

Gene Therapy Medicinal Products

(preventive, therapeutic, in-vivo diagnostic use)

genetically modified human cells

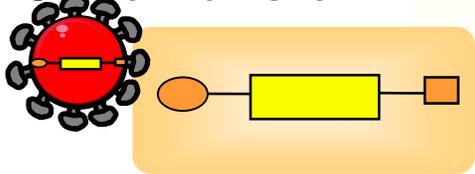
vectors, nucleic acids, replicating micro-organism (not including live vaccines)

cell line

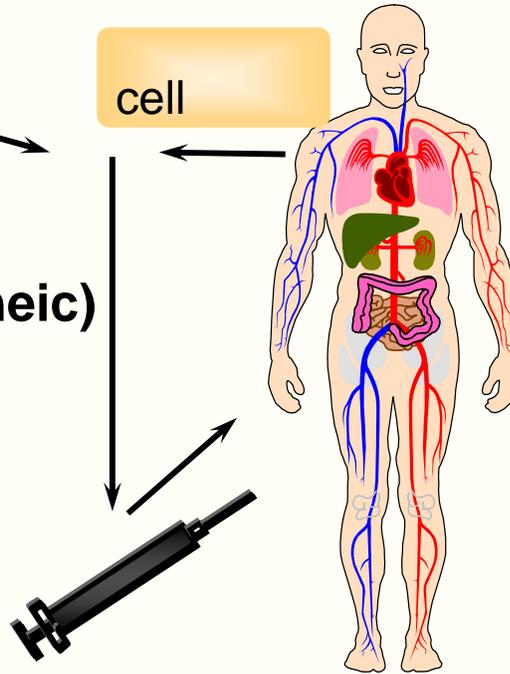
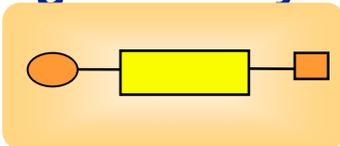
cell

1) Isolation of the target cells (autologous or allogeneic)

2) Gene transfer

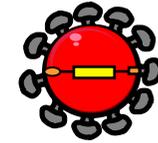


3) Re-Infusion of the genetically modified cells

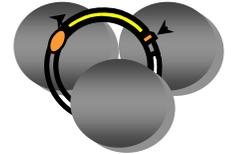


Direct application:

viral vector



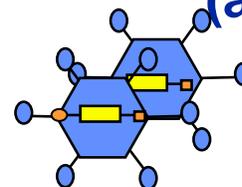
non-viral vector



naked DNA

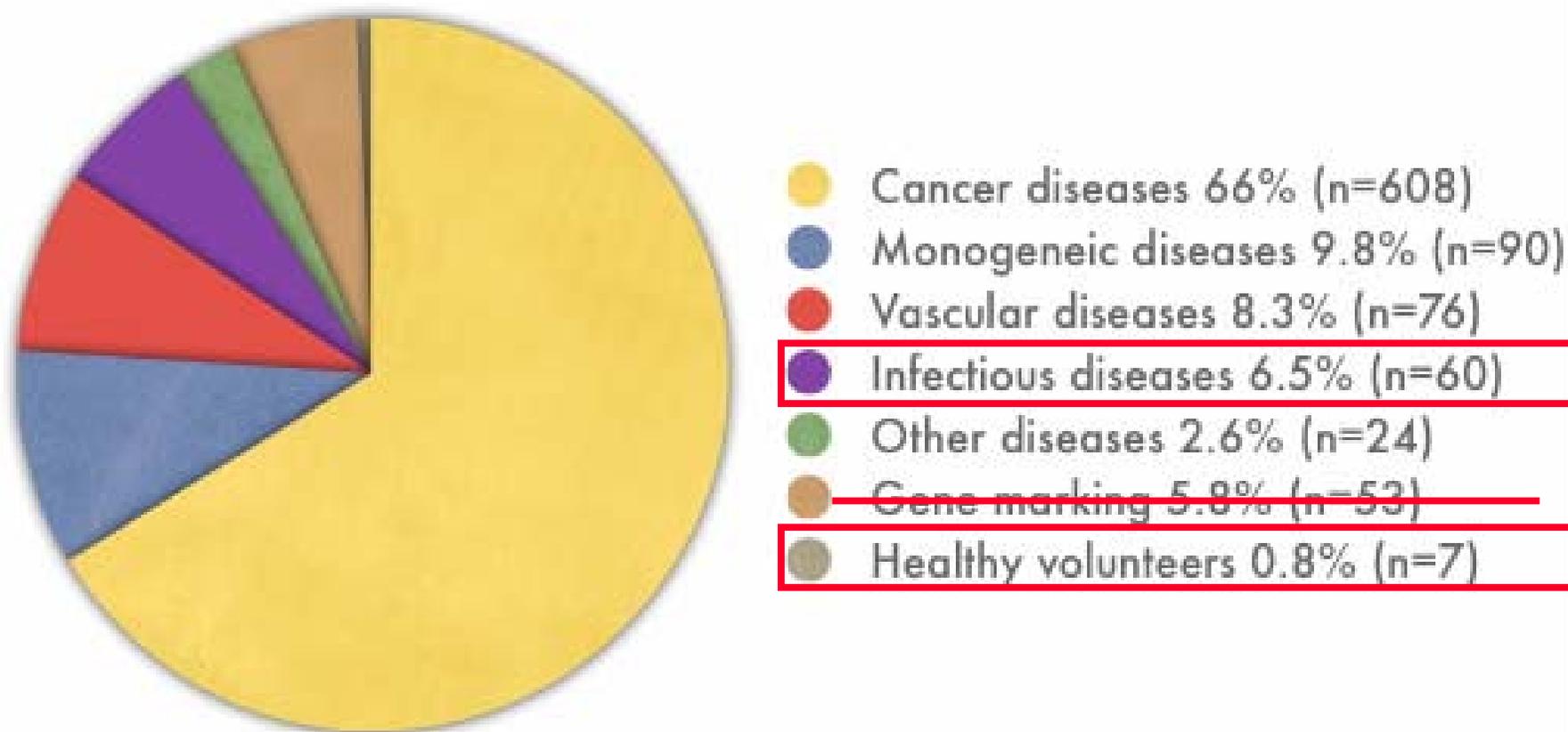


replicating rec. micro-organism (adenovirus, Salmonella)



