II FÓRUM DE HIV E HEPATITES VIRAIS DA SOCIEDADE PAULISTA DE INFECTOLOGIA

24 de outubro de 2015

É possível atingir a cura da infecção pelo HIV?

"*novas estratégias*"

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Declaração de conflitos

Para a presente apresentação:

“nada a declarar”

Interação HIV *versus* hospedeiro humano

vantagem para o vírus

Fases da complexa interação "*vírus/ser humano*":

- **Fase inicial : parcialmente controlada pela resposta imune do hospedeiro embora robusta, não suficiente para erradicar o vírus**
- **Fase assintomática (crônica): período inicial de certo "*equilíbrio*" entre o vírus e o sistema imune, lentamente progredindo a favor do vírus**
- **Fase tardia - AIDS: previsível para a maioria dos pacientes sem tratamento**

A questão da cura da infecção pelo HIV tem sido apontada como uma "tarefa hercúlea":

enorme complexidade da interação "*vírus/ser humano*"

Meta final: completa erradicação do vírus - "*cura esterilizante*"
Meta mais factível: obtenção de "*controle livre de ARV*" sem progressão - "*cura funcional*"

Shytaj IL & Savarino A: Retrovirology 2013

TARV: obtenção de "infecção crônica controlável" – *SUCESSO!*

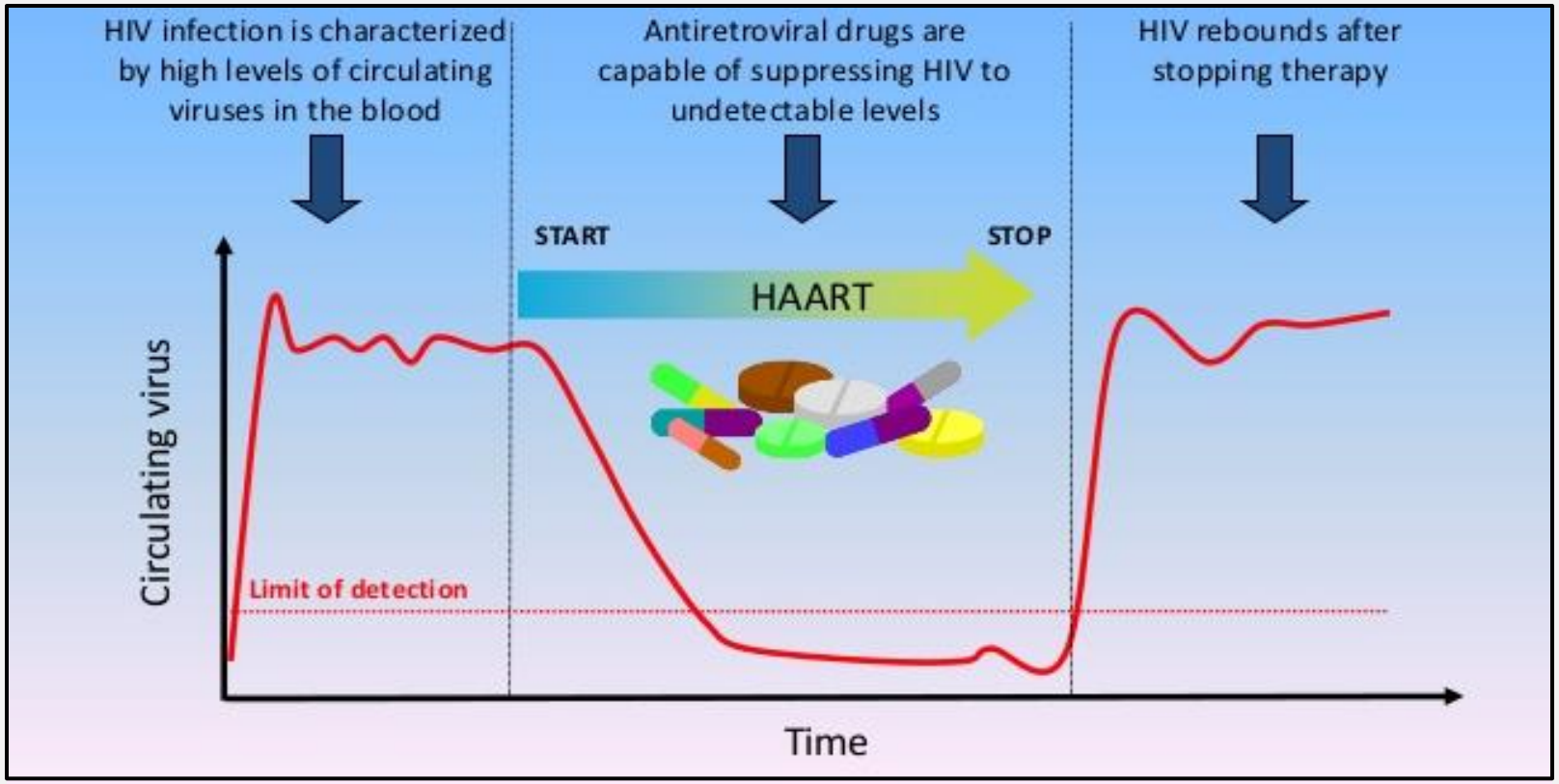
A TARV sistematicamente reduz a viremia a níveis abaixo do limite de detecção dos testes da rotina clínica e retarda a deterioração imune, mas não é suficiente para:

- ***extinguir os "reservatórios" do HIV***
- ***induzir resposta eficaz contra o HIV***

Os "reservatórios virais" são formados precocemente durante a infecção aguda e são fontes estáveis de "persistência viral", abrigando cópias latentes de vírus integrados, que são "invisíveis" ao sistema imune e não afetadas pela TARV

