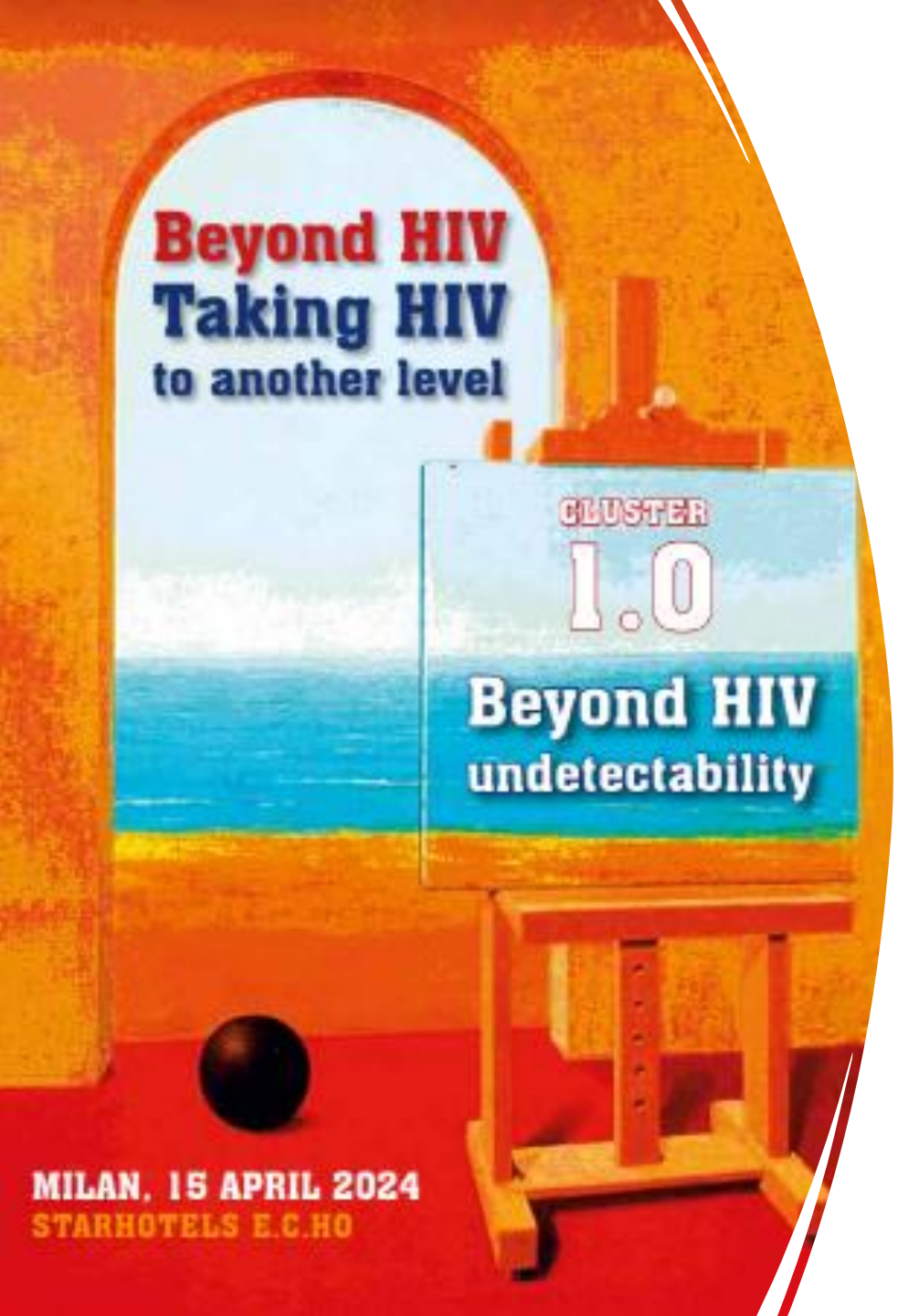


Therapeutic intervention for an HIV cure: from drug mechanisms to therapeutics and back

Relatore: Ilaria Vicenti

University of Siena

Disclosure: None to declare

UNIVERSITÀ DI SIENA
1240

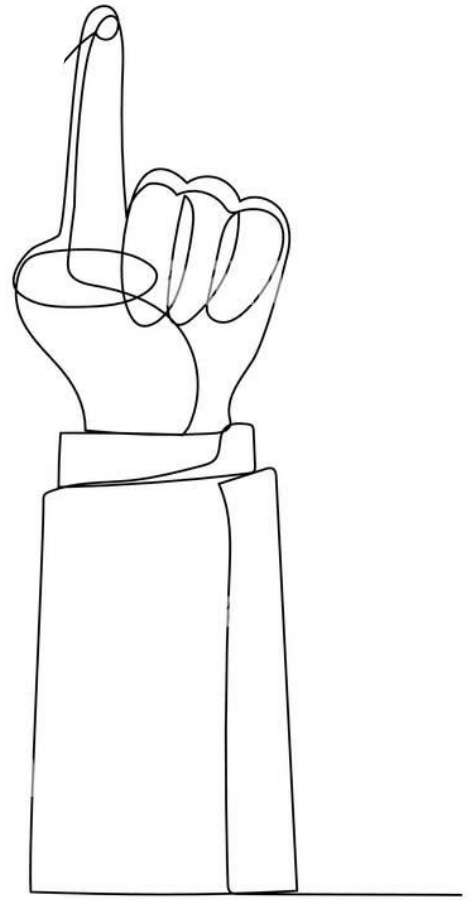
Towards eradication…

Understanding HIV-1 Latency

Eradication strategies

Evaluation of HIV-1 eradication strategies in vitro and in vivo

HIV-1 eradication



Improvement of Methodologies

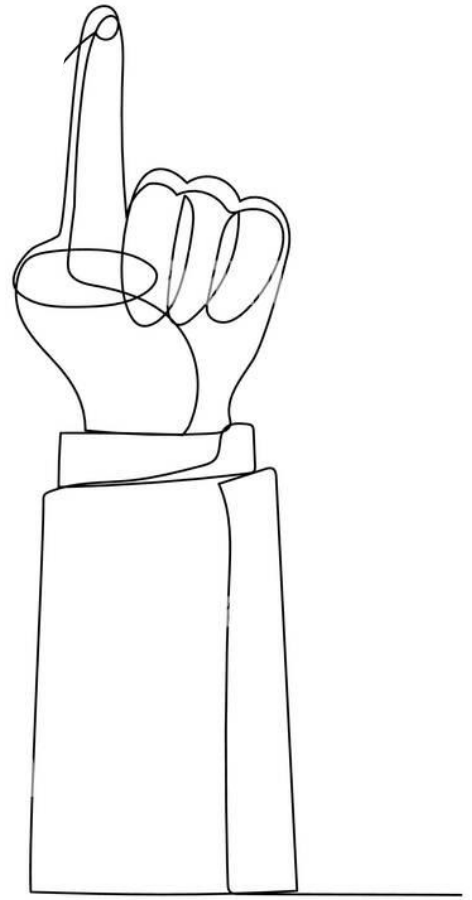
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Improvement of Methodologies

HIV reservoir

HIV reservoir: Cells infected with replication-competent HIV in the blood and at various anatomical sites that persist despite long-term ART

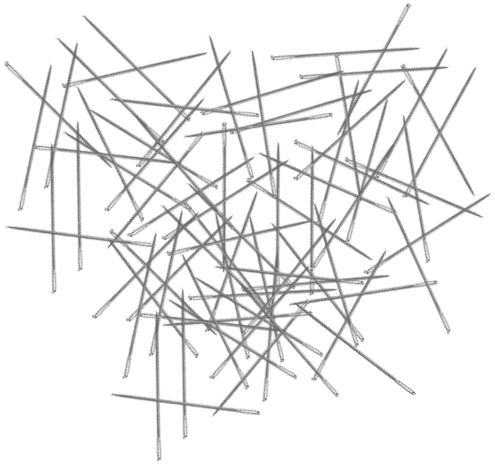
Infected cells are very rare!



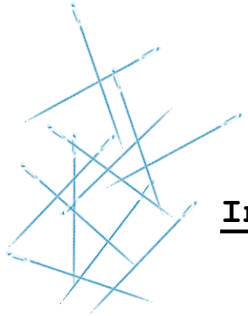
~1 in 10,000 cells is infected

'Needle in a haystack'

A lot of viruses are defective!



Defective viruses



Intact viruses

Ratio Provirus Intact/inducible = 40/1



Rebounding viruses

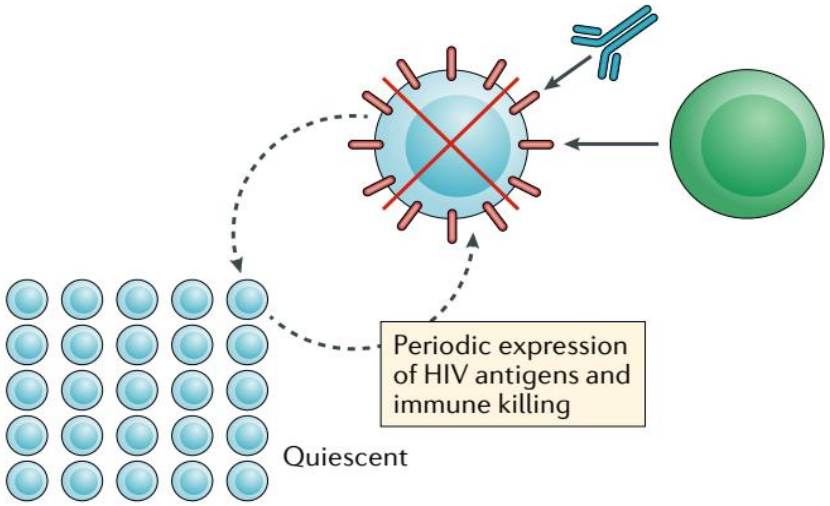
~95% of the viruses are defective

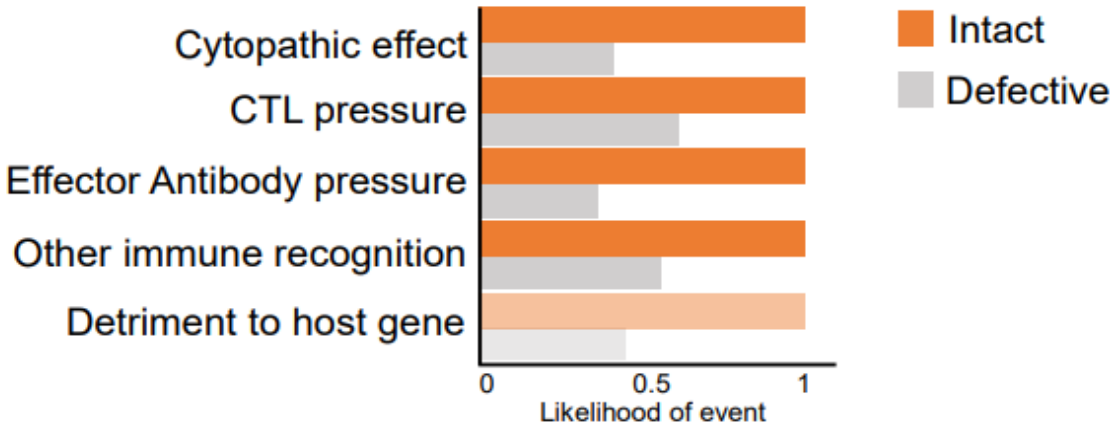
The reservoir has a high genetic

Adapted by Linos Vanderckove EMHH

Reactivation of latent HIV infection during suppressive ART and reduction of the reservoir

Latent cells may periodically express HIV antigens and thus be susceptible to immune killing. Defective proviruses are less likely to be negatively selected





