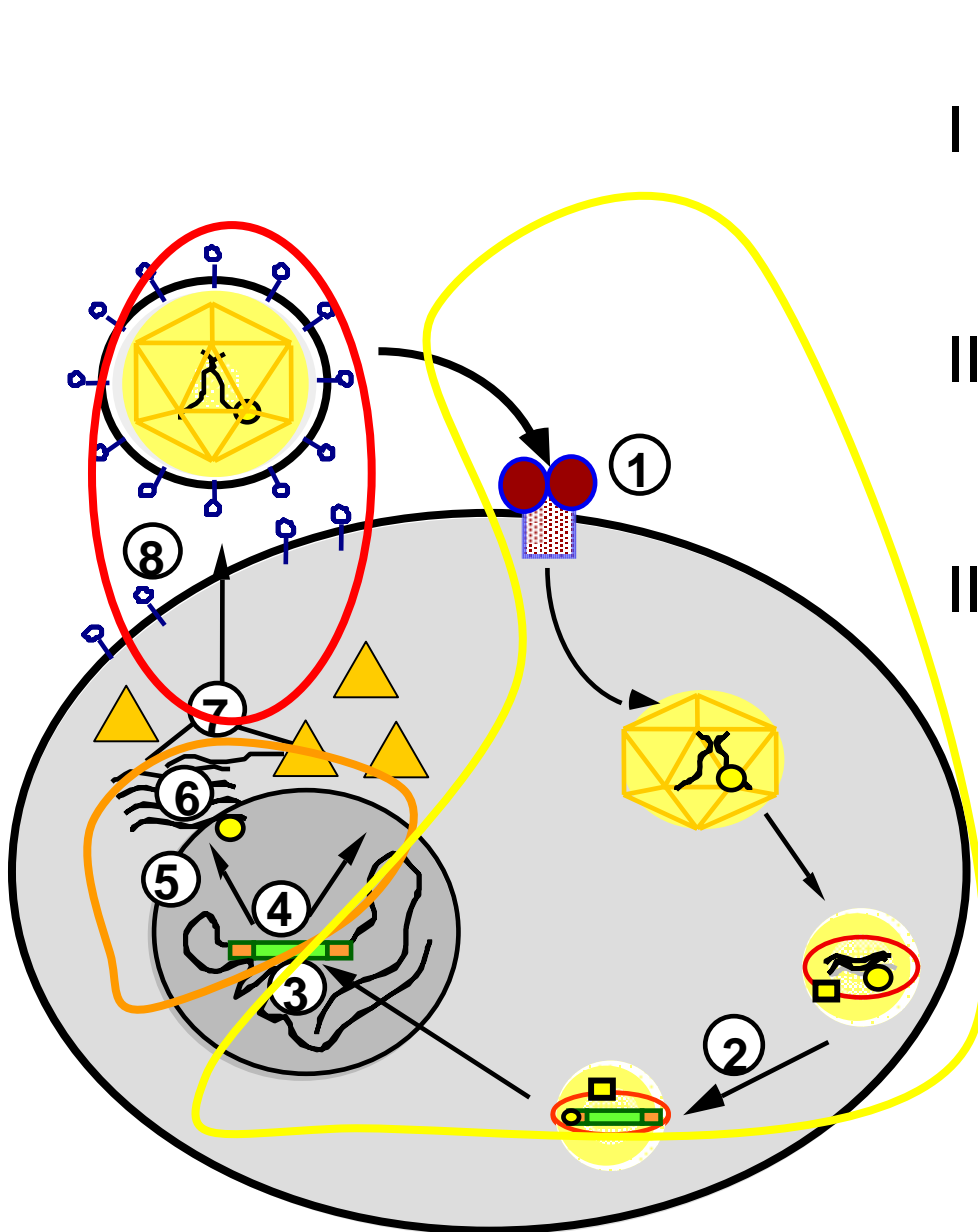
- the transferred gene confers a selective advantage to the gene-modified cell.

