

Choosing Optimal Viral Vector for T-cell Transduction

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PACT Webinar

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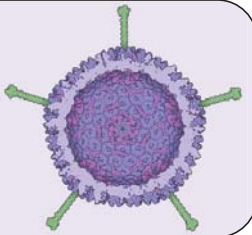
Viral vectors for blood cells

Short/long term gene expression

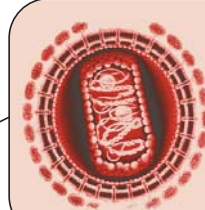
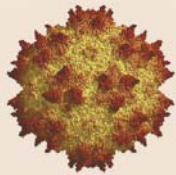
T cell

Stable gene expression

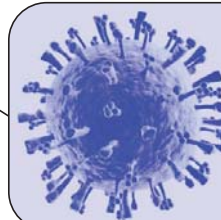
Adenoviral
vectors
(AdV)



Adeno-
associated
viral vectors
(AAV)



Gamma-
retroviral
vectors



Lentiviral
vectors

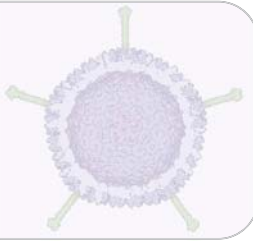
Non-integrating

Integrating

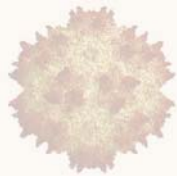
Viral vectors for blood cells

Short/long term gene expression

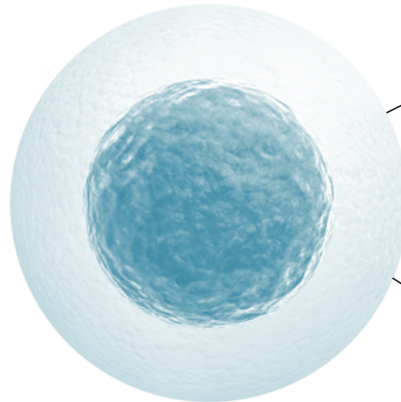
Adenoviral vectors (AdV)



Adeno-associated viral vectors (AAV)

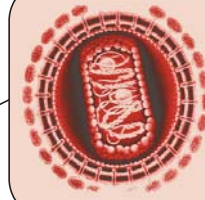


T cell

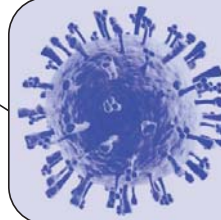


Stable gene expression

Gamma-retroviral vectors



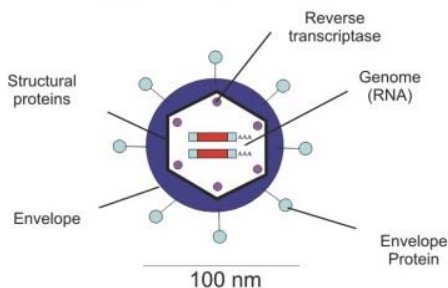
Lentiviral vectors



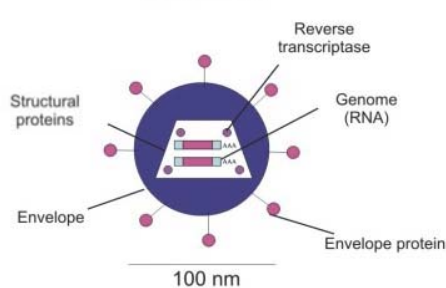
Retroviruses

Retrovirus

Gammaretrovirus



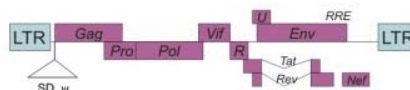
Lentivirus



- ssRNA viruses capable of **integrating** into host genome
- Can be modified to efficiently transduce T cells



