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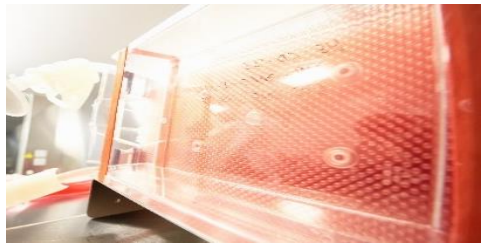




Quality aspects during manufacturing of retroviral vectors for cell therapy

Overview

- Some facts about retroviral vectors
- Summary of different manufacturing methods for retroviral vectors
- Potential riscs involved in retroviral vector manufacturing and how to adress them

Gamma retroviral vectors – an old horse that can still jump

- first clinical tool for stable gene transfer
- causal for insertional oncogenesis in early stem cell trials
- transduce only dividing cells
- mostly replaced by lentiviral vectors in stem cell gene therapy

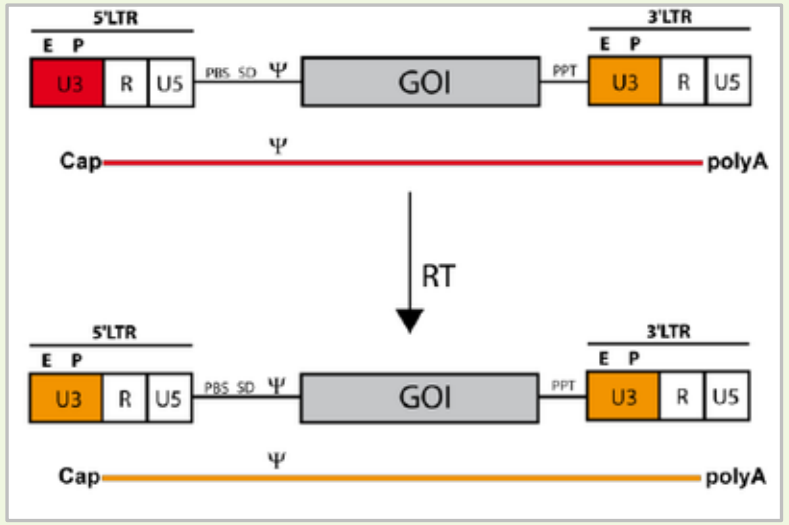
BUT

- much better safety profile in SIN configuration
- exellent safety track record in T cells
- at least one log lower GMP production costs compared to lentiviral vectors

=> still an exellent choice for stable transduction of T cells and NK cells (as long as the cells are expanded as well)

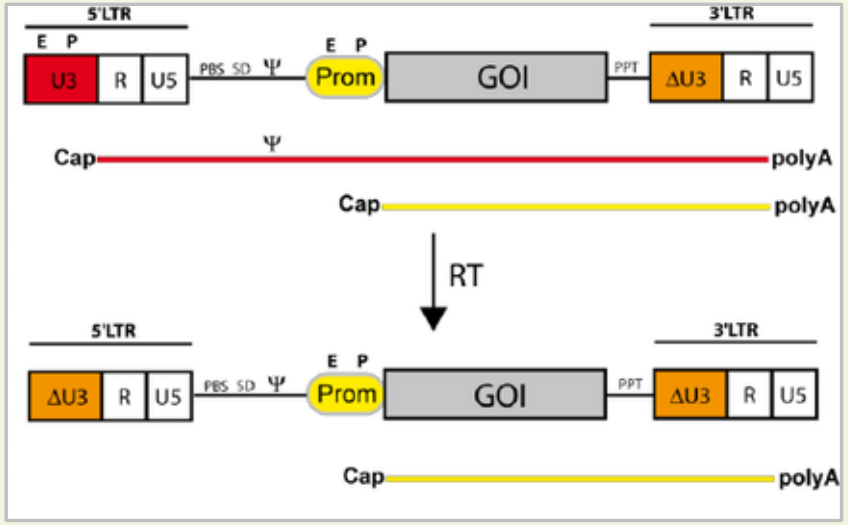
LTR driven vs SIN vectors

LTR driven VECTOR



- Decades of experience
- Easy to produce in stable packaging cell lines
- Can be produced at high titers
- **BUT:** insertional mutagenesis in stem cells due to enhancer and promotor elements

