Broadening the Applicability of Antigen-specific T-cell Immunotherapy for HIV-1 Infection

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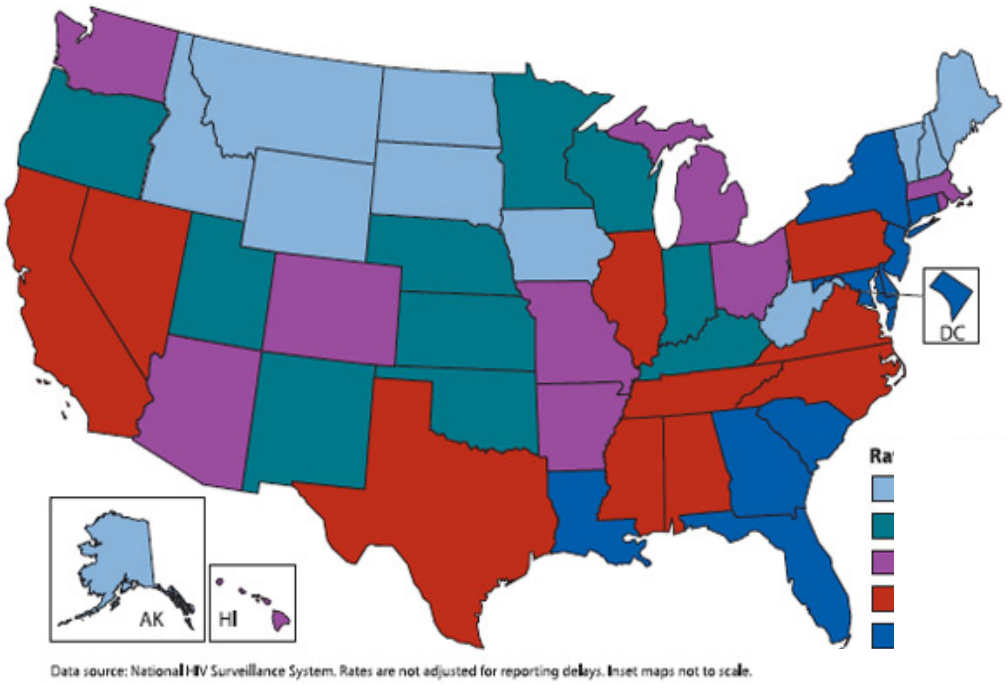
Children's National

THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

Prevalence of HIV



Per 100,000

- 40.1 - 129.3
- 129.4 - 202.4
- 202.5 - 319.3
- 319.4 - 428.0
- 428.0 - 3365.2

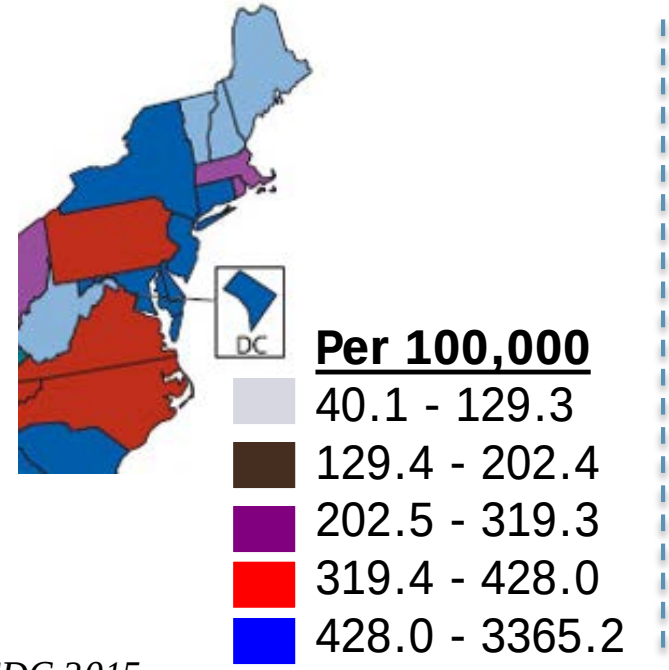
1.2 million people living in the United States with HIV

Estimated 36.9 million people infected worldwide

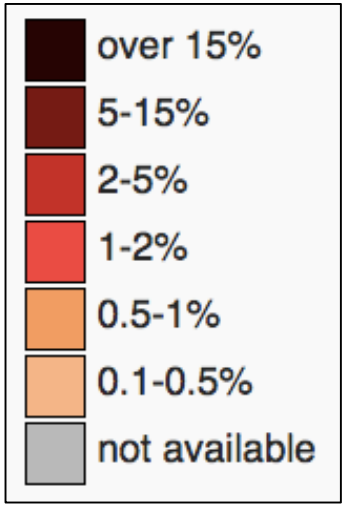
CDC 2015

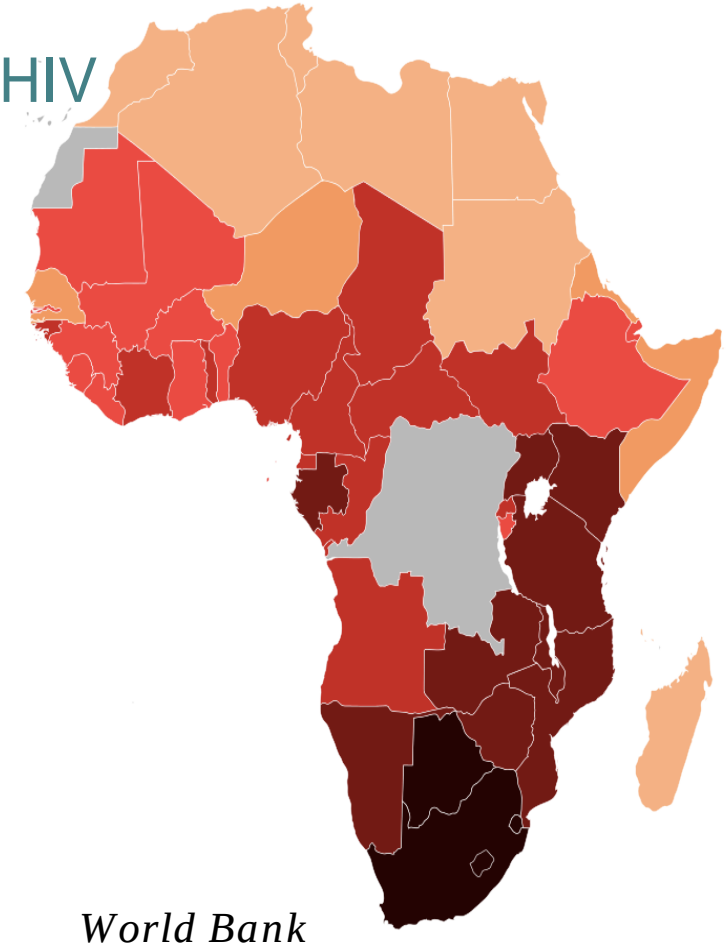
Prevalence of HIV in DC

- Epidemic is considered >1% of population
- Incidence of HIV in DC = 2.5% of adult residents
  - Over 16,000 people infected with HIV
- Compared to African countries



CDC 2015





World Bank

Antiretroviral Therapy

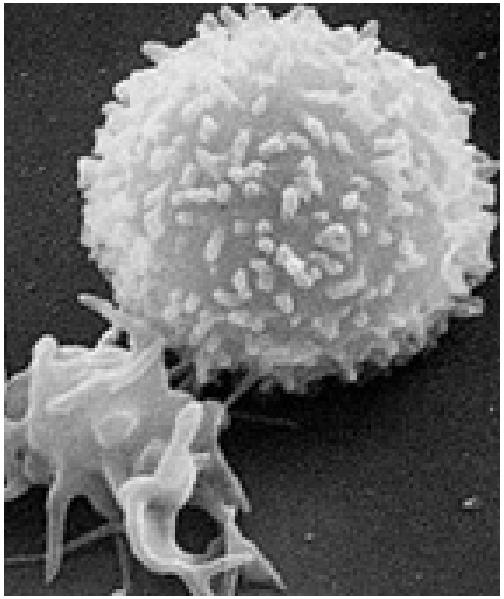
- Successful in suppressing HIV and stopping disease progression
- Cost - $24K annually
- Not curative
- Social stigma

Viral control depends on Robust T-cell immunity

- T-cell deficiency impacts susceptibility to severe viral infections
  - HSCT
  - Primary Immunodeficiency
  - Iatrogenic immune suppression due to autoimmunity, cancer.

Adoptive T-cell Immunotherapy restores antiviral immunity

- **Transfer of virus-specific T-cells (VST) from a donor to recipient**
  - Utilizes selection or *ex vivo* expansion
  - Highly successful in post-HSCT period
    - >400 patients treated internationally
    - Minimal risk of GVHD