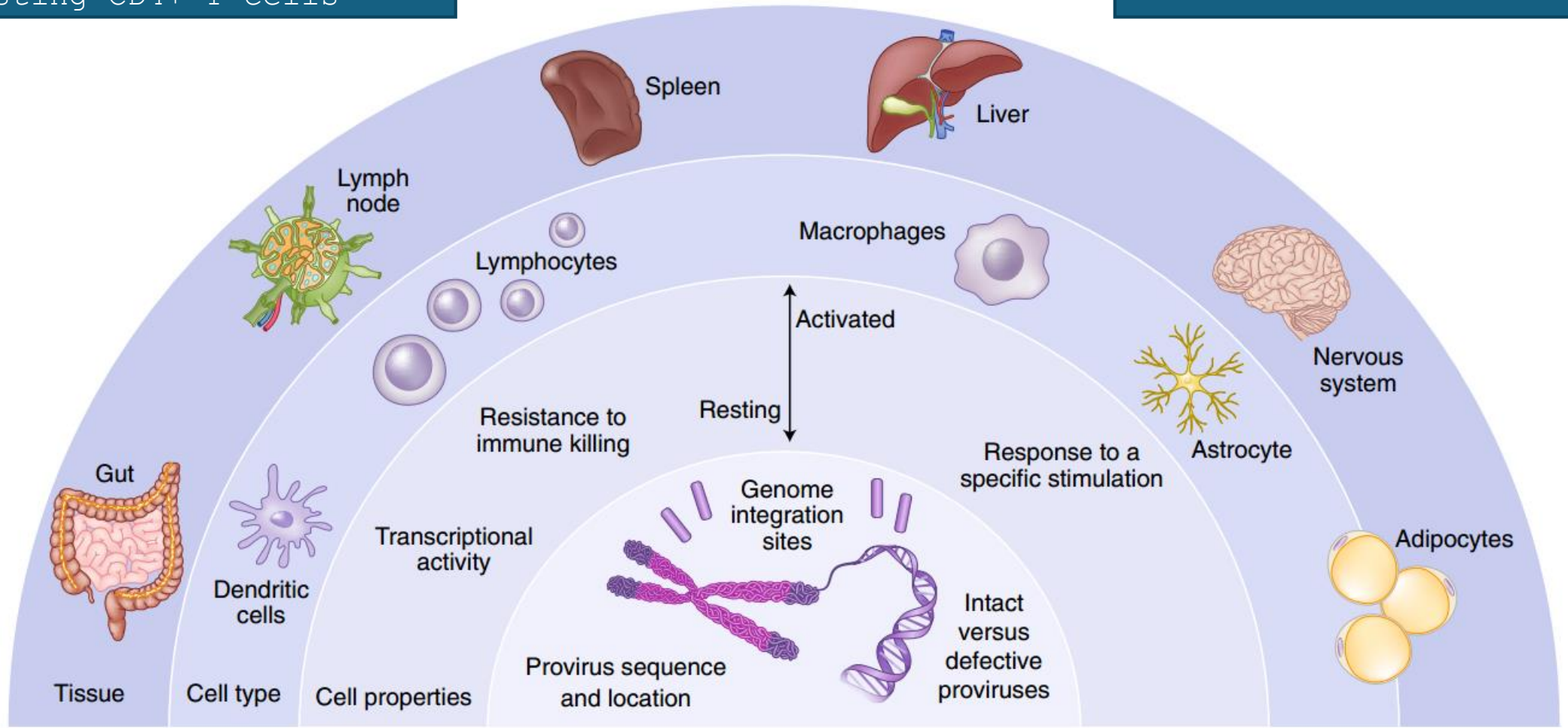
Depending on the proportion of cells that express antigen and are killed each year, it will take different amounts of time until the reservoir is notably reduced (estimated eradication time: 73.4 years on ART)

Davenport, Nat Rev Immunol 2018, Cho, 2022

Barrier to eradication: compartmentalization of reservoirs

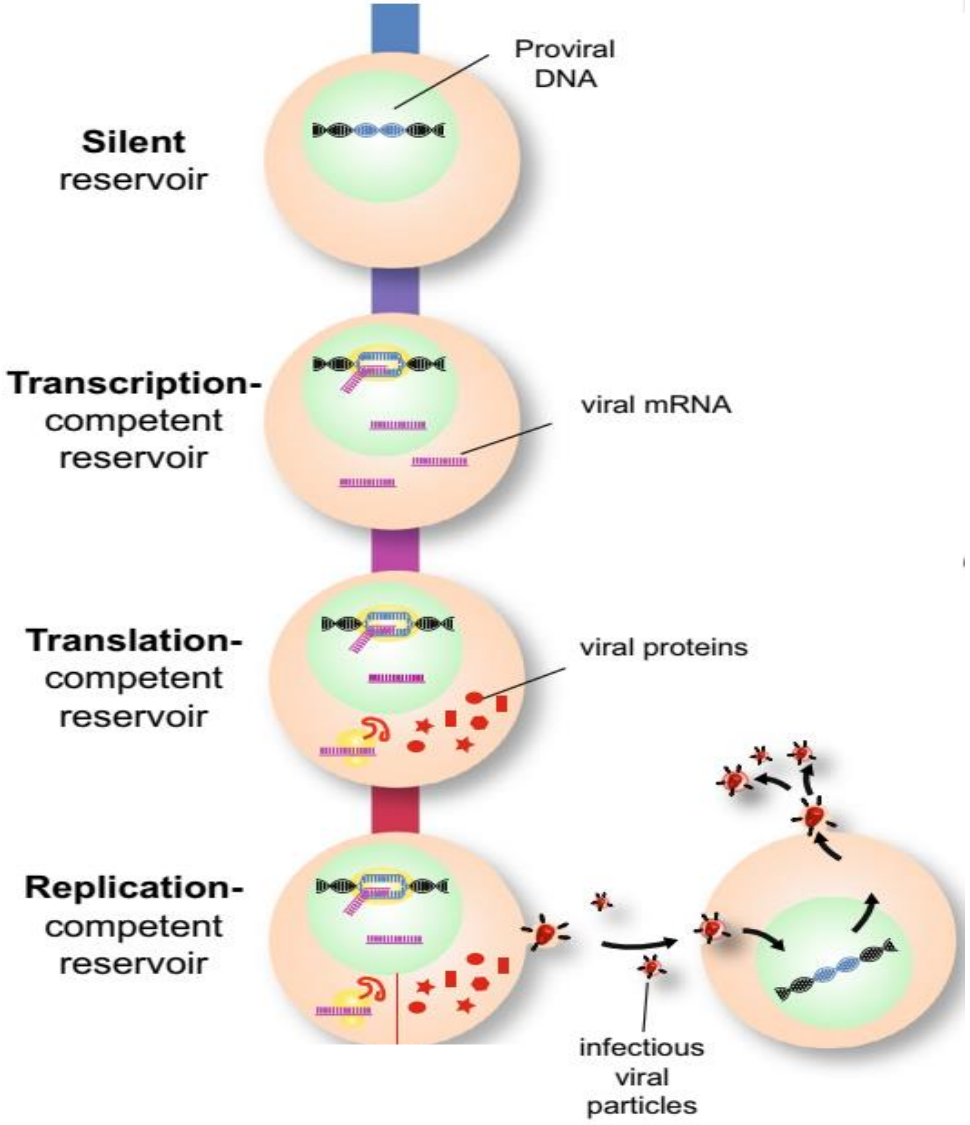
Latent reservoir is prevalently identified in resting CD4+ T cells

Blood is more characterized than GALT and macrophages



Deeks et al., Nature

Different assays targeting different marker



qPCR/ddPCR of total HIV DNA	→ Marker of persistence
Quantification of intact HIV DNA (e.g IPDA)	→ Marker of latent reservoir
Quantification of cell associated HIV RNA	→ Marker of active reservoir
Quantification of replication competent HIV (qVOA)	→ Marker of infectious reservoir

Phenotypic characterization of HIV-1 infected cells/tissues must be improved

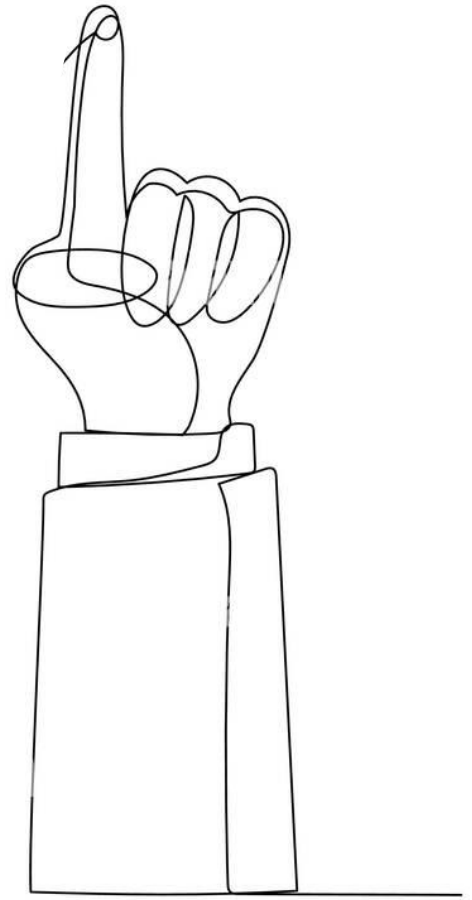
Towards eradication…

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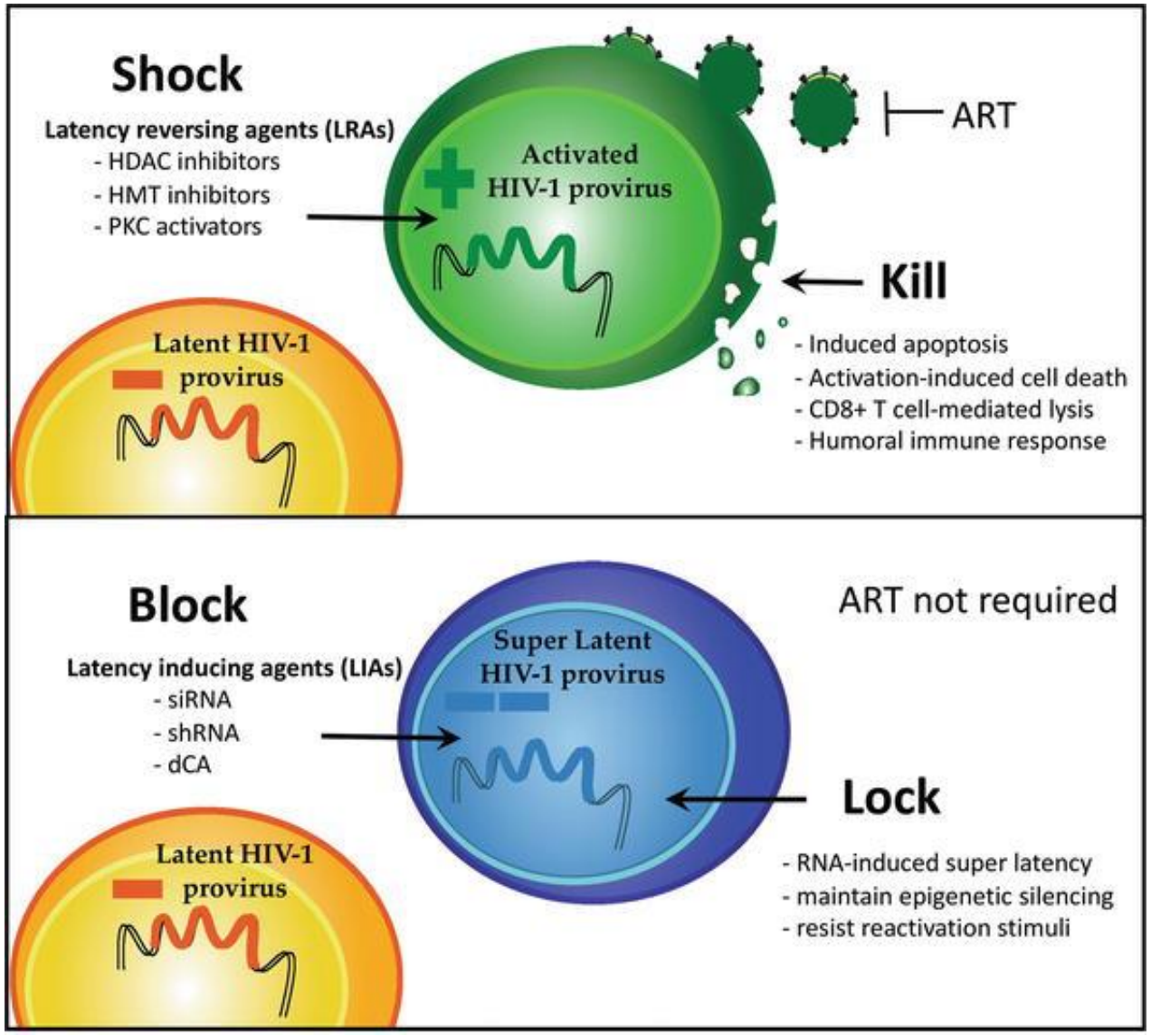
Evaluation of HIV-1 eradication strategies in vitro and in vivo

HIV-1 eradication



Improvement of Methodologies

Strategies for HIV-1 eradication



Sterilizing cure: eradication of reservoir

Critical: >10,000-fold reductions may be required to prevent rebound

Functional cure: control of HIV-1 infection without ART requirement

Thomas, Front. in Microb.