SIN VECTOR



- Less mutagenic due to loss of viral enhancers
- Possibility to target expression by tissue-specific promotors in internal expression cassette
- **BUT:** more complicated to produce in stable packaging cell lines

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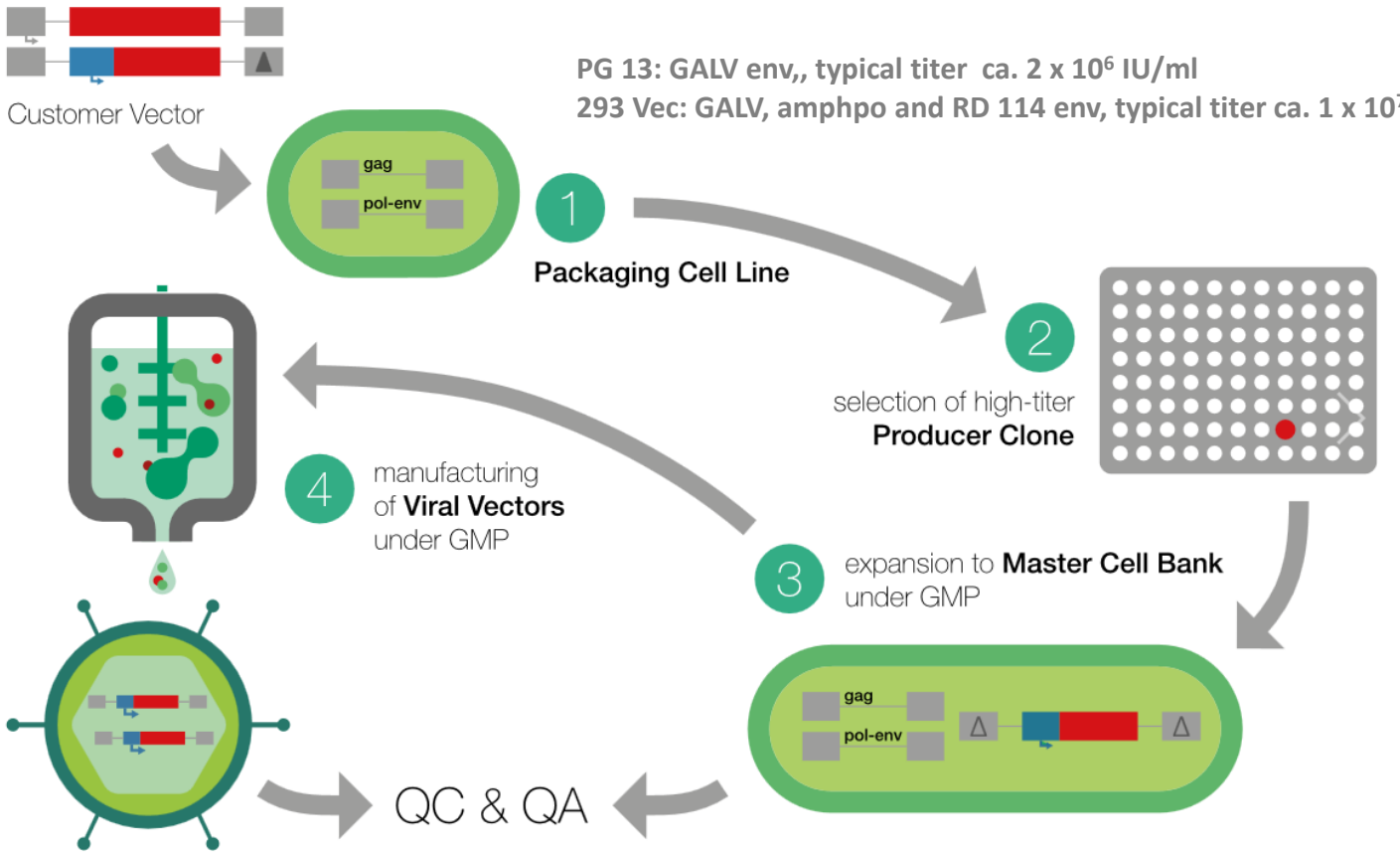
STATE OF THE ART: STABLE RETROVIRAL VECTOR PRODUCTION



PG 13: GALV env,, typical titer ca. 2 x 10^6 IU/ml
293 Vec: GALV, amphpo and RD 114 env, typical titer ca. 1 x 10^7 IU/ml