-> ***in vivo selection***

In vivo selection of transduced cells

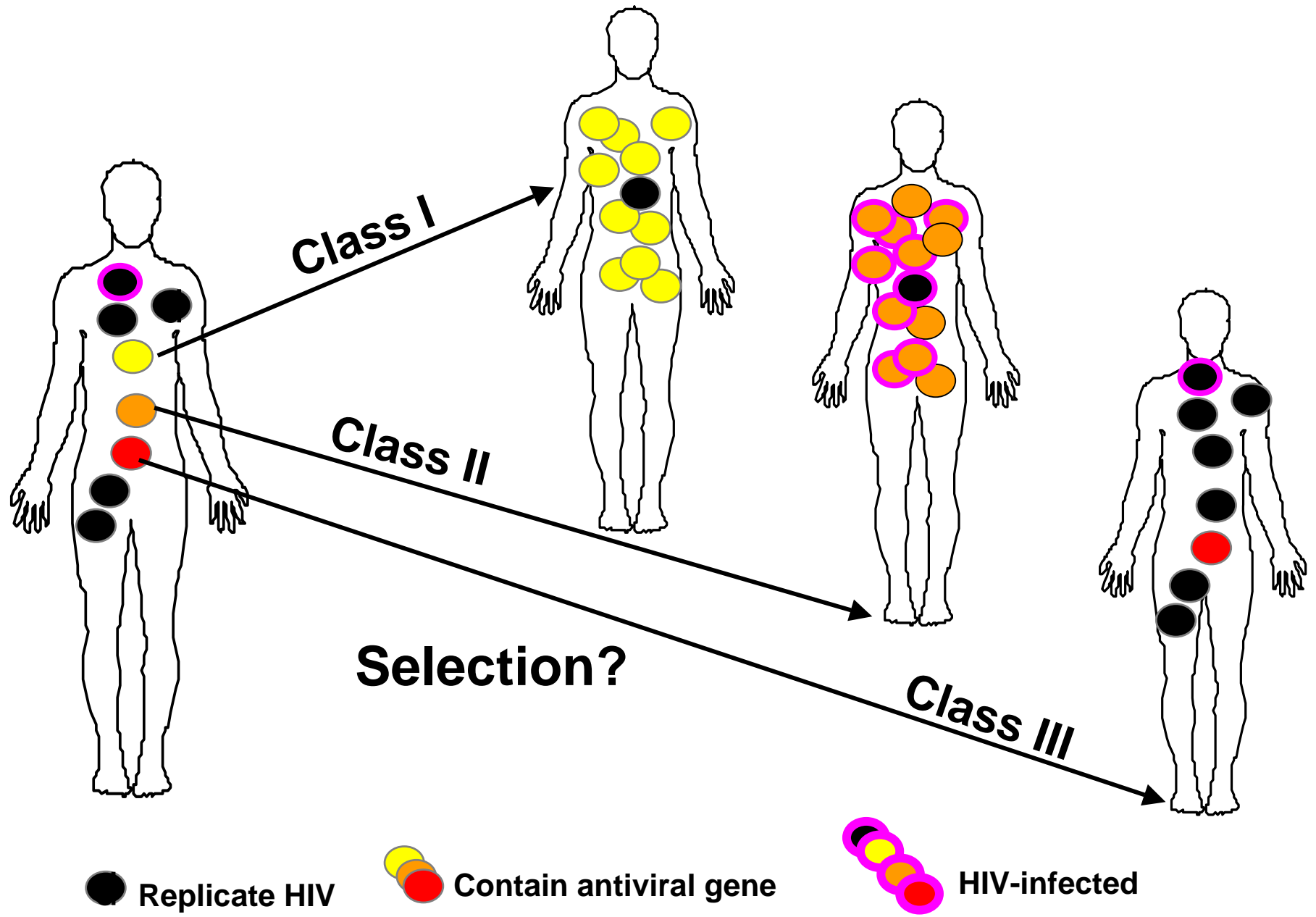


Antiviral genes

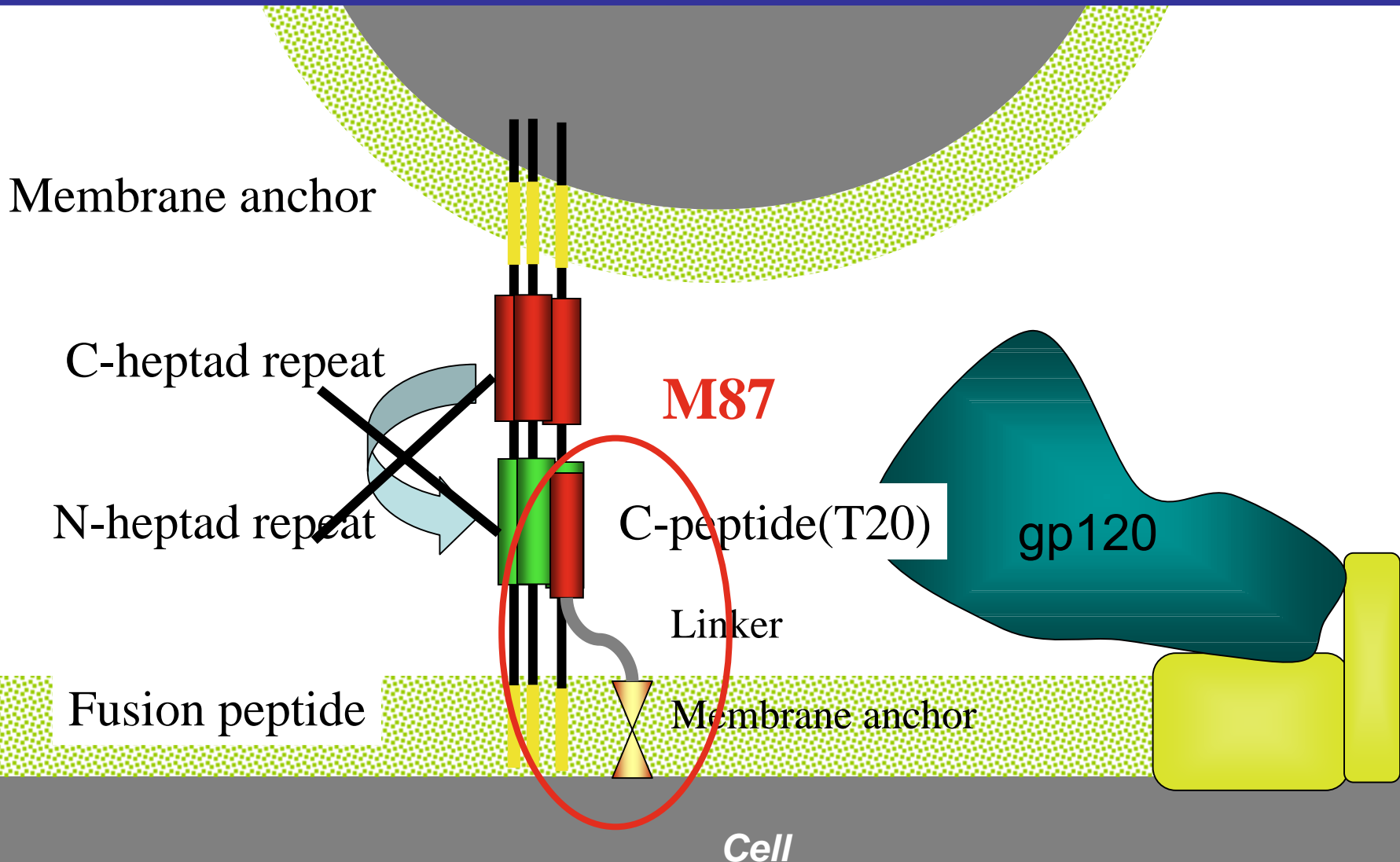


- I {
 - 1. Secreted neutralizing mabs
 - 2. membrane-anchored peptides
 - 3. scFv to RT
 - 3. scFv to IN
- II {
 - 4. Td Tat, TAR decoy
 - 5. Td Rev, RRE, mEIF-5A
 - 6. Anti-sense RNA, Ribozymes
- III {
 - 7. td Gag, pbs decoy
 - 8. Gag-Nuclease

Accumulation of gene-modified cells?



The membrane-anchored fusion inhibitory peptide M87: Mode of action



Structure and components of the ma peptide

Components:

Fusion inhibitory peptide

Derived from C-term heptad repeat

C46: Env aa 636-671

C36: Env aa 646-671

,linker'