A: Virus-exposed donors

PBMC

Hexon
Penton

EBNA-
LMP-2
IE-1
pp65

G-Rex

IL-4
IL-7

VST in 10-12 days

B. Cord blood / Virus-naïve donors

CBMCs or Naïve T-cells

Dendritic Cells

IE-1
pp65
Hexon
Penton
EBNA-1
LMP-2

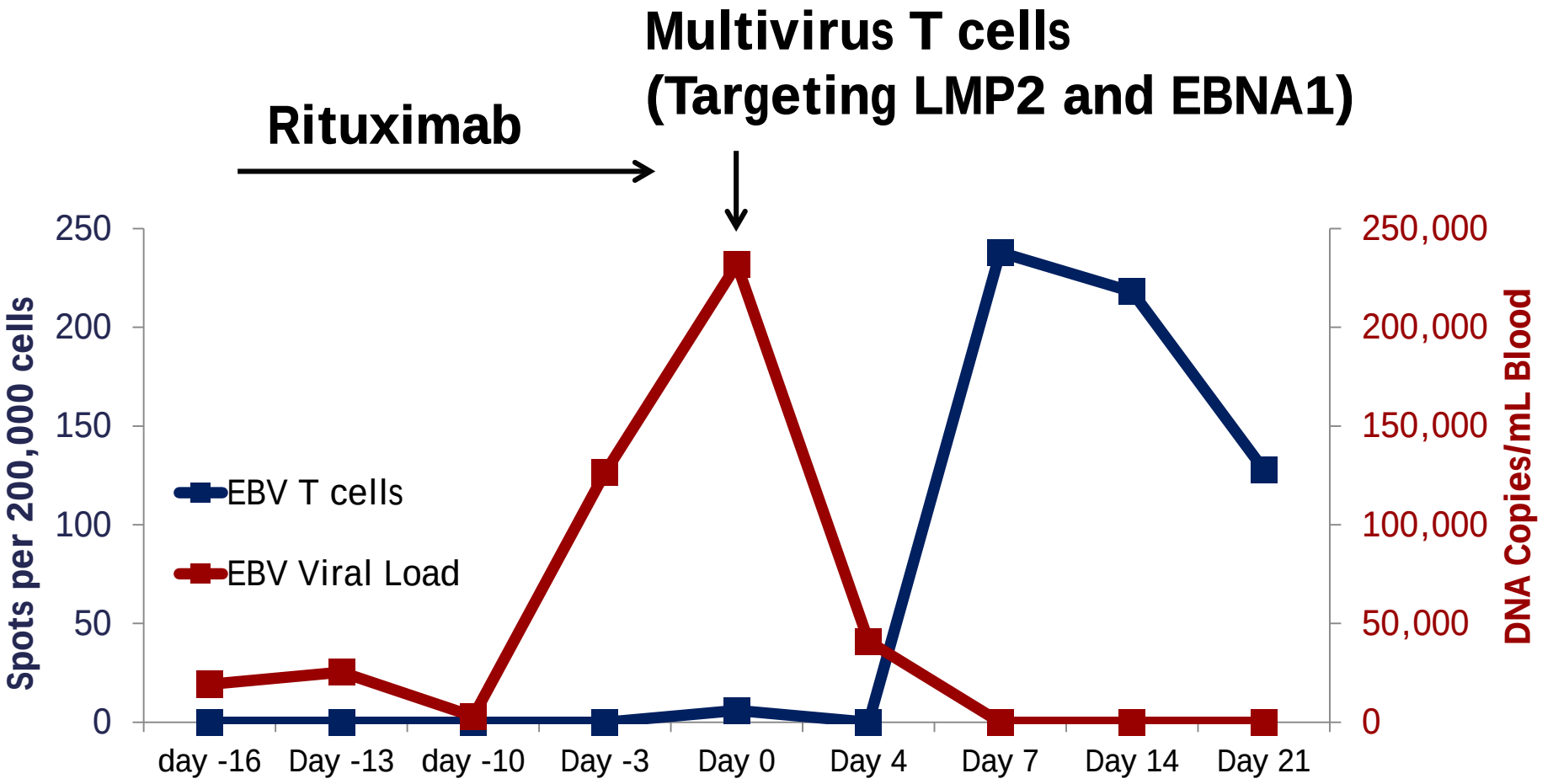
G-Rex

IL-7
IL-12
IL-15

EBV-LCL

VST in >28 days

LMP/EBNA specific T cells Effectively Treats EBV-PTLD Post Allo BMT



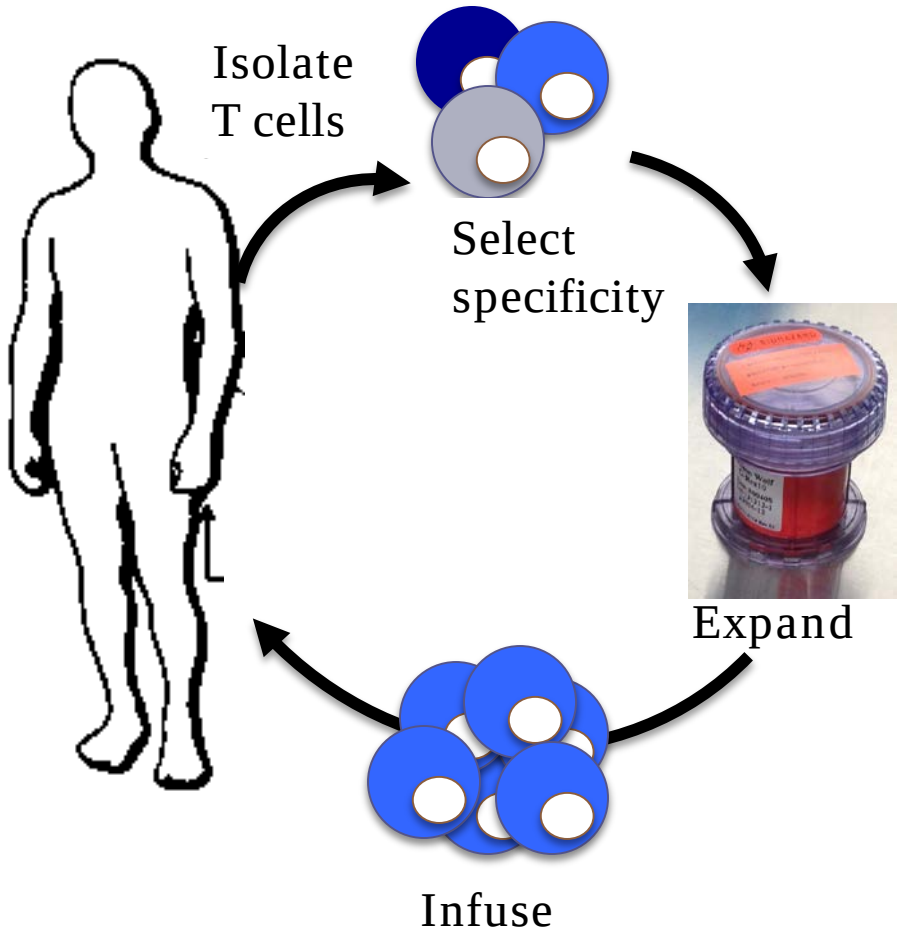
Expansion of Sources and Targets has increased applicability of VSTs

- **Expanded Donors**
  - Cord Blood VST (CMV/EBV/Adv): ACTCAT1/2 trials
  - CMV-seronegative adults: MUSTAT trial (Children's Natl)
- **Expanded targets**
  - ARMS protocol: CMV/EBV/Adv/HHV6/BK VST
  - Upcoming targets: Human parainfluenza-3, HPV
- **Protective regardless of source of VSTs (naïve or memory-derived)**

Leen et al, Nature Medicine 2006
Bollard et al, JCO 2014
Hanley et al, Science TM 2015

Developing T Cell Therapeutics for HIV: Previous trials

Antigen specific T cell therapy



- Safe in both HIV+ and other settings
- Less off-target effects
- Proliferate and migrate *in vivo*
- Potentially long-lasting immunity due to memory

Antigen-specific T cell studies for HIV

Study	Method	Dose	n	Results
Lieberman *et al*., *Blood* 1997	HLA-A02 restricted clonal T-cells	$1x10^8$ x 1 dose	6	• Transient increase in CD4 count and decrease in viral load
Tan *et al*., *Blood* 1999	Autologous expanded HLA-A02-restricted CD8+ clones (Gag, Pol-specific)	$3x10^9$ x 1 dose	1	• No change in CD4 count or viral load
Brodie *et al., Nat Med* 1999	CD8+ Gag-specific T-cell clones	3 doses, up to $3x10^9$	3	• Decrease in productively infected CD4+ T-cells • No impact on viral load.
Chapuis *et al.*, *Blood* 2011	HIV-specific CD8+ T-cells specific for HIV clade B peptides; **central memory enriched**	$3.3x10^9/m^2$ x 1 dose	7	• Clones persisted for up to 84 days • found to traffic to rectal mucosa in 4/7 patients for >100 days • HIV replication not accessed

CARs for HIV

- Phase II trial - CD4zeta CAR -extracellular domain, binds to HIV Env glycoprotein.
- T-cells expressing CD4ζ become activated upon binding HIV gp120 envelope protein on infected cells
- 24 HIV+ individuals → single infusion +/- IL-2.
- CD4+ and CD8+T cells trafficked to rectal tissues → > 0.5 log decrease in rectal tissue-associated HIV but not plasma
- T cells detected at 1 year

Mitsuyasu, R.T., et al., Blood, 2000. ***96****(3): p. 785-93.*
Cell Genesys Inc in collaboration with Hoechst Marion Roussel.
UCLA, UCSF, Uni Colorado

CARs for HIV

- UCSF and U Penn
- Phase II randomized study CD4zeta-modified CD4+ and CD8+T cells.
- 40 HIV+ subjects on HAART received T cell infusions (20 gene-modified, 20 unmodified).
- All subjects received $1x10^{10}$ T cells x3
- Decrease in HIV burden of patients infused with unmodified T cells
- CD4zeta-modified CAR T cells in 98% of samples up to 11 years

Deeks, S.G., et al., Mol Ther, 2002. ***5****(6): p. 788-97.*
Scholler, J., et al.,. Sci Transl Med, 2012. ***4****(132): p. 132ra53.*

Summary – Previous T cell therapies for HIV

Therapy	Approach	Limitations
CD8 clones[1]	Select high reactivity clones and expand	• Short persistence • No CD4 help • Limited specificity
High-affinity T cell receptors (SLY)[2]	Find conserved epitopes and express artificial receptors in T cells	• HLA-A*0201 restricted • Have to generate new receptor for every HLA type • Safety concerns of Artificial TCRs
CD4 CAR T cells	Targets HIV infected CD4 T cells – contains the extra cellular domain of human CD4; binds to HIV env	Long term persistence in some Decrease in HIV burden None cured ?toxicity concerns

Patel, Jones and Bollard, Cytotherapy 2016

Developing T Cell Therapeutics for HIV Targeting Multiple Antigens

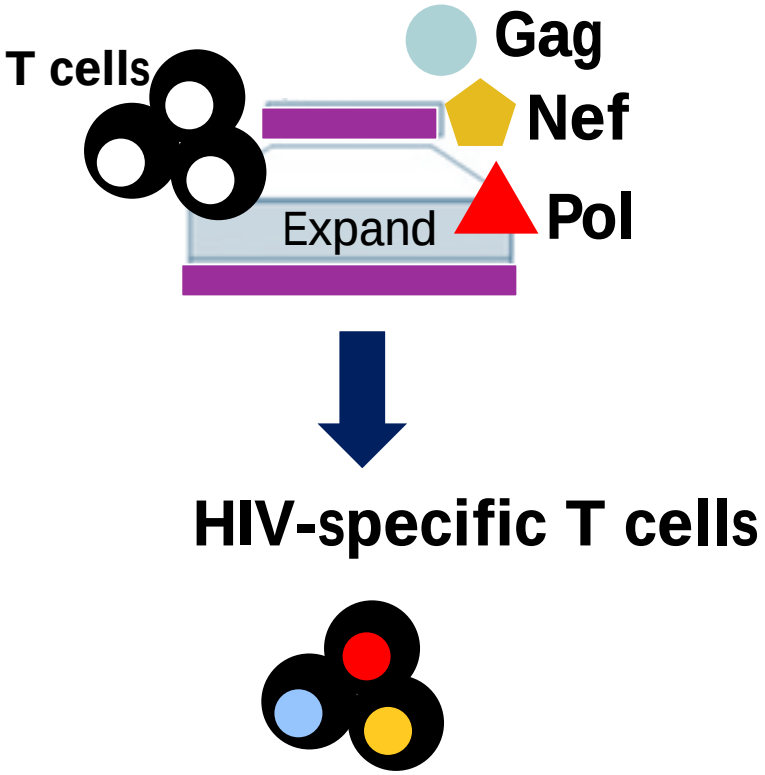
Objective

- Determine whether we can generate HIV-specific T cells (HXTCs), derived from both HIV^{+} and HIV-negative donors

- Multi-antigen HIV-specific T cells
  - Prevent immune escape
- Include both CD8+ and CD4+ T cell subsets
  - Improve persistence of infused T cells

HIV Antigens

- Choosing HIV Antigens:
  - More conserved than envelope proteins[1]
  - Target early and late infection stages
    - Nef (early) & Gag, Pol (late)
  - Dominant Gag-specific T cell responses found in elite controllers[2]



[1]*Rolland et al, PLoS Path 2007*

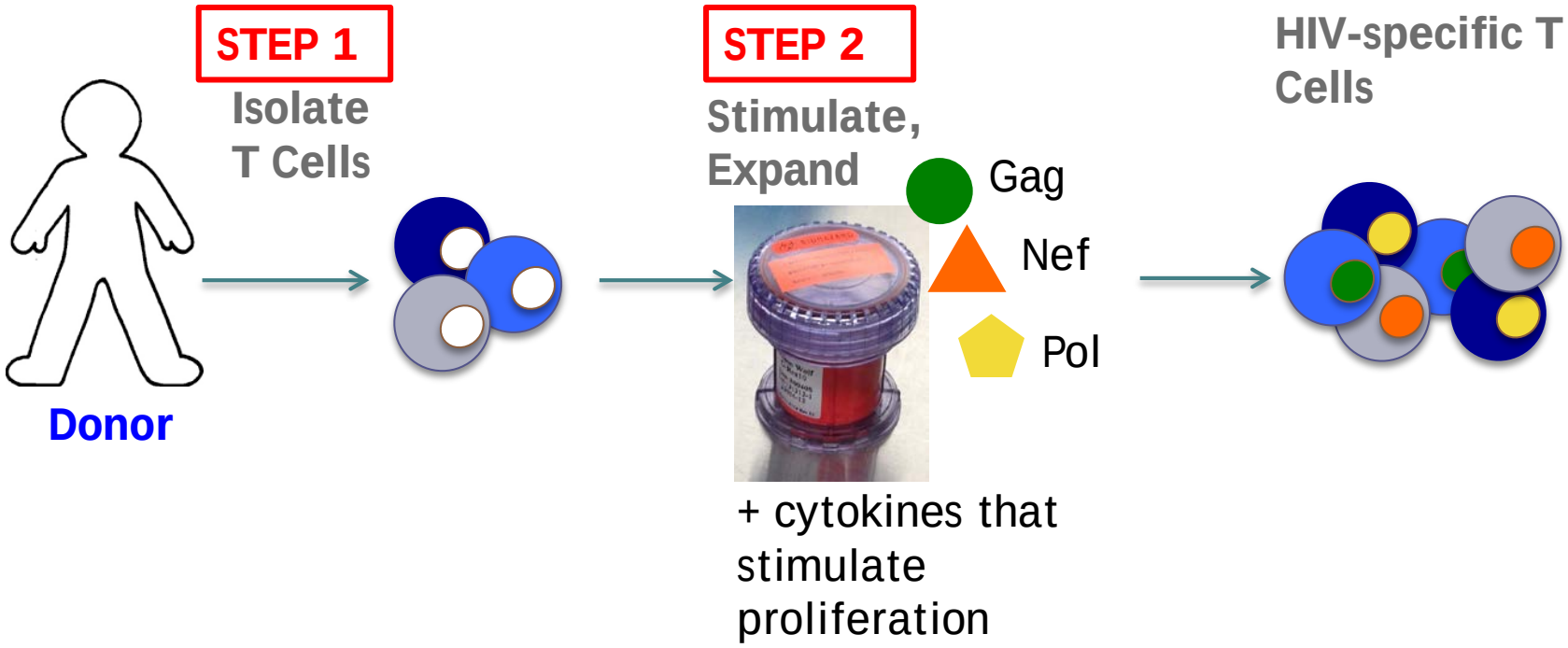
[2]*Saez, Cirion, et al. J Immunol, 2009.*

Generating HIV- T cells (HXTCs) from HIV+ Individuals

- 7 HIV+ subjects with either acute (2) or chronic infection (5)
- All on ART with viral suppression ≥3 years

Lam, et al. Molecular Therapy, 2015.