Target diseases

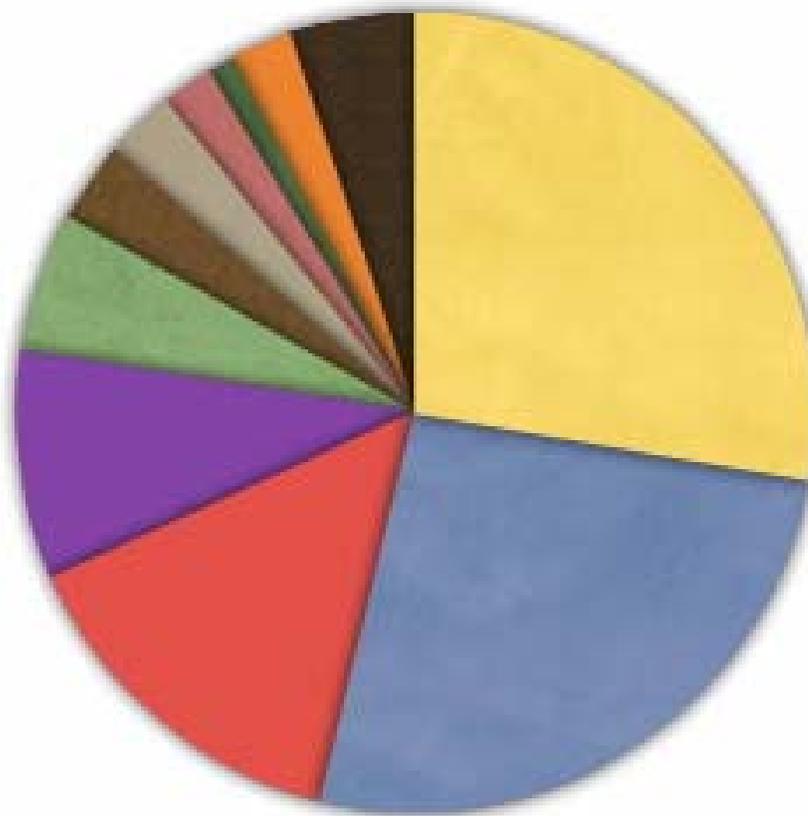
Indications Addressed by Gene Therapy Clinical Trials



Gene transfer vehicles (ex vivo and in vivo)



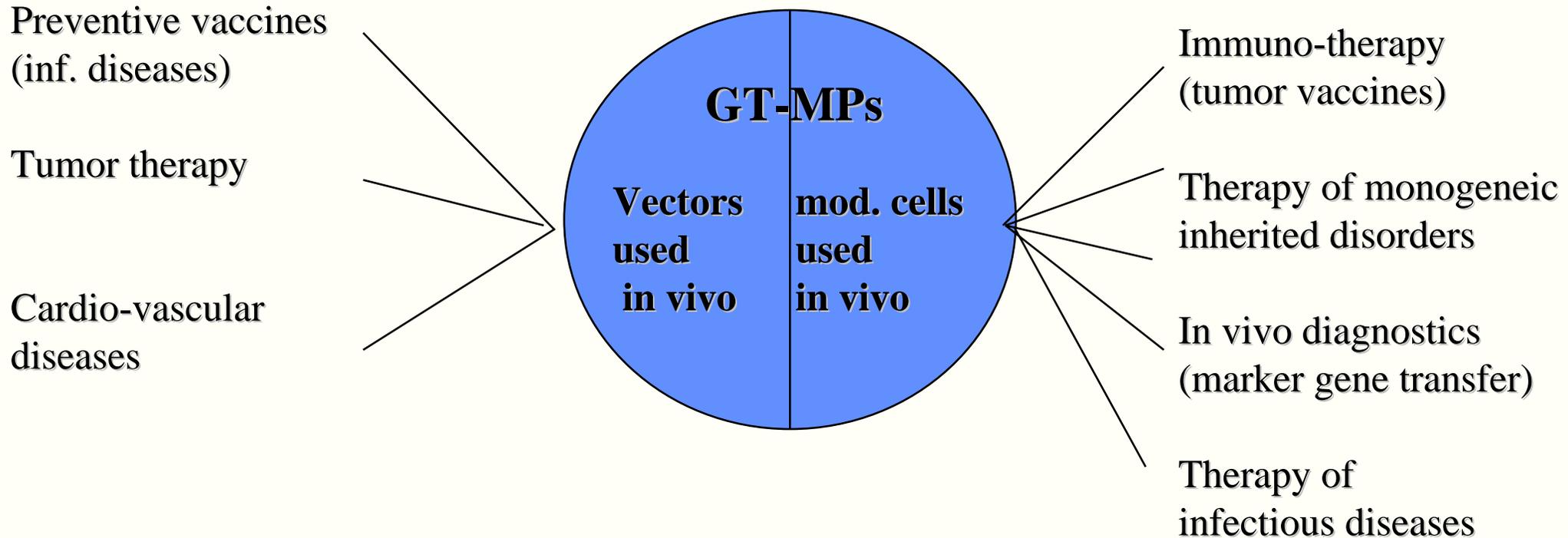
Vectors Used in Gene Therapy Clinical Trials