mu IgG2

hu IgG1, **IgG2**, IgG4

Synthetic linker

Membrane anchor

hu dLNGFR

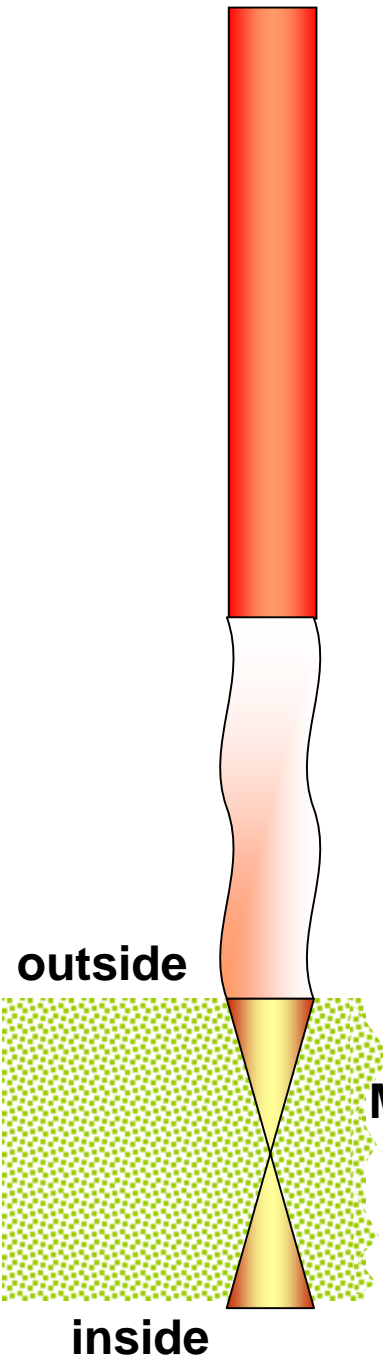
hu tCD34

2 hu GPI anchors

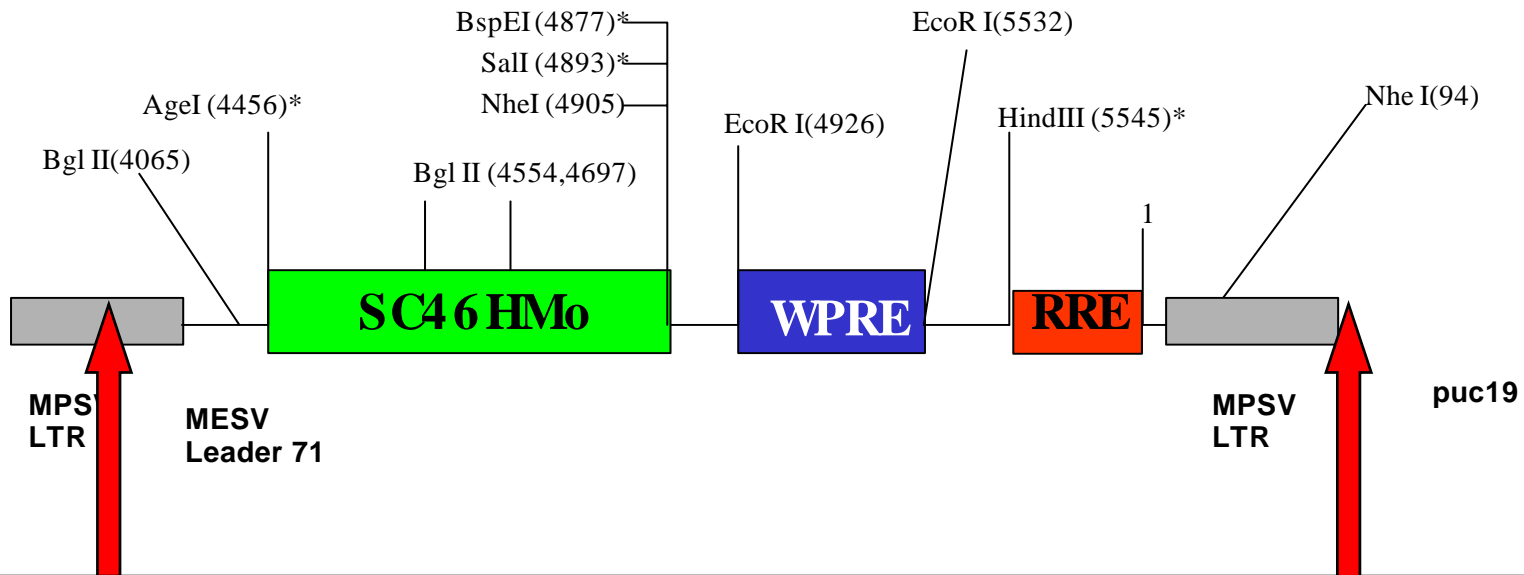
hu zeta chain

Basic construct

Optimized construct



The vector



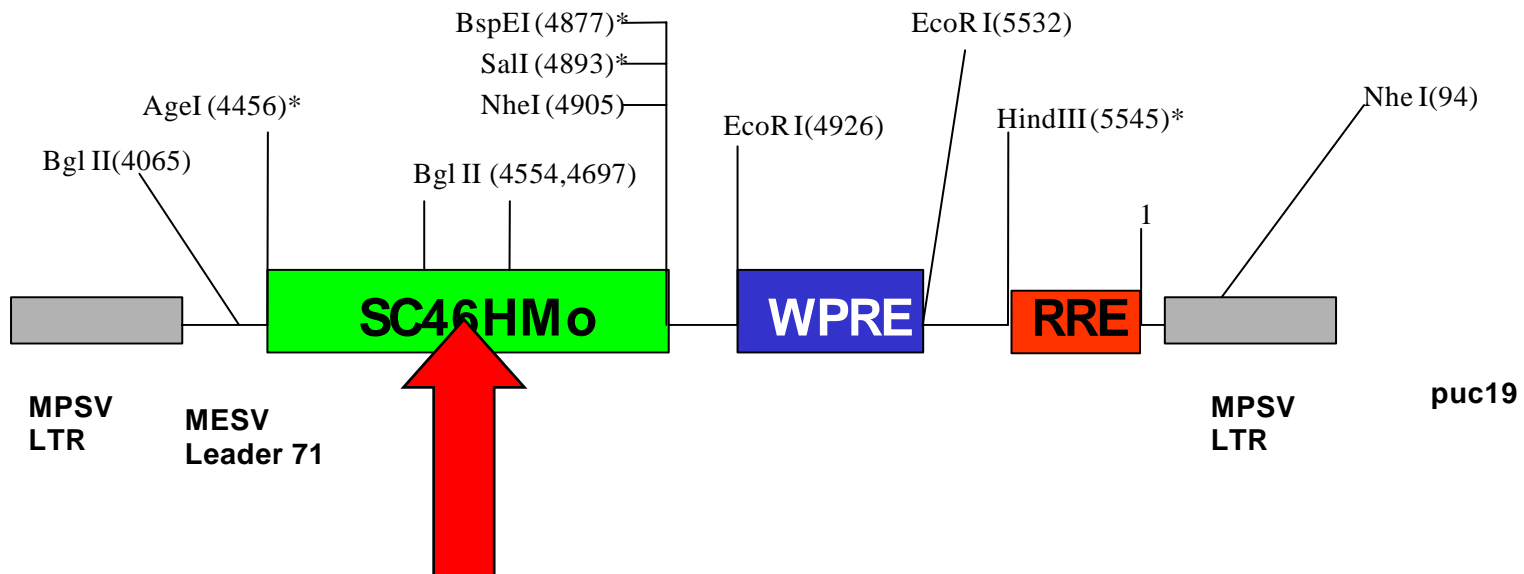
MPSV LTR = „long terminal repeats“ of MPSV

(Myeloproliferative Sarcoma Virus = related to murine leukemia virus)

→ **initiation of transcription**

→ **important for „activation of genes“**

The vector

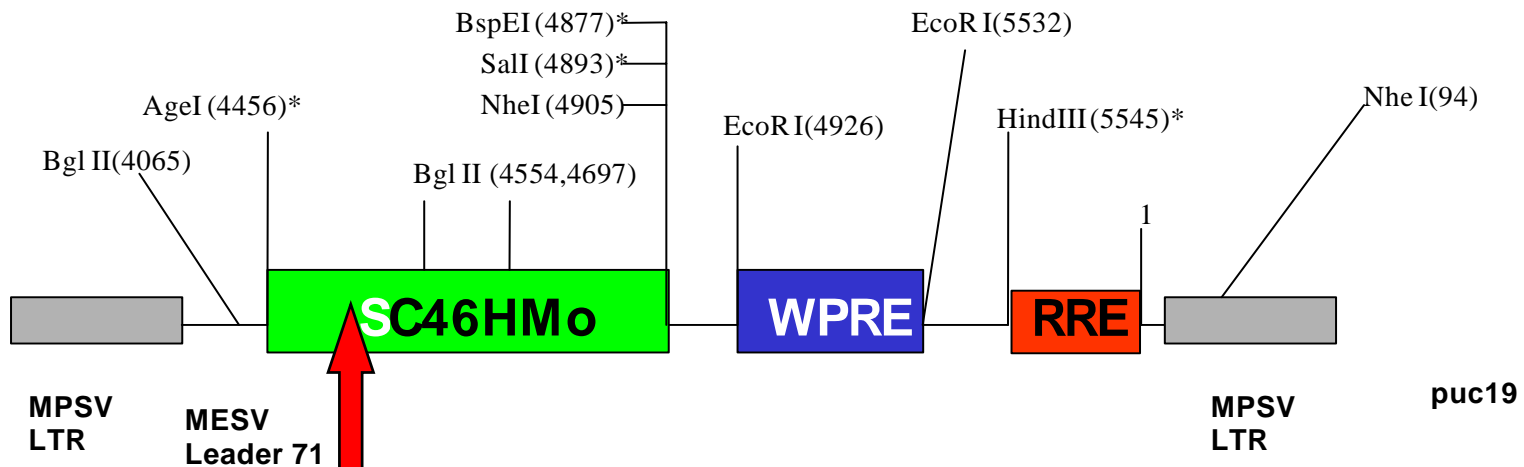


SC46HMo = first antiviral principle

⇒ encodes for peptide, which inhibits fusion of HIV and cell membrane – close to T-20