Progress in HIV eradication

Eradication strategies

Pharmacological (LRA, LPA)



Gene therapy (CRISP)





Immunological (self- not self)





HIV reservoir elimination

Methodology improvement

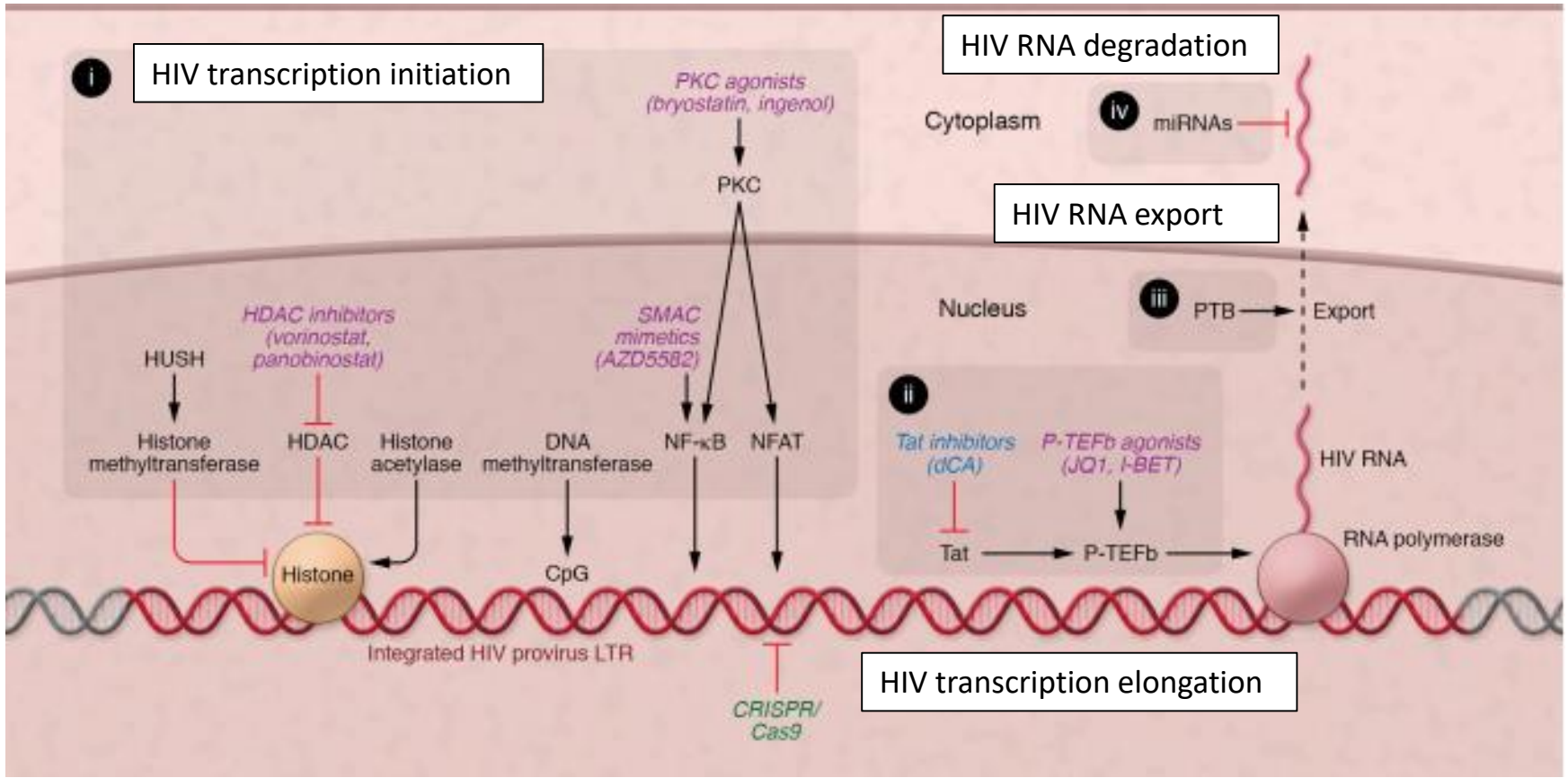
Delivery platforms

Drug potency

Discover of new biomarkers targeting HIV reservoir

Pharmacological approach

Pharmacological modulation of epigenetic or signalling pathways involved in HIV transcription to **reactivate** latent HIV or to **permanently silent** HIV transcription



HIV latency is heterogeneous thus a combination of LRA/LPA targeting various transcription pathways may be necessary

Black arrows: Pathways promoting HIV expression

Red arrows: Pathways inhibiting HIV expression

LRAs: **purple**

LPA: **blue**

Gene editing: **green**

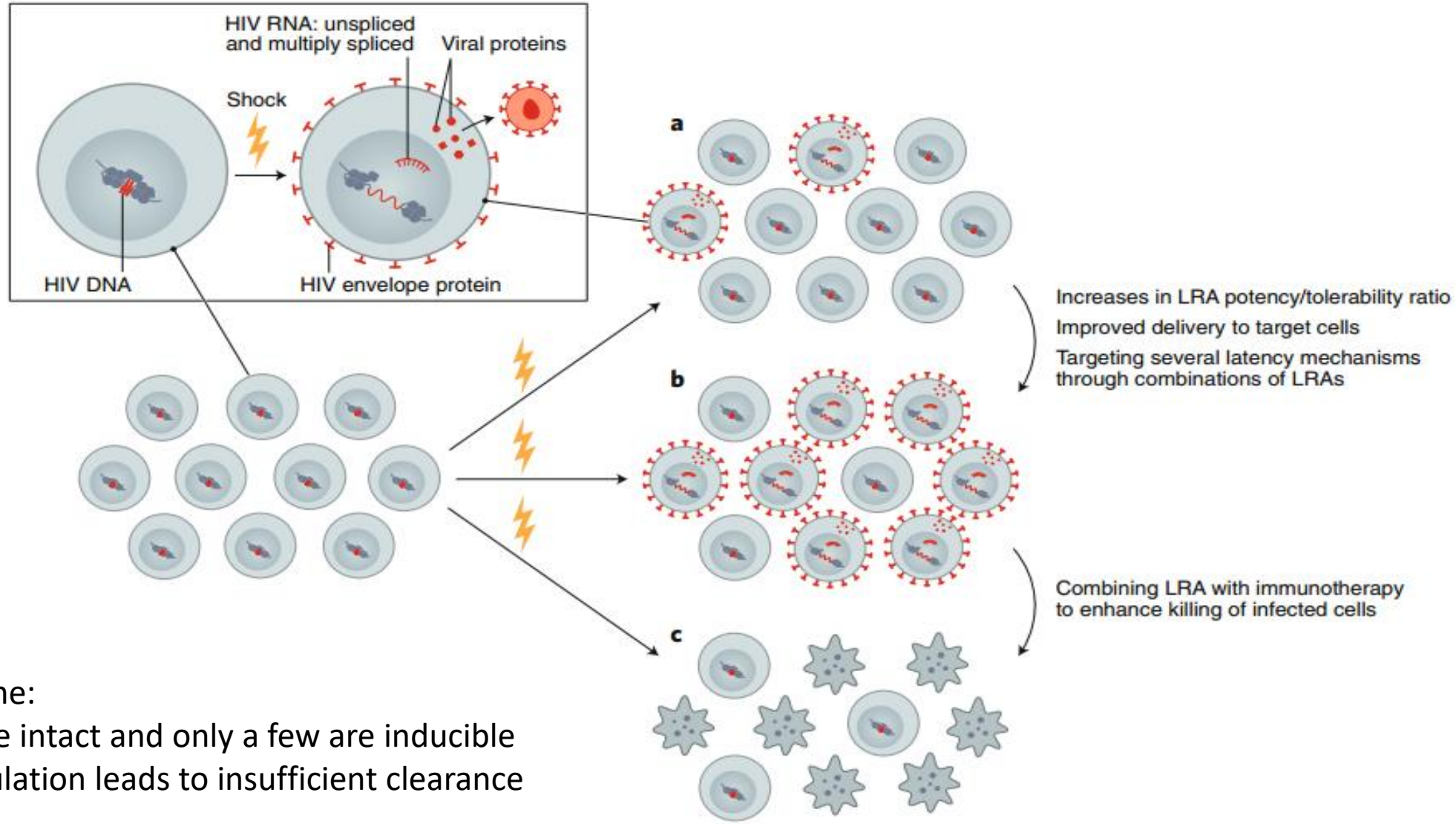
Tested in vivo: *italic*

PTB: polypyrimidine tract-binding protein

LPA: Efficacy in mouse models, persistence and delivery can be improved

LRA: Poor results *in clinical trials* also when used in combination with immunological approaches

Optimizing latency reversal



LRA limits to overcome:

- Few proviruses are intact and only a few are inducible
- Low antigen stimulation leads to insufficient clearance
- Off-targets effects
- Cells containing reactivated latent HIV may be resistant to killing by cytotoxic CD8 T cells