- **State of the art process**
- **Allows for large and consistent vector batches**
- **BUT: timeline ~ 15 months**

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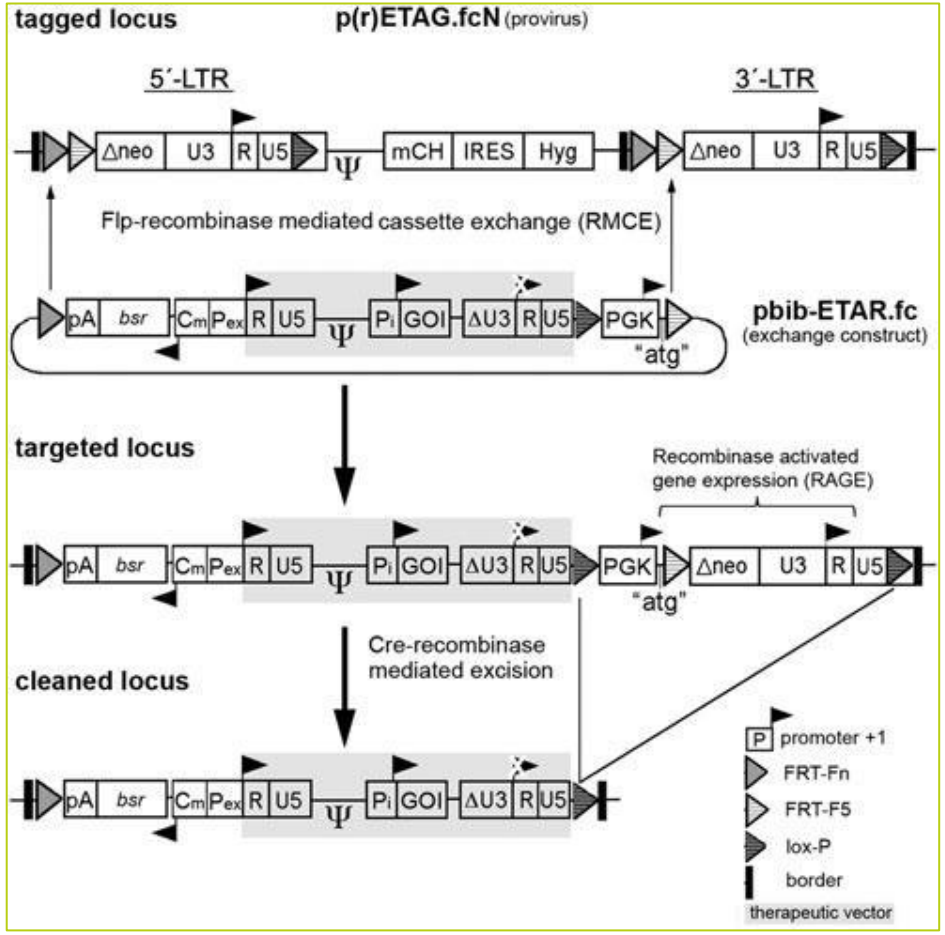
Concept for generating stable producer cell lines for SIN retroviral vectors

- Problem 1: If integrated as provirus, vector does not produce genomic RNA that can be packaged
- Problem 2: If integrated after plasmid transfection, genotype and production capacity of the resulting producer clone is not stable
- Solution: Integration of vector into pre-selected locus by „Recombinase Mediated Cassette Exchange“ =RMCE (Hennig et al., Hum Gene Ther, 2014)



Results in producer cell clone that has only one productive integration !