- Retrovirus 28% (n=254)
- Adenovirus 26% (n=240)
- Naked/Plasmid DNA 14% (n=132)
- Lipofection 9.3% (n=85)
- Pox virus 5.7% (n=52)
- Vaccinia virus 3.3% (n=30)
- Herpes simplex virus 2.8% (n=26)
- Adeno-associated virus 2.1% (n=19)
- RNA transfer 1.1 (n=10)
- Others 3% (n=23)
- N/C 5,1% (n=47)

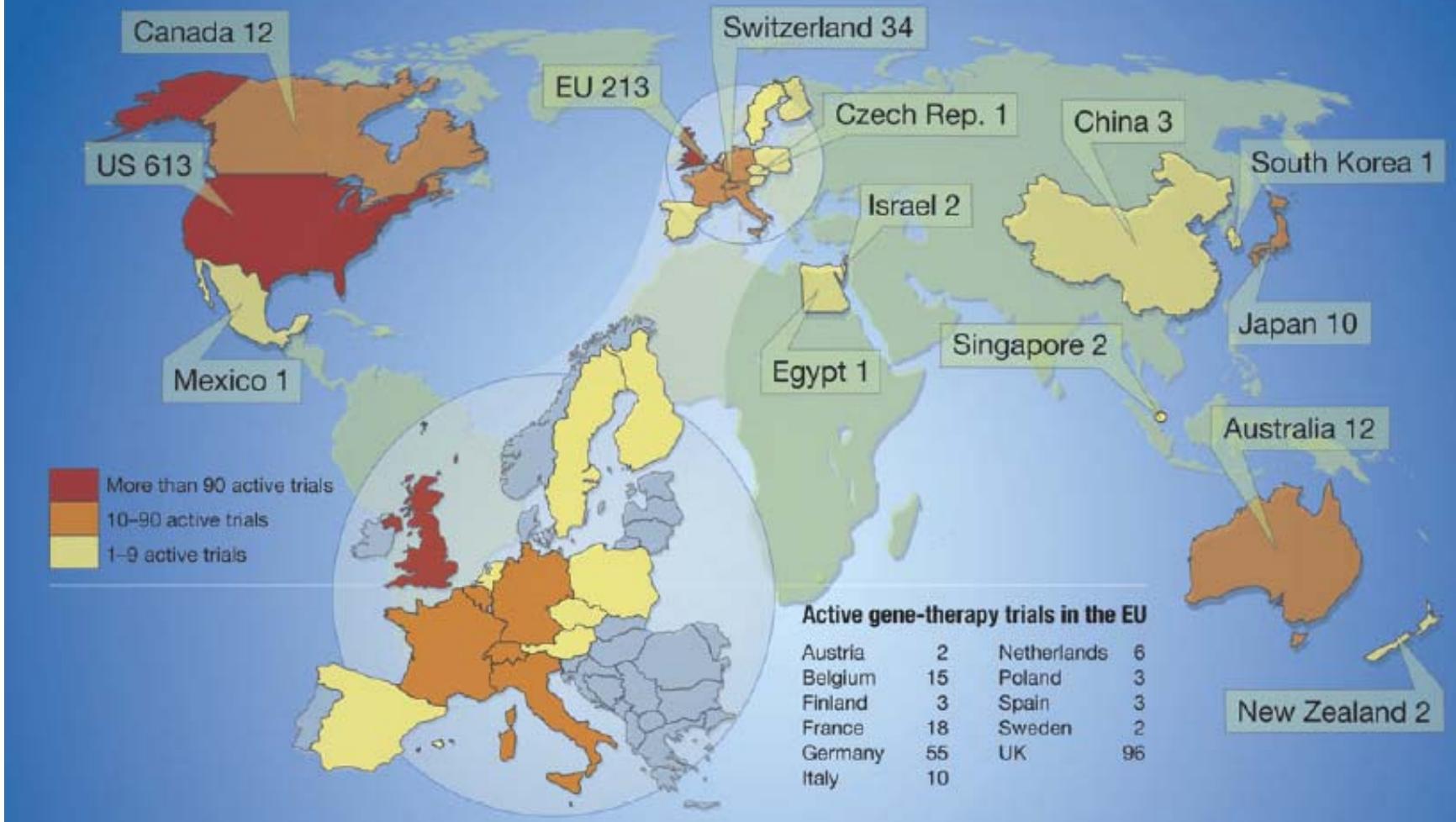
Also
used as
preventive
vaccines.

GT products are modified cells or vectors



5.000 patients were treated with GT-MPs, ~ 600 in Europe (260 in Germany)

Number of active gene-therapy trials



Lessons learned from gene therapy

- Gene therapy includes preventive vaccination
- Theoretical and practical concerns with respect to chromosomal vector integration:
 - leukemias in SCID-X1 gene therapy
 - DNA vaccines
- Considerations with respect to qualification of non-diploid cell lines and vectors