Deeks et al., Nature Medic

Genetic approach

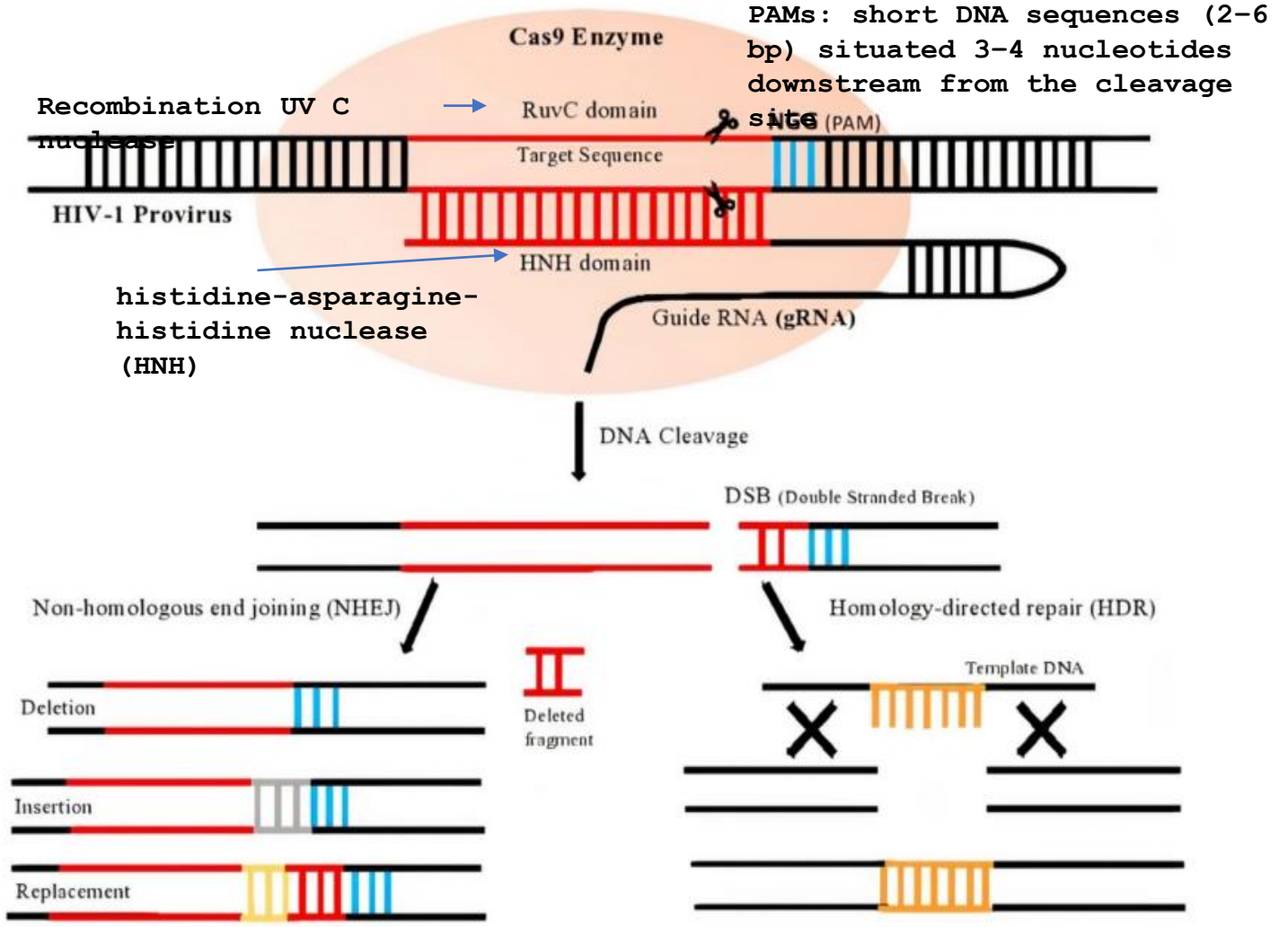
Replacement of HIV and/or host genetic regions

Endonuclease therapy:

- ZFNs (zinc finger nucleases)-> directly cut DNA
- CRISPR-CAS9 (Clustered Regularly Interspaced Short Palindromic Repeats)-> require a single guide RNA. **One clinical trial** is ongoing (**NCT05144386**), most studies performed in humanized mice

Pay attention to:

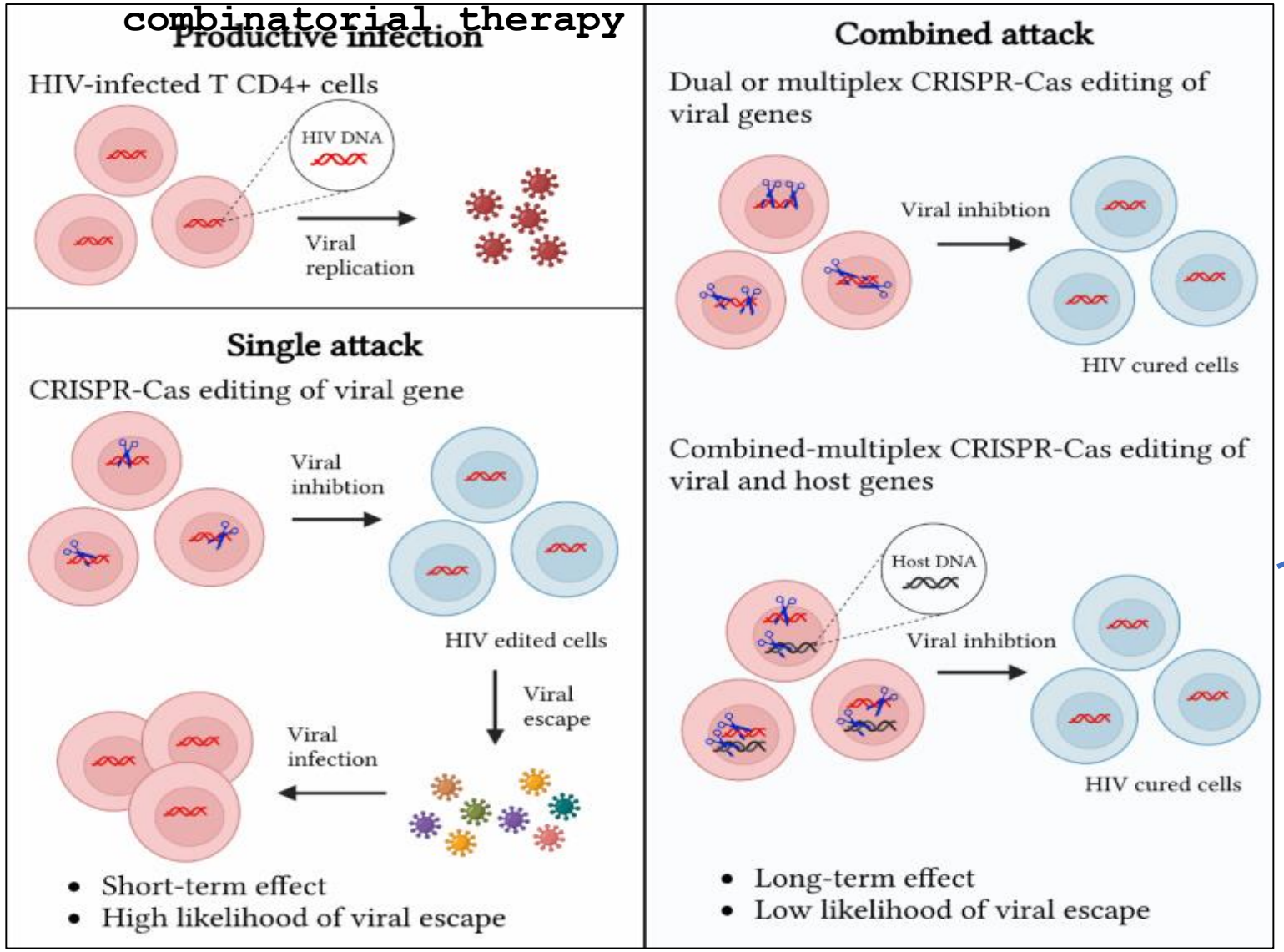
- HIV high mutational rate (reduced or impaired Cas9 cleavage activity)
- Off-target effects (low specificity)