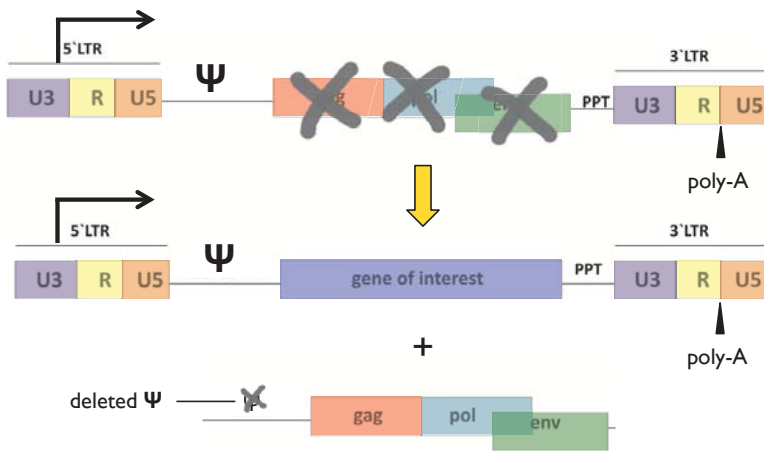
MoMLV, MSCV etc



HIV, FIV, SIV etc

Gammaretroviral vector

Gammaretroviral genome

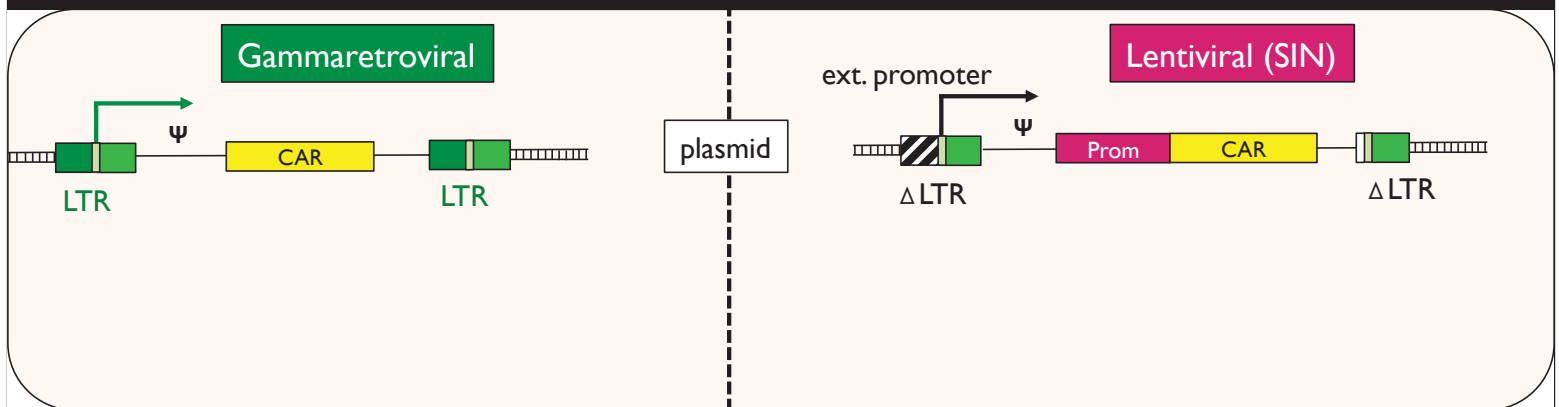


Packaging plasmids
(not included in the virion)