Approach for HIV+ derived HXTCs



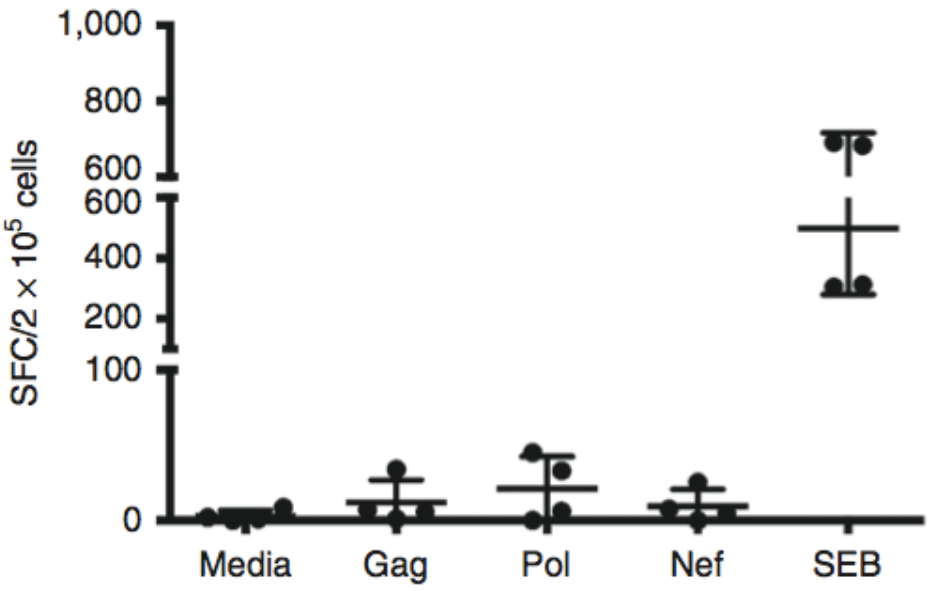
*Cells are grown in amprenavir→raltegravir and indinavir

The PepMixes consist of 150 15-mer peptides. Based on a proprietary algorithm held by JPT to provide the broadest coverage across all clades of HIV.

HXTCs Display Multi-HIV Antigen Specific Activity

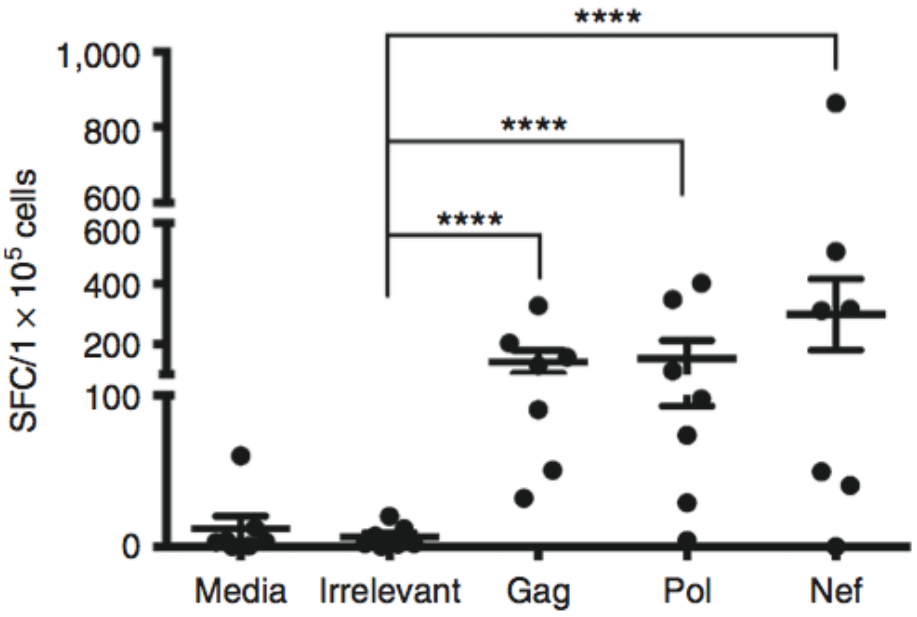
Unexpanded

Day 0



Expanded

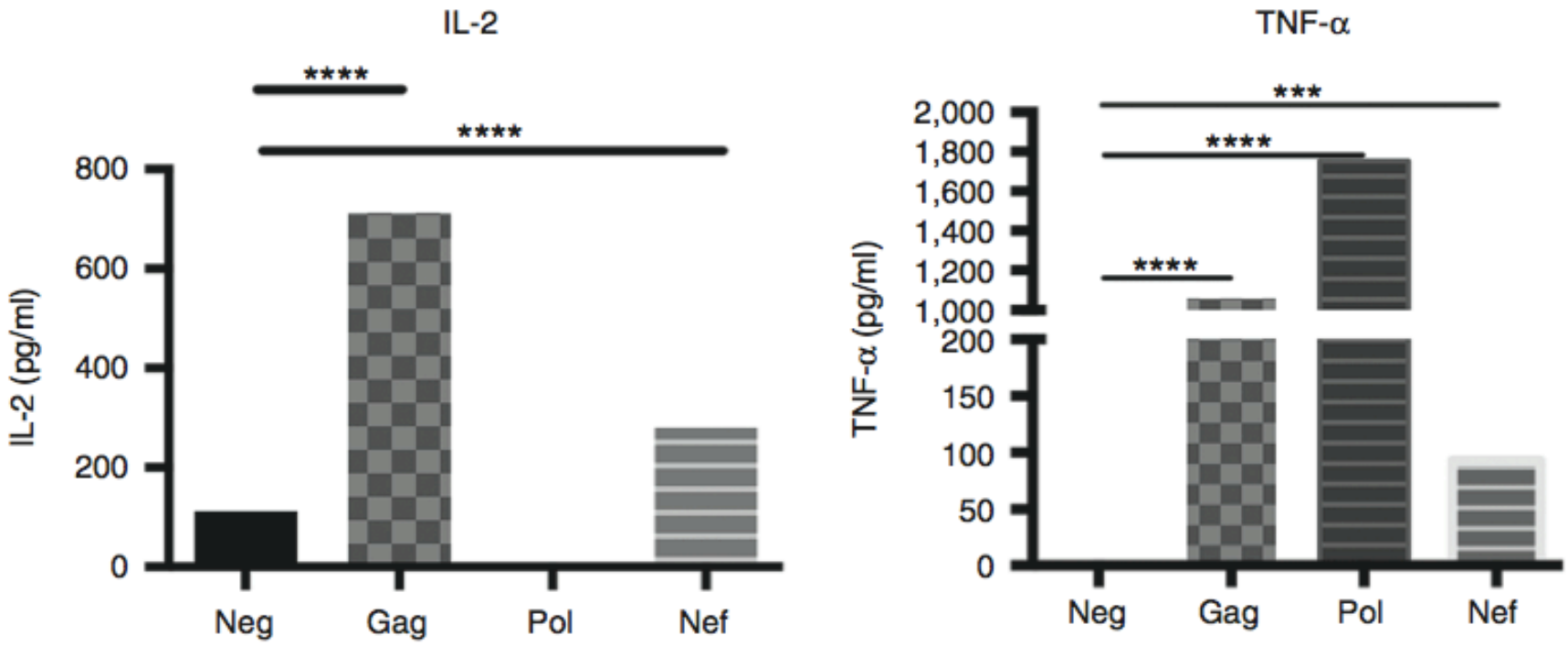
Day 24



Spot Forming Cells (SFC) Secreting IFNgamma

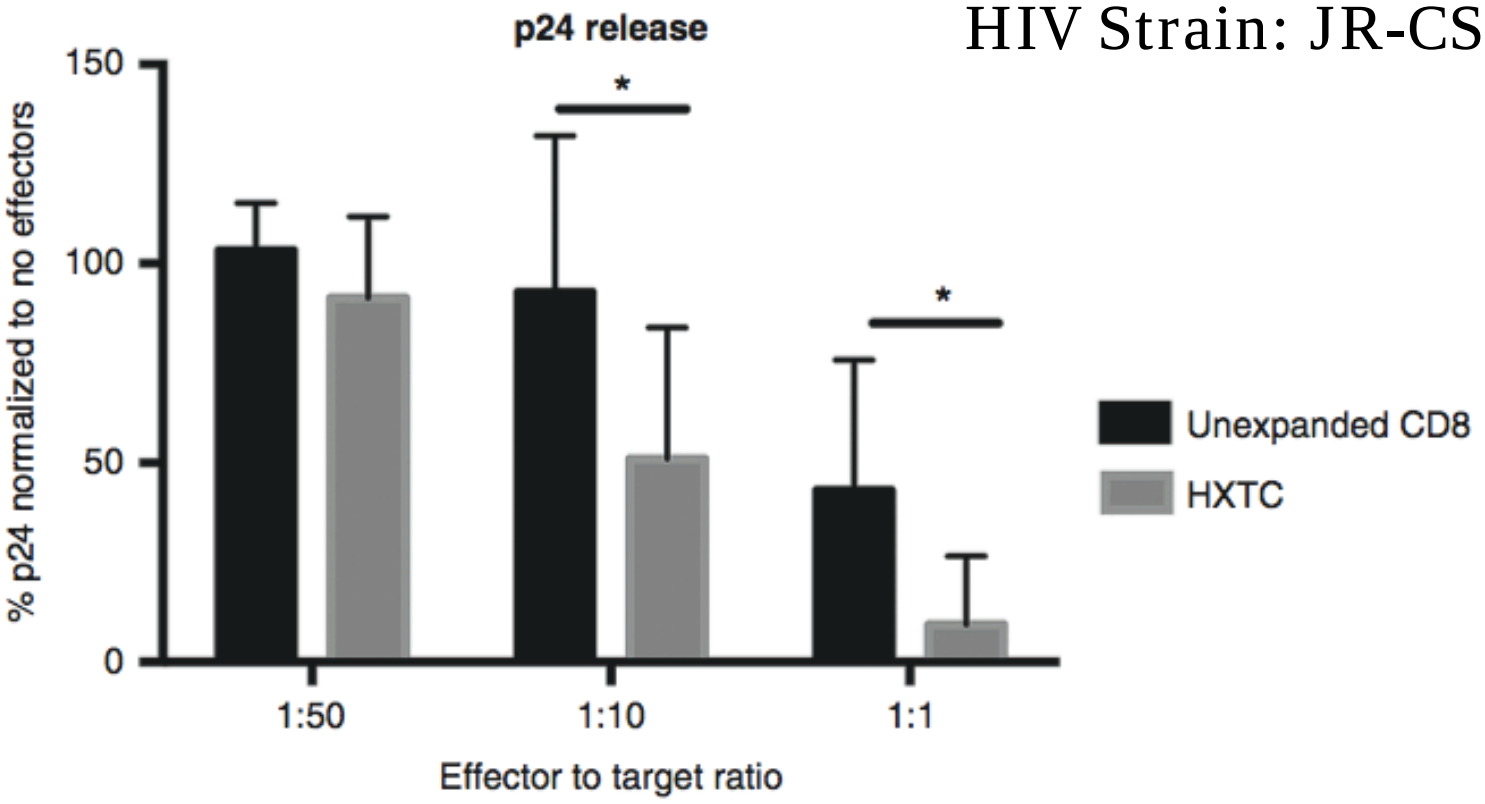
Lam, et al. Molecular Therapy, 2015.