Shytaj IL & Savarino A: Retrovirology 2013

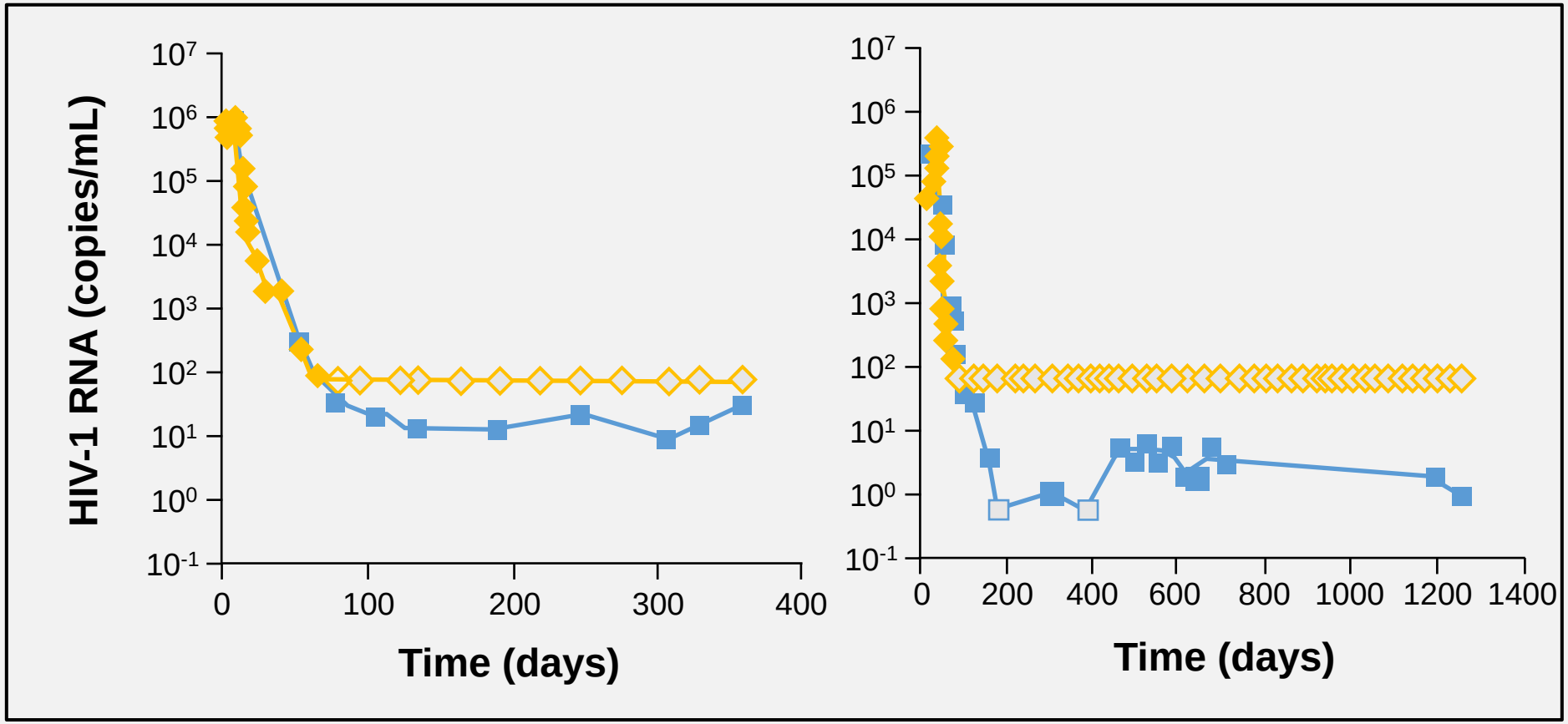
Persistência do HIV durante a HAART



- **Infecção pelo HIV: elevados níveis de replicação viral (plasma "*viral load*")**
- **HAART: reduz a "*viral load*" a níveis indetectáveis (testes em uso clínico)**
- **Interrupção da TARV: reaparecimento da replicação viral**