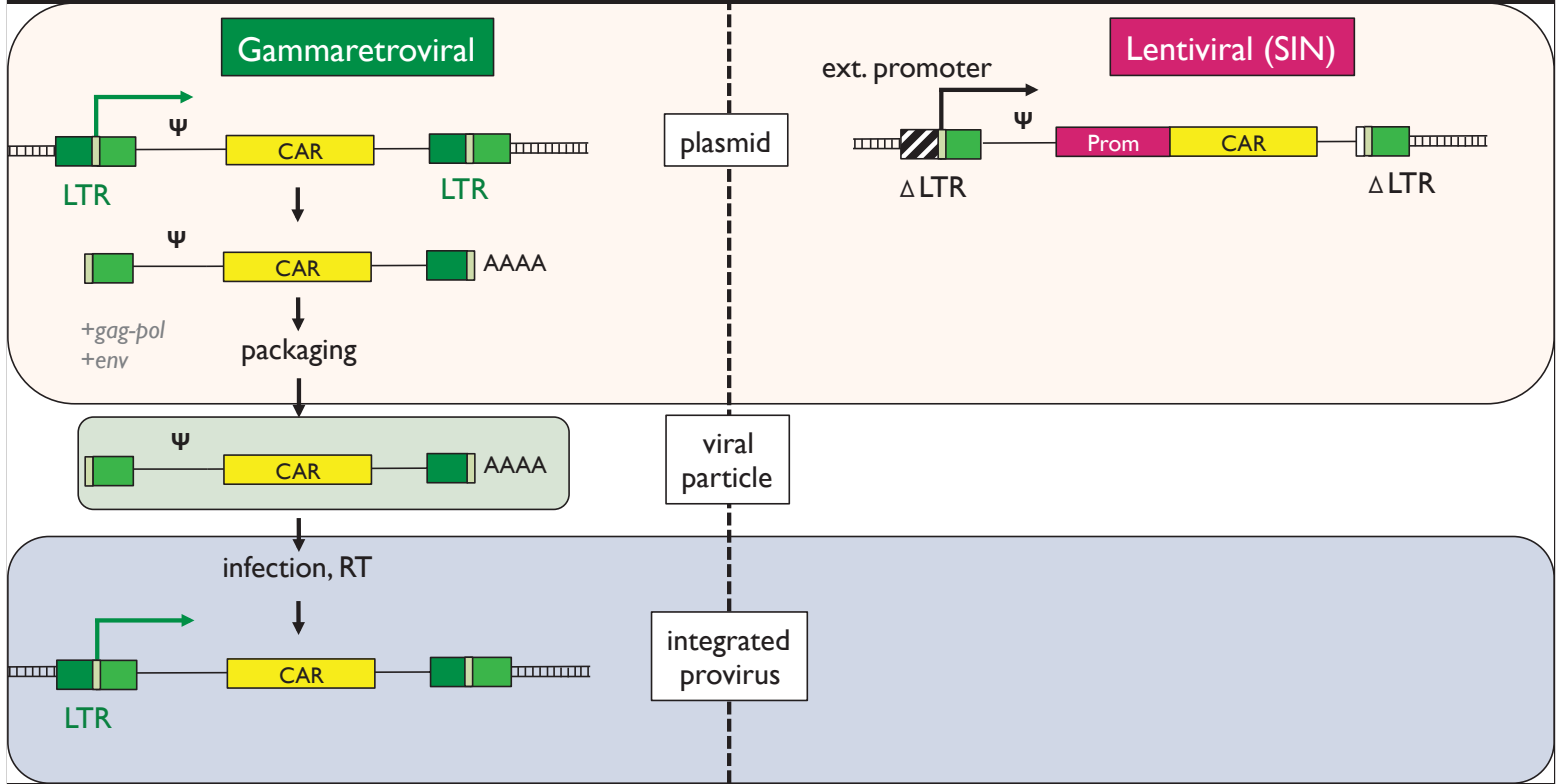
Adapted from Intechopen.org

- In vectors most viral genes are **deleted** to make room for transgene
- *Gag*, *pol*, and *env* are provided in separate plasmids in packaging cells
- Host specificity is dictated by *env* genes