⇒ composed of three parts

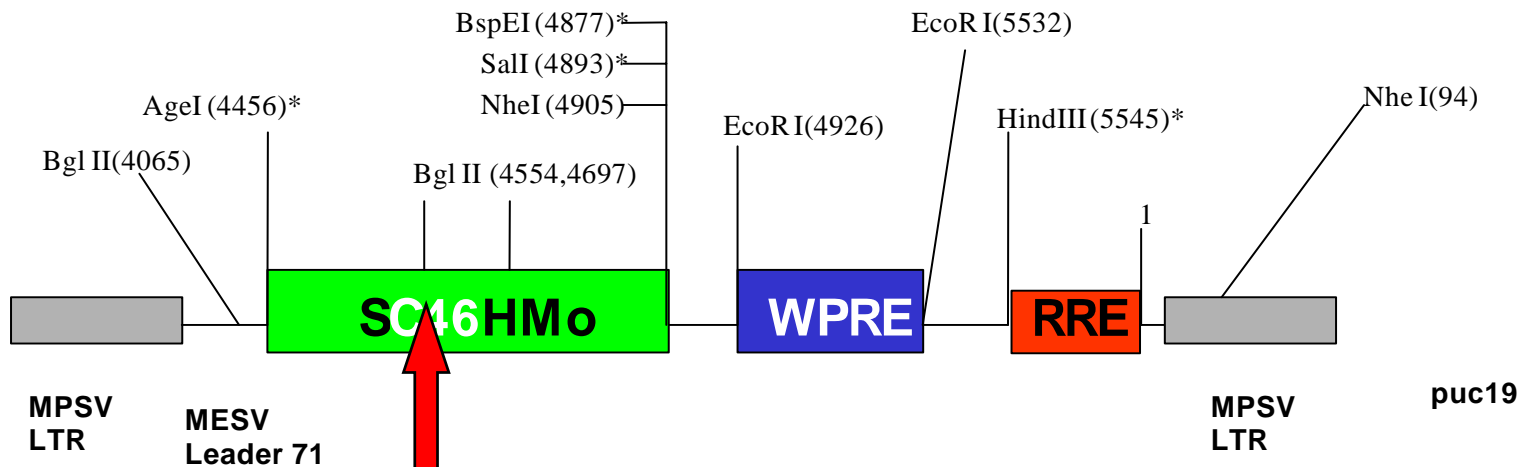
The vector



S = signal peptide

⇒ induces translocation of C46-peptide to cell membrane

The vector



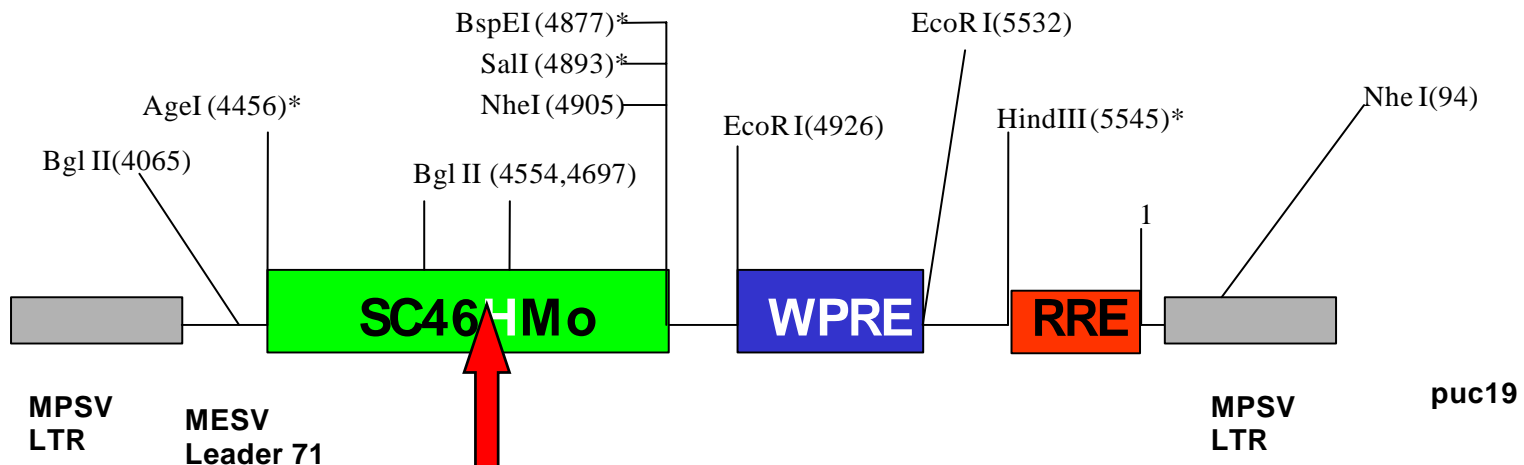
C46 = fusion inhibitor

⇒ originates from C-domain of HIV-gp41

⇒ analogue of T-20, but 10 AA longer

⇒ inhibits T-20 resistant mutants

The vector

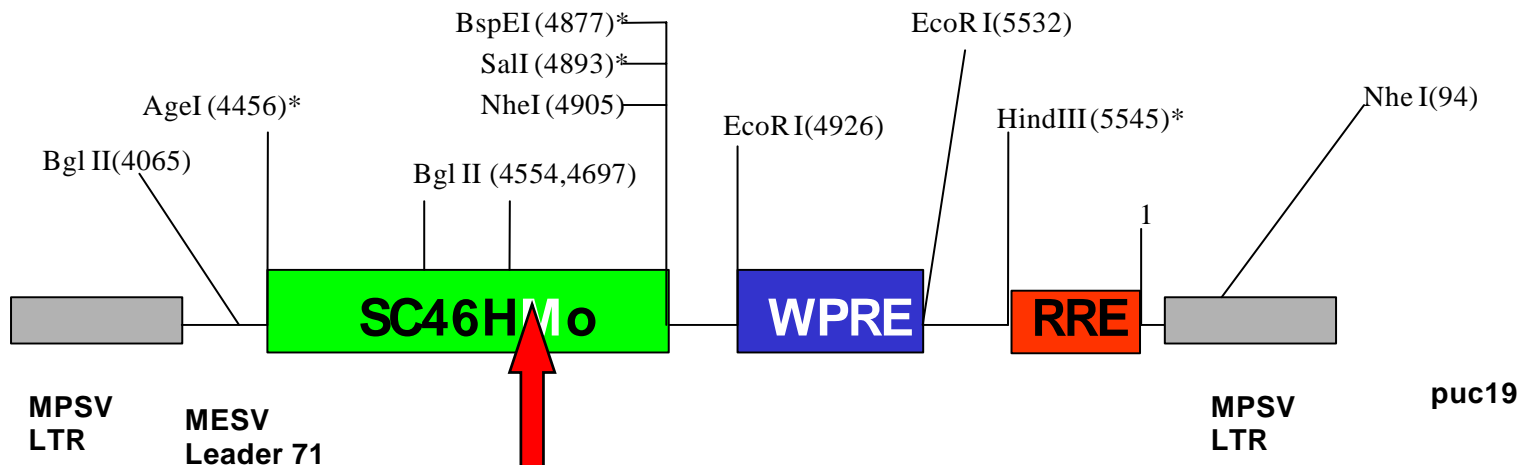


H = „linker“

⇒ analogue of the hinge-region of human IgG-2

⇒ facilitates steric flexibility of C46

The vector

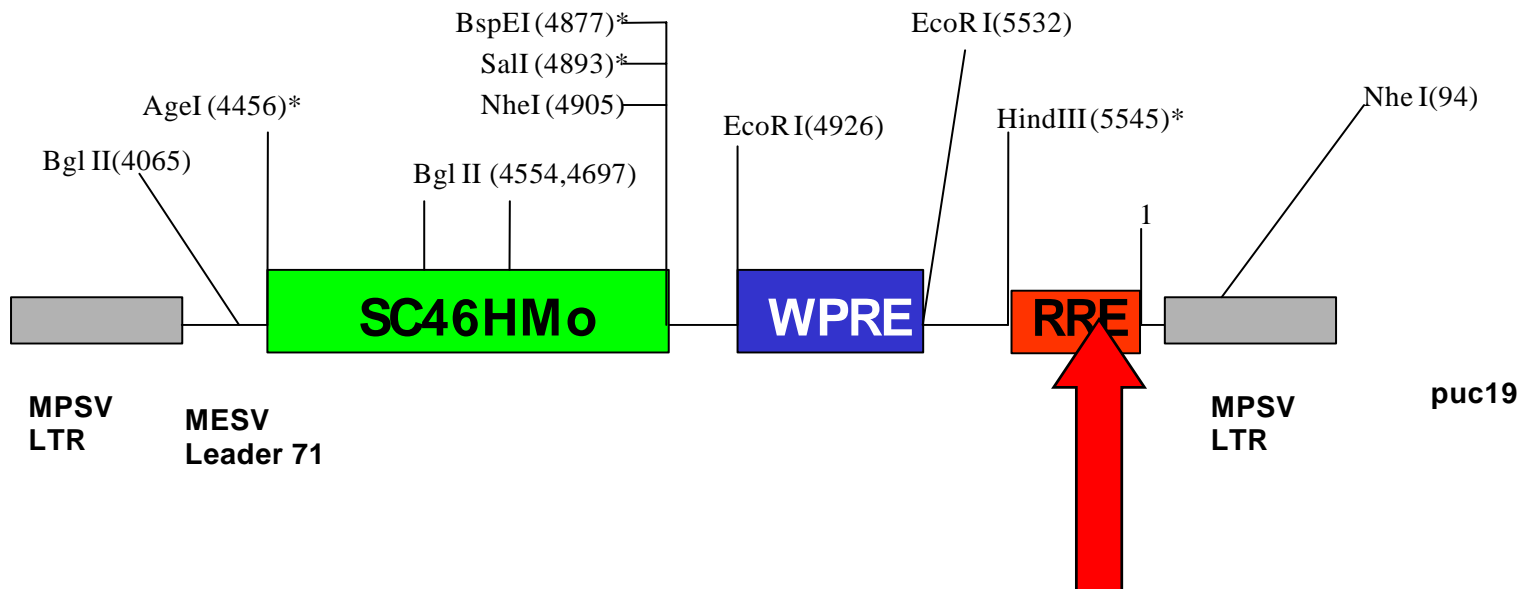


M = „membrane anchor“

⇒ analogue of the transmembrane domain of human CD34

⇒ anchors C46 into the cell membrane

The vector

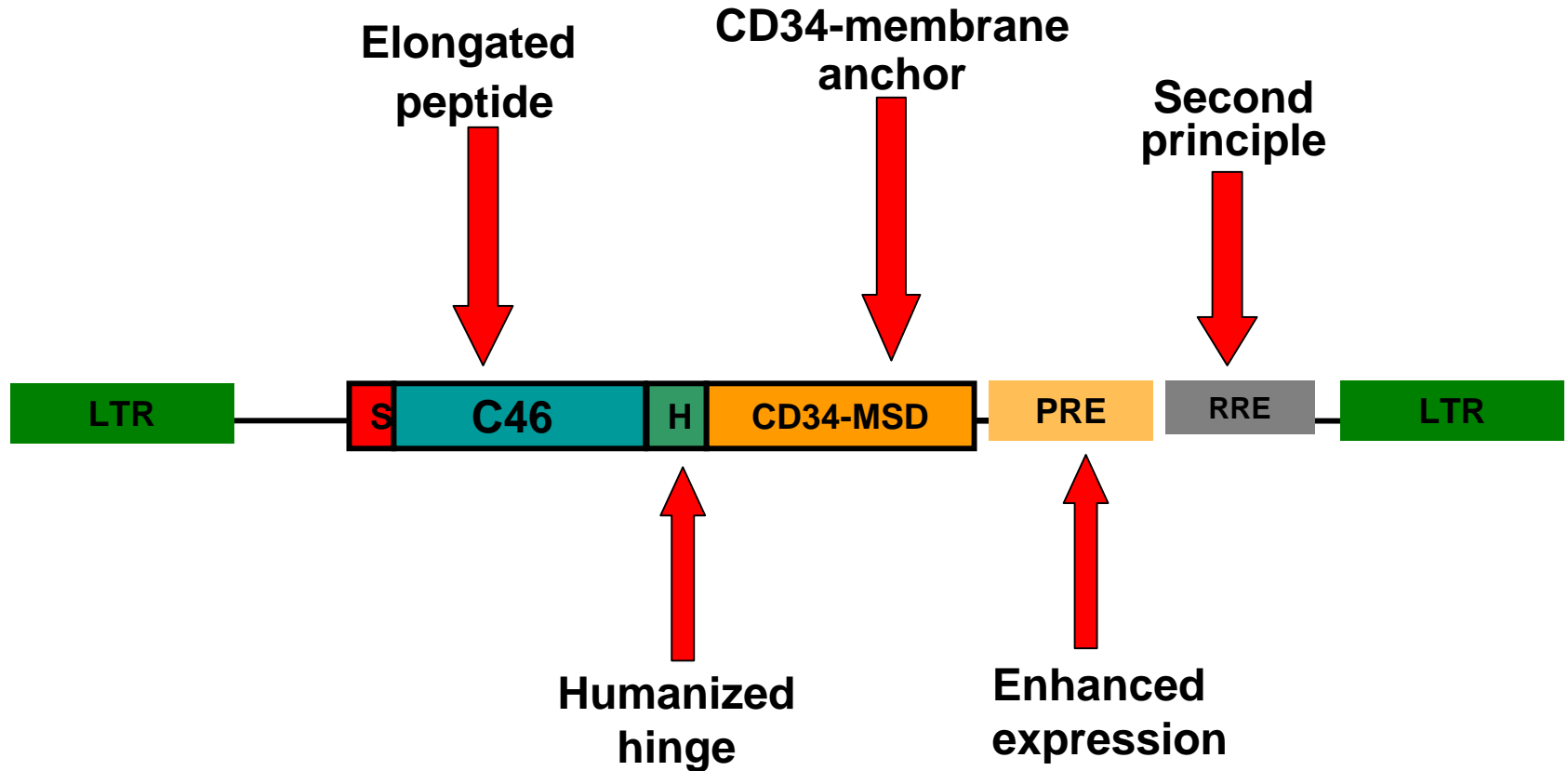


RRE-decoy = second antiviral principle

⇒ binds HIV-rev in the nucleus and inhibits it

⇒ unspliced HIV-mRNA cannot be transported from the nucleus into the cytoplasm → replication inhibited

A retroviral vector expressing membrane-anchored C-peptide and an RRE decoy: M87o



Preclinical toxicity studies

- **Toxicity in cell lines: long-term growth**
- **Tox. in primary T cells: ELISpot, surface marker, gene expression analysis**
- **Transforming potential of M87o in Rat-1 cells by soft agar colony growth**