HXTCs Produce Polyfunctional Response to HIV Antigens



Lam, et al. Molecular Therapy, 2015.

HXTC Suppress HIV *in vitro*



Lam, et al. Molecular Therapy, 2015.

Phase I Study

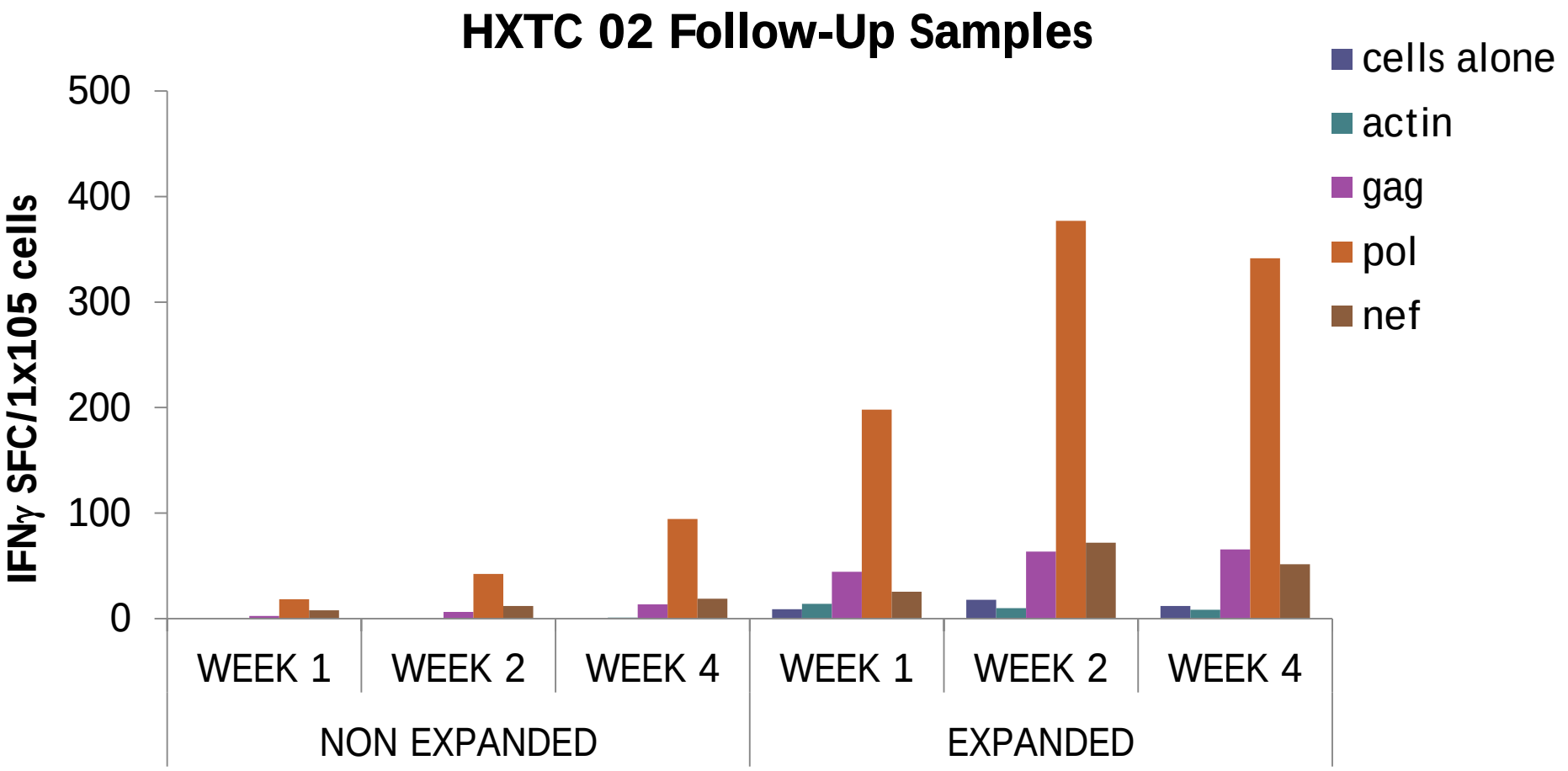
- Collaboration with UNC (NCT02208167)
- Phase I single-site study of HXTC Therapy
  - Evaluate the safety, immunologic, and virologic response
  - HIV+ participants on ART with chronic or acute HIV
  - Patients receive 2 HXTC infusions

  ($2x10^7$ cells/m^2 each) given 2 weeks apart

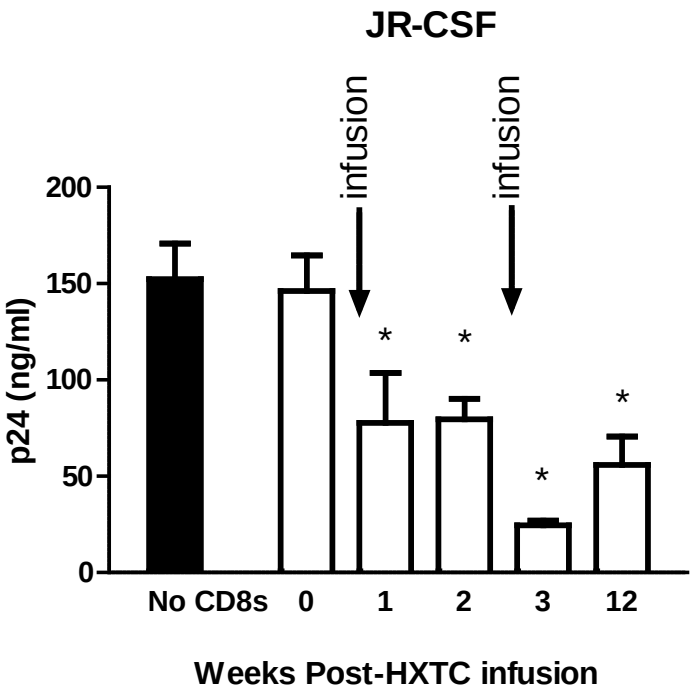
Preliminary Clinical Data

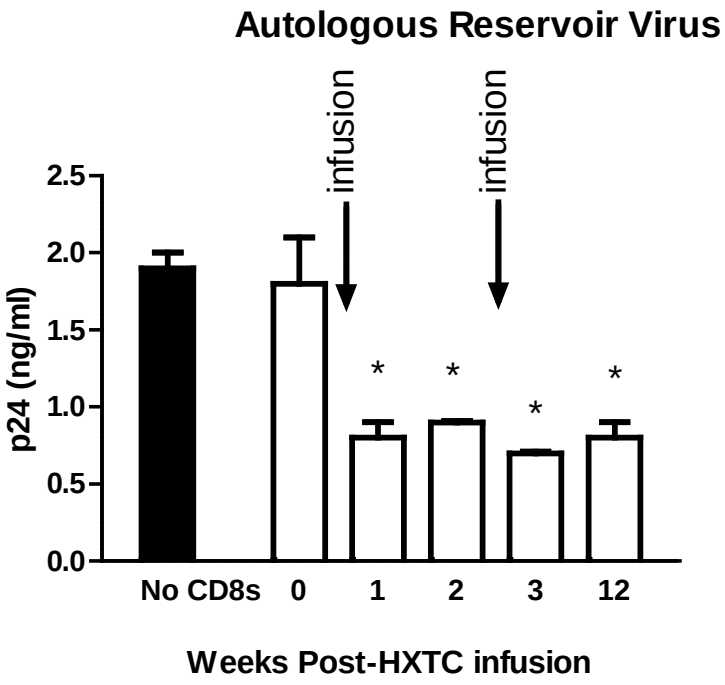
- Autologous HXTCs generated from 3 HIV^+ subjects
- 2 subjects received 2 doses of HXTCs

HXTCs Expand *In Vivo*

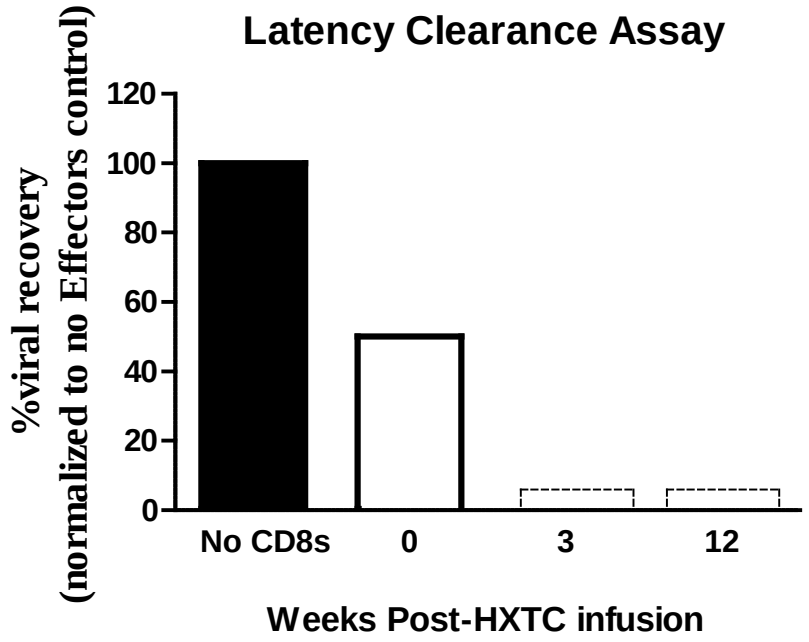


HXTCs Suppress HIV Replication Following Infusion (n=3)





No Viral Recovery possible Post-HXTC Infusion

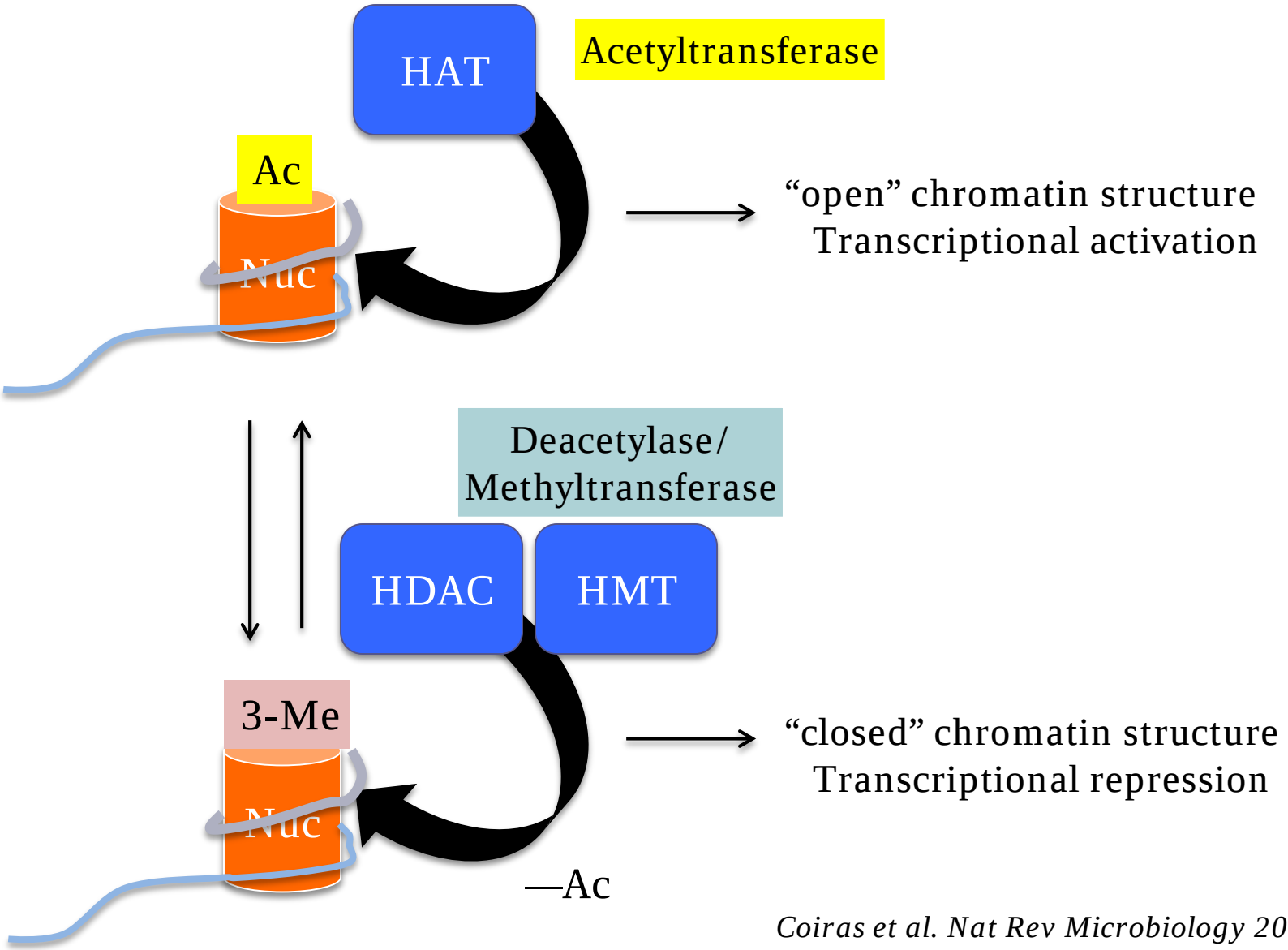


Enrolled patients still on ART – therefore without doing a controlled treatment interruption unable to understand role of HXTCs as a cure strategy