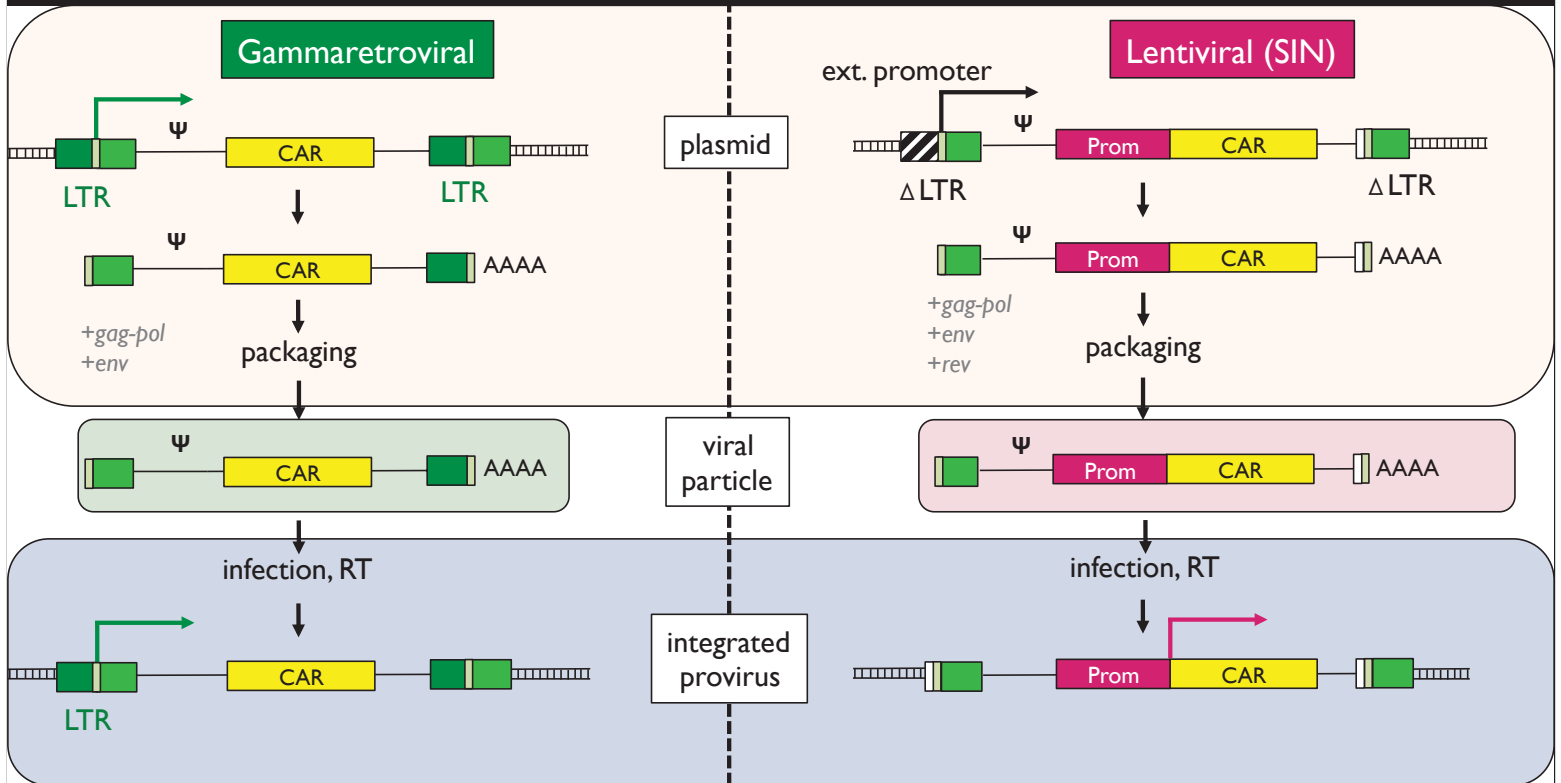
Gammaretro- vs SIN lentiviral vectors



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Gammaretro- vs SIN lentiviral vectors

Gammaretroviral

Lentiviral (SIN)

- High efficiency of T cell transduction
- Pseudotypes: RD114, GALV
- Cheap(er) to manufacture in cGMP
- Stable producer cell lines available
- Integrates in dividing cells only
- Smaller genome -> limited cargo (~4-5Kb)
- Transgene expression is driven by LTR
- Tends to integrate near promoters, potential for insertional mutagenesis (HSC)

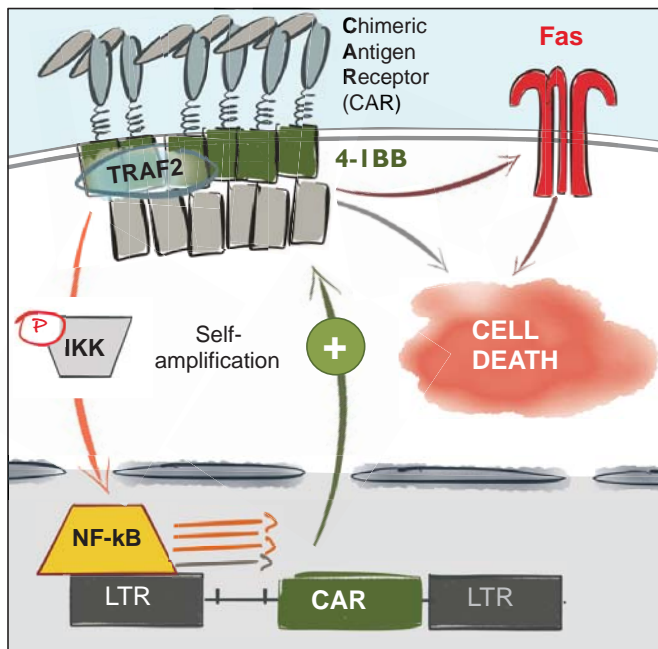
Gammaretro- vs SIN lentiviral vectors

Gammaretroviral

Lentiviral (SIN)

- | | |
|--|---|
| <ul style="list-style-type: none">• High efficiency of T cell transduction• Pseudotypes: RD114, GALV• Cheap(er) to manufacture in cGMP• Stable producer cell lines available• Integrates in dividing cells only• Smaller genome -> limited cargo (~4-5Kb)• Transgene expression is driven by LTR• Tends to integrate near promoters, potential for insertional mutagenesis (HSC) | <ul style="list-style-type: none">• Lower rates of transduction• Mainly VSV-G pseudotype• More complex production• No cGMP-grade producer lines yet• Dividing and non-dividing cells• Higher cargo capacity• Transgene expression is driven by an internal promoter• More random integration in gDNA, self-inactivating LTR promoter |
|--|---|