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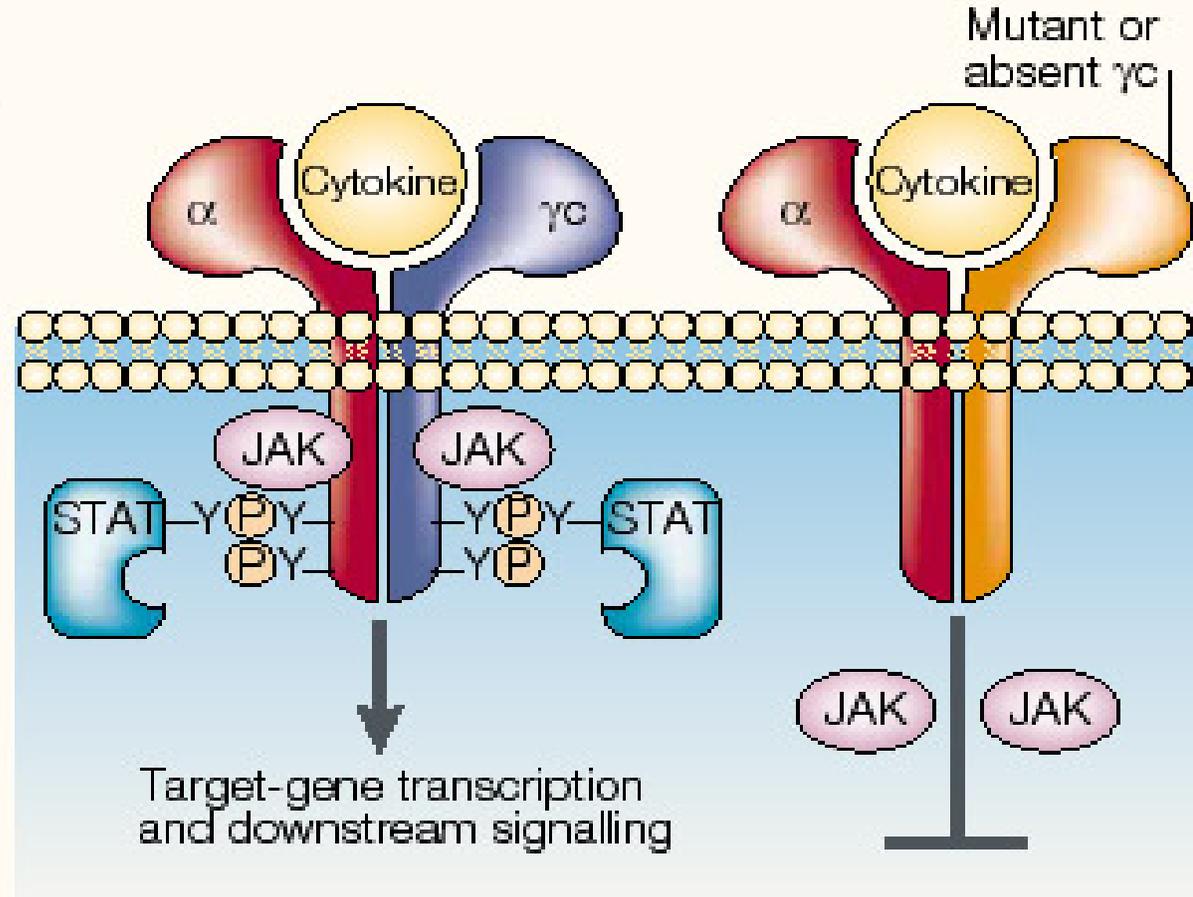
SCID-X1



Kinder mit Immunkrankheiten können die Außenwelt nur geschützt erkunden

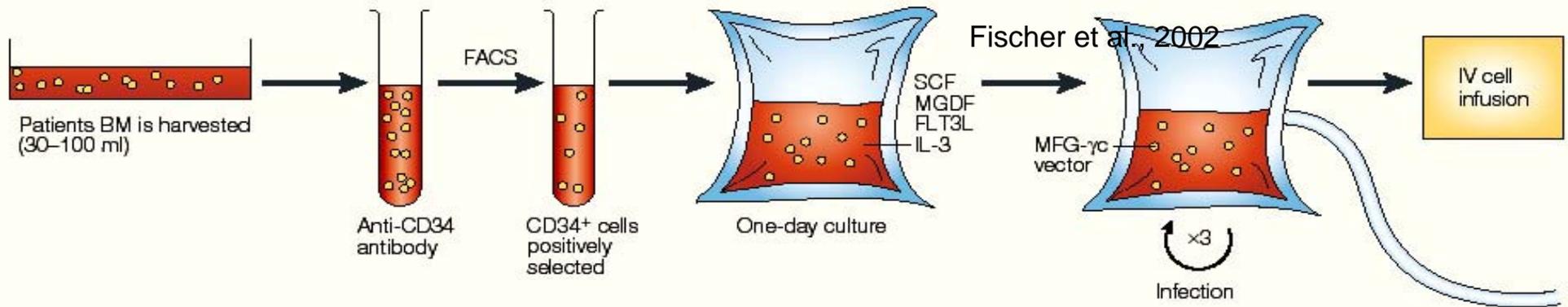
„Severe Combined Immunodeficiency“

- genetic defect in γ c-chain
- cytokine receptor mutated or absent (IL-2R, -4, -7, -9, -15, -21)
- defect in T- and B-cell development
- high sensitivity to infections
- BMT from haploidentical donor