Bhowmik 2022 AIDS Research and Therapy

CRISPR-Cas9 Genetic approach: HIV

Multiplex gRNAs or application of combinatorial therapy

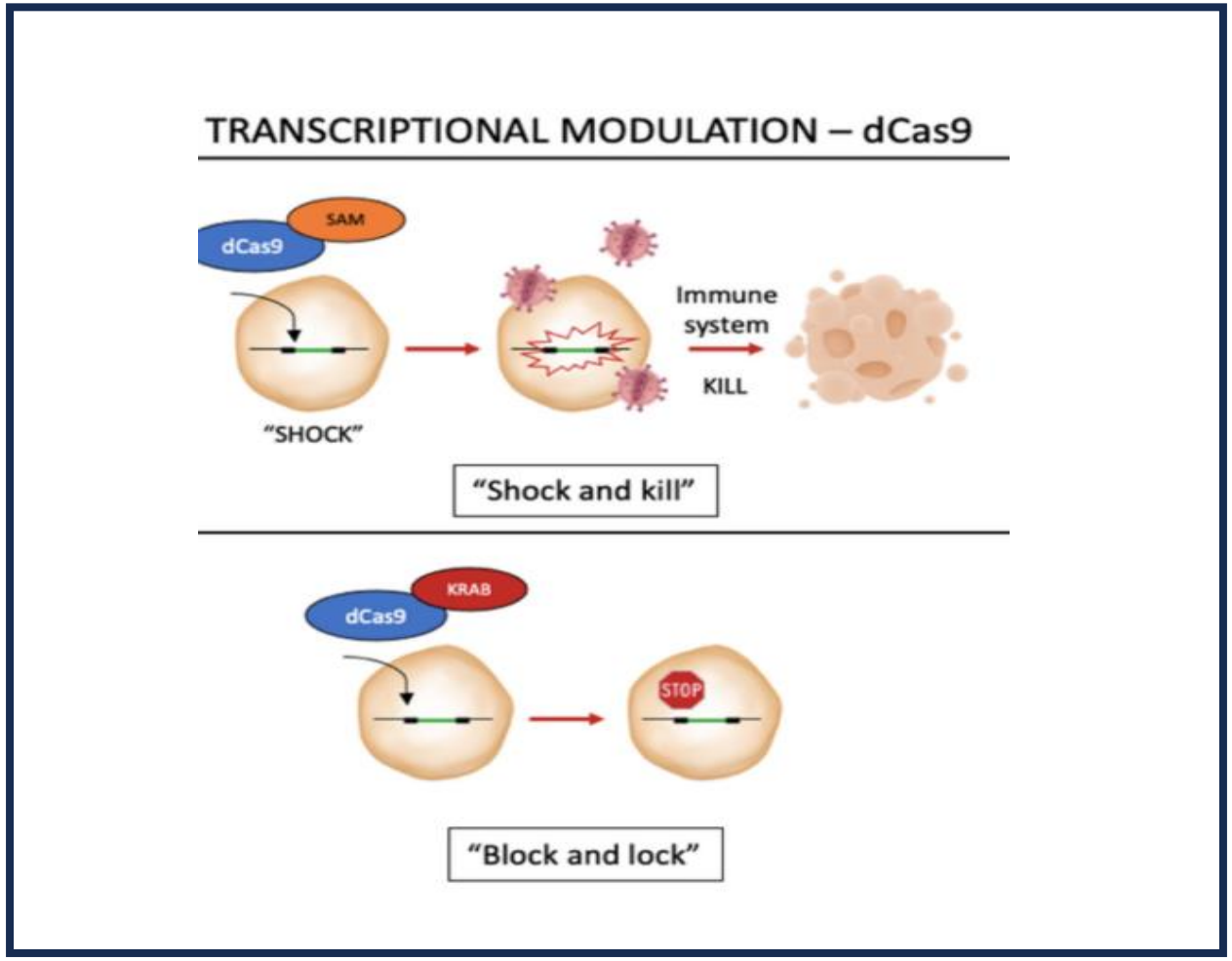


Host gene:CCR5 receptor. Halting of viral entry

Hussein IJMS 2023

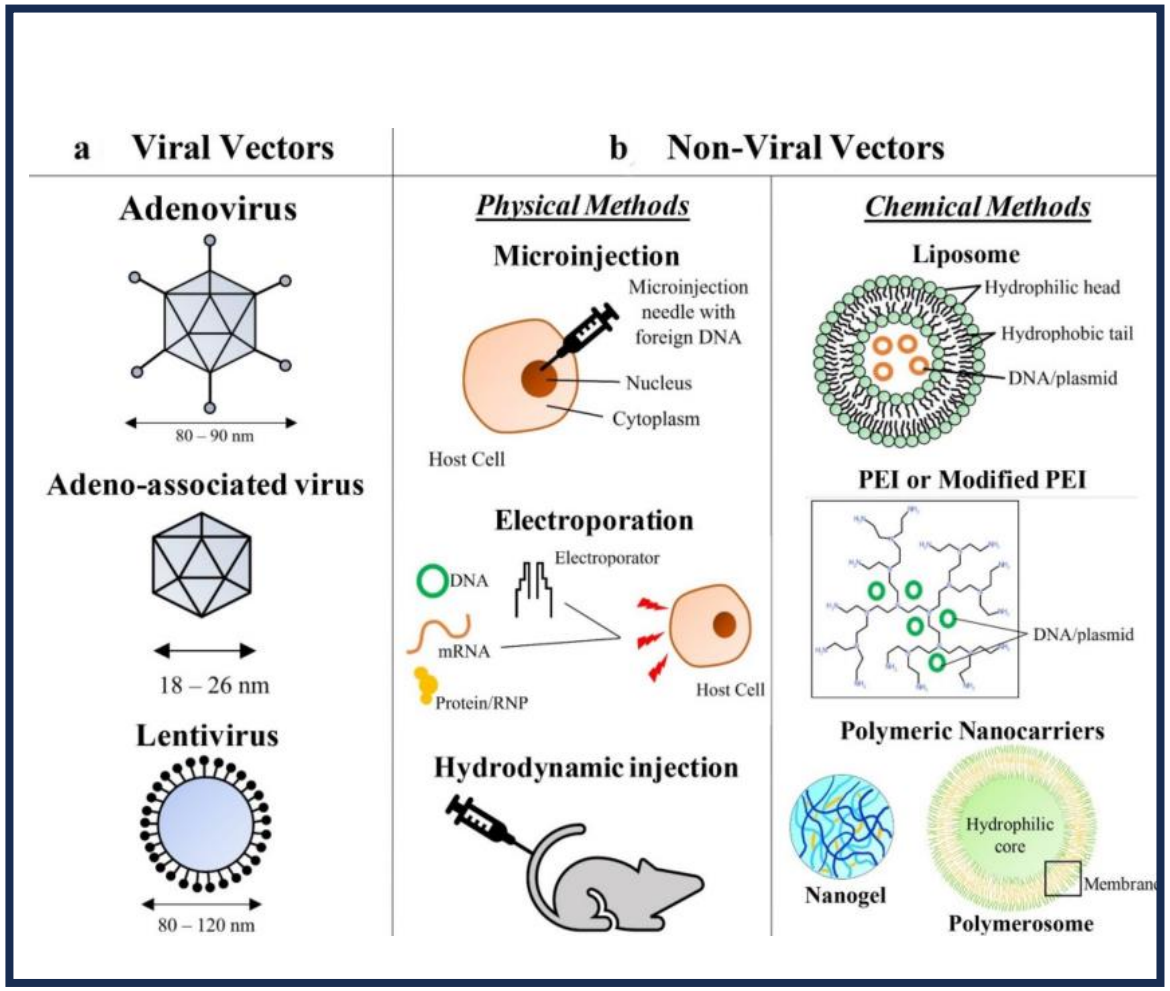
Genetic approach: Future developments

Novel Multiplex Cas9 Approaches



Magro Pathogens 2022

New delivery platform



Hussein IJMS 2023

Immunological approach

Enhance **«self»** immune response (CD8+ T cells)

Not self: Introduce artificially modified immune cells

Dendritic Cell vaccines

Used in combination with LRAs in different clinical trials

Cells are transfected to produce HIV antigens and other immunomodulatory proteins (clinical trials and mouse models)

To improve:

- Durability of response (frequent vaccination is required)
- Potency (immune enhancement) -> research of new immunogens (Bailon et al., Nat Med 2022)

Antibodies (large scale production by B-cell cloning techniques)

- Fc effectors
- bNAbs

CAR-T cells (T cells engineered to express chimeric antigen receptors)

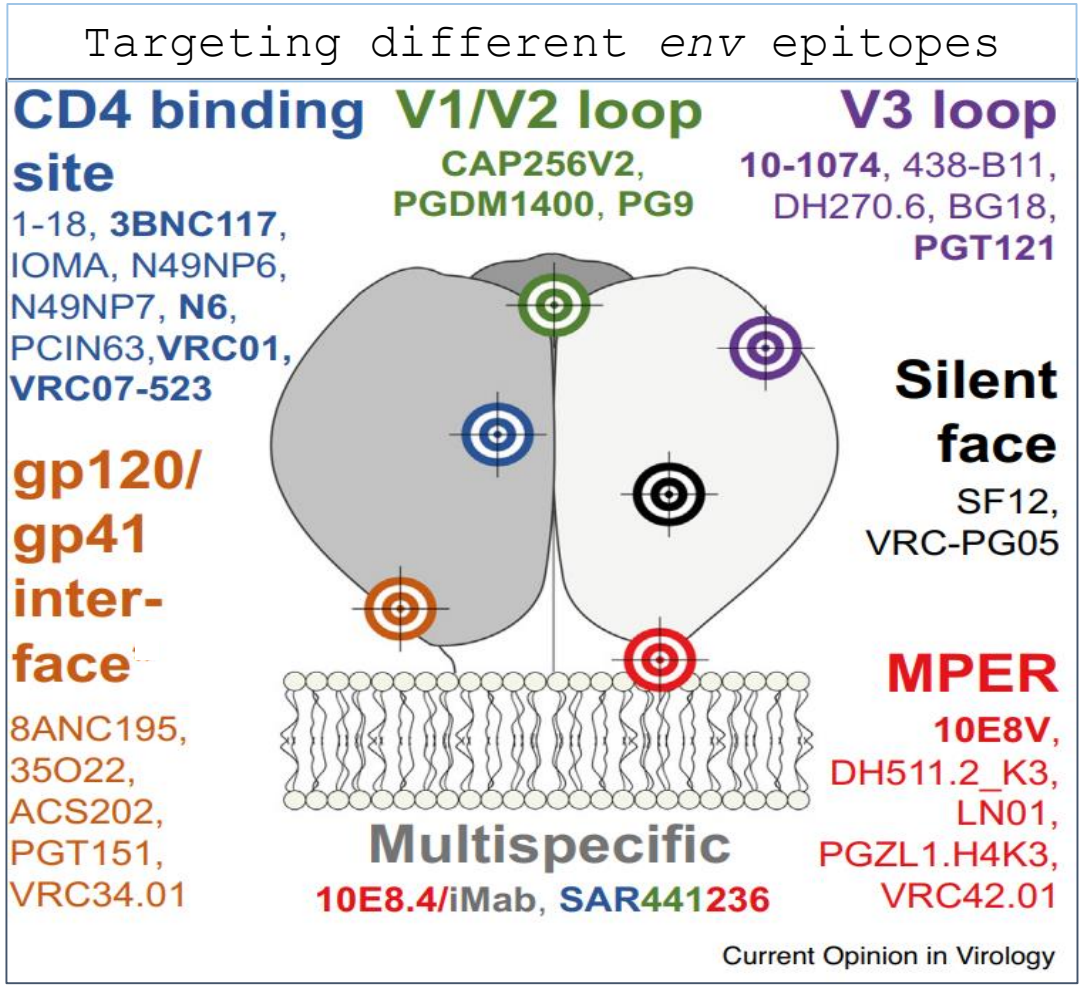
- Evolution toward bi/tri specific CAR

Immunomodulatory therapy

Immune checkpoint molecules (i.e. PD-1, LAG-3, TIGIT) are overexpressed on HIV infected cells, their inhibition can restore T cell exhaustion

Broadly neutralizing antibodies (bNAbs)

