Hot topics and advances in cure basic research

Najla Nasr

HIV Immunotherapeutic Lab

The University of Sydney, Sydney, Australia

The Westmead Institute for Medical Research

I have no financial interests or relationships to disclose

HIV reservoirs and latency

- Long-lived HIV reservoirs include proliferating CD4 T cells and macrophages that persist on ART
- Latency is maintained by epigenetic repression (histone deacetylation), low levels of transcriptional host/viral factors (P-TEFb/Tat & Rev), integration in non-coding genomic regions
- Reverse transcriptase error rate, host factors (APOBEC3G, RNA Pol II) drive viral diversity leading to HIV mutants that escape immune recognition & resist ART/Abs
- Anti-apoptotic mechanisms enable the survival of reactivated and latent cells (Ren et al., JCI 2020)
- Immune activation
    - Loss of gastrointestinal mucosal integrity followed by microbial translocation
    - Increased pro-inflammatory cytokine (IL-1b, TNF-α, IL-6)
    - Alteration in the balance of CD4 T cells and NK subpopulations
    - Active and non-active HIV reservoir under ART (Takata et al., Cell Host and Microbe 2023; Dube et al., Cell Host and Microbe 2023) – No transcriptional inhibitors yet!
    - High & active HIV reservoirs under ART sustained a high CD8 T cell numbers that are exhausted (Takata et al., Cell Host and Microbe 2023 and similar to Dube et al., Cell Host and Microbe 2023
    - Exhaustion of HIV-specific CD8 T cells and impaired function of NK cells
- CD8 T cells in LN, lungs and duodenum of PLH are cytokine-producing rather than cytotoxic (Papadopoulos et al., Cell report 2025, Harper et al., JCI 2022, Mvaya et al., JCInsight 2022)
    - This prevents tissue damage and inflammation in healthy controls and in PLH but limits their ability to clear HIV reservoirs (Mvaya et al., JCInsight 2022, Niess et al; Sci Immunol 2022)

Requirement for successful HIV Cure

- **Kick and Kill**
  - Latency reversing agents (LRA)
  - Sensitising reactivated cells to cell death
  - Immune mediated clearance via:
    - CAR T cells
    - NK cells
    - Broadly neutralizing antibodies (bnAb)