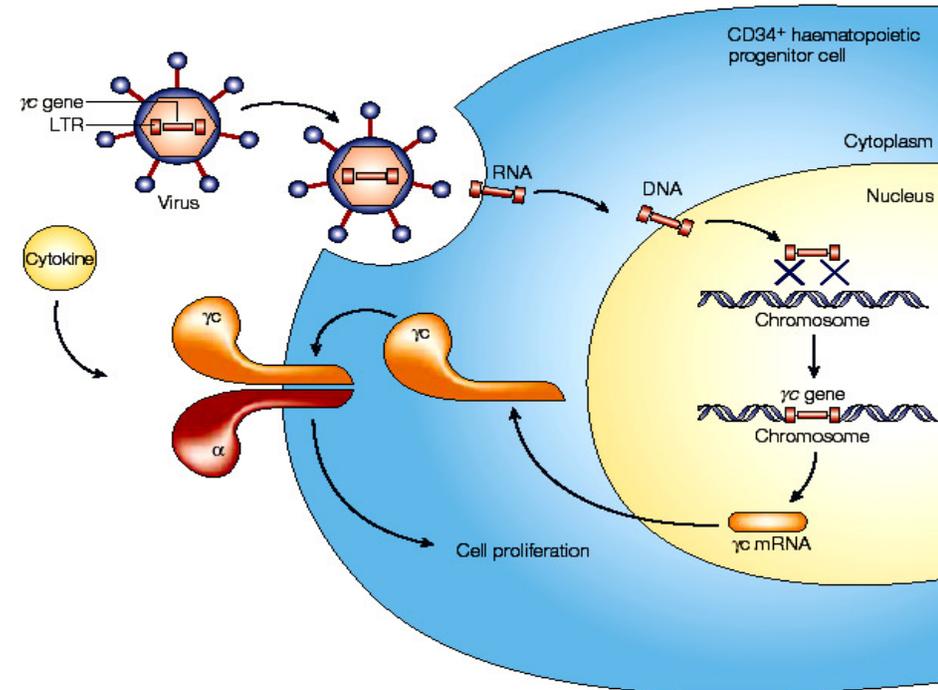


X- SCID gene therapy

Fischer et al., 2002, Nature Rev Immunol. 2, 615



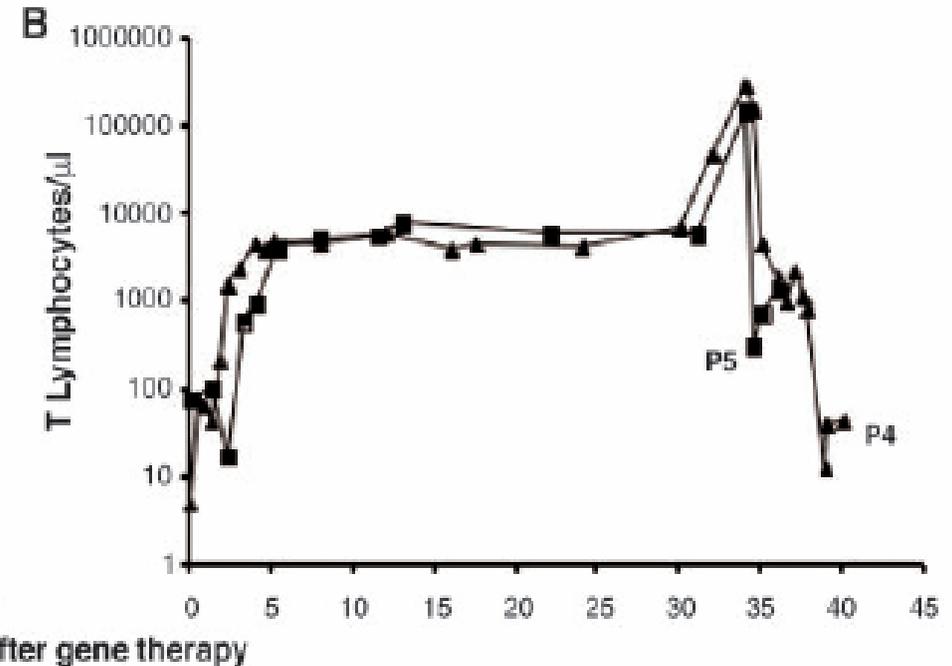
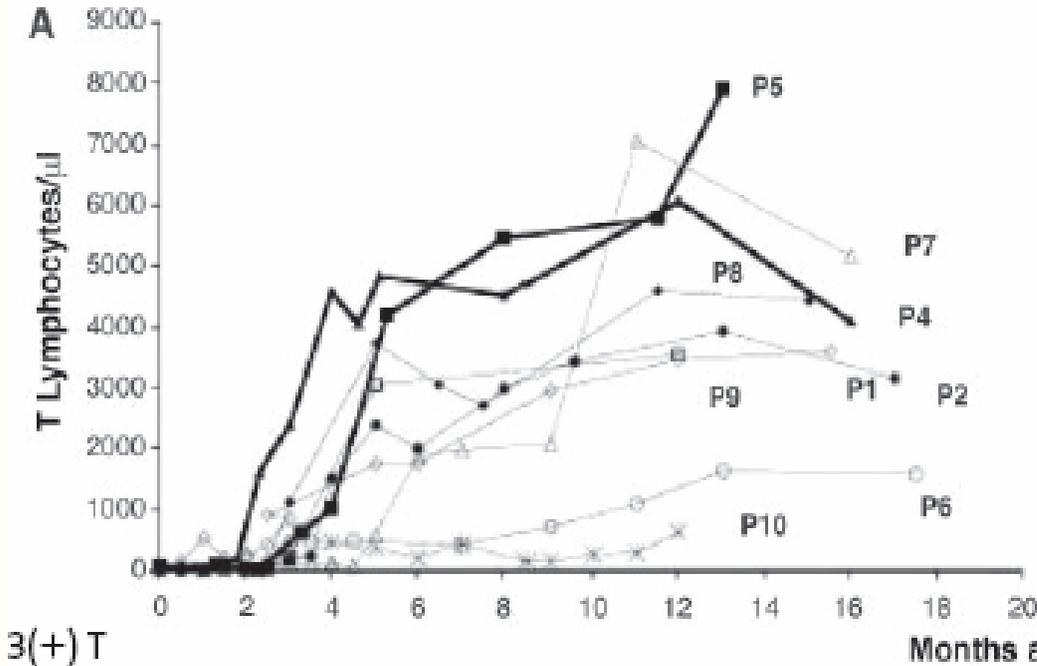
- investigator-driven approach
- children < 1 year old with no available matched donor
- retroviral modification of CD34⁺ bone marrow cells ($\sim 2 \times 10^8$ cells per kg)
- MLV-derived replication-incompetent vector (MLV(ampho) or MLV (GaLV))
- γ c-chain gene transfer (e.g. IL2-receptor)
- theoretical risk of insertional oncogenesis