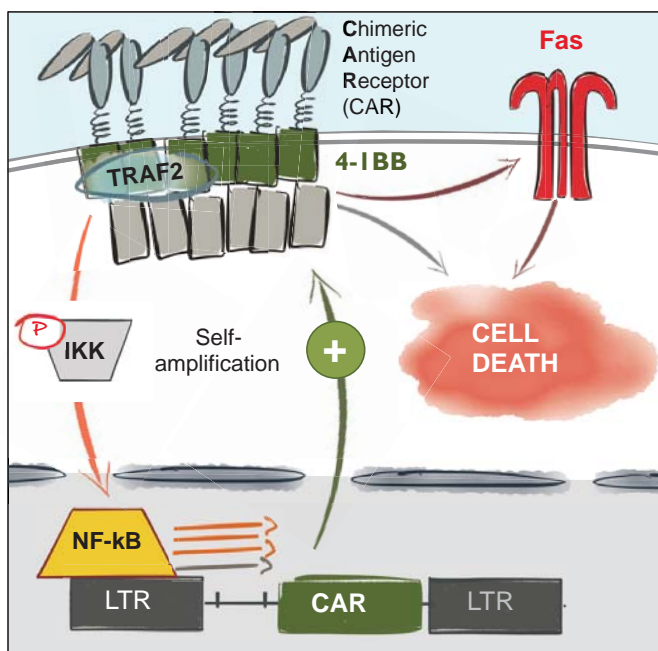
Interaction between the viral vector and its CARgo



Gomes-Silva et al. Cell Rep 2017

- When expressed from a gammaRV vector, 4-1BB.zeta CARs may enhance their own expression and amplify tonic signaling
- High tonic CAR signaling can reduce viability and anti-tumor activity of CAR T cells

Interaction between the viral vector and its CARgo



Gomes-Silva et al. Cell Rep 2017

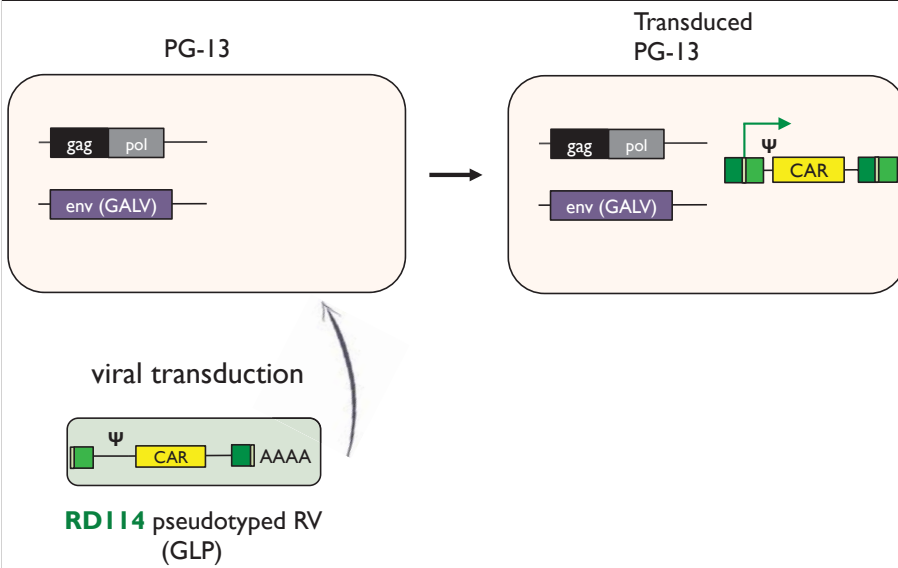
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SOLUTIONS