Low-level viremia (< limite de detecção) durante supressão viral sob HAART

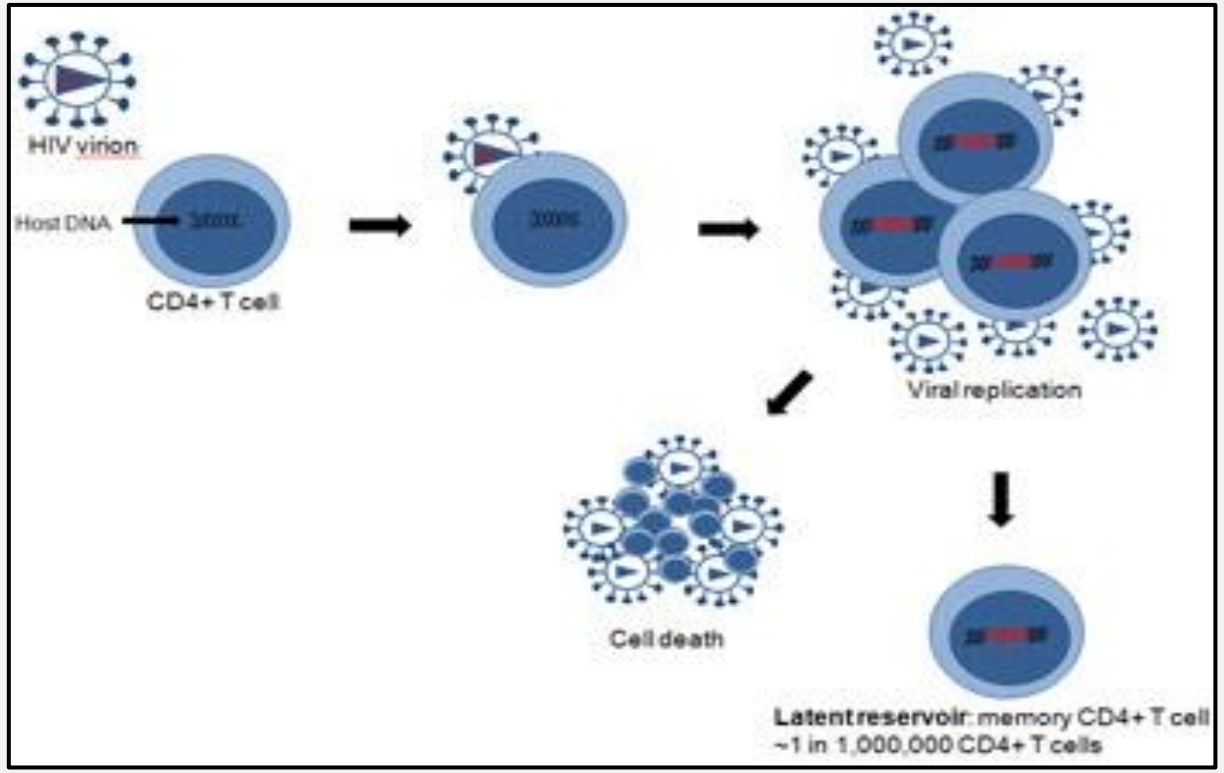
80% pacientes com viremia detectável – média 3,1 cps/ml



Maldarelli F *et al.:. PLoS Path*. 2007; 3:e46

Palmer S et al.: *Proc Natl Acad Sci USA*. 2008;105:3879-3884

HIV: *reservatórios latentes*

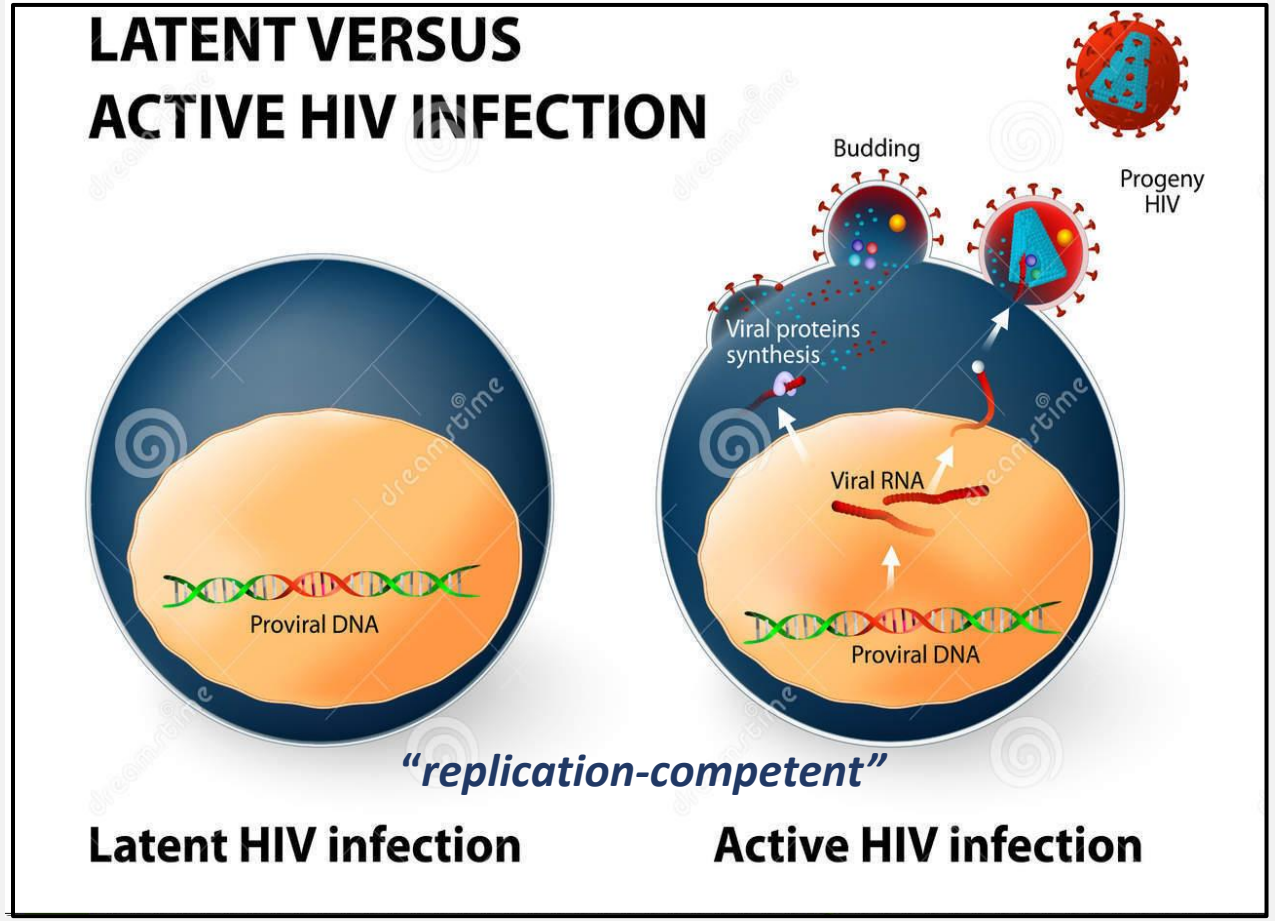


~1 / 1.000.000 CD4

A infecção de linfócitos CD4 pelo HIV resulta em morte celular/apoptose na maioria das células. Mas, algumas células retornam a um estado de quiescência, como células CD4 de memória. Elas contêm uma cópia integrada do genoma viral (*red)* dentro de seu DNA (*black*) e são transcripcionalmente silenciosas, com expressão viral ausente