T lymphocyte development and proliferation

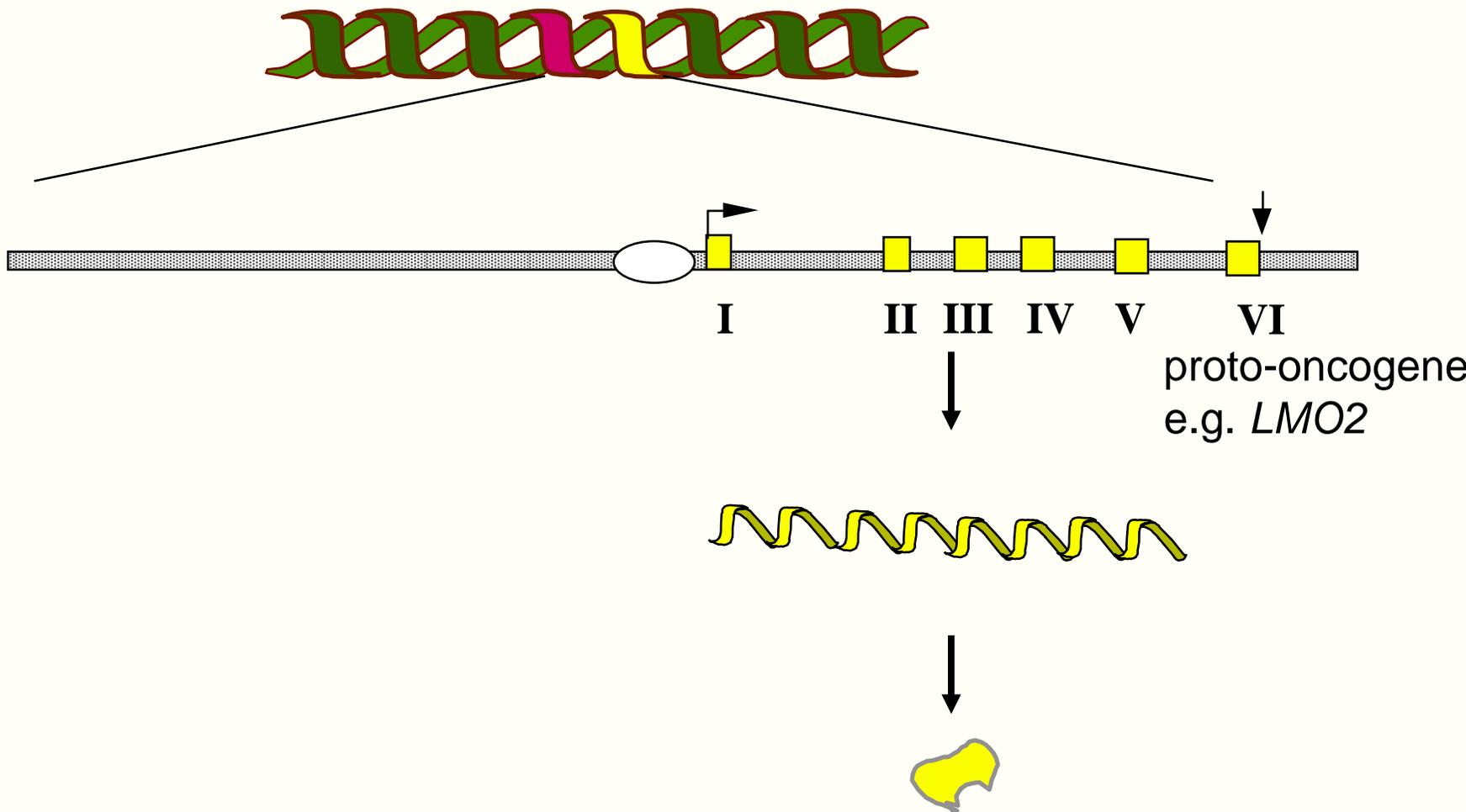
9 of 10 patients successfully treated

P4 and P5 treated < 3 months old developed leukemias



- CD3+ counts in blood with time
- P4 and P5 developed leukemias 30 and 33 months post treatment
- conventional treatment lead to remission

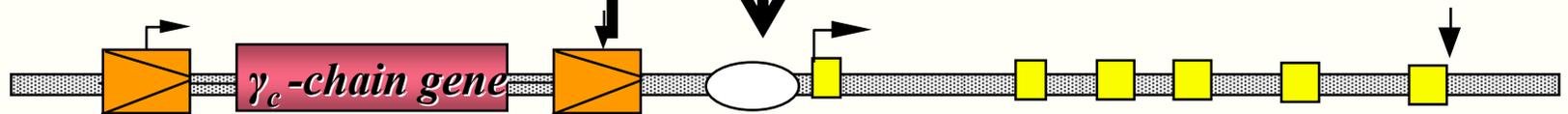
2x leukemia cases due to insertional mutagenesis mediating insertional oncogenesis



p-*onc* gene over-expression due to chromosomal integration of the retroviral expression vector