Summary to date

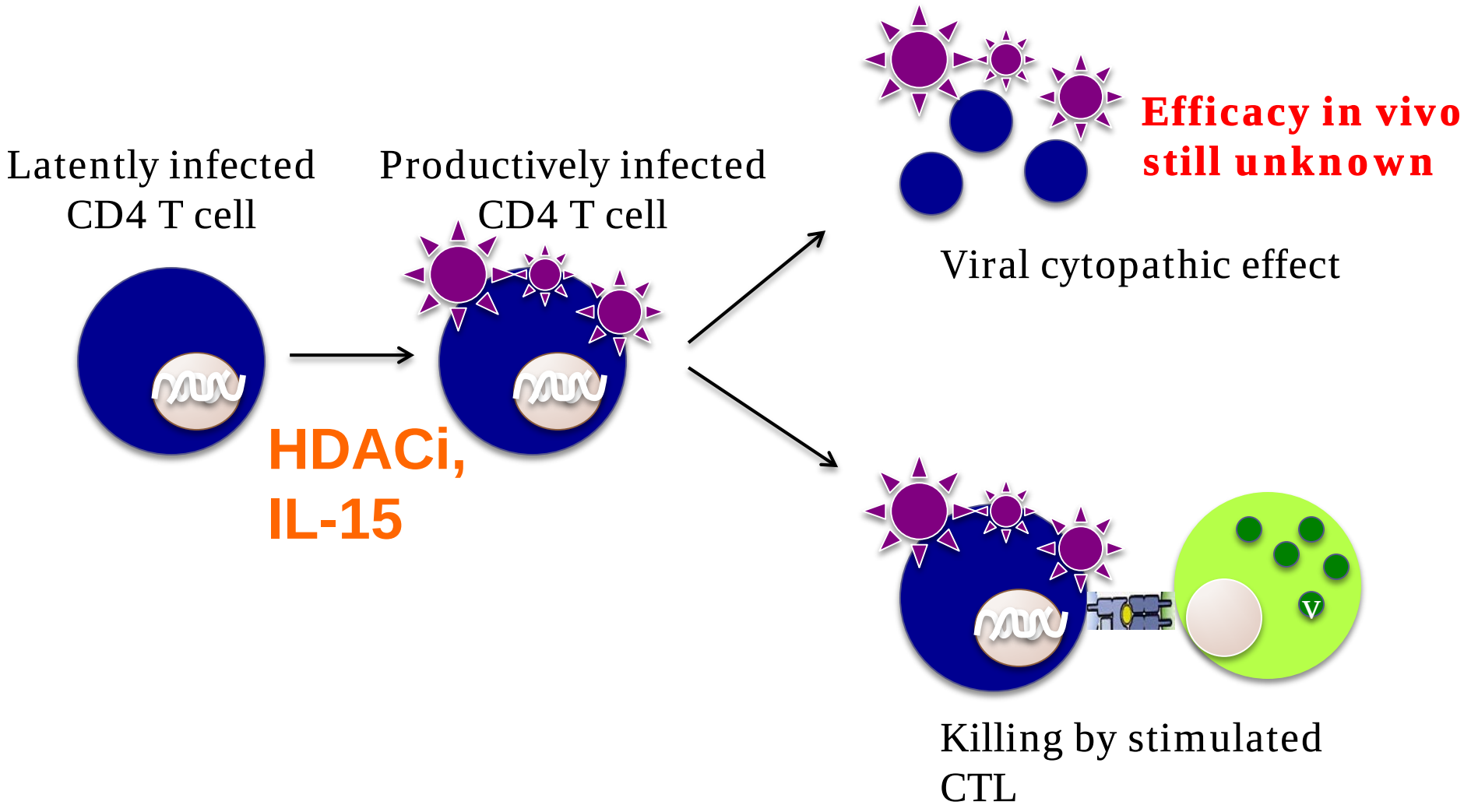
- Autologous HXTCs may aid HIV control
- ***Subjects currently remain on ART***
  - Unclear if autologous HXTCs have a role as a cure strategy
- **Unclear if HXTCs can reach latent viral reservoir**

Latency is controlled by epigenetic regulation



Coiras et al. Nat Rev Microbiology 20

HIV “purging” strategies



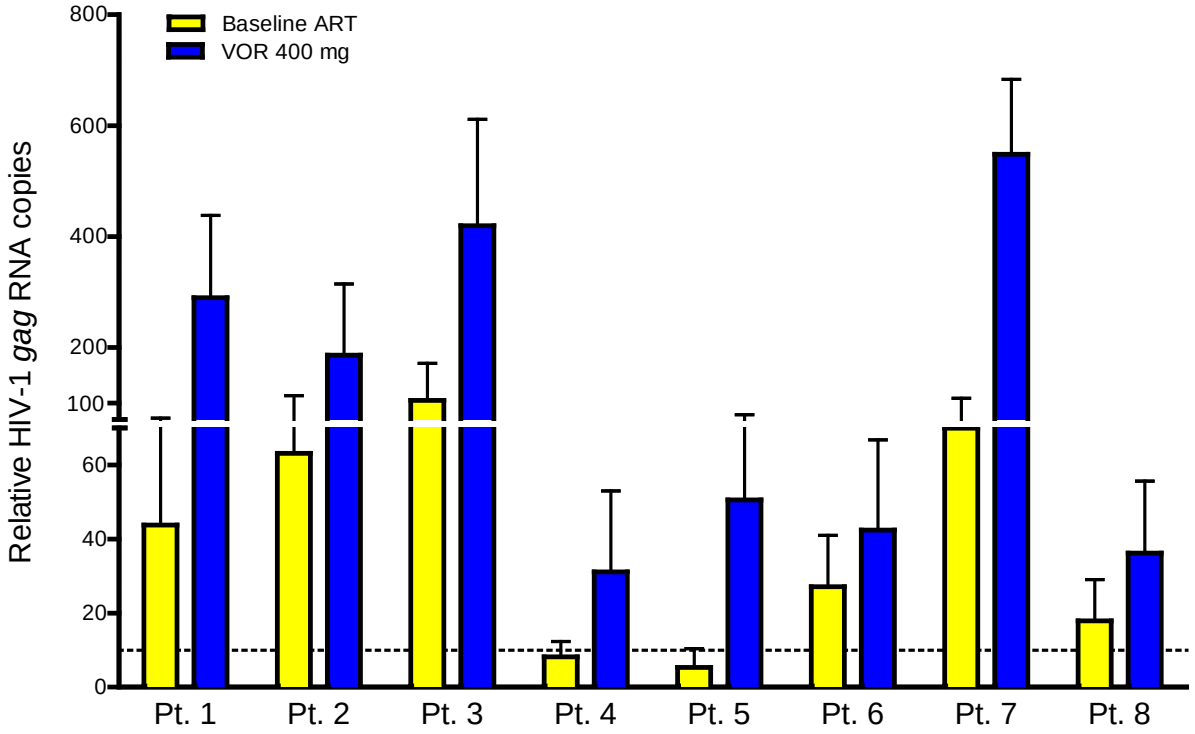
Migueles and Conners. Immunity. 2012

LETTER

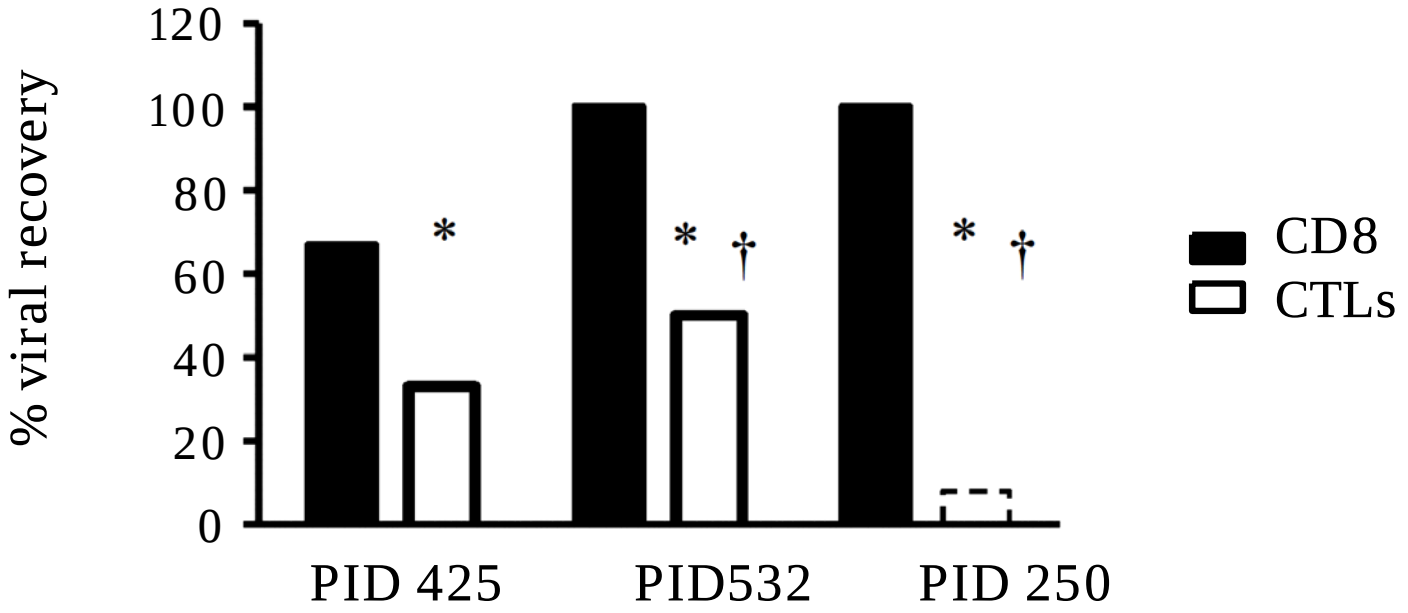
doi:10.1038/nature11286

Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy

N. M. Archin[1], A. L. Liberty[1], A. D. Kashuba[1], S. K. Choudhary[1], J. D. Kuruc[1], A. M. Crooks[1], D. C. Parker[1], E. M. Anderson[2], M. F. Kearney[2], M. C. Strain[3], D. D. Richman[3], M. G. Hudgens[1], R. J. Bosch[4], J. M. Coffin[2], J. J. Eron[1], D. J. Hazuda[5] & D. M. Margolis[1]