Persistência do HIV: um desafio para a cura

Integração do DNA viral no genoma da célula hospedeira



The diversity of the tissues and cellular types in which HIV persists, as well as the multiplicity of the molecular mechanisms contributing to HIV persistence, complicate the efforts to develop a safe, effective, and globally accessible cure for HIV

Kulpa DA & Chomont N: J Virus Erad 2015; 1:59-66

Persistência do HIV sob HAART

- **Linfócitos CD4:** principal alvo da infecção pelo HIV

  - **Infecção ativa/produtiva:** caracterização de replicação: HIV-RNA
    - significativamente reduzida pela HAART (mas não eliminada)
    - persistente em "santuários"

    "residual levels of viral replication not fully suppressed in drug-previleged anatomical compartments"

  - **Infecção latente:** caracterização de latência: HBV-DNA

    CD4 cells carrying integrated viral genome that can reactivate and reignite infection

- **Outras células:** monócitos/macrófagos, microglia...

Kulpa DA & Chomont N: J Virus Erad 2015; 1:59-66

HIV persistência sob HAART: múltiplas razões

- **replicação viral residual não completamente suprimida:**
  compartimentos anatômicos privilegiados
- **persistência de pequeno *"pool"* de *"resting latent cells"*: albergam**
  genomas integrados silenciosos capazes de reativar a infecção
- **disfunção imune persistente: incapaz de controlar a replicação residual**
  e a reativaçao das células latentemente infectadas

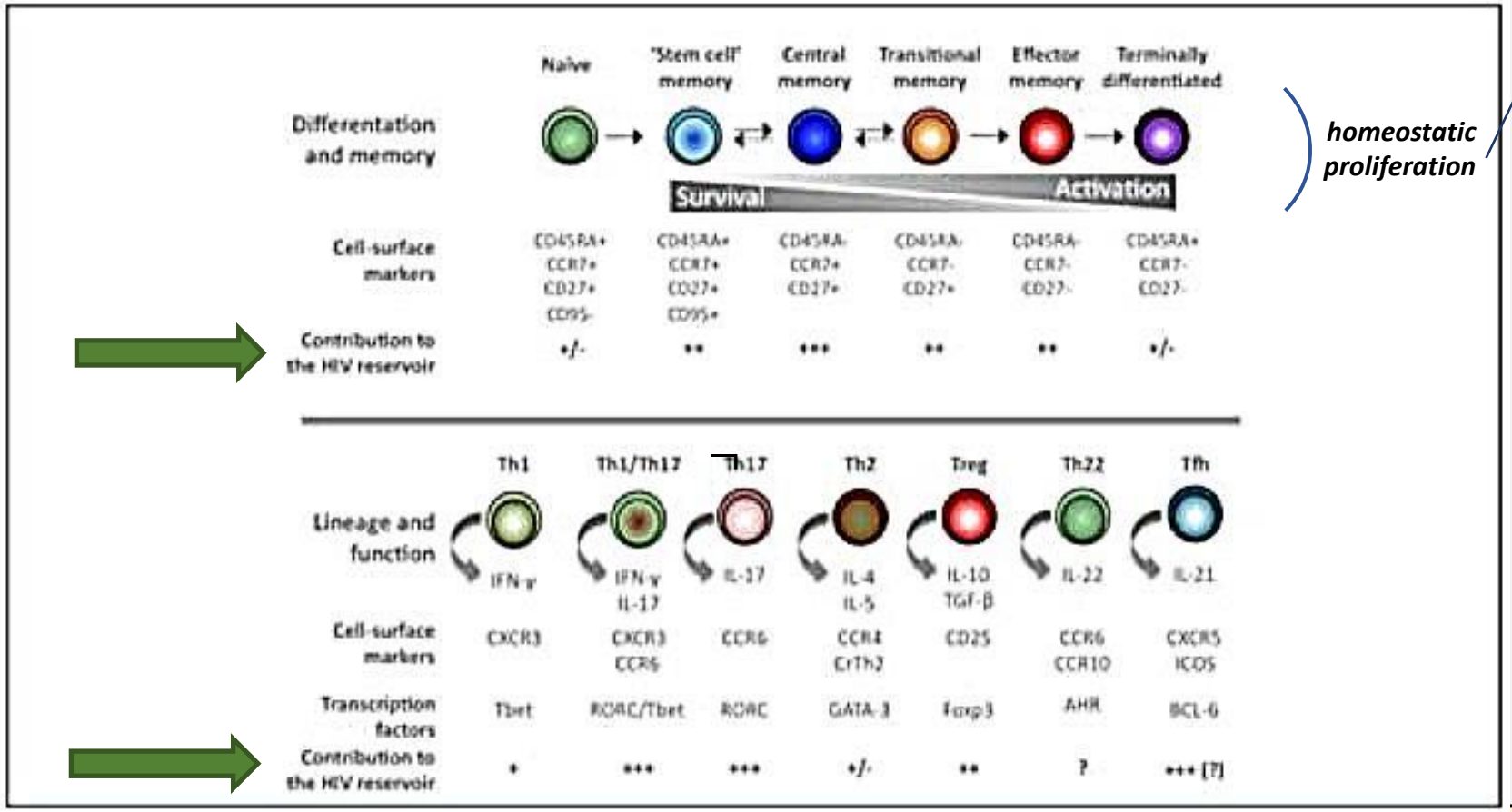
"*viral reservoirs*": cell types or anatomical sites in association with persistent "*replication-competent virus*"