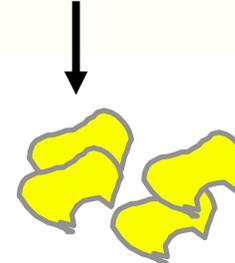
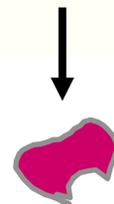
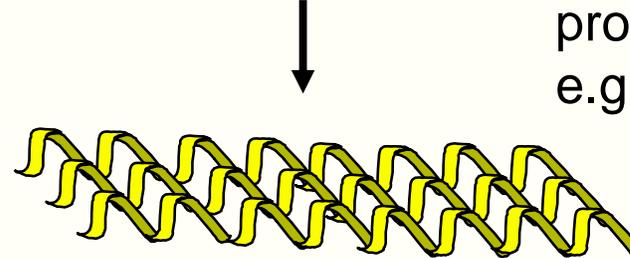
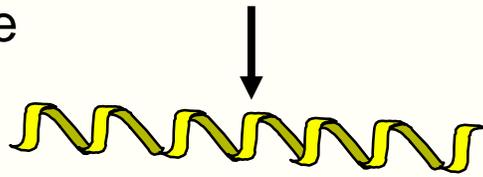


enhancer
effect



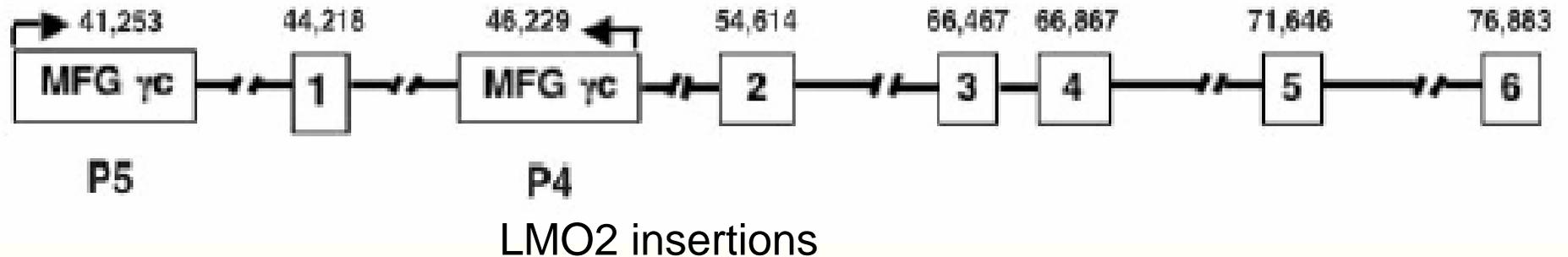
vector expressing
the γ_c chain gene

over-expressed
proto-oncogene,
e.g. *LMO2*



Chromosomal vector integrations in malignant T cell clones: Insertional mutagenesis and enhancer-mediated p-onc activation

C



- no RCR
- no VL30 integration



Lessons learned from insertional oncogenesis (1)

- Seems to be a practical issue for SCID-X1 gene therapy, not others.
- Points to theoretical concern of insertional mutagenesis, which may be induced not only in animals, but also in humans.
- More than one step required, but one insertion is sufficient for initiation, in the absence of virus replication.
- Probability of risk may depend on
 - vector mode of integration,
 - preference for particular integration sites,
 - transcriptional control element in expression vector,
 - selection of modified cells.
- Points at necessity to check absence or level of chromosomal integration for expression vectors able to enter the nucleus of cells.



Lessons learned from insertional oncogenesis (2)

- Synergy between two activated oncogenes may suffice to induce leukemia in 2 out of 10 children (LTR-activated LMO-2 and γ c-chain gene).
- Theoretical risk assessments may not predict practical risks, here those real for children <6 months of age.
- Mouse model was a good predictor for the risk of oncogenesis (evi-1 + LNGFR combination; Li et al, Science).
- Mouse model may provide a general model to assess the risk of leukemia presented by MLV vector-modified bone marrow stem cells.
- Calculations have to be paralleled by experimental observations.



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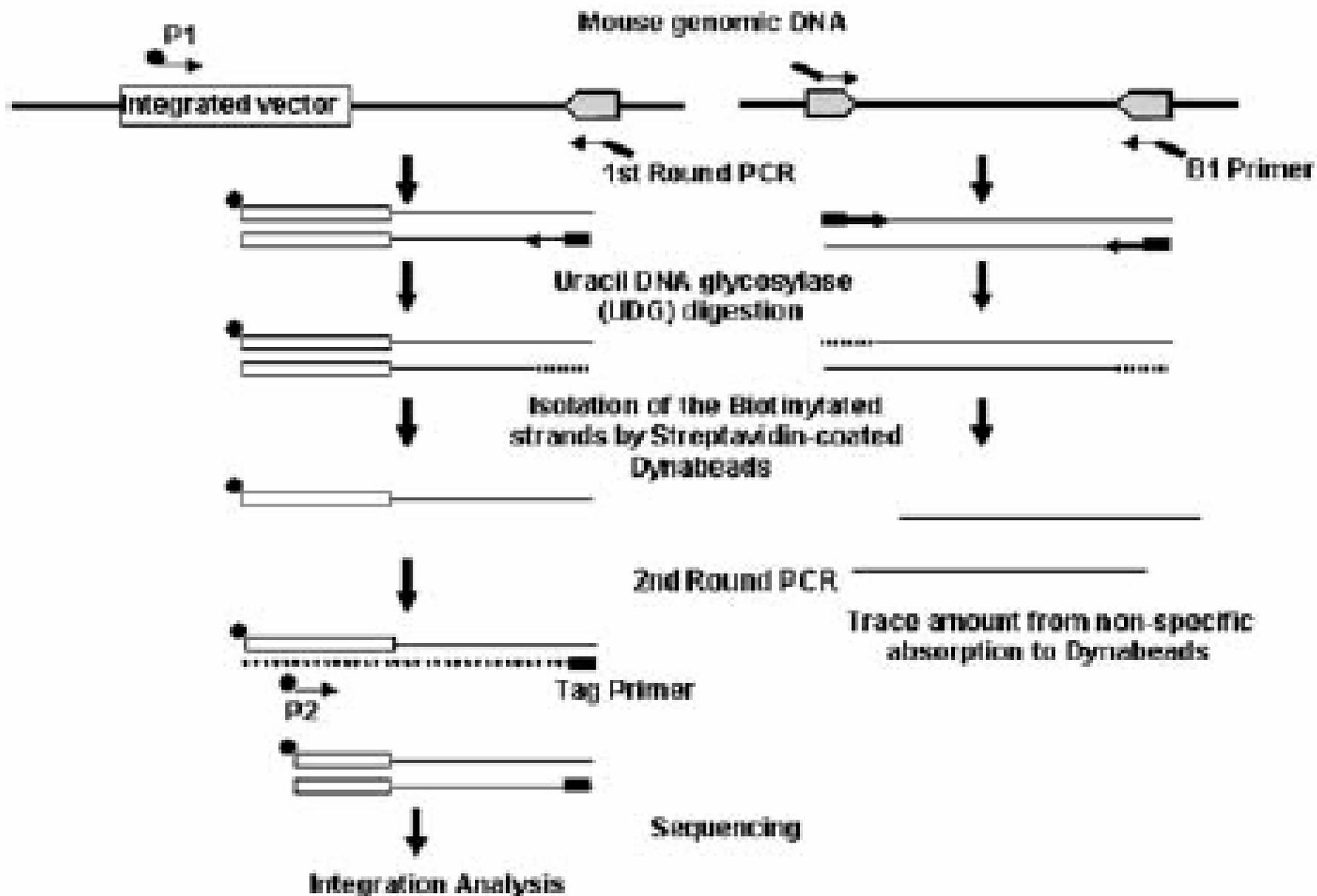