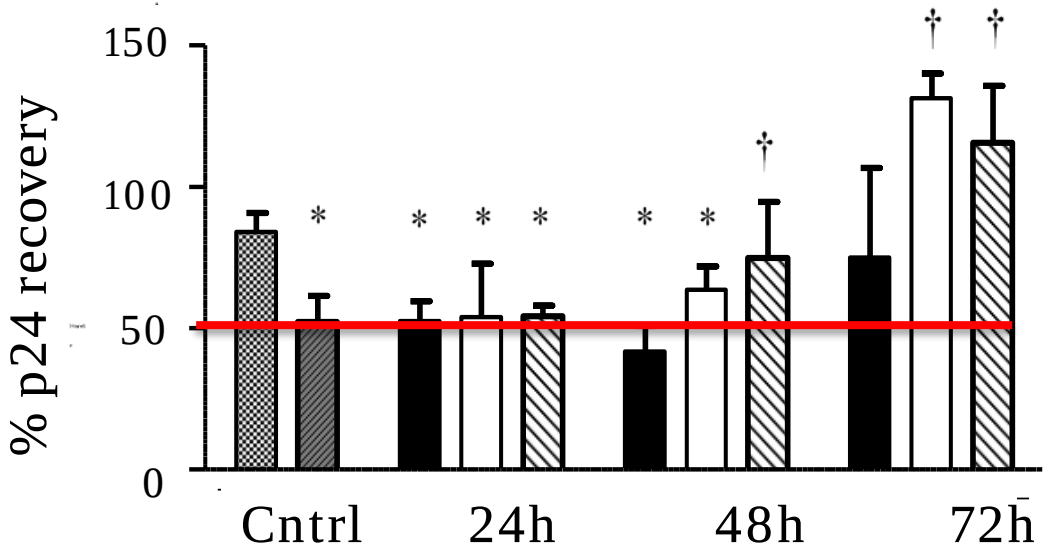
HXTCs suppress viral recovery after SAHA-induced reactivation



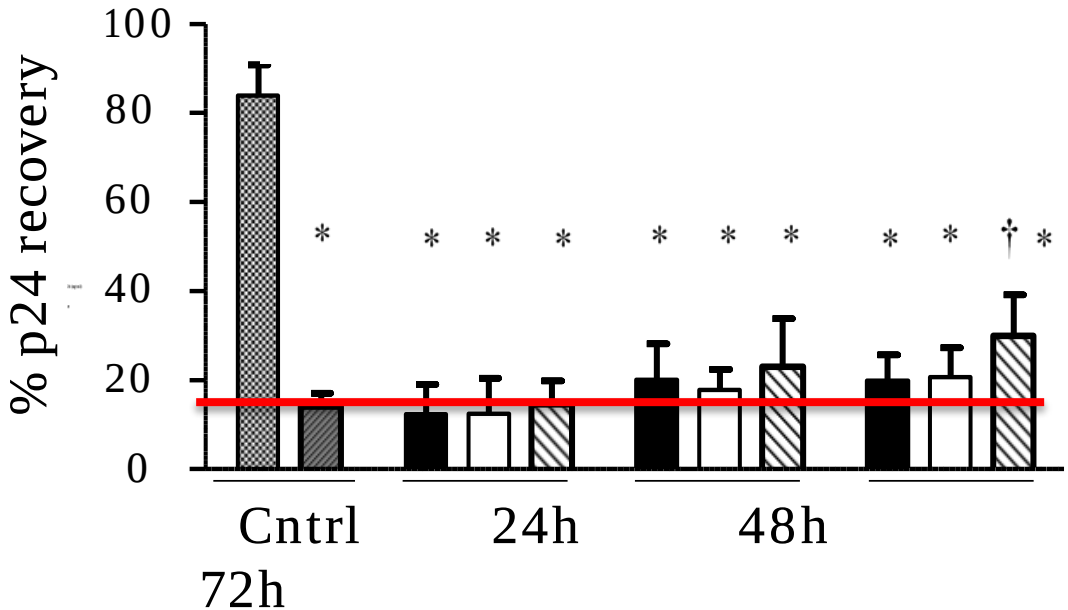
Sung, Lam et al. JID 2015

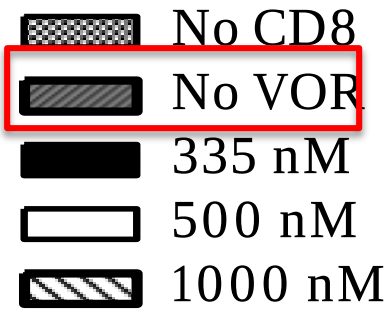
Vorinostat impairs CD8 T cells only after 48 hrs of exposure

ET 1:10



ET 1:1



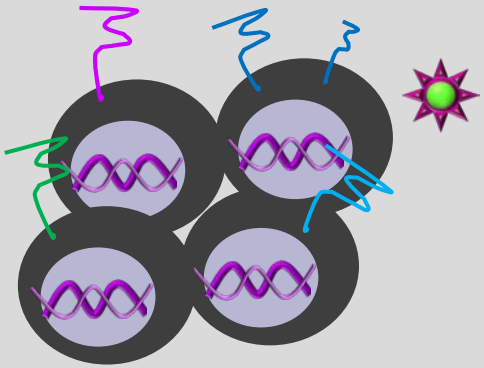


Sung, Lam et al. JID 2015

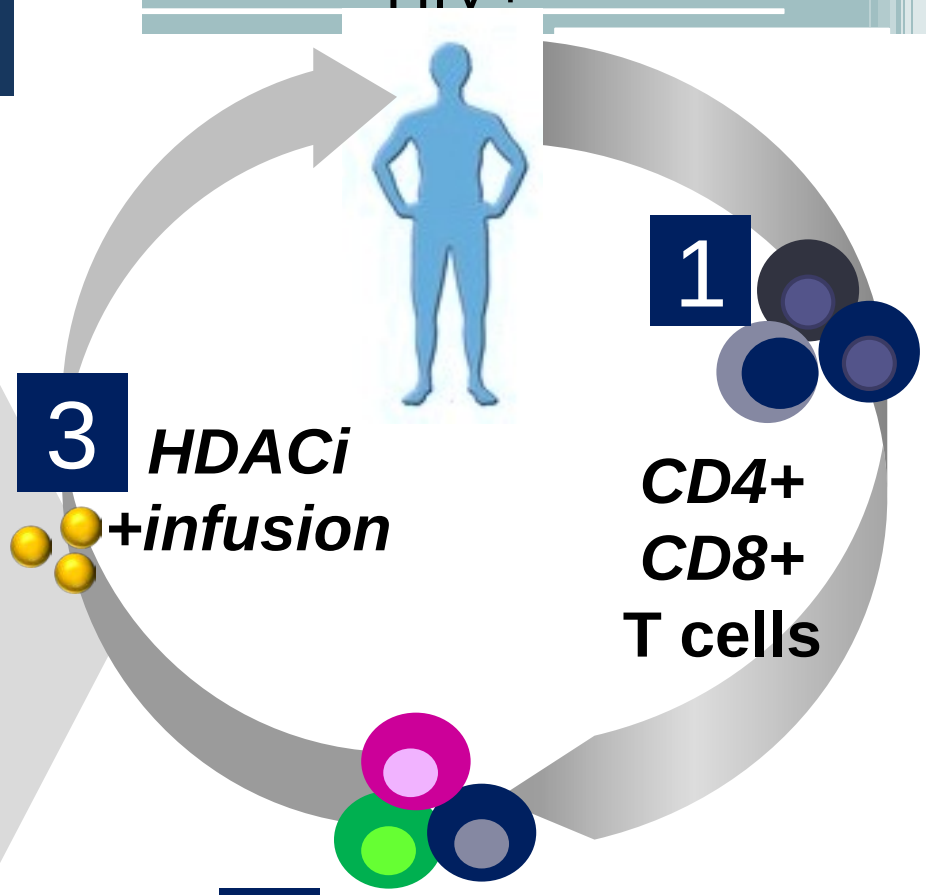
Caveats and Future Directions

- Does vorinostat actually increase HXTC mediated viral suppression?
  - Measure increased antigen expression, if any
- Do HXTCs maintain anti-viral function in presence of other latency reversal drugs?
  - Jones et al. suggested impairment with other HDACi

Overall Summary

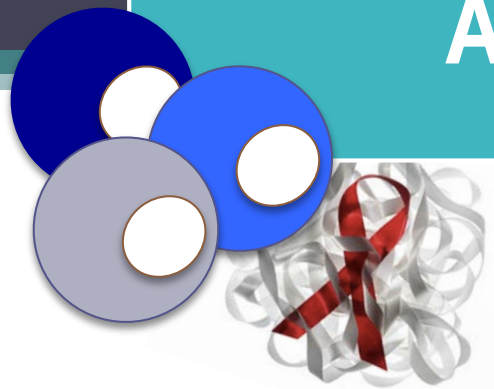


Latent reservoirs



HIV-Specific T Cells:

A Cure Strategy Post-Allogeneic HSCT



J1331 Clinical Trial

- Hypothesis: The combination of the allogeneic effect and continuous ART can reduce or completely eradicate HIV reservoirs

- Study Population:
  - HIV+, BMT for cancer

JH HIV Allo-BMT: 7 allo-BMT patients

N=7	1	2	3	4	6	7	9
Age	34	53	38	50	51	46	50
Cancer	HL	DLBCL	AML	AML	DLBCL	DLBCL	AML
Survival, oncology outcomes	Died at week 49, liver failure	Alive, 127 weeks	Died at week 64, GVHD	Alive, 84 weeks	Alive, 46 weeks	Alive, 36 weeks	Alive, 24 weeks
% donor	100%	80%	100%	77%	100%	100%	100%

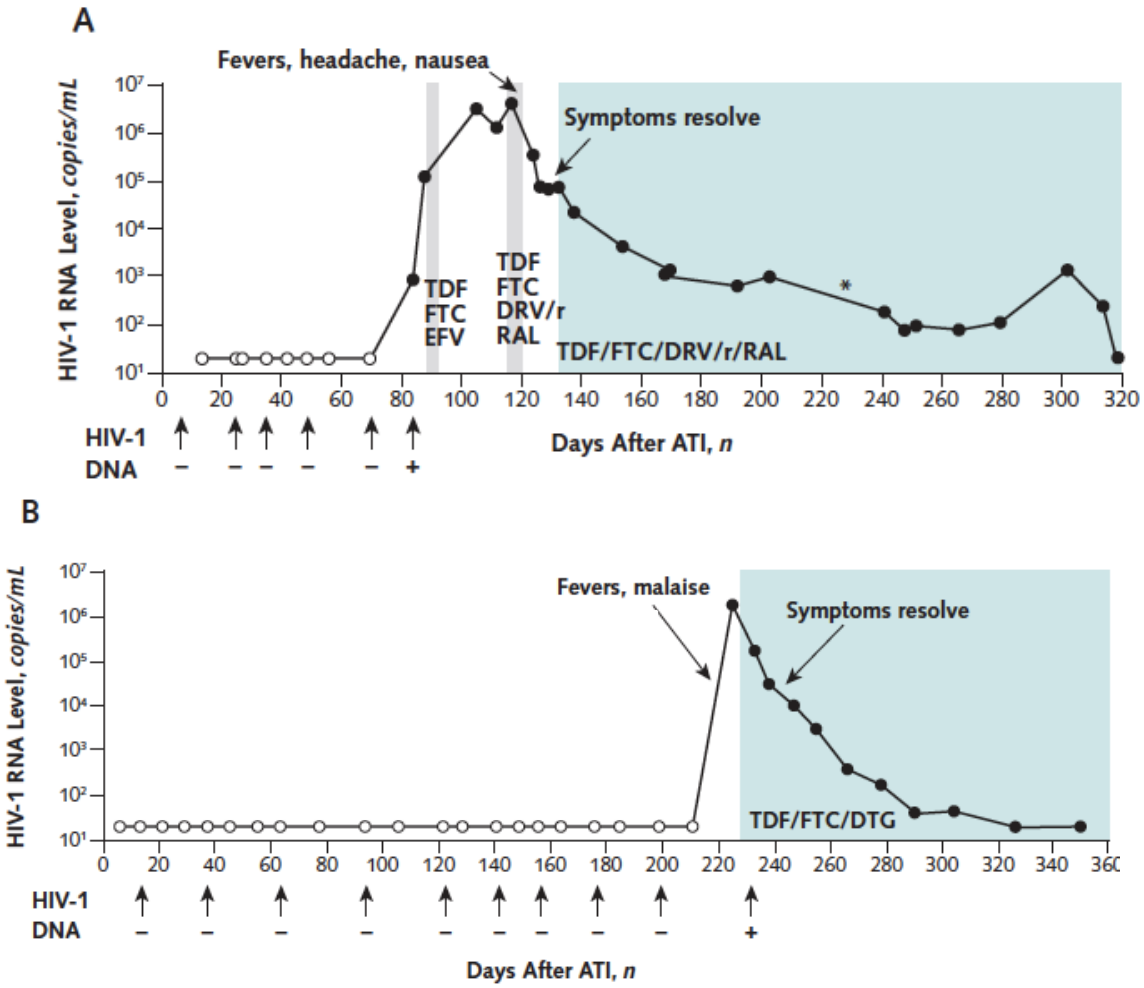
JH HIV Allo-BMT: 7 allo-BMT patients

- The HIV reservoir not detected post transplant in any patient with 100% donor chimerism
  - When only donor cells were detected in blood, no HIV reservoir detected by **Quantitative Viral Outgrowth Assay**