- **Toxicity in mice: >tx of transduced bone marrow, 2nd tx, 8 mice**
 - > tx of transduced T cells, 8 mice**
- **Toxicity in rhesus macaques: T cell transfer in 2 animals**

In none of the studies toxicity or immunogenicity of M87o or the M87o-transduced cells was observed

Human Phase I/II Study Design

- Screening
- Lymphapheresis
- Ex vivo proliferation with anti-CD3/CD28
- Transduction with vector
- Harvesting & biosafety testing
- Retransfusion & follow-up
- Gene marking, CD4, virus load
- Change of ART possible 3 mths. after Tx.



Current Status of Clinical Study Phase I/II

- ▶ Number of patients treated: 10
- ▶ Data available for 44 (36-48) months median follow-up
- ▶ No SAE`s, all patients well and alive
- ▶ Mean total number of T-cells infused: 7.20×10^9



Phase I/II Study Inclusion Criteria

Main inclusion criteria

- Male and female patients aged 18 to 60
- Documented HIV infection
- HAART experienced with resistance and/or intolerability
- Pre-therapy with 3 drug classes, MDR viruses
- Stable ART >3 mths. before screening
- Viral load >5000 copies per ml
- CD4+ counts <200 per μ l or below 15 % of total T-cells
- Karnofsky performance >80%, life expectancy >6 months