Figure 1 Strategy of the RAIC PCR assay. The left panel represents the

No integration following i.m. injection of 100 ug DNA in mice (W. Nichols, 1995)

Table 8. Integation studies in mice using V1Jns-HPV16 E7 plasmids: comparison of wild-type versus mutant E7 genes

Tissue ^a	E7 gene ^b	Plasmid level (pre-gel) copies/ μ g DNA ^c	Rounds of gel purification (steps) ^d	Plasmid level (post-gel) copies/ μ g DNA
Quadriceps	Wild-type	100–800	5 (TAE, TBE, TAE, TBE, ee, CHEF, ee)	<1
	Mutant	100–800	5 (TAE, TBE, TAE, TBE, ee, CHEF, ee)	1–8
Skin (injection site)	Wild-type	10–40	2 (TAE, TBE, ee)	0
	Mutant	10–20	2 (TAE, TBE, ee)	0
Inguinal node	Wild-type	≤ 1	2 (TAE, TBE, ee)	0
	Mutant	≤ 1	2 (TAE, TBE, ee)	0

^a Tissues were taken approximately 6 weeks after dosing. Analysis was limited to the tissues shown.

^b Mice were injected with 50 μ l per quadriceps of 3.0 mg/ml V1Jns-HPV E7 plasmid, containing either a wild-type (active) or mutated (inactivated) E7 gene from HPV type 16.

^c Plasmid level was estimated using conventional PCR, using dilution analysis for samples with >16 copies/ μ g DNA.

^d Gel purification procedures are abbreviated as described in Methods. ‘ee’ refers to electroelution and to a stopping point in the multiround gel purifications.

Lessons learned from i.m. inoculation of naked DNA

- At most 1 copy of plasmid DNA per 1 ug chromosomal DNA found (= 150,000 diploid cells)
 - following i.m. injection of 100 ug of pure DNA,
 - 1 day or several months after injection,
 - Given the high spontaneous uptake of naked DNA by murine muscle.
- Hundreds of patients treated with naked DNA: no tumors have been linked to the use of DNA.



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Cell substrates used for manufacture of preventive gene therapy vaccines

- AAV
- Adv
- Plasmid DNA
- Vaccinia, MVA, ALVAC



Cell substrates used for manufacture of preventive gene therapy vaccines

- | | | |
|------------------------|--------------|----------------|
| • AAV | 293 cells | non-diploid |
| • Adv | Per.C6 cells | non-diploid |
| • Plasmid DNA | E. coli | not applicable |
| • Vaccinia, MVA, ALVAC | CEF | diploid |



Issues associated with preventive gene transfer vaccines

- Activated oncogene DNA can be transmitted to normal human somatic cells and predispose cells to malignancy.
- Unknown agents or micro-organisms in the cells could be tumorigenic.
- Vector preparation itself could contain any of the two materials listed above.



Issues associated with preventive gene transfer vaccines

- Activated oncogene DNA can be transmitted to normal human somatic cells and predispose cells to malignancy.
 - Test malignancy of the production cell line in nude mice.
 - Test oncogenicity of the cell DNA in primary rat embryofibroblasts.
 - Test tumorigenicity of the cell DNA in nude mice (s.c. inoculation).
- Unknown agents or micro-organisms in the cells could be tumorigenic.
 - Test cell supernatant for oncogenicity.
 - Test cell supernatant for tumorigenicity.
- Vector preparation itself could contain any of the two materials listed above.
 - Test vector prep. for absence of activated oncogenes known to be present in the production cell line.
 - Minimise the amount of cellular DNA in the vector prep. (<10 ng per dose).

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Lessons learned from gene therapy

- Chromosomal integration of vector DNA may result in leukemia under special circumstances.
- About 300 patients have been treated with the same regimens and have not suffered from leukemia.
- Use of naked DNA and “integrating” vectors has led to no other cases of tumor development, although more than 2000 patients have been treated, some of them more than 10 years ago.

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Innovative biotechnology medicinal products

Gene Transfer Medicinal Products (vectors, DNA, gen. mod. cells, micro-org.)

Somatic Cell Therapy, MSCs (human cells, including stem cells)

Xenogene



Licens
scientific

Dev. of NfGs

Gene Therapy E
(EU-GT)

WHO Clinical G
Monitoring

cal trial,
ufacture

mission of
Gene Therapy

rial approval

ections

Gene therapy

(AIDS and tumor gene therapy)

Cell therapy

(Signal transduction, stem cell diff.)