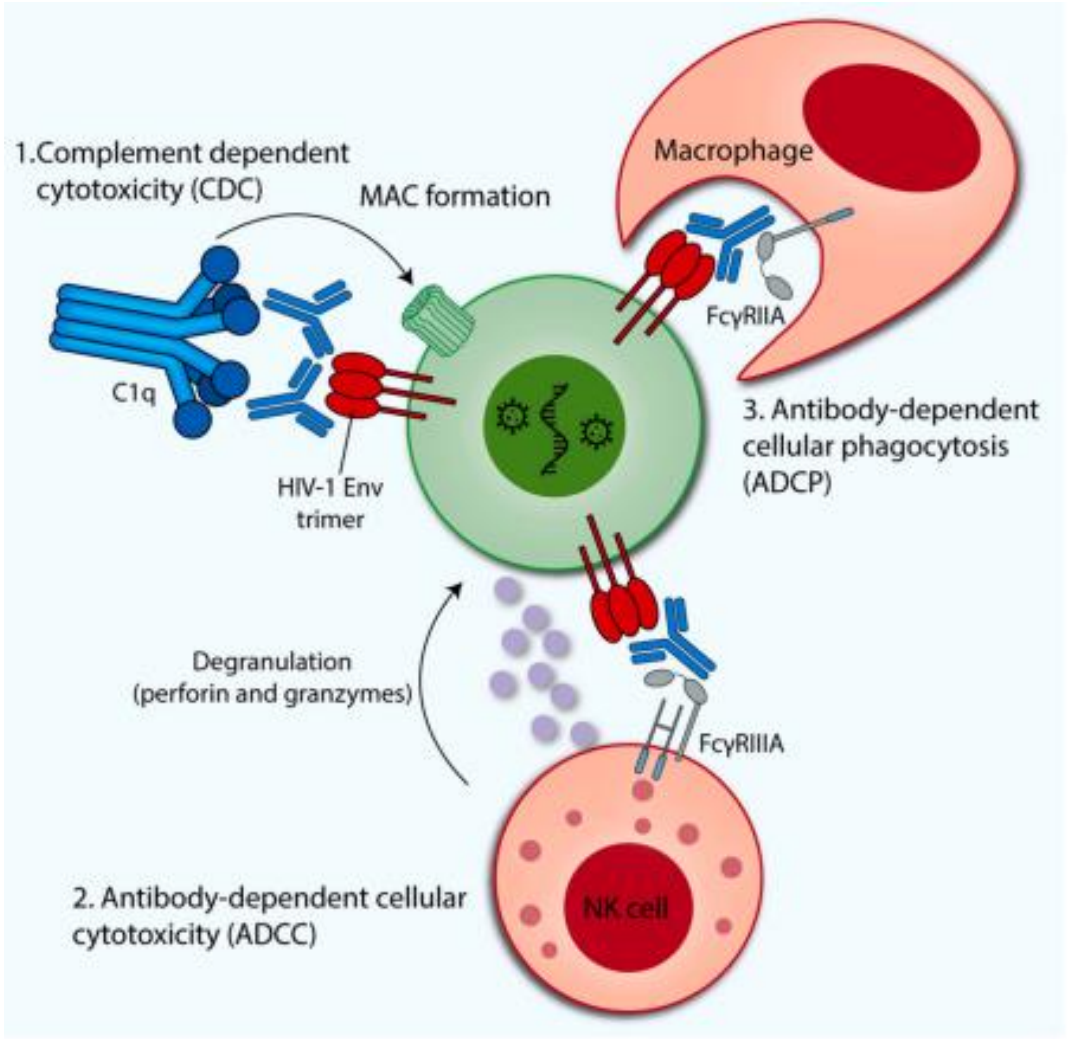
Targeting different *env* epitopes



MPER: membrane-proximal external region of gp41

Gruell 2022

Antibodies in **bold** have advanced into clinical trials



Schriek, Antiviral Reseach 2024

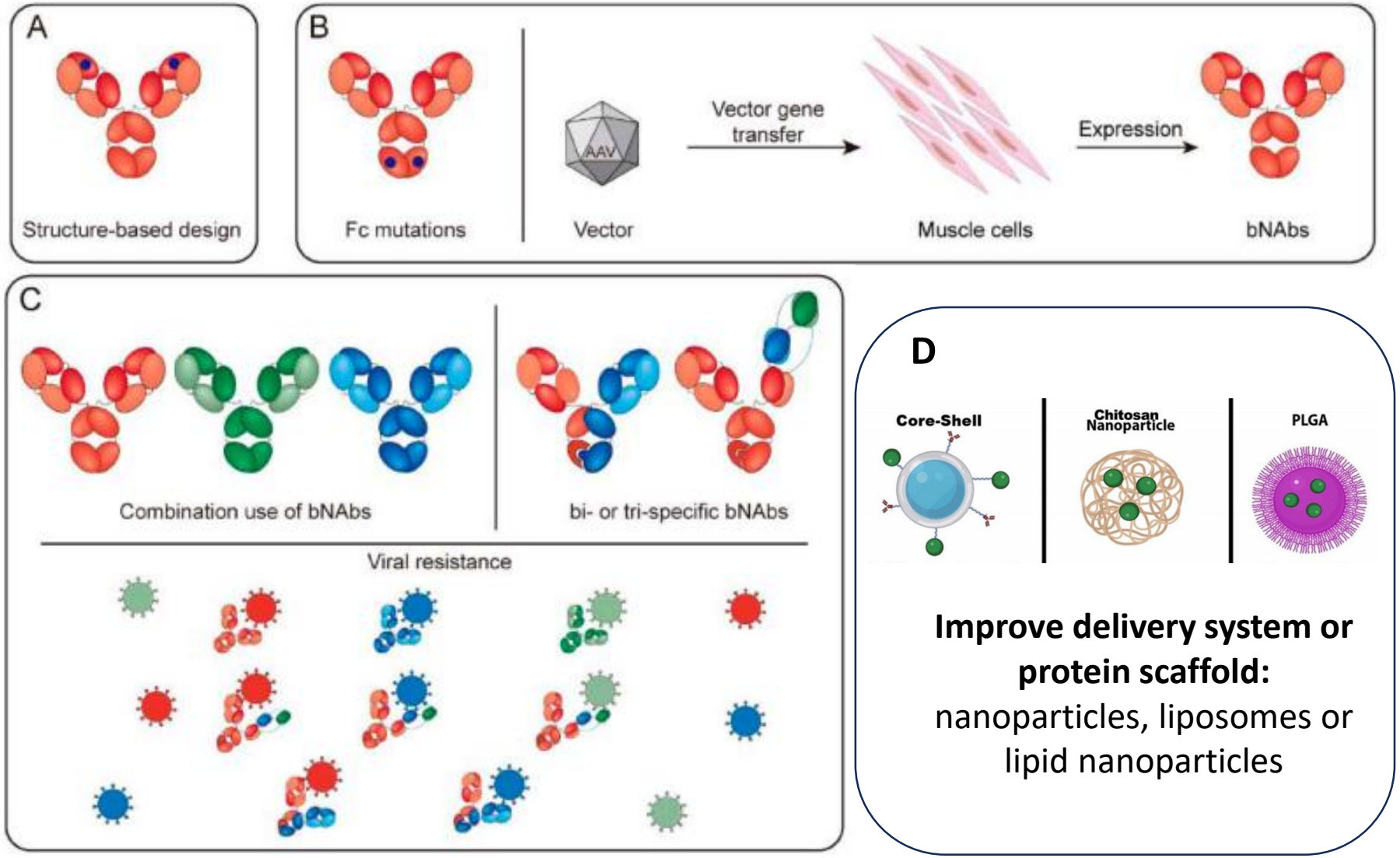
Antibody Fc-mediated effector functions

Strategies to improve bNabs efficacy

bNAbs limits

- Potency or breadth
- Low half-life
- Viral resistance (acquired and pre-existing)
- *Env* density and heterogeneity (mono/trimeric, truncated)
- No effects on transcriptionally si

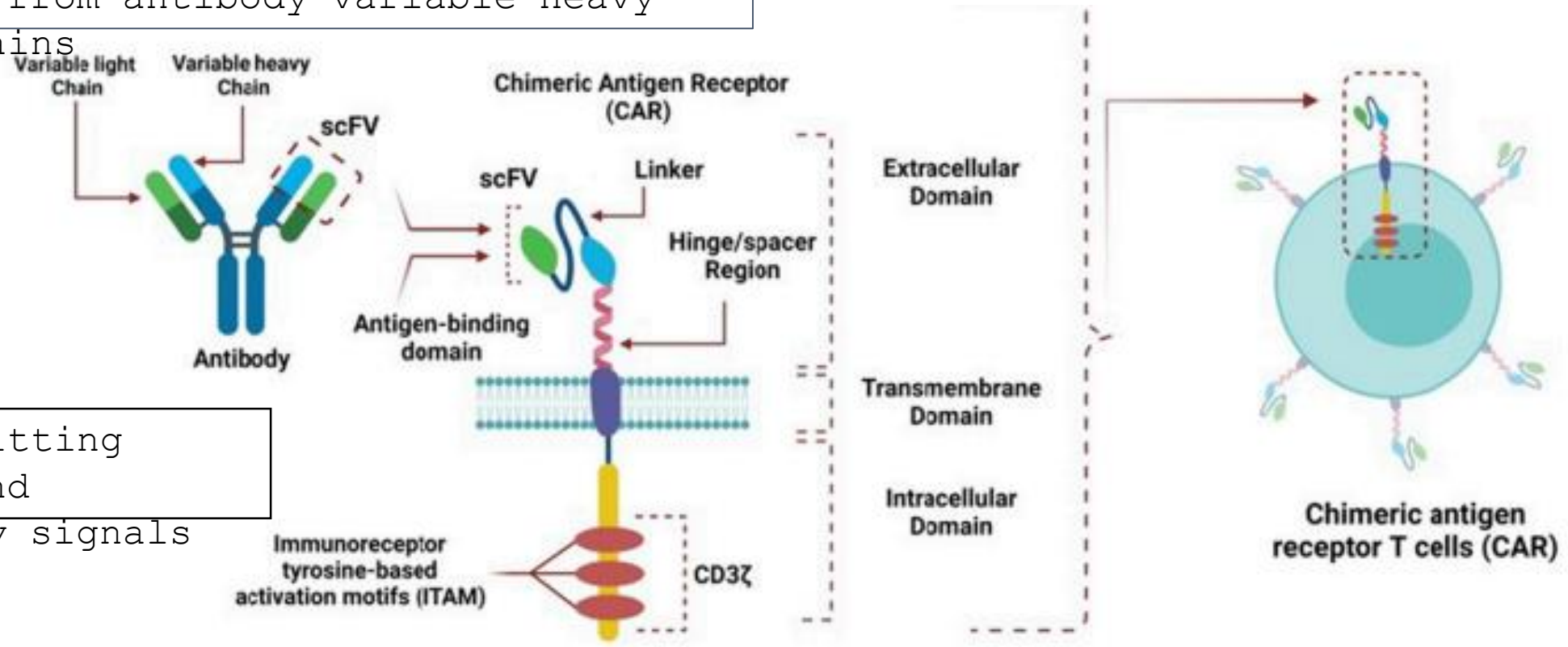
HIV cellular markers universally expressed on latently infected cells are not yet available



Liu 2020 Emerging Microbes and Infe

CAR T cells

scFV derived from antibody variable heavy and light chains

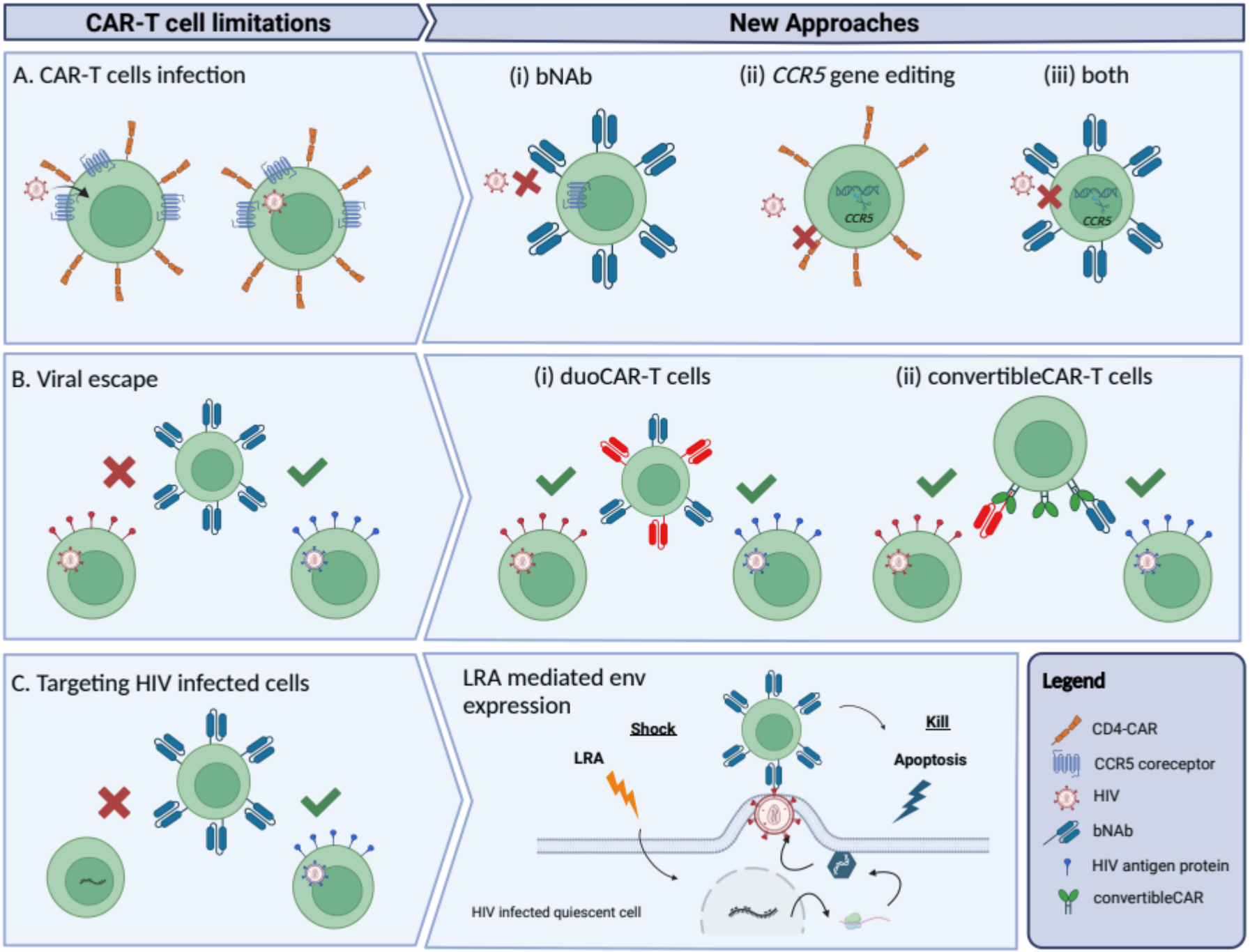


ITAM: transmitting activating and costimulatory signals to T cells

Extracellular domains contains hetero-trimeric glycoprotein gp120/gp41 and consist of either a CD4+ extracellular domain or a VH/VL segment derived from bnAbs

T cells can be transduced to express 1 (monoCAR), 2 (duoCAR) ore more unique CAR molecules