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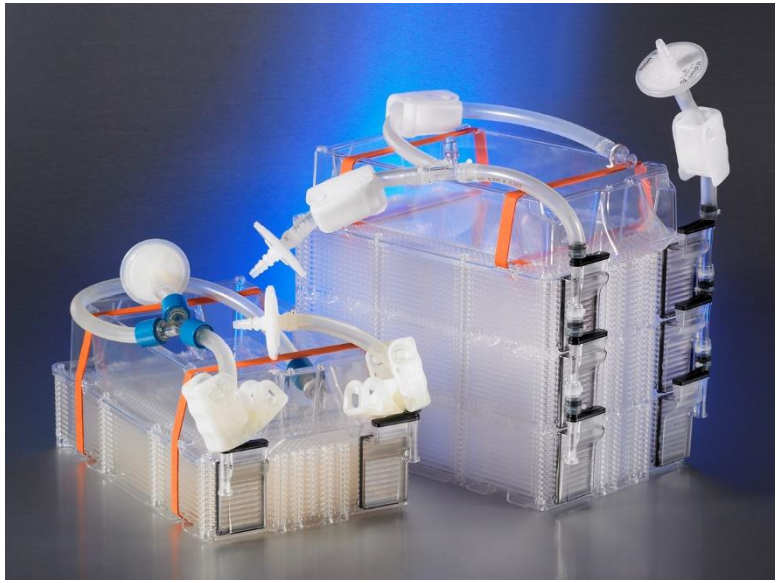
Evolution of GMP Manufacturing Processes for RV from stable Producer cells

Version 1

- Harvest in Hyperestack results in 4 x 4 l of sup
- 0,45 µm filtration of harvests, pooling and aliquoting (50 to 100 ml in plastic bags)
- Cryopreservation and QC (uses ca. 25 % of material)
- Typical titer 5 x 10^6 IU/ml

=> **ca. 5 x 10^{10} IU for clinical use** (e.g. 100 patients if MOI 5, 10^8 cells/patient)



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Evolution of GMP Manufacturing Processes for RV from stable Producer cells

Version 2

- Harvest in Hyperestack (2 in parallel) results in 4 x 4 l of sup
- Concentration/purification of harvests by TFF resulting in ca 1.2 l concentrated sup
- pooling and aliquoting (5 ml in vials)
- Cryopreservation and QC (uses ca. 25 % of material)
- Typical titer 5 x 10^7 IU/ml

=> **ca. 5 x 10^{10} IU for clinical use** (e.g. 100 patients if MOI 5, 10^8 cells/patient

Advantages to version 1:

- Less process related impurities
- Less volume of vector preparation in transduction process
- Better logistics

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Evolution of GMP Manufacturing Processes for RV from stable Producer cells

Version 3

- Multiple harvests in iCellis nano bioreactor results in up to 12 x 4,5 l of sup
- Concentration/purification of harvests by TFF resulting in ca. 3.6 l concentrated sup
- pooling and aliquoting (2 - 5 ml in vials)
- Cryopreservation and QC (uses ca. 20 % of material)
- Typical titer 1 x 10^8 IU/ml

=> **ca. 3 x 10^{11} IU for clinical use** (e.g. 600 patients if MOI 5, 10^8 cells/patient)

Additional advantages to version 1:

- Higher overall yield, possible for commercial production
- Mostly closed and automated process allows parallel manufacturing in one clean room

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TRANSIENT RETROVIRAL VECTOR PRODUCTION

- Transfection of 293T cells, seeding in 2 Hyperstacks
- 2 harvests of supernatant (total of ca. 8 l)
- Concentration/purification of harvests by TFF resulting in ca. 1.2 l concentrated sup
- pooling and aliquoting (5 ml in vials)
- Cryopreservation and QC (uses ca. 25 % of material)
- Typical titer 5 x 10^7 IU/ml

=> **ca. 4 x 10^{10} IU for clinical use** (e.g. 80 patients if MOI 5, 10^8 cells/patient)

Advantages to production from stable cell lines:

- Less time involved for first in man clinical trials

Quality Aspects: Producer Cell Cone

Keep in mind: This clone will serve as starting material for your product throughout the whole life cycle!

- Establishment can be done under R&D conditions, BUT document everything (batch numbers of all reagents and media, passages, operators, special emphasis on reagents of animal origin ….)
- Use certified FBS from BSE free countries (e.g. Australia)
- Origin of parental cell bank has to be documented
- Archive CoAs of all media/reagents
- Define the number of vector integrations and their sequence

Quality Aspects: Master Cell Bank

General:	post thaw recovery, confluency, cell count, viability, morphology
Potency:	infectious titer determined by FACS on HT1080
	physical particles determined by p30 ELISA
Identity:	Presence of Transgene (FACS)
	Vector Sequence (Sanger Sequencing)
	human cell line (HEK293) (DNA Fingerprinting (Multi Locus Probe 33.15)))
Safety:	Endotoxin
	Sterility
	Mycoplasma
	detection of Mycobacteria
	detection of replication competent retrovirus
	detection of BPyV, Circovirus, HEV, HSV, B19, EBV, SV40, hCMV, HHV6, HHV7, HHV8, HIV, HBV, HCV, HTLV, HAV, HPV, HPyV (PCR)
	detection of porcine and bovine viruses (in vitro)
	detection of human and primate adventitious viruses (in vitro and in vivo)
	detection of adventitious agents in cells by TEM