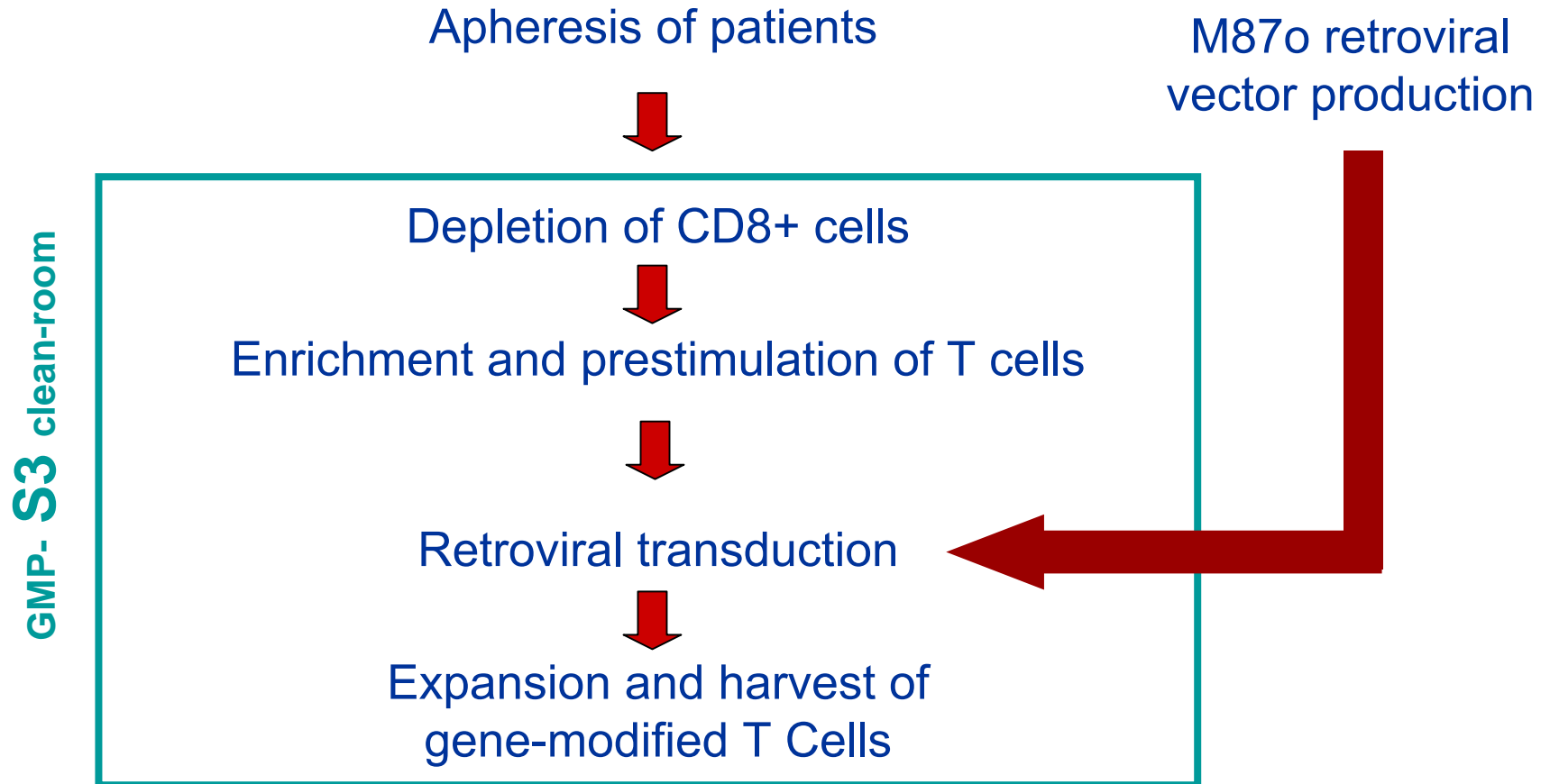


Baseline characteristics

n	10 pts. (planned: 15 pts.)
sex	male: 10/10
age (median)	45,5 yrs. (33-54)
Full blown AIDS (CDC-C)	7/10
CD4+ absolute (median)	115 (40-250)
CD4+ percentage (median)	9 (3,0-13,8)
HIV-viral load (median)	5,1 log₁₀ (4,6-5,8)

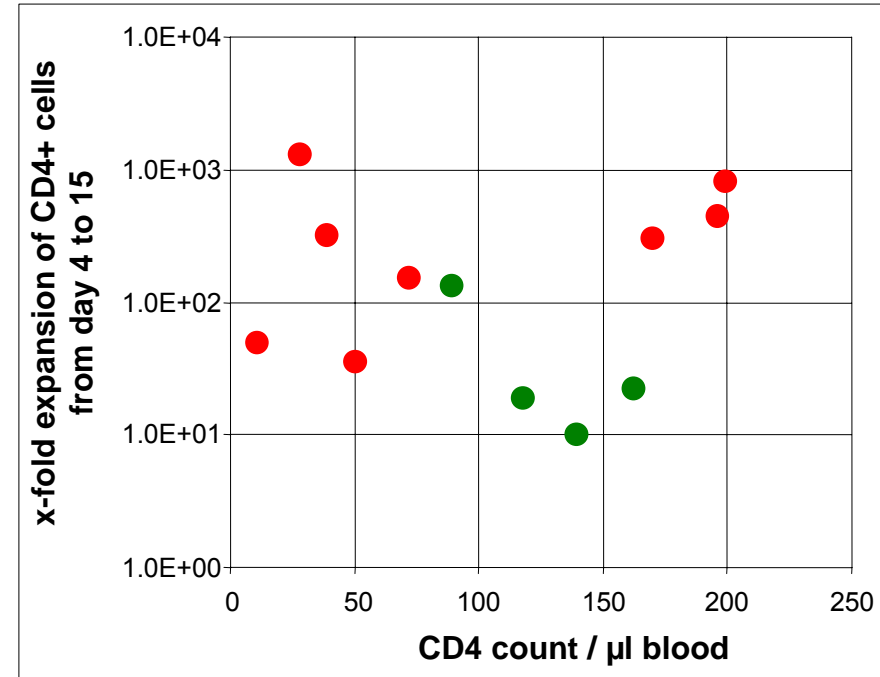
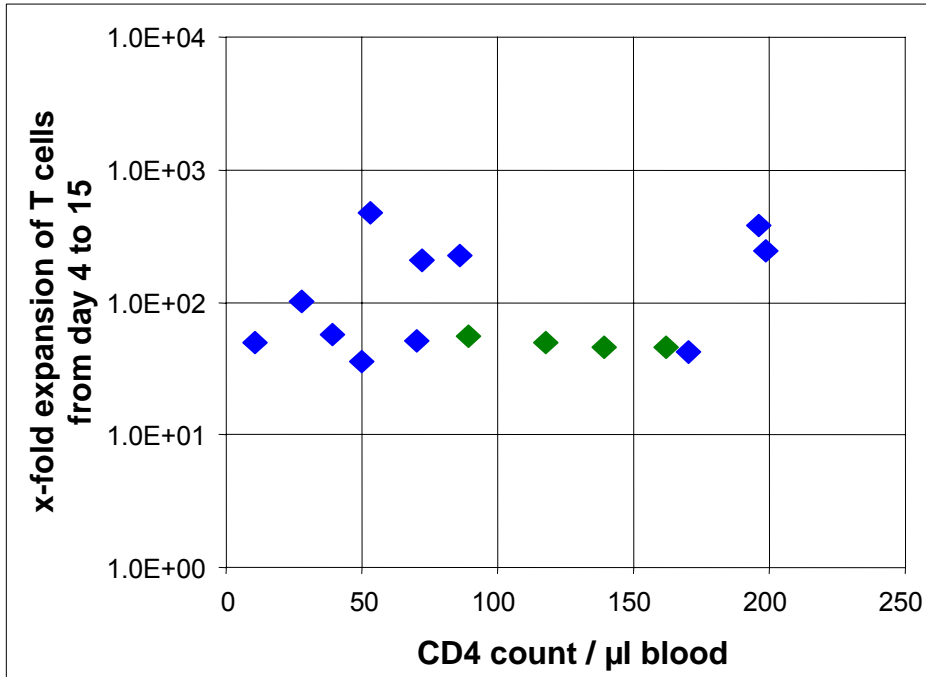


Generation of study medication



No correlation between CD4 count and proliferation

HIV positive donors - small scale



- ◆ T lymphocytes (CD8+ T cells and CD4+ T cells)
- CD4+ T cells
- ◆ and ● with magnetic concentration of CD3+ cells

- Transduction efficacy (large scale): 25-30%
- Fold-expansion after 10-12 days: 50 to 100-fold -> minimum of 10^9 M87o+ T-cells


A substantial number of T cells can be infused into patients

		1 st T-cell infusion	2 nd T-cell infusion	Total
Total volume of T-cells infused (mL)	Mean±SD	68.8±2.30	163.1±78.56	231.8±78.82
	Median	70.0	140.0	210.0
	Range	63.0 – 70.0	68.0 – 280.0	138.0 – 350.0
Total number of T-cells (x10 ⁹)	Mean±SD	0.4±0.99	7.5±4.44	7.6±4.44
	Median	0.10	7.58	7.68
	Range	0.1 – 3.2	2.4 – 15.0	2.5 – 15.1
Total number of T-cells modified with M87o (x10 ⁶)	Mean±SD	46.5±80.39	1432.6±985.19	1269.0±876.03
	Median	19.0	1170.0	1037.0
	Range	8.9 – 260.0	380.0 – 3200.0	396.0 – 3225.0