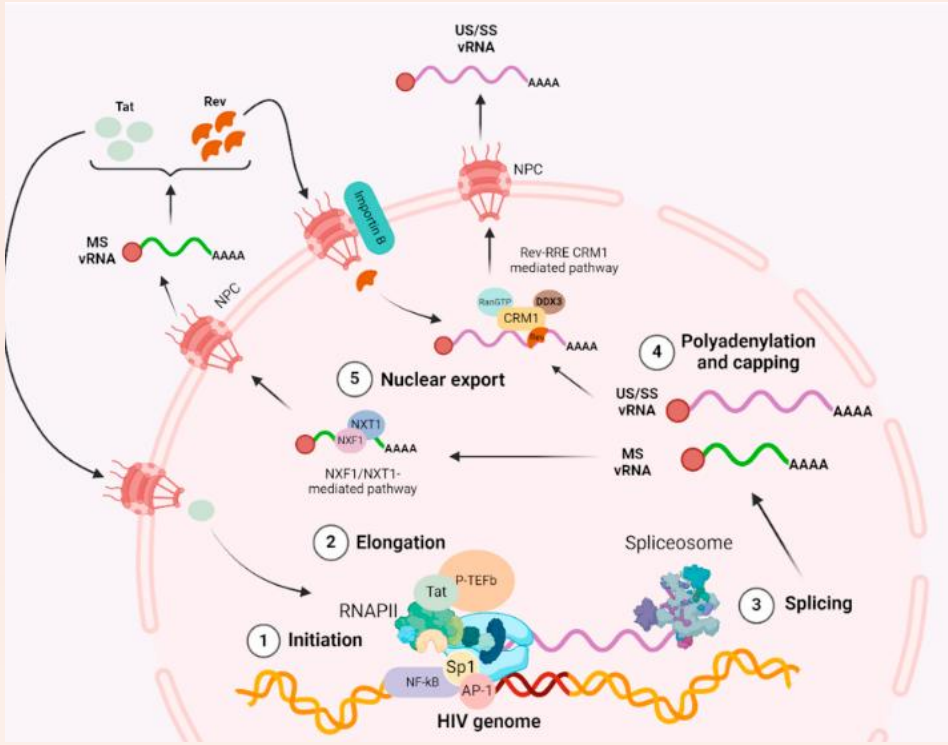
- **Block and Lock**

First generation LRAs were too weak or too toxic to reactivate HIV

- **HDAC inhibitors** (e.g., vorinostat, panobinostat) loosen chromatin - modest reactivation in vivo without killing reactivated T cells
- **PKC agonists** (e.g., bryostatin-1) activate NF-κB - cause broad immune activation, inflammation, and bystander T-cell death
- **TLR agonists -** Modest latency reversal in humans; risk of immune overactivation
- **Cytokines**
  - IFNγ, IL-2, IFNα2 had no effect on HIV viral load post ART interruption
  - IL-15 reactivated silent HIV and enhanced the survival, proliferation, cytotoxicity of HIV specific CD8+ T cells and NK cells (Manganaro et., Proc Natl Acad Sci 2018)
    - IL-15 receptor agonist, N-803, developed by ImmunityBio to maximize its tissue distribution. N-803 is safe in clinical studies, but there is a risk of induced proliferation of infected T cells (IAS 2025)

HIV cure requires strong HIV reactivation

- HIV latency is maintained by roadblocks along the different transcription stage (Yulk et al., 2018) - elongation, splicing and completion (poly A). Therefore, HIV is not visible to the immune system
- Need a combination of first generation LRAs to reverse different blocks to HIV transcription (Darcis et al., 2015)
    - HDAC inhibitors reverse block in elongation while PKC agonists reverse block to splicing and completion
- LRAs are not HIV-specific, have off target/adverse effects- some inhibit CD8 T cell function!
- None has reduced the size of the HIV reservoir
- Research is focused on finding a specific, safe and strong reactivating LRA



Izquierdo-Pujol et al; Microorganisms 2024

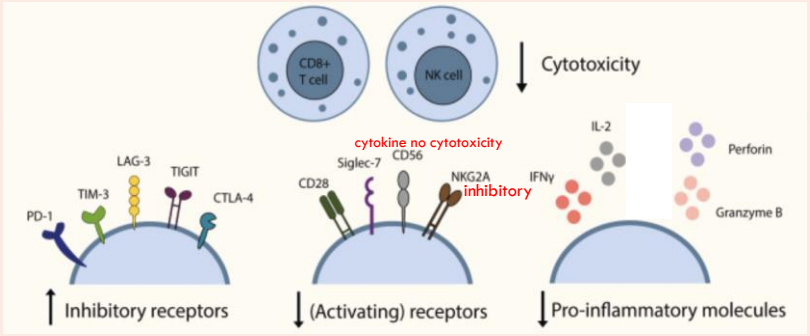
Tat mRNA reversed latency but did not induce killing of reactivated T cells

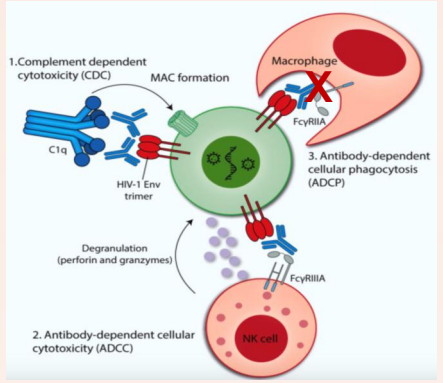
(Pardons et al., Nature Comm 2023; Cevaal et al., Nature Comm 2025)

- Tat is essential for transcriptional elongation from HIV LTR
- Novel lipid nanoparticle formulation (LNP) delivered ex vivo Tat mRNA into $CD4^+$ T cells of PLH on ART
- Tat-LNPs enhanced HIV transcription at all stages and increased virion production
- However, there was no cell death or decline in the HIV reservoir - consistent with latently and reactivated cells overexpressing pro-survival proteins such as BCL2 (Ren et al., JCI 2020)
- Need to combine potent LRAs with additional interventions: sensitise infected cells to death and/or enhance immune-mediated clearance
  - Venetoclax, an apoptosis drug used to treat cancer, has been found to antagonise BCL2 by selectively killing HIV infected cells ex-vivo & in mice (Arandjelovic et al., cell Report Med 2023)
  - **AMBER** Clinical trial started to assess whether upon ATI, Venetoclax will lead to an HIV cure