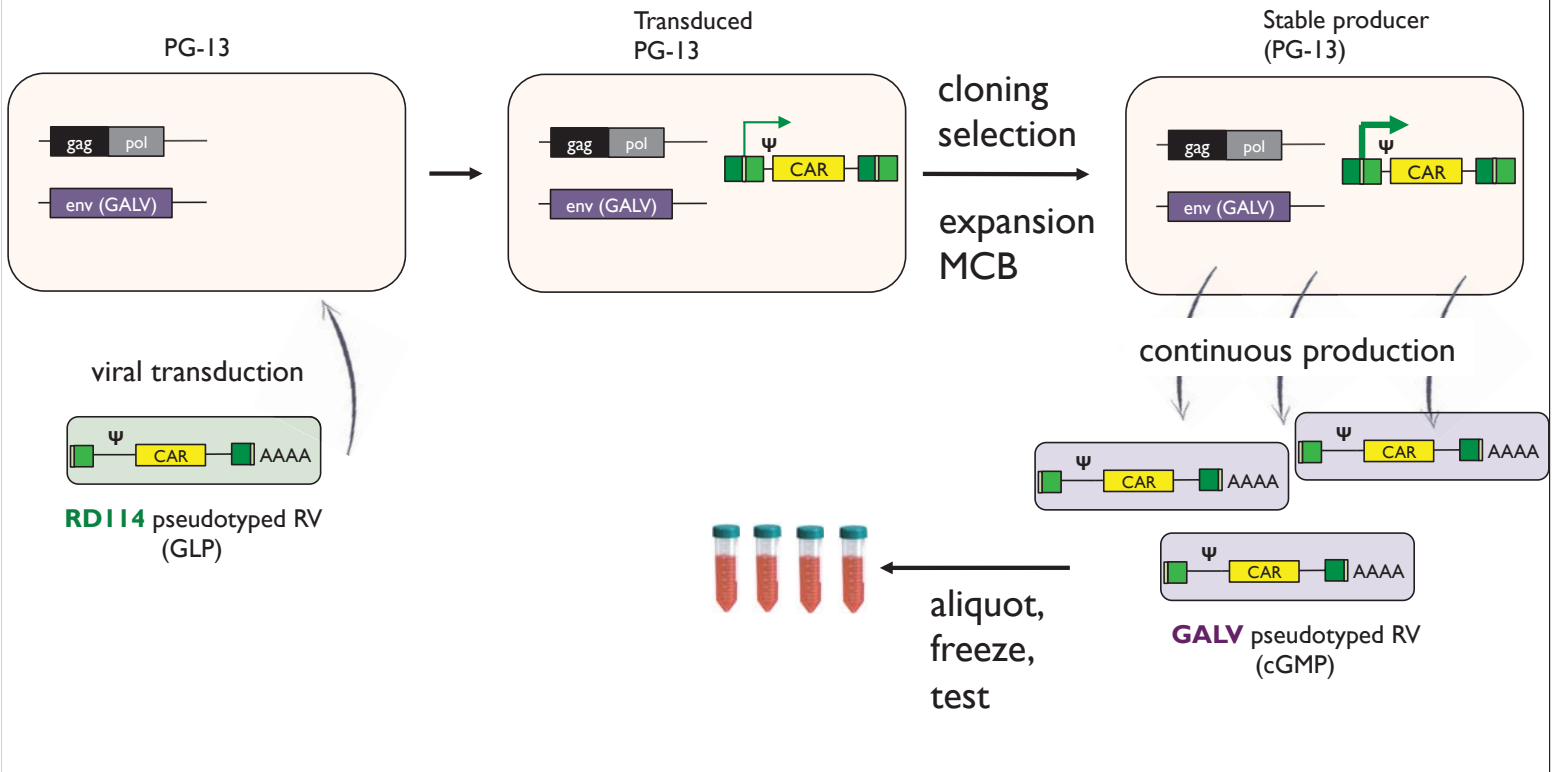
- Reduce CAR expression
- Modify CAR structure to reduce tonic signaling
- Use non-LTR (SIN lentiviral) vectors

cGMP production of gamma RV and LV vectors

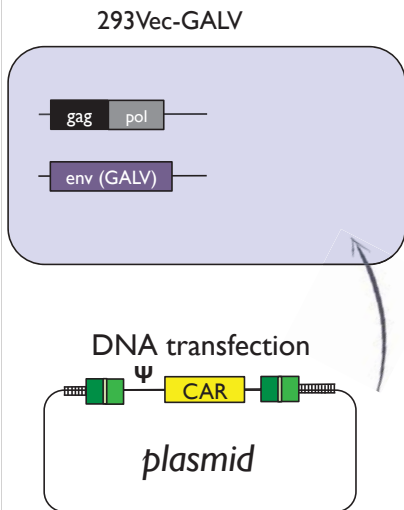
cGMP production of gammaRV vectors – stable producer line