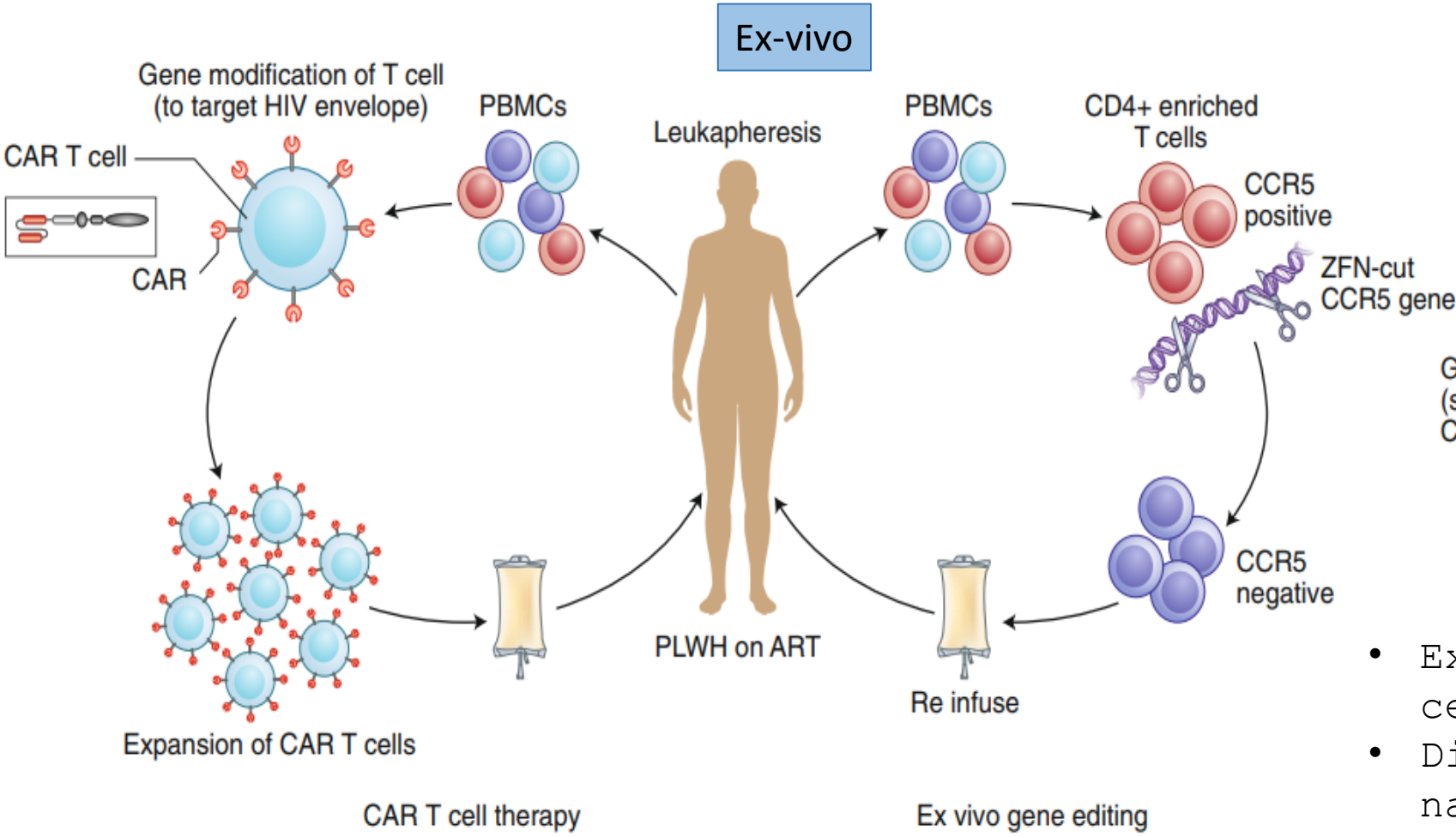
Alnefaie et al., Frontiers in Bioengiinering and

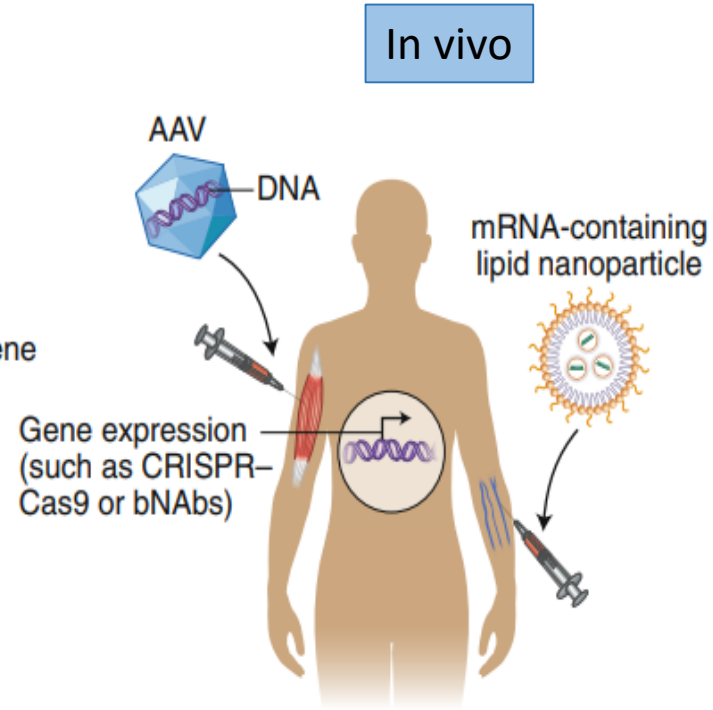


Main limitations of CAR-T cells as a therapy for HIV and new approaches to overcome this

Campos-Gonzalez et al. Viruses

Strategies for in vivo gene expression

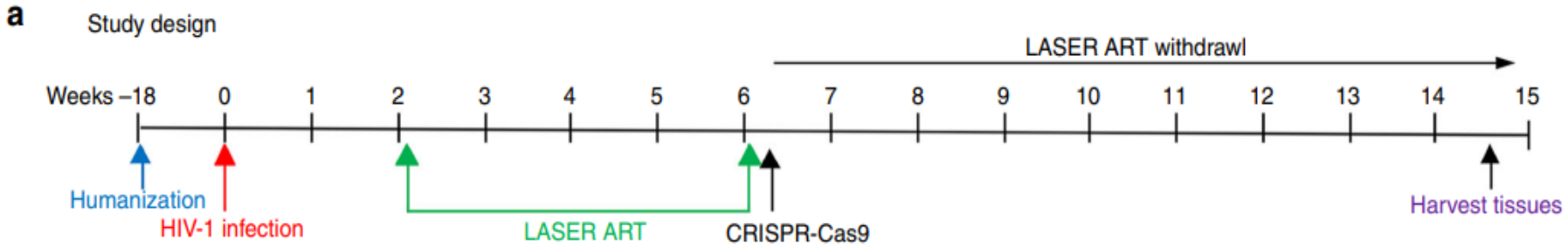




- External manipulation of cells is not required
- Direct administration of nanoparticles or viral vectors encapsulating mRNA or DNA, respectively

Deeks et al. Nature Medici

Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice

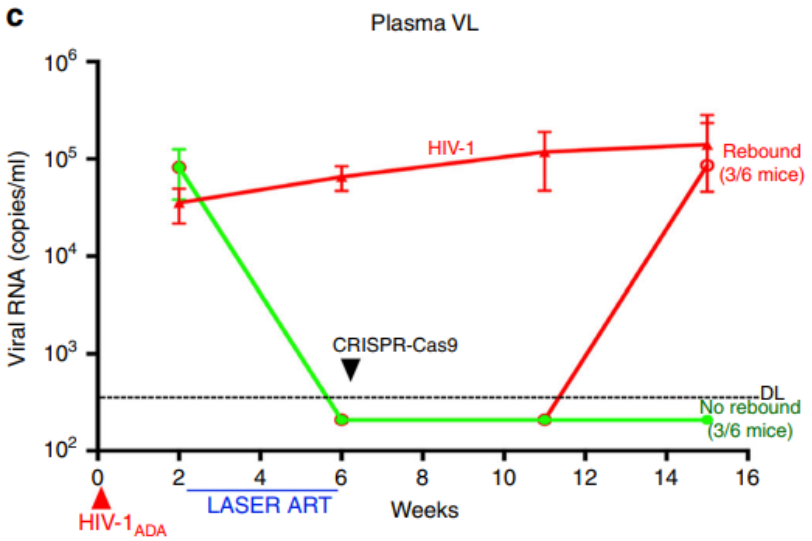


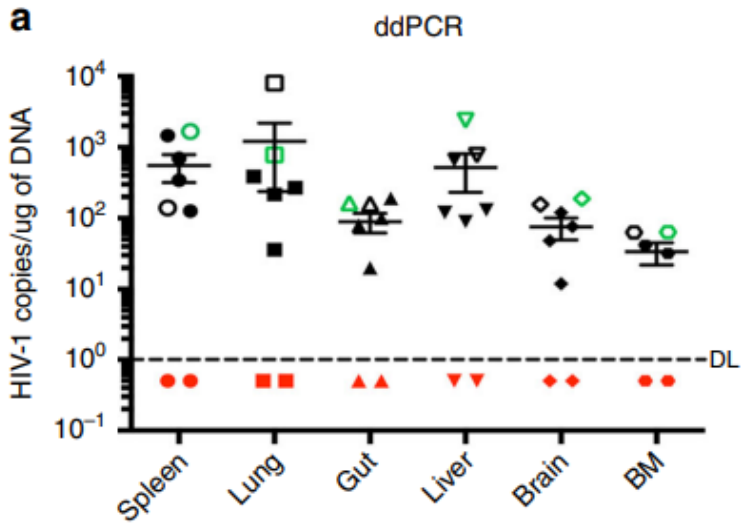
Infected animals were divided into 3 groups:

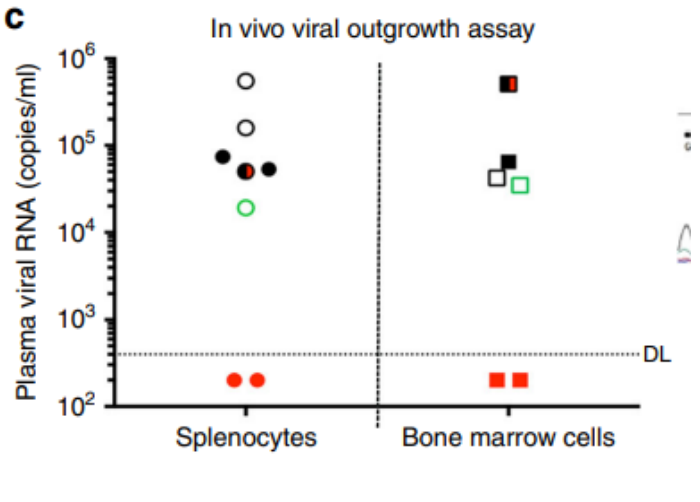
- **no treatment** infected group (n=4)
- received **LASER ART with CRISPR-Cas9** (n=6) or **without** (n=7).