Immune-mediated clearance

- Immune checkpoint blockade on CD8 T cells did not significantly delay or reduce rebound HIV upon ART interruption – variable results in PLH (Amancha et al., 2023, J immunology)
- HIV mutates to escape recognition (10- 21 days pi) in response to potent T cell responses (Ferrari et al., Plos Path 2011, Deng et al, Nature 2015)
- Downregulation of MHC-I on productively infected CD4 T cells preventing HIV presentation to CD8 T cells (Duette et al., JCI 2022)
- Microenvironmental factors such as IL-10 prevent CD8 TRM cytotoxicity (Harper et al., JCI 2022)
- Elevated HLA-E on Tfh cells engaged NKG2A-expressing CD8 T cells to downregulate granzymes/perforin and cytotoxicity (Papadopoulos et al., Cell report 2025)





Schriek et al., Antiviral Research 2024

Therefore, we need immune boosting after an LRA or upon ATI via:

- T Cell Immunotherapy: engineer CD8 T cells with **CAR** such as **Abs** targeting conserved regions of HIV Env (work independent of MHC-I)
- **Potent bnAbs** to induce killing of cells expressing HIV Env via **NK cells**

Abs via their interaction with FcγRs facilitate effector functions such as ADCC (NKs), ADCP (MQs) or CDC

Immune-mediated clearance via CAR T cells

- **CAR T cells were** assessed in two recent clinical trials
- One failed within 5 weeks of ART interruption due to pre-existing & emergence of viral mutants to a single bNAb (VRC01) expressing CAR T cells (Liu et al; JCI 2021)

Second Trial was conducted under ART and an LRA (Mao et al; Cell Discovery 2024)

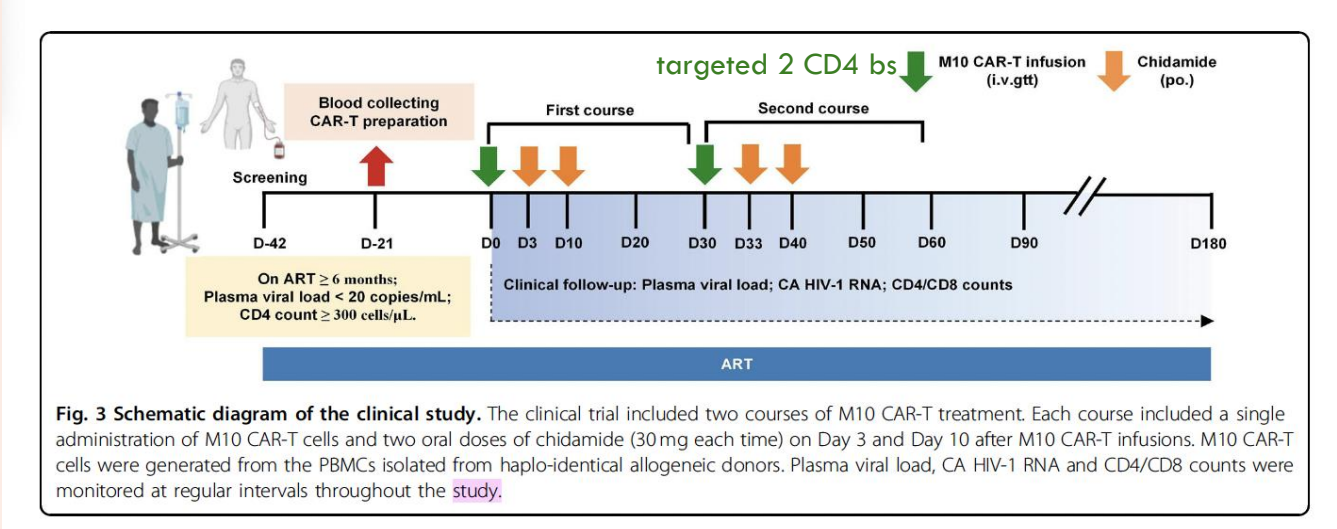
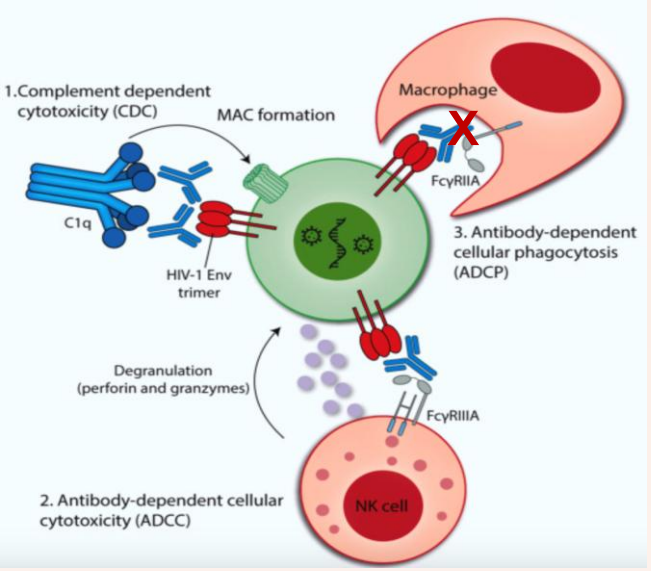