Summary Safety Data

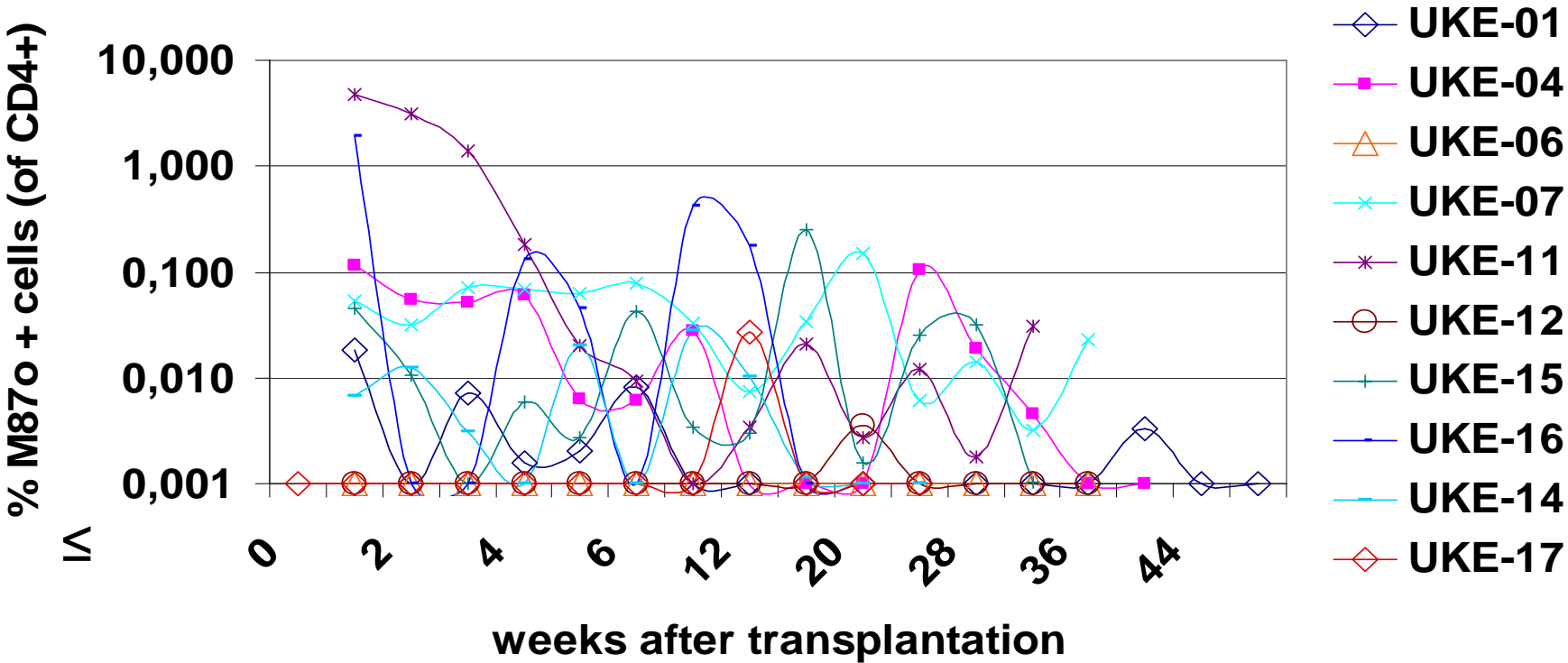
- no treatment-related SAEs, no malignancies (insertional mutagenesis)
- minimal, clinically not relevant changes of safety laboratory parameter
- six patients were considered by investigator to have possibly and two patients probably related AEs (e.g. dizziness, fever; timely related to infusion), mild or moderate, no additional treatment require



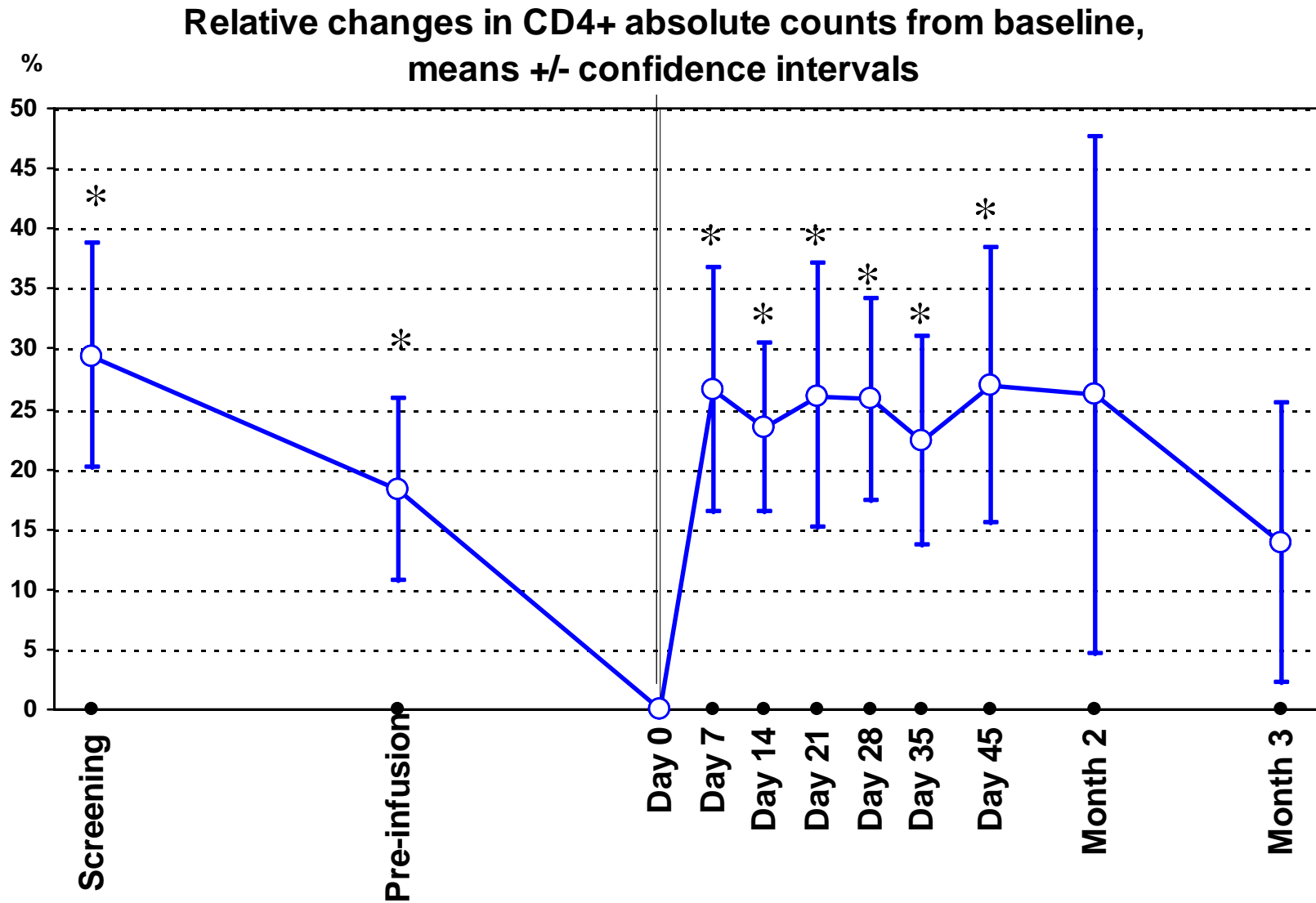
Good tolerability of autologous gene-modified T-cells to treat HIV infection shown in 10 patients with a median follow-up of 44 months