LASER ART*: long-acting slow-effective release antiviral therapy. Highly hydrophobic antiretroviral slow drug dissolution, enhanced lipophilicity, improved bioavailability and limited off-target toxicities

*Fatty-acid modified prodrugs synthesized for dolutegravir (DTG), lamivudine (3TC), abacavir (ABC) and rilpivirine (RPV)



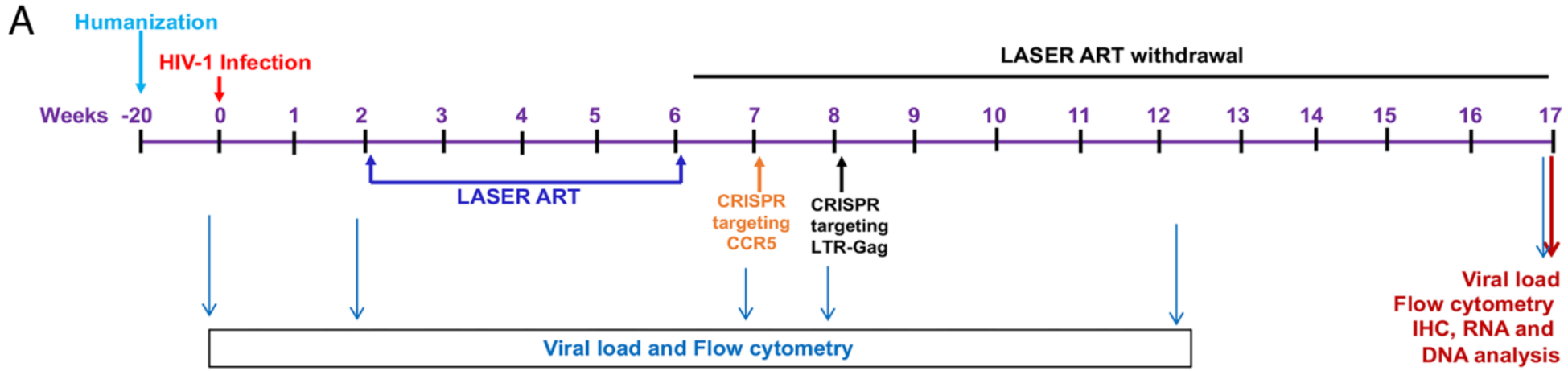


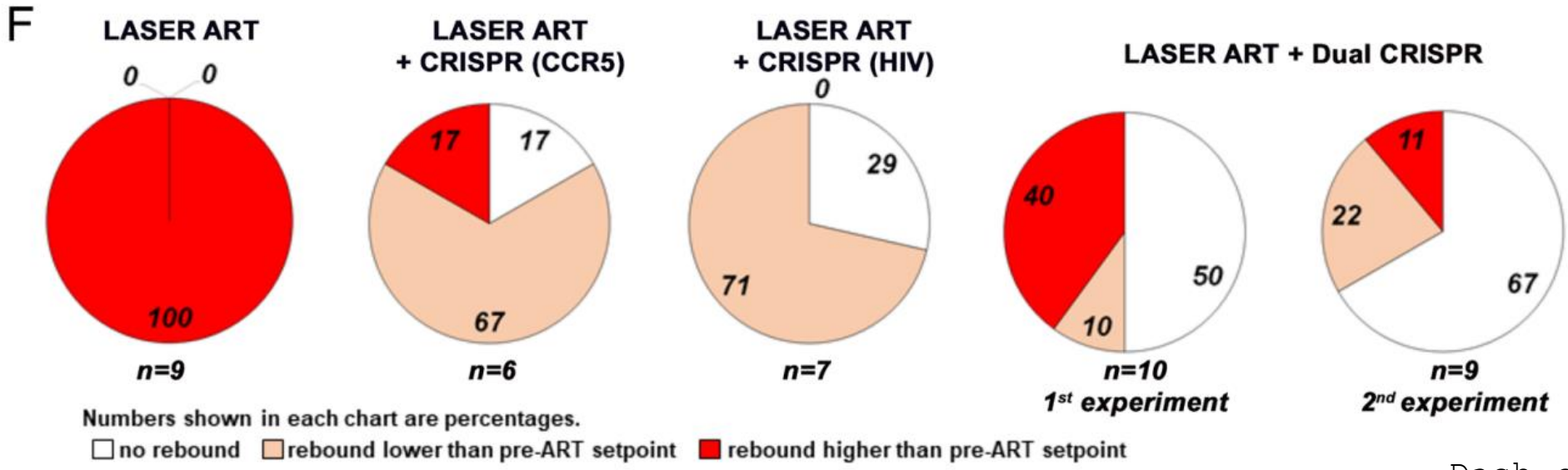


Continuous viral replication observed in untreated animals and treated with only LASER ART. 3/6 LASER ART and CRISPR-Cas9-dual-treated animals had no viral rebound and virus could be not detected in tissues

Dash. Nature Communications 2022

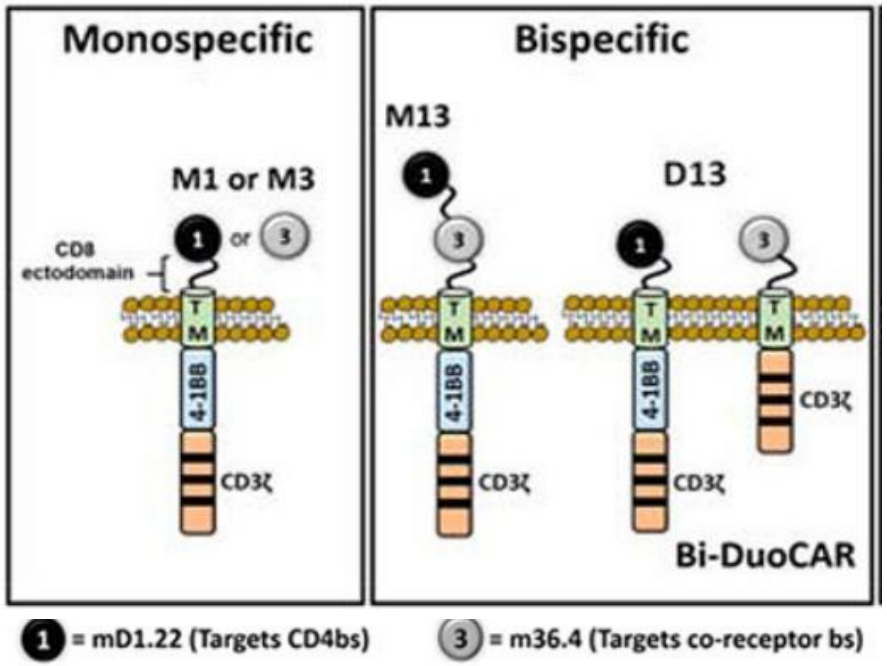
CRISPR editing of CCR5 and HIV-1 facilitates viral elimination in antiretroviral drug-suppressed virus-infected humanized mice

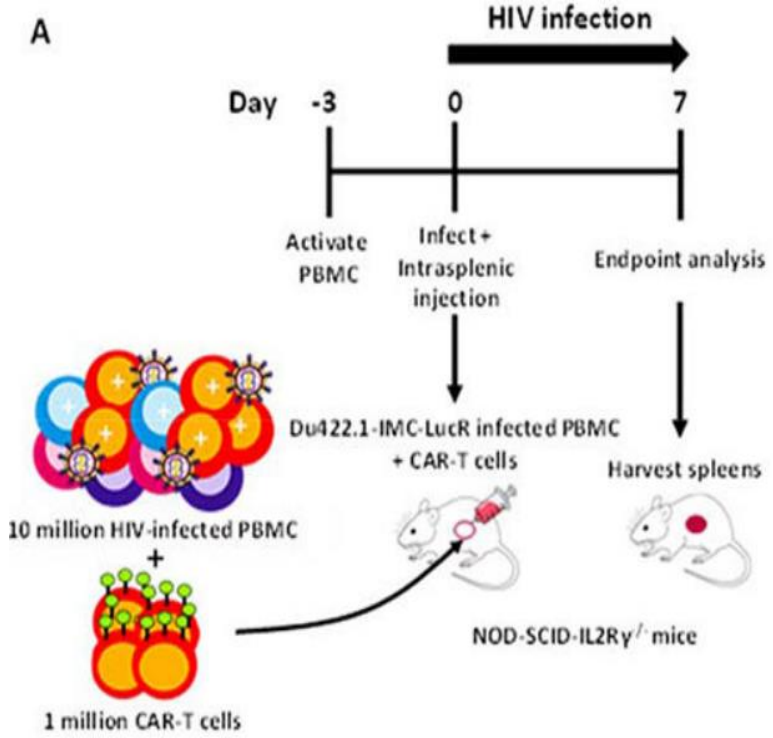


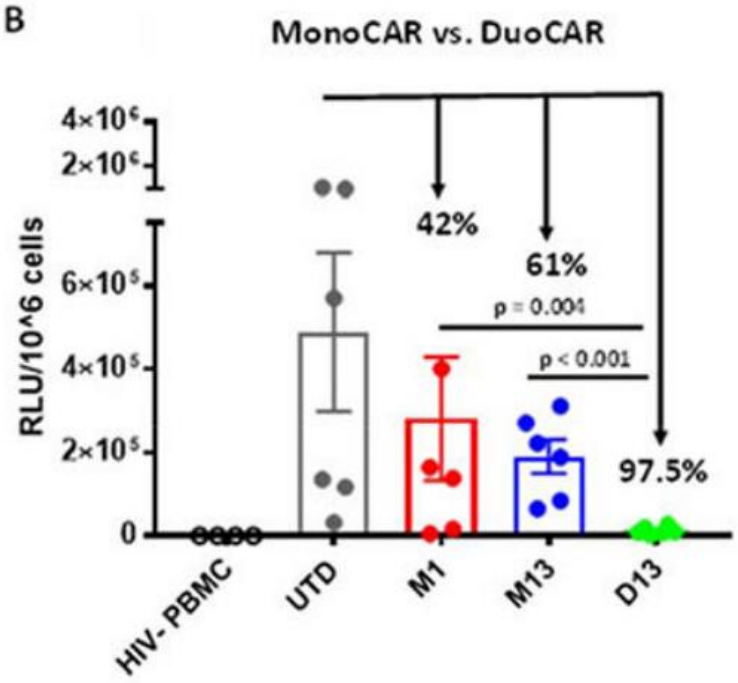


Dash et al., PNAS 2

Multispecific anti-HIV duoCAR-T cells in mouse models







- Transduction with lentiviral vectors encoding multispecific anti-HIV duoCARs confers primary T cells with the capacity to potently reduce cellular HIV infection by up to 99% in vitro and >97% in vivo
- The transduced T cells are protected from genetically diverse HIV-1 strains
- Multispecific anti-HIV duoCAR-T cells demonstrated long-term control of

Anthony-Gonda Science Trans. Med. 2019

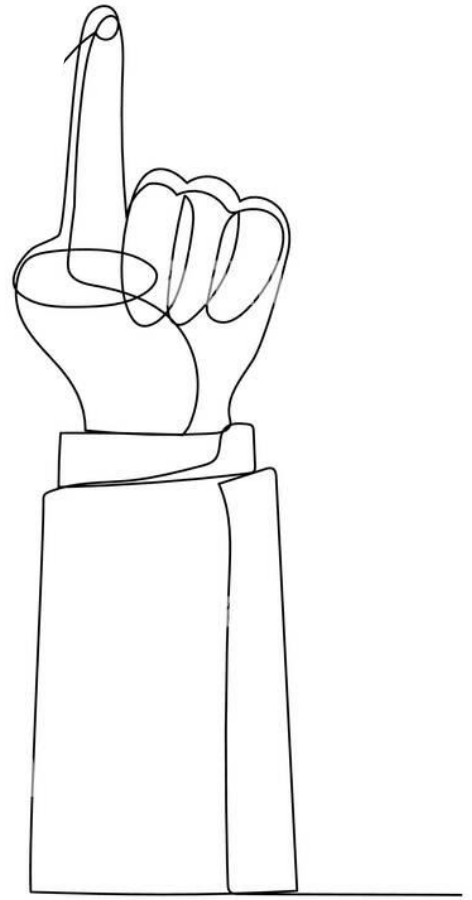
Towards eradication…

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HIV-1 eradication



Improvement of Methodologies

How to evaluate HIV cure strategies?

- *In vitro* assays:
  - Different methodologies to measure latency and reverse of latency are available.
    - High throughtput assay are needed.
    - Methods developed are subtype B specific.
  - HIV-1 RNA transcription pathways are complex, further studies are necessary.
  - Poor phenotypic markers of latency
- *In vivo* assays:
  - Mouse models
    - Different good humanized mice models have been developed (Fraker, AIDS Review 2022)
  - Non-human primate models
    - Barrier to access and high cost
  - Limits
    - ART duration (no more than 2 year)
    - Treatment related toxicities
    - Response to Simian or mouse adapted viral strain is different with respect to HIV (i.e. bNAbs)
- Human clinical trials:

Conclusions and perspectives

- Understanding the HIV reservoir
  - Size, distribution and activity
  - Mechanisms of HIV latency
- Eradication strategies
  - Expanded breadth across multiple HIV subtypes
  - Improved delivery strategies and durability
  - Combination of multiple approaches
- Evaluation of eradication strategies
  - Human organoid models
  - Improved mouse and non-human primate models
  - Reliable biomarkers associated with remission/cure to avoid ATI

All these goals are tightly related!!

Grazie per l’attenzione