Fig. 3 Schematic diagram of the clinical study. The clinical trial included two courses of M10 CAR-T treatment. Each course included a single administration of M10 CAR-T cells and two oral doses of chidamide (30 mg each time) on Day 3 and Day 10 after M10 CAR-T infusions. M10 CAR-T cells were generated from the PBMCs isolated from haplo-identical allogeneic donors. Plasma viral load, CA HIV-1 RNA and CD4/CD8 counts were monitored at regular intervals throughout the study.

- CARs targeted two HIV binding sites, secreted a bnAb, and expressed reservoir homing markers
- Despite efficient HIV control by CAR T cells, long-term efficacy remains unknown as PLH never stopped ART
- Once the "current dominant Env" strain was suppressed, more ancient variants were detected
- Chidamide reactivates the latent T-cell reservoir. Its ability to reverse viral latency in macrophages is unclear

Immune-mediated clearance by NK cells

- Long lived HIV-infected macrophages are resistant to CD8 T and NK cell killing (Clayton et al., Nat Immunol 2018 ; Clayton et al., cell Host & microbe 2021)
- NK/CD8 T cell interactions with infected macrophages skew the response to cytokine production, rather than cytolytic effector function
- Strategies to enhance macrophage killing are essential for HIV cure
  - NKs are impaired in chronic HIV - stimulation with IL-15 agonist to activate them
  - NKs have low affinity FcR (CD16-FcγRIIIa) – genetically modified to express the high-affinity FcR (CD64- FcγRIa) thus increasing their retention of loaded bnAbs to enhance their capacity to target infected cells via ADCC – evidence of killing CD4 T cells but not yet macrophages (Tomescu et al., J Immunol 2025)
  - Assess which anti-HIV Abs best recognize HIV-infected macrophages
  - Identify the most lethal subset of NKs, which will be recruited by Abs to kill infected macrophages



Immune-mediated clearance by bNAbs

- Passive administration have a transient and insufficient effect to sustain viral control
- bnAbs efficacy is hampered by:
  - Ab half-life (7-21 days!)
  - High production costs, scalability and affordability
  - Env density: Pair bNAbs with LRA (or ART interruption) to increase Env density
  - Viral diversity/resistance: Triple bNAb therapy only delayed viral rebound due to pre-existing resistance and bNAb rapid decay (Thavarajah et al., Viruses 2024)
  - Fc engineering for improved FcγR binding of bnAbs & effector functions of NKs
    - Fc point mutations, reduced Fc glycolysation (Edwards et al., JV 2021 Ackerman et al., JCI 2013; Anand et., JV 2021)
    - improved antiviral activity in vitro/animal models, their impact on reservoir clearance is yet to be tested
- Combining TLR7 or IL-15 receptor agonist with 2 bnAbs upon ATI: control of HIV for < 1 year (IAS 2025)

Challenges and outlook for an HIV cure

- **Key challenges**
  - Viral diversity & escape mutations: engineering dual/triple? targeting cellular therapies
  - CD8 T cells skewed toward inflammatory cytokines rather than cytotoxic functions in tissues
    - LN directed CD8 T cells did not prevent SIV rebound during ART interruption, even with IL-15 and blocking PD1 (Pampena et al., Proc Natl acad Sci, 2025)
    - Would targeting the HLA-E/NKG2A interaction boost cytotoxicity and immune-mediated clearance of HIV in LN? (Papadopoulos et al., Cell report 2025)
  - Safety & scalability
    - Allogenic stem cell transplantation showed cure is possible but is too risky/unscalable
    - Excising integrated HIV from infected cells, but only if it is safe, precise, and durable

- **Progress toward an HIV cure is accelerating**
  - Basic research is advancing across multiple fronts — from novel delivery systems like mRNA-LNPs to cutting-edge genetic editing and immune-based strategies
  - **Combined approaches and specifically Immune cell-based therapies** offer heightened specificity, and prolonged efficacy to significantly reduce the HIV reservoir, short circuit the need for lifelong ART, limit its side effects, resistance, and improve quality of life