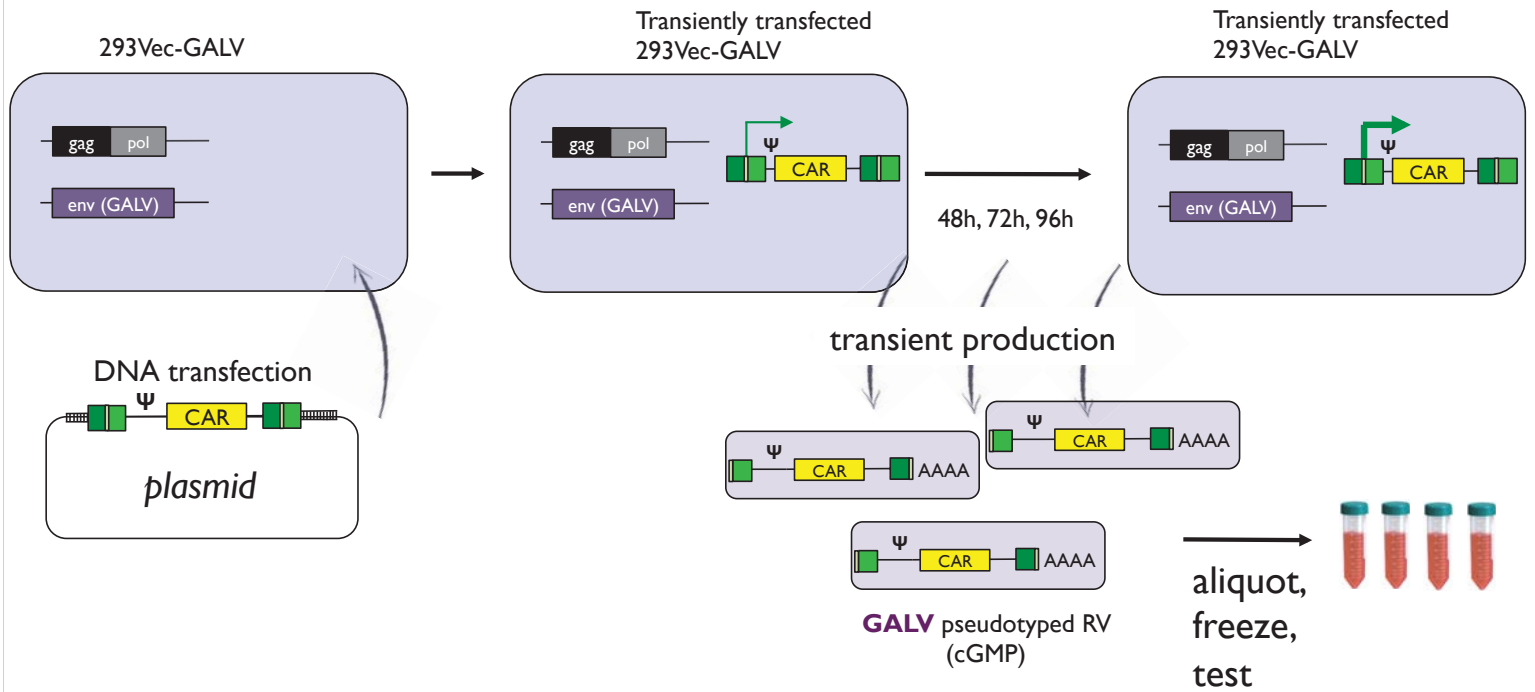
cGMP production of gammaRV vectors – stable producer line



cGMP production of gammaRV vectors – transient transfection



cGMP production of gammaRV vectors – transient transfection

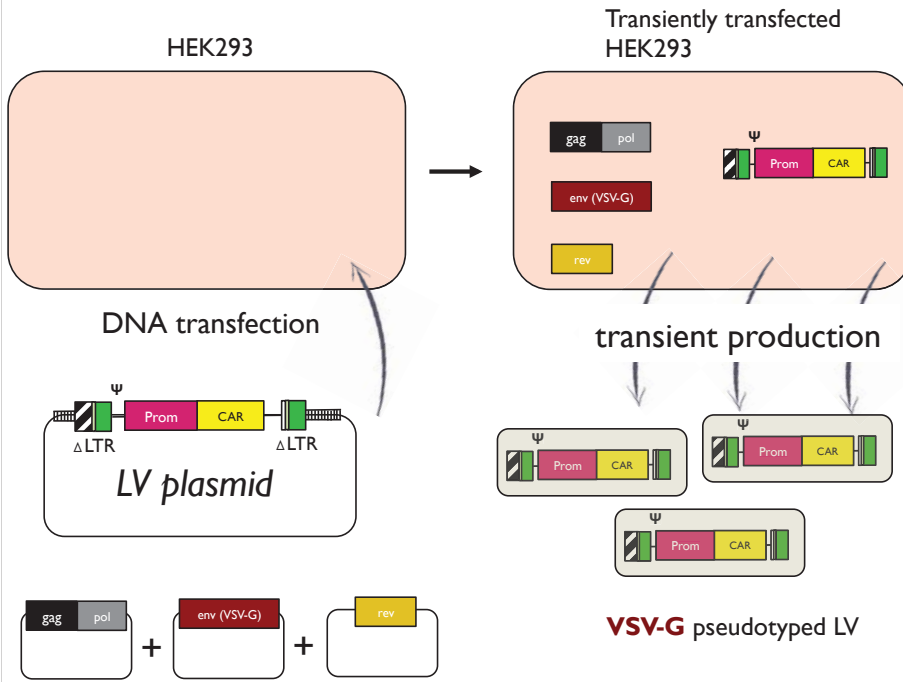


Phase I CD5 CAR T cells (MAGENTA)