- ***"latent reservoirs"*: estabelecidos durante a fase inicial da infecção – "*long life span*"**
  "*resting*" T CD4, macrófagos, "*resident*" macrófago/microglia do SNC
  & GALT "*gut-associated lymphoid tissue*"/macrófagos

Kulpa DA & Chomont N: J Virus Erad 2015; 1:59–66

Kumar A et al.: Clinical Epigenetics 2015
DOI 10.1186/s13148-015-0137-6

Contribution of CD4+ T cell subsets to the HIV reservoir



homeostatic proliferation

preserves immunologic memory

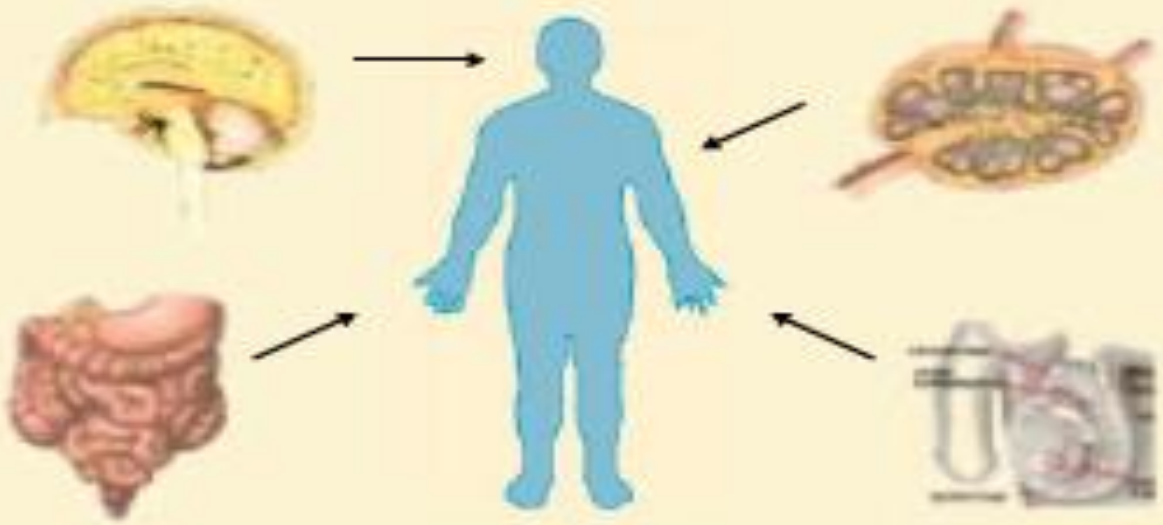
CD4+ T cell subsets: memory status (top) or effector functions (bottom)
Cell-surface markers & production of specific cytokines: identify each individual subset
The relative contribution of each subset to the HIV reservoir is indicated (arrows)

Kulpa DA & Chomont N: J Virus Erad 2015; 1:59-66
Geginat et al.: Semin Immunol 2013; Front Immunol 2014

HIV: ongoing viral replication & persistence

cellular reservoir & anatomical sanctuary sites

Another source of residual viraemia is represented by HIV persistence and replication in tissues where the penetration of antiretroviral drugs is suboptimal



d'Arminio Monforte A et al.: AIDS 2014, 28:1-7

Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues

ARV therapy can reduce HIV to undetectable levels in peripheral blood
Replication in **lymphoid tissue reservoirs:**
lymph node samples before & during 6 mo of treatment => tissue concentrations of 5 frequently used ARV drugs => much lower than in peripheral blood

Lower concentrations correlated with:

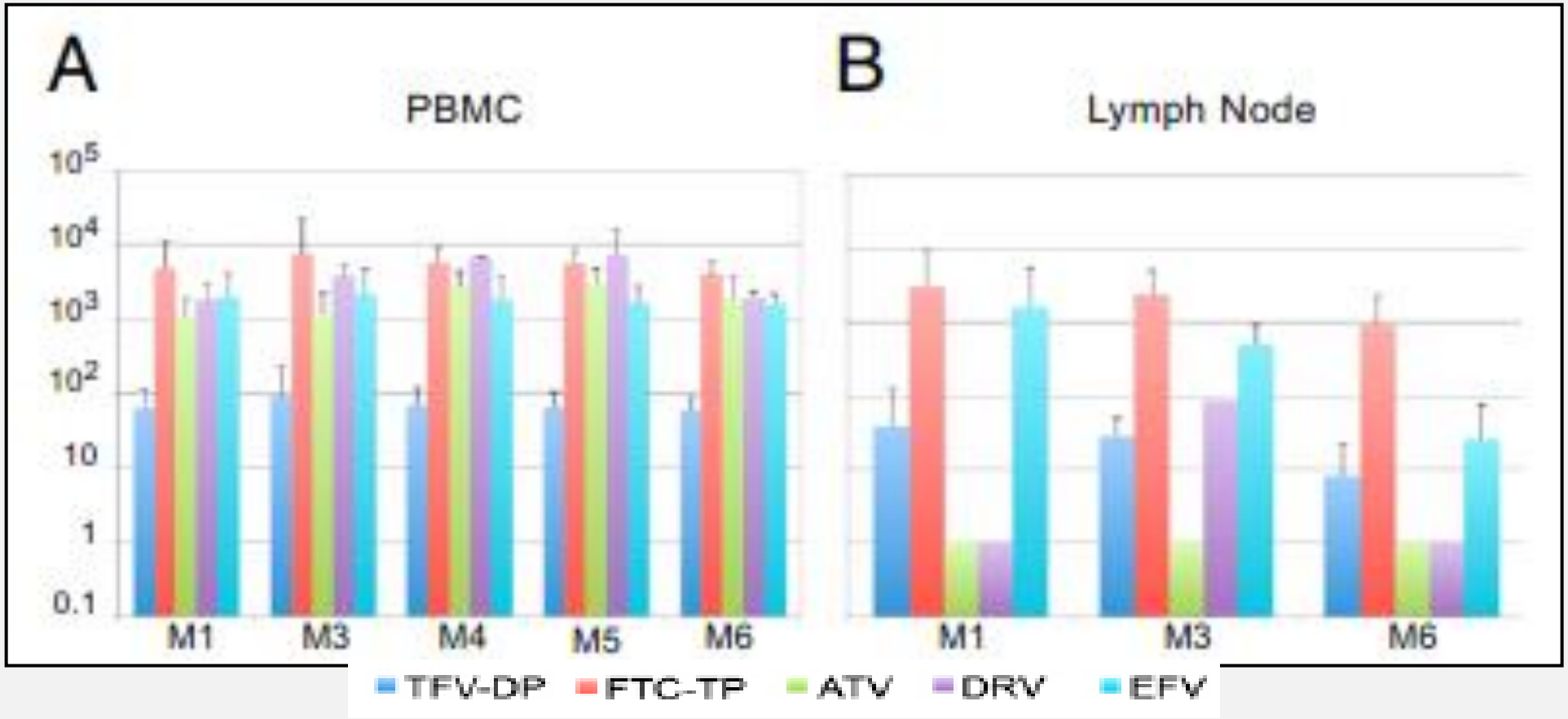
a) continued virus replication (slower decay or increases in the follicular dendritic cell network pool of virions)
b) detection of viral RNA in productively infected cells

The persistent replication associated with apparently suboptimal drug concentrations argues for development and evaluation of novel therapeutic strategies that will fully suppress viral replication in lymphatic tissues **and could avert the long-term clinical consequences of chronic immune activation driven directly or indirectly by low-level viral replication to thereby improve immune reconstitution.**

Fletcher CV et al.: Proc Natl Acad Sci USA 2014

Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues

Decreased Drug Concentrations in Lymphatic Tissue lymph node, ileum, rectum



Fletcher CV et al.: Proc Natl Acad Sci USA 2014

HIV/AIDS: *caminhos para a "cura"!*

1. *HIV infecção:*

a. HIV: ***cura "esterilizante"*** *(cura virológica; ou biológica)*

b. HIV: ***cura "funcional"***

- ***experiências clínicas de sucesso (limitado)***
- ***latência & reservatórios do HIV***

  estratégias para erradicação do HIV
  ou contrôle do HIV ("drug-free control")

2. *HIV doença: AIDS*

eliminação/controle da doença *("end of epidemic AIDS", "AIDS-free world")* – ***UNAIDS*** *90-90-90 treatment: targets for 2020*

HIV: estratégias cura funcional & *esterilizante*

observações & intervenções...

- **HAART: intensificação - tratamento precoce**
- **Transplante de "*stem cell*"**
- **Manipulação gênica – "*knockout*" do coreceptor CCR5**
- **Reversão da latência viral – "*purging*"**
  "kick & kill"
- **"*Enhancement*" da resposta imune - vacinas terapêuticas**
- **etc.**

HIV: estratégias cura funcional & *esterilizante*

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- **etc.**

HIV: estratégias cura funcional & *esterilizante*

os caminhos...

Terapia antirretroviral combinada (*cART*) de elevada potência (*HAART*)

elevado potencial de inibição da replicação viral => regra: obtenção de *"viral load"* não detectável (plasma) pelos testes de uso clínico

a. *HAART*: permanente...