Relatively low levels of gene marking were observed

Marking of CD4+ cells with M87o

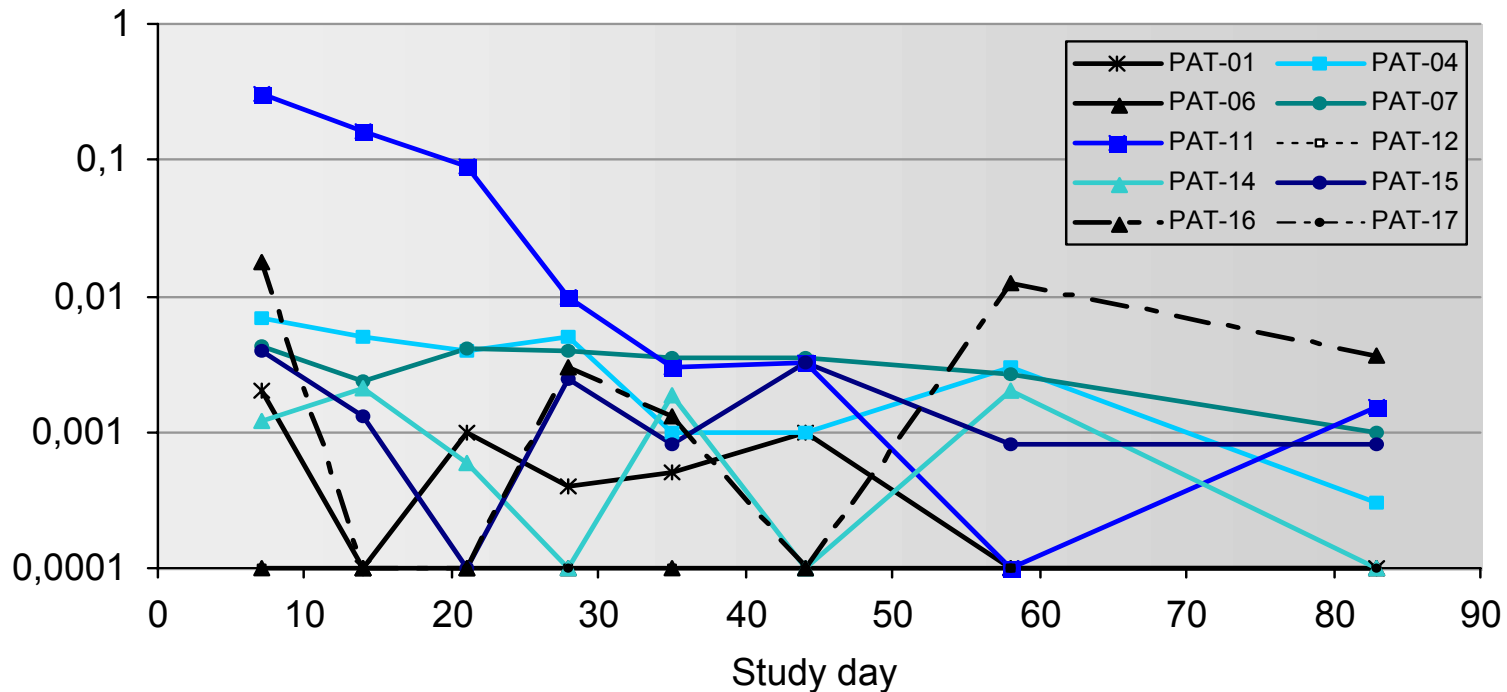


CD4 cells increase significantly after transfer of ex vivo expanded M87o-modified T cells



* Difference to baseline statistically significant, $p < 0.05$

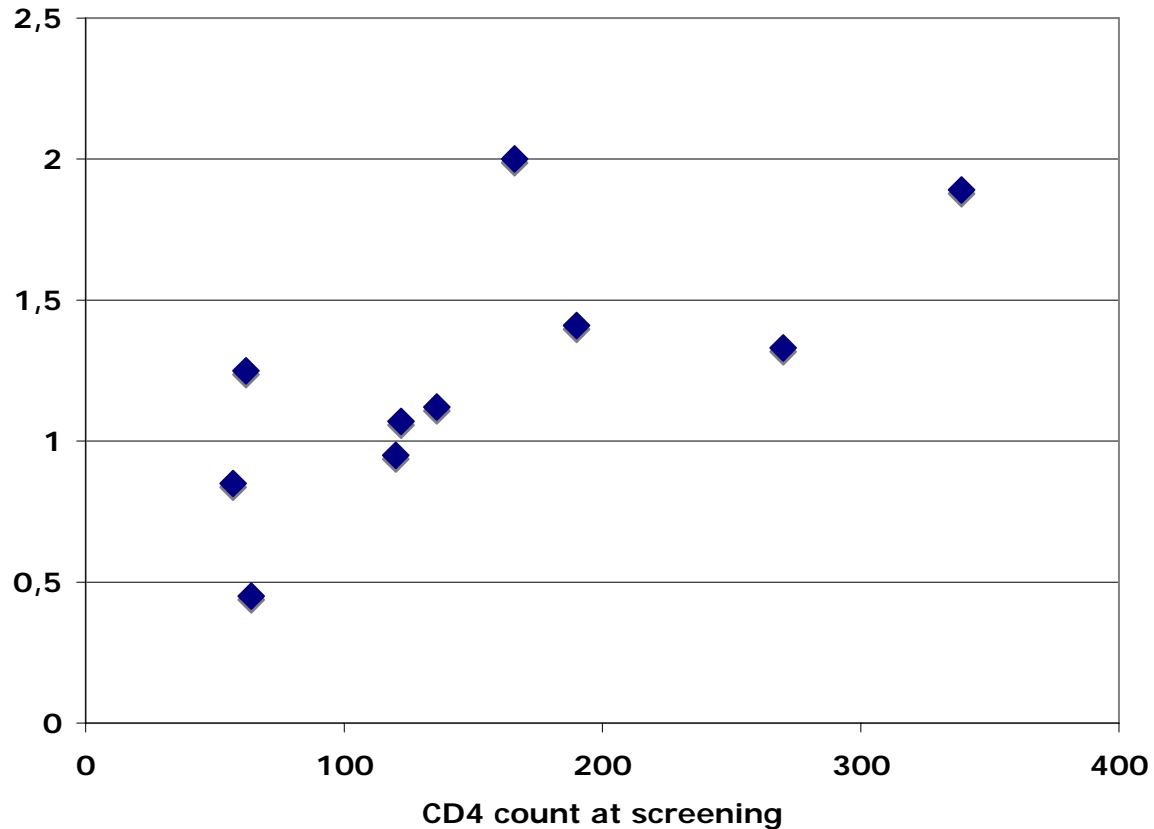
Percentage of transduced CD4+ T cells after transfusion



(% of M87o copies per genome; all patients, colored lines = best CD4 response)

Mean relative CD4 increase @ w12: 43%

Relative increase in CD4 correlates with CD4 count at screening



$P < 0.05$ also at days 7, 14, 28, 35

Conclusions Phase I Study

- CD4 counts rise significantly after cell infusion (mean 43%).
- Patients with higher CD4 counts before treatment tend to show more pronounced relative increase in CD4 ($p < 0.05$).
- Patients with high gene marking tend to show a more pronounced relative increase in CD4 ($p < 0.05$).
- Gene marking is low relative to the CD4 rise.
- No effect observed on viral load.

Summary

- An antiviral gene (M87o) was constructed that inhibits HIV entry into the target cell with great efficiency in cell lines and in primary T cells for a broad range of different virus isolates.
- Infection was inhibited at the level of membrane fusion.
- M87o had no major toxicity in cell lines, primary cells and in animals.
- A phase I clinical trial with autologous transfer of gene-modified T cells was initiated in late-stage patients with HAART failure.
- A significant increase in the number of T-helper cells was observed, despite gene marking levels below 1%.

Acknowledgements

- **UKE, Hamburg:**

Tobias Glaunsinger
Ingrid Stahmer
Hans-Jürgen Stellbrink

- **Fresenius/EUFETS:**

Alexander Alexandrov
Klaus Kühlke

- **GSH, Frankfurt:**

F. Hermann
Dorothee von Laer

- **Primary treating physicians:**

Christoph Mayr
Ari Moll
Hans Heiken
Elke Lauenroth-Mai
Dirk Schürmann
Albrecht Stoehr

Thanks to the very brave patients!