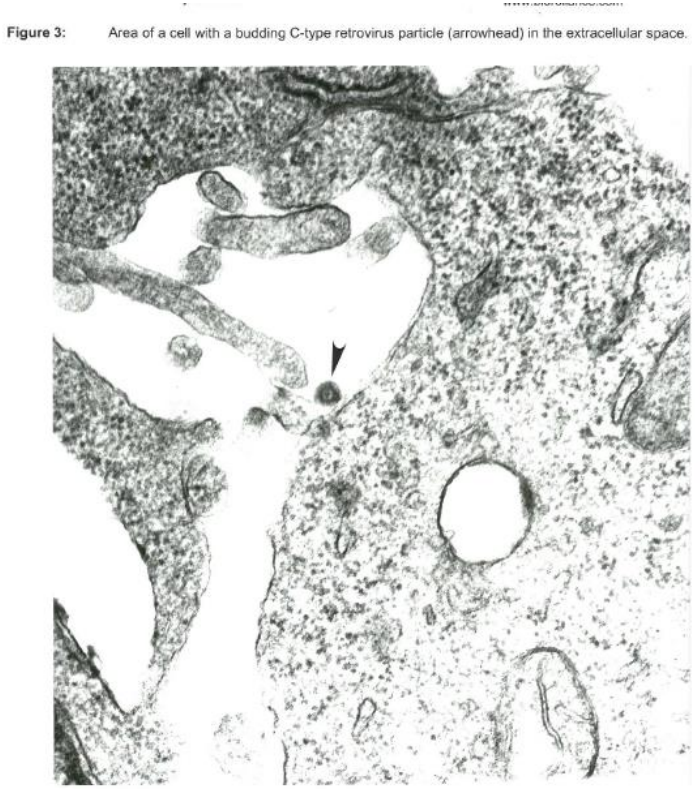
Figure 3: Area of a cell with a budding C-type retrovirus particle (arrowhead) in the extracellular space.

Also important: Stability of cell line over max # of passages

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Quality Aspects: Working Cell Bank

General:	post thaw recovery, confluency, cell count, viability, morphology
Potency:	infectious titer determined by FACS on HT1080
	physical particles determined by p30 ELISA
Identity:	Presence of Transgene (FACS)
	Vector Sequence (Sanger Sequencing)
	human origin (CO1 Barcode)
	human cell line (HEK293) (Karyotyping, STR Analysis)
Safety:	Endotoxin
	Sterility
	Mycoplasma
	detection of Mycobacteria
	detection of replication competent retrovirus
	detection of BPyV, Circovirus, HEV, HSV, B19, EBV, SV40, hCMV, HHV6, HHV7, HHV8, HIV, HBV, HCV, HTLV, HAV, HPV, HPyV (PCR)
	detection of porcine and bovine viruses (in vitro)
	detection of human and primate adventitious viruses (in vitro and in vivo)
	detection of adventitious agents in cells by TEM

Quality Aspects: Vector batch

- Release testing in accordance with ICH, USP and EP
- main focus: patient safety
- Stability study required with several pull points for several years, to show that potency and safety are stable, container closure integrity for each pull point (nothings comes into the primary packaging and nothing leaks out)
- Next to releases testing: do tests on primary cells to ensure, that the vector has a sufficient transduction efficiency
- Validation of aseptic processing (media fills) has to be shown

General:	determination of pH and osmolality
Potency: HT1080	infectious titer determined by FACS on
	physical particles determined by p30 ELISA
	Biological potency (cell based assay)
Identity:	Presence of Transgene (FACS)
	Vector Sequence (Sanger Sequencing)

Safety:	Endotoxin Sterility Mycoplasma detection of replication competent retrovirus (on VSN and EPC) detection of porcine and bovine viruses (in vitro), BPyV (PCR) detection of human and primate adventitious viruses (in vitro and in vivo)
Impurities:	host cell DNA Host cell Proteins HEK293 host cell gene target E1A BSA (surrogate for FBS)