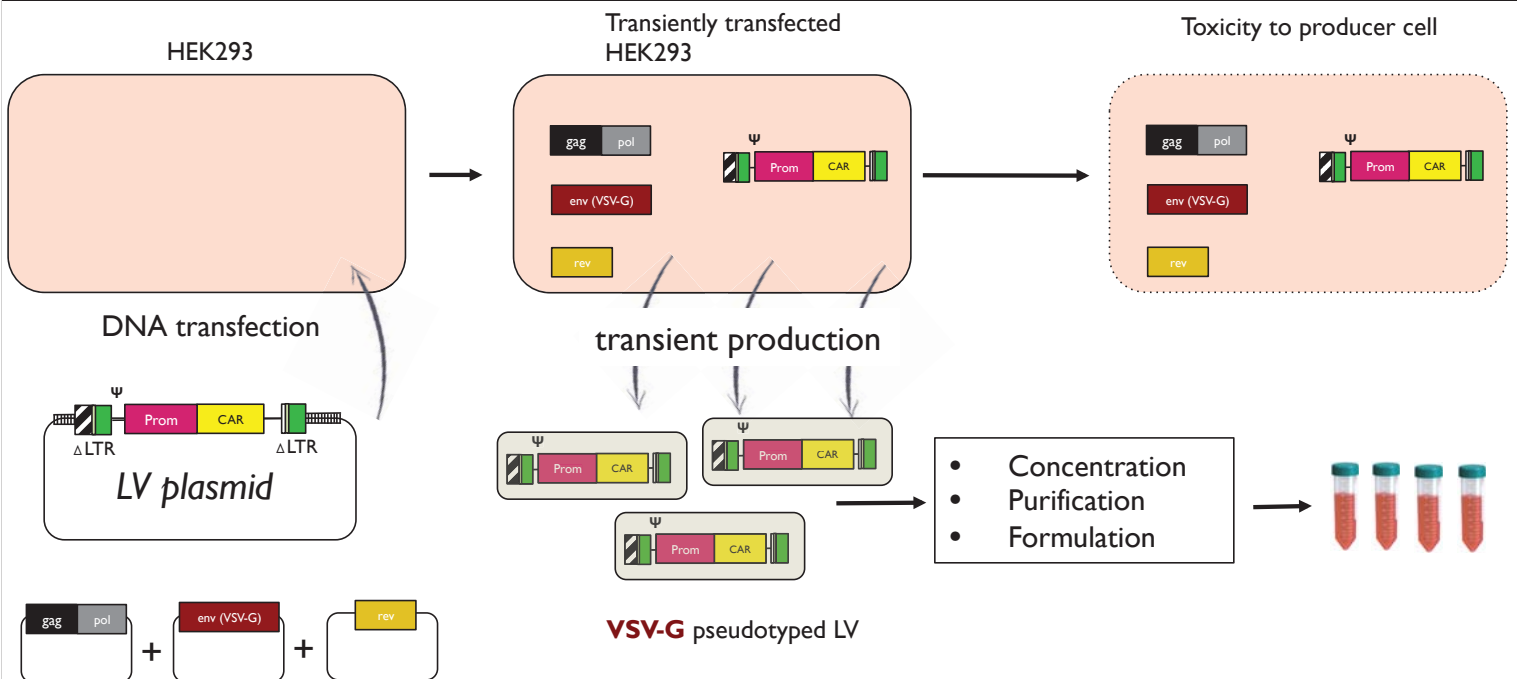
Stable producer lines vs transient transfection

	Stable producer	Transient Tf
Time to clinic	>>>	>>
Cost (incl. testing)	>>>	>>
Consistency between batches	Stable	May vary
Amount of vector generated	High	Low (enough for Phase 1)

cGMP production of lentiviral vectors – transient transfection



cGMP production of lentiviral vectors – transient transfection



Gammaretroviral vs lentiviral vectors

	gammaRV	LV
Division Status	Dividing cells only	Diving or non-dividing
Cargo Capacity	++	+++
Transduction Efficiency	60-90%	20-80%
Transgene Promoter	LTR	Internal (SIN)
Transgene Expression Level	May fluctuate	Stable
cGMP Production	Stable producer line or transient transfection	Transient transfection
Pseudotype	RD114 or GALV	VSV-G
Cost	\$ - \$\$	\$\$\$