b. *HAART*: intensificação

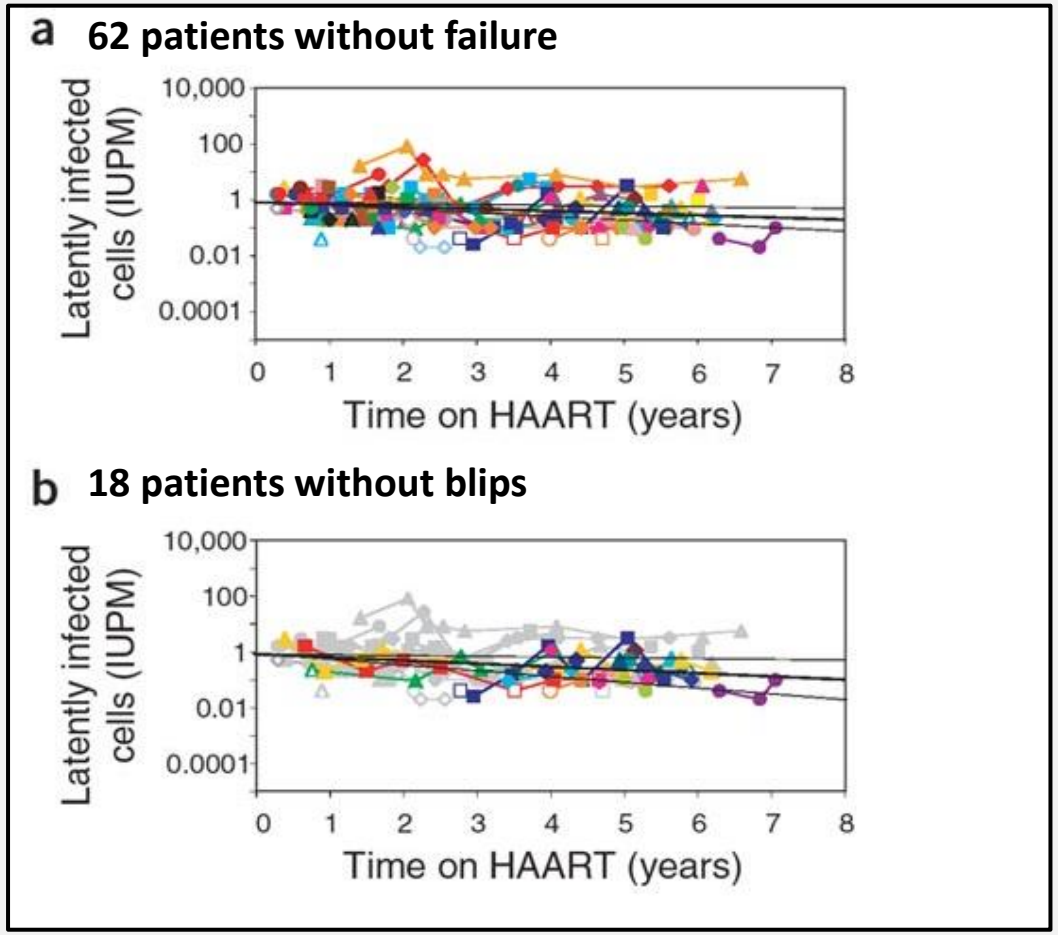
c. *HAART*: início precoce

racional:

redução (mesmo eliminação?)
dos
reservatórios virais
(inclusive os "latentes")

Extremely slow decay of the latent reservoir

in patients on HAART



Heavy black line: mean decay rate; light black lines: 95 CI

Decay of latent infected cells in adult patients on cART with suppression of viremia
(resting CD4 T cells harboring replication-competent HIV)

Decay: half-life approx.
44 months
eradication based on cART would take up to
73,4 years

Siliciano JD et al.: Nature Medicine 2003; 9:727-8

HAART: intensificação do tratamento

- substancial quantidade de estudos avaliaram o efeito da intensificação da cART sobre o resíduo viral (HIV-RNA & HIV-DNA)
- inclusão de diversos ARVs: enfuvirtida, IP/r (ATV, LPV, DRV) e raltegravir em adição a regimes já supressivos
- desapontamento: como regra tais estudos falharam em demonstrar cabalmente a ocorrência de declínio da viremia HIV-RNA residual ou do HIV-DNA celular

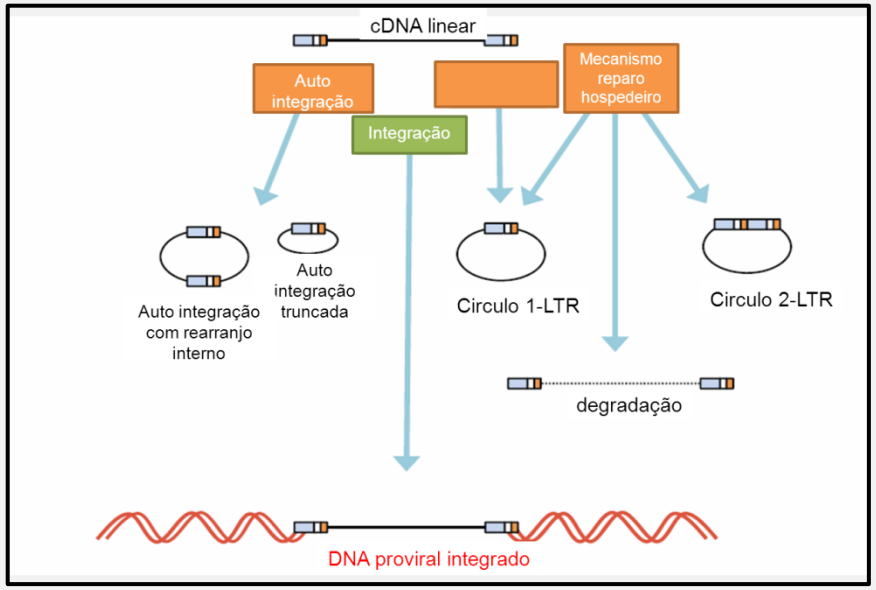
detalhe:

- raltegravir: conjunto dos estudos sugerem um mínimo efeito sobre a viremia "*low-level*" persistente e o nivel de HIV-DNA em sangue ou tecidos

Josefsson L et al.: Curr Opin ID 2010; 23:628-32; Maldarelli F: Curr Opin HIV AIDS 2011; 6:49-56; Doyle T & Geretti AM: Curr Opin ID 2012; 25:17-25; Blanco JL & Martinez-Picado J: Curr Opin HIV AIDS 2012; 7:415-21

HAART: intensificação do tratamento

- **maraviroc (antagonista de CCR5): um recente pequeno estudo, não randomizado – achados intrigantes: maraviroc por 48 sems. adicionado a cART supressiva em 10 pacientes:**
- **aumento do HIV-RNA (ensaio de 1 cp/ml) & de círculos de "2-LTR" de HBV-DNA epissomal & significante redução do HBV-DNA (teste: IUPM)**
- **única intervenção revelando diminuição no número das "latently infected cells" usando intensificação de cART supressiva**
- **mecanismo: não claro**