HIV Meningoencephalitis following ART non-compliance at 5 months

- Week 20: fevers and a change in mental status
  - LP: 28 WBC, Protein 150, glucose 50
  - Consistent with Meningoencephalitis
  - HIV plasma viral load = 25,518 copies/ml
  - The CSF viral load = 17,000 copies/ml
- Recovered with parenteral HIV therapy

Boston Patients—BMT with Antiretroviral Therapy ➔Interruption ➔Aggressive Viral Rebound

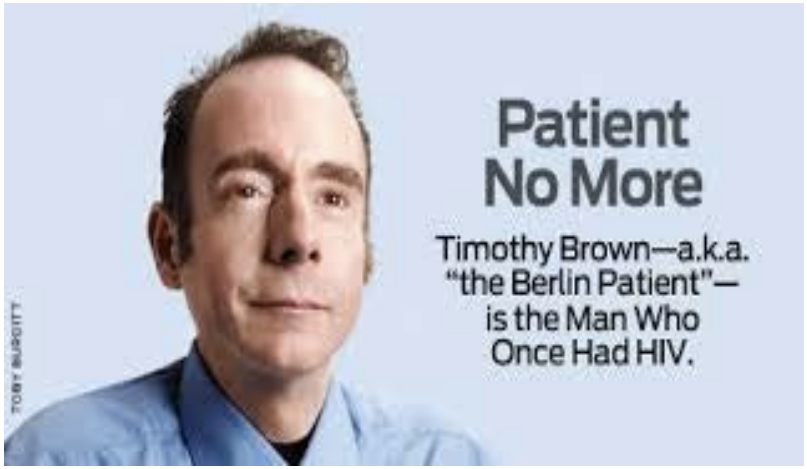


Summary: Allo-BMT with antiretroviral therapy

- Clears measurable viral reservoir when full donor chimerism achieved
- Puts patients at risk for a syndrome of aggressive viral rebound when antiretroviral therapy is interrupted

The Berlin Patient

- SCT: A curative approach to HIV?
  - Received SCT from a homozygote CCR5-delta32 donor
  - ‘HIV cured’: Unable to detect HIV in blood or biopsies after discontinuation of ART



CCR5-delta32

- Homozygotes: 1% caucasians
  - No CCR5 expression
  - Largely resistant to infection
- Heterozygotes: 10%
  - Reduced CCR5 expression
  - Slowed viral progression (before antiretroviral therapy)

Not repeated because identifying perfect HLA matches and CCR5-delta32 homozygous donors is difficult

Autologous CCR5 Gene Editing

- NCT00842634, Tebas, *et al.* NEJM 2014
- ZFN-modified (CCR5 targeting) autologous CD4+ T cells.
- 12 participants on HAART open label, nonrandomized, uncontrolled study.
- Single dose of 10x10e8 ZFN-modified CD4+ T cells.
- ZFN-modified CD4+ T cells detected up to 42 months.
- HIV DNA decreased in most patients.

Replicating the "Berlin Patient"

- Genetic engineering—works well in vitro.. but no one has been successful in achieving immune reconstitution with most cells protected from HIV in humans
- Current phase I protocol for CCR5 gene editing of HSCs for allogeneic BMT (City of Hope)
  - Lentiviral siRNA approach

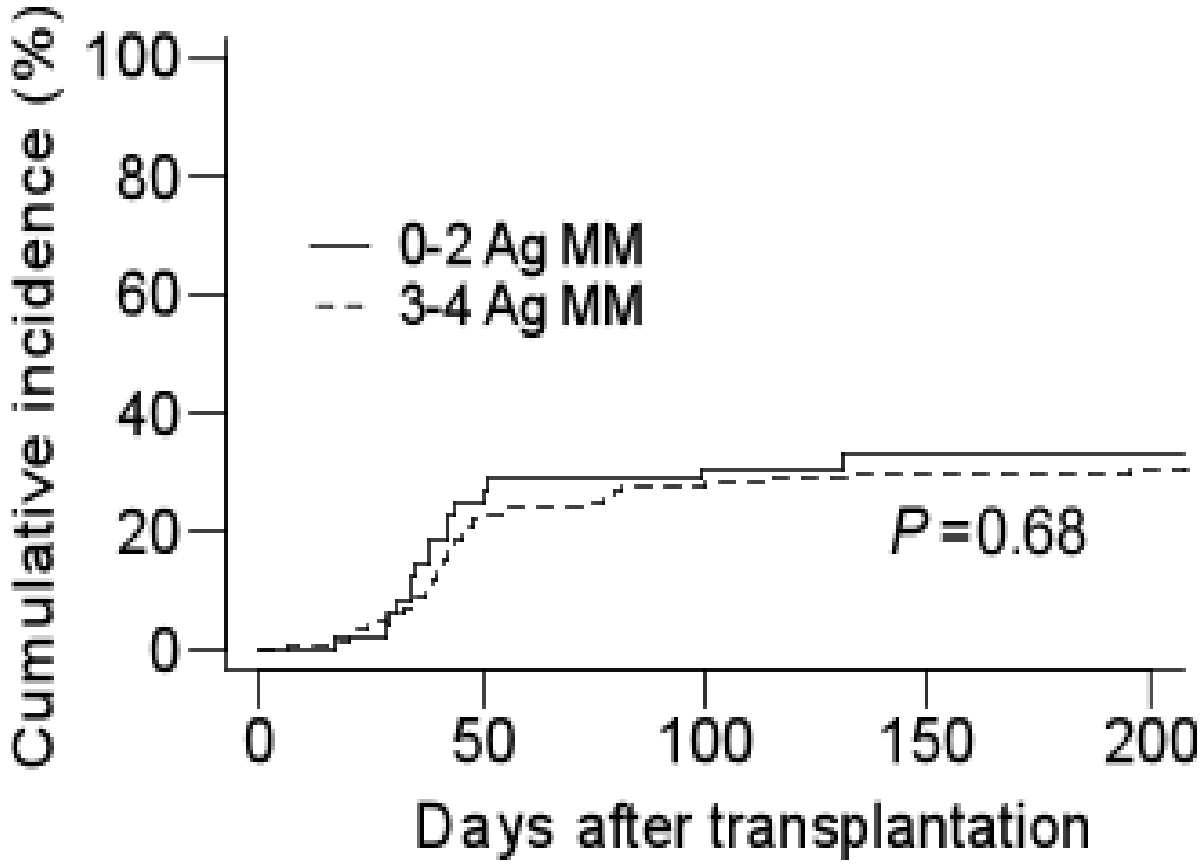
Peterson….Kiem Blood. 2016 Mar 15. Blood

Replicating the “Berlin Patient”

- Cord blood—several hundred cord blood units that are HIV resistant have been identified
- Several cord blood transplants done for HIV^+ patients, no long term cure to date.

New HSCT Protocols Permit Greater HLA mismatch

- **HaploBMT protocol with post-HSCT cytoxan**
  - **Increasing HLA mismatch does not worsen outcome**



Kasamon *et al BBMT* 2010

Some Patients Don't Have Matched Unrelated Donors or Related Haplo Donors

- At Hopkins, about 5% of patients fall into this category
- →trial with non-myeloablative prep and post-transplant cy that accepted unrelated donors mismatched at up to 5 of 10 loci.

JHH preliminary results (ASH 2015)

- No prohibitive toxicities or TRM despite up to 5 mismatched loci (n=16)
- No acute grade 3-4 GVHD

➔ Unrelated partially mismatched allo-BMT donors should be considered acceptable

JHH HIV+ allo-HSCT

N=7	1	2	3	4	6	7	9
Age	34	53	38	50	51	46	50
Cancer	HL	DLBCL	AML	AML	DLBCL	DLBCL	AML
Survival, oncology outcomes	Died at week 49, liver failure	Alive, 127 weeks	Died at week 64, GVHD	Alive, 84 weeks	Alive, 46 weeks	Alive, 36 weeks	Alive, 24 weeks
Donor	MUD	MUD	Matched sib	Matched sib	Haplo	Haplo	Mismatched unrelated

DKMS

- ~2 million donors typed at CCR5
- ~20,000 CCR5delta32 resistant donors

Hypothesis

If we prioritize CCR5 delta32 HIV resistant donors over best HLA match, it should be possible to identify HIV resistant donors for a substantial fraction of patients.

This should protect patients from the aggressive HIV rebound syndrome and may cure some patients of HIV.

Adoptive T Cell Therapy for HIV After SCT

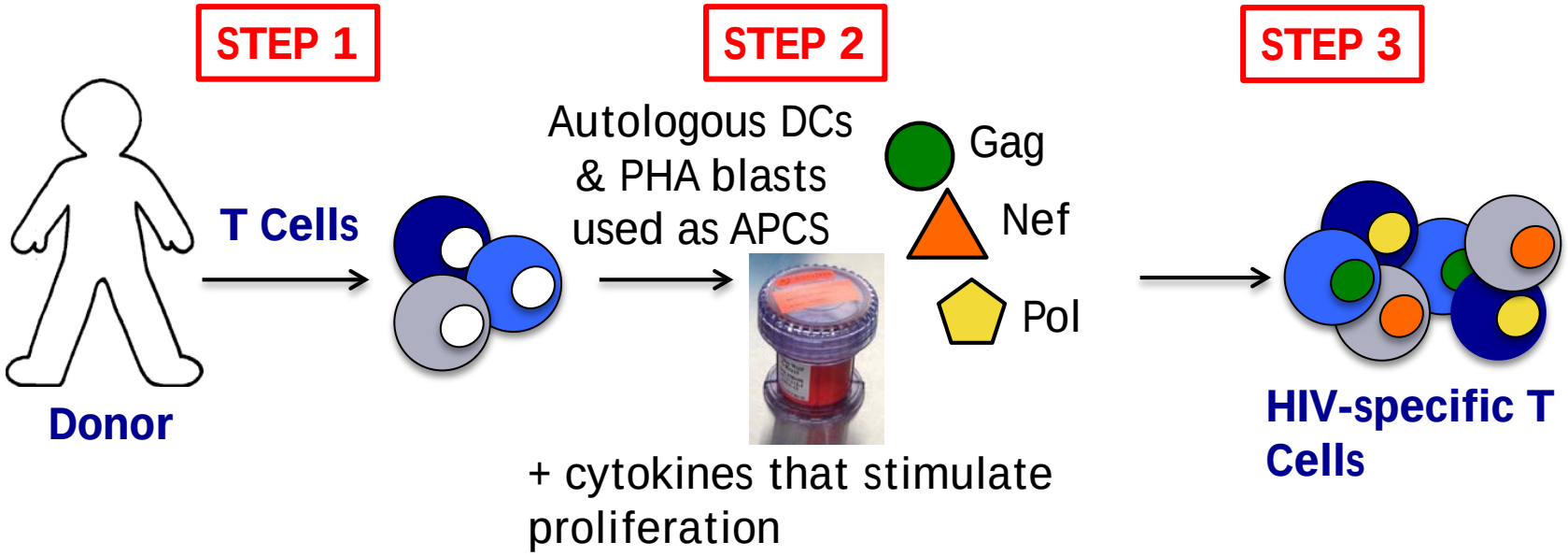
- **Adoptive T cell therapy** can be used to restore antiviral immunity post-transplant (SCT)
  - Donor-derived HIV-specific T cells could be used to target HIV reservoirs after SCT

Developing a dHXTC Product for use after SCT

- Determine if HIV-specific T cells can be generated from HIV seronegative donors (dHXTCs) and suppress HIV replication *in vitro*

- Ultimate goal: Infuse dHXTCs from eligible HIV-seronegative CCR5Δ32 HSCT donors to effect a cure post-transplant

Generation of dHXTCs

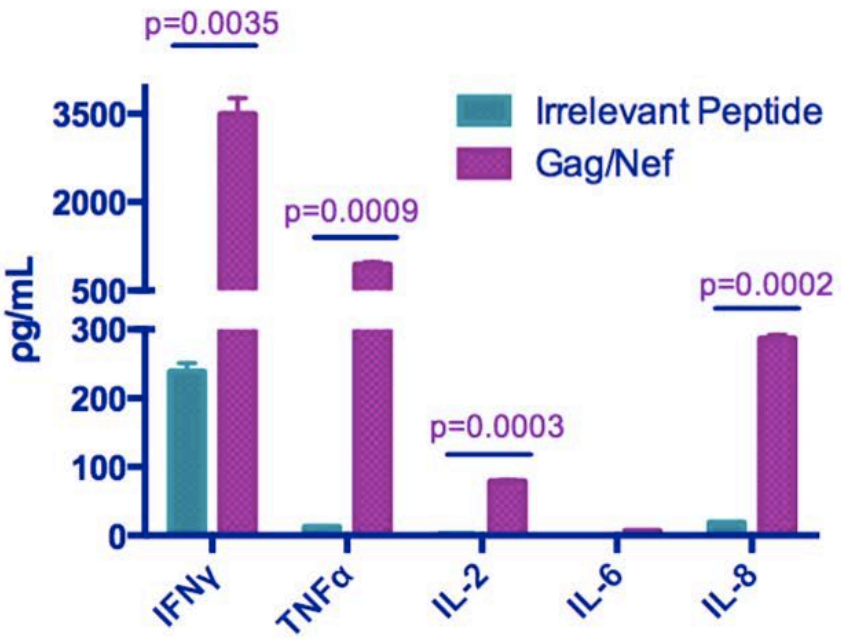


Choosing HIV Antigens:

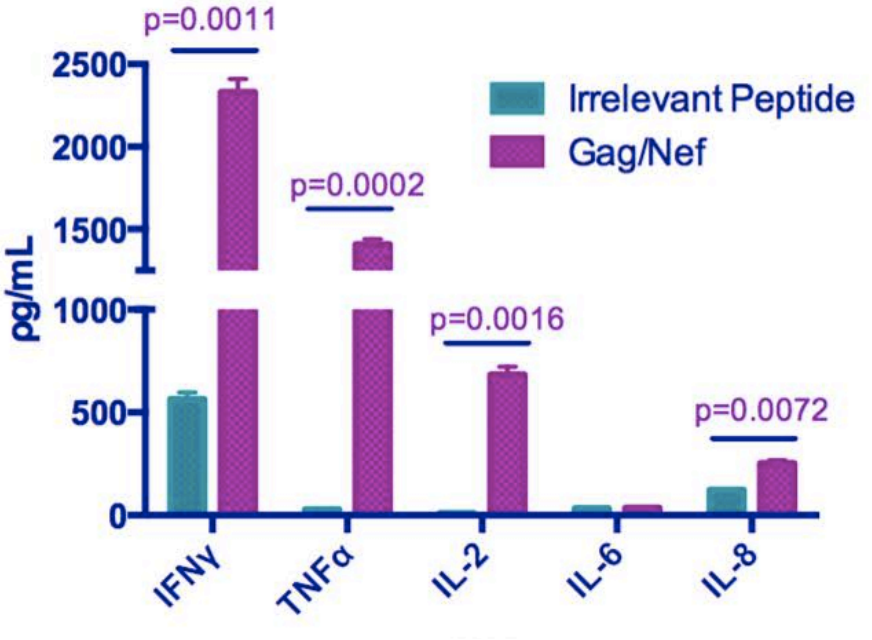
- Less mutable/more conserved than envelope proteins[1]
- Target early and late infection stages
  - Nef (early) & Gag (late)
- Dominant Gag-specific T cell responses found in elite controllers[2]

[1]*Rolland et al, PLoS Path 2007* [2]*Saez, Cirion, et al. J Immunol, 2009.*

dHXTCs Produce Polyfunctional Response to HIV Stimulation



Seronegative donor 6

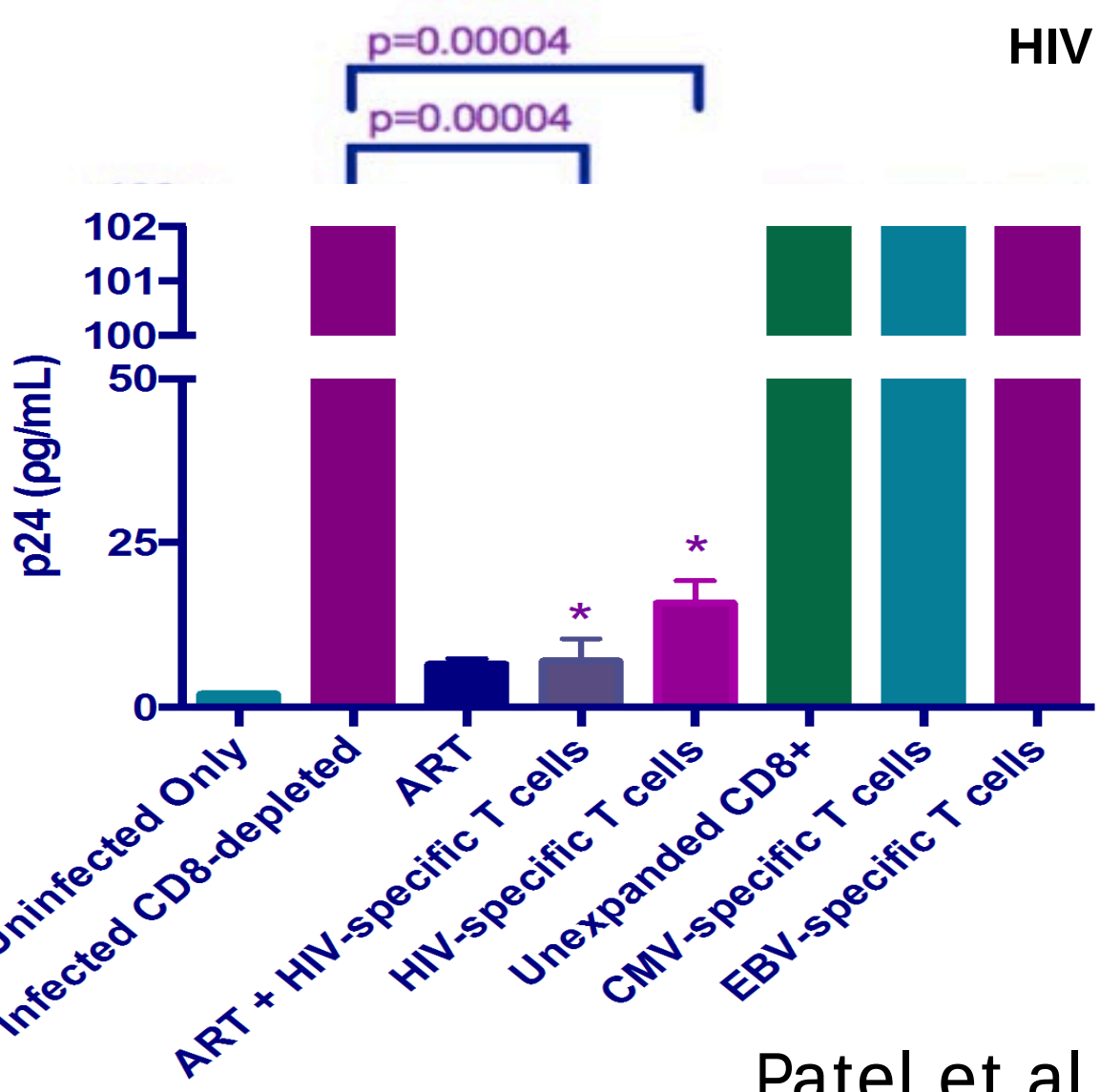


Seronegative donor 7

Patel *et al*, *BBMT* 2016

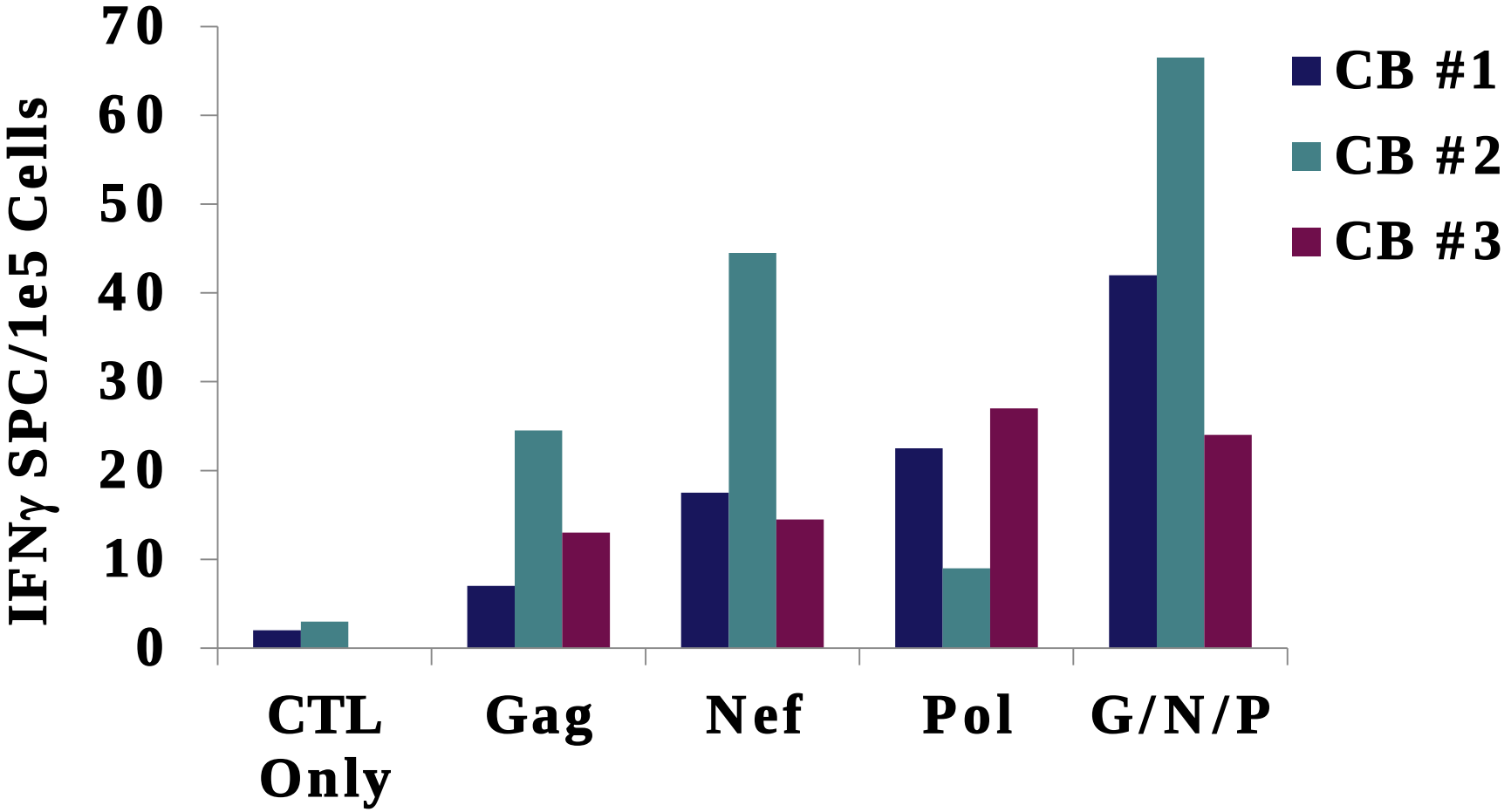
dHXTCs Suppress HIV *in vitro*

HIV Strain: SF162



Patel et al, BBMT 2016

HXTCs can be generated from Naïve Cord blood T-cells



S Patel, ASGCT 2016

Summary of Virus-naïve dHXTCs

- **Seronegative-derived dHXTCs have the ability to:**
  - Recognize multiple HIV antigens: Gag/Nef
  - Produce a polyfunctional immune response
    - IFNγ, IL-2, TNFα, IL-6, IL-8, perforin
  - Recognize Class I and II restricted HIV peptides
  - Suppress HIV replication *in vitro*

Future Directions

- Utilize donor-derived HXTCs as a curative strategy post allo SCT

- On dHXTCs:
  - Knockdown CXCR4 and CCR5 HIV co-receptors using gene editing approaches: CRISPR/Cas9, ZFN, or TALEN

    - CCR5-delta32 mutation donor bank for SCT and for dHXTC generation

Concluding Remarks

HIV specific T cells in the autologous setting may need to be combined with latency reversing agents

HIV specific T cells in the post allo BMT setting may offer a curative strategy if rendered resistant to HIV infection

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