LTR = long terminal repeat

IUPM *(infectious units per milliom):* measures cell capacity to produce infectious virus by dilution co-culture assay; heavy technique, high amount of blood (180 ml)

equiv. VOA *(viral outgrowth assay)*

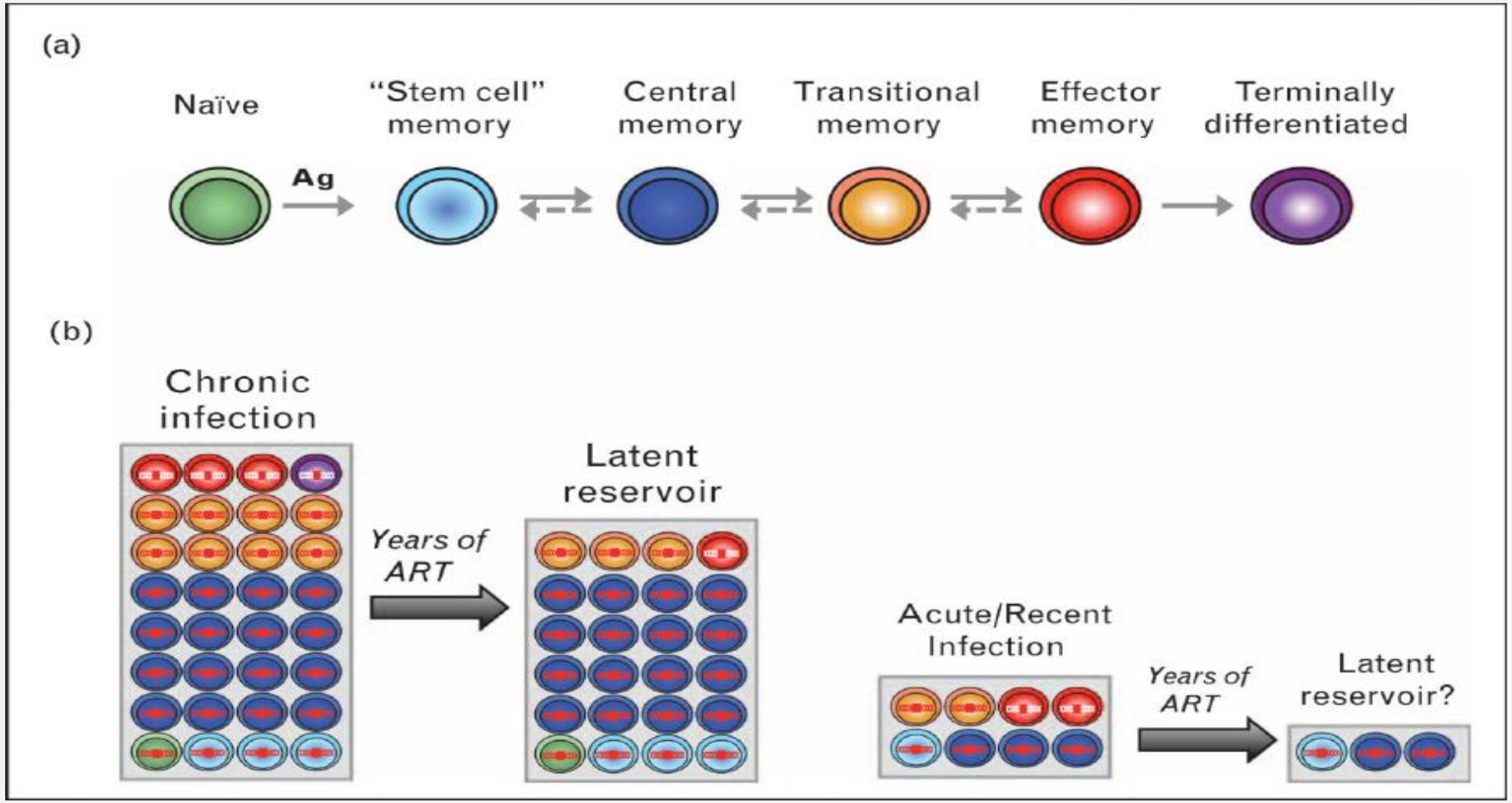
HIV-DNA integrado & não integrado:

produto da transcrição reversa o DNA linear além da integração tem outros destinos: várias formas circulares (1-LTR & 2-LTR) presentes nas células infectadas

Sloan RD & Wainberg MA: Retrovirology 2011; 8:52

How does the timing of ARV therapy initiation in acute infection affect HIV reservoirs?

Early ARV therapy limits the size & alters the distribuition in CD4 T-cell subsets of the latent HIV reservoir – may be the first critical step for remission or cure by limiting the HIV reservoir



Ananworanich J et al.: Curr Opin HIV AIDS 2015; 10: 18–28

The Mississippi Baby

The **Mississippi baby** became **infected** by her mother around the time of her **birth.** Physicians initiated **highly active antiretroviral therapy** within **30 hours of birth.** The child appeared to have achieved a "**sterilizing**" cure after the rapid initiation of antiretrovirals and **treatment was withdrawn**. However, 27 months after treatment had been **discontinued,** the child experienced a viral **rebound** and physicians **reinstated antiretroviral therapy**.

Conclusão: "cura funcional de duração transitória !

Persaud D et al.: N Engl J Med 2013

"*Estudo VISCONTI*": racional

- *HIV contollers:* espontaneamente controlam a replicação viral, com indetectabilidade por muitos (indefinidamente?) anos
- Questão: é factível para outros pacientes alcançarem um *status* símile? ou seja: remissão e cura funcional

Evidências recentes: tratamento precoce durante a infeçcão primária proporciona *long-term* benefícios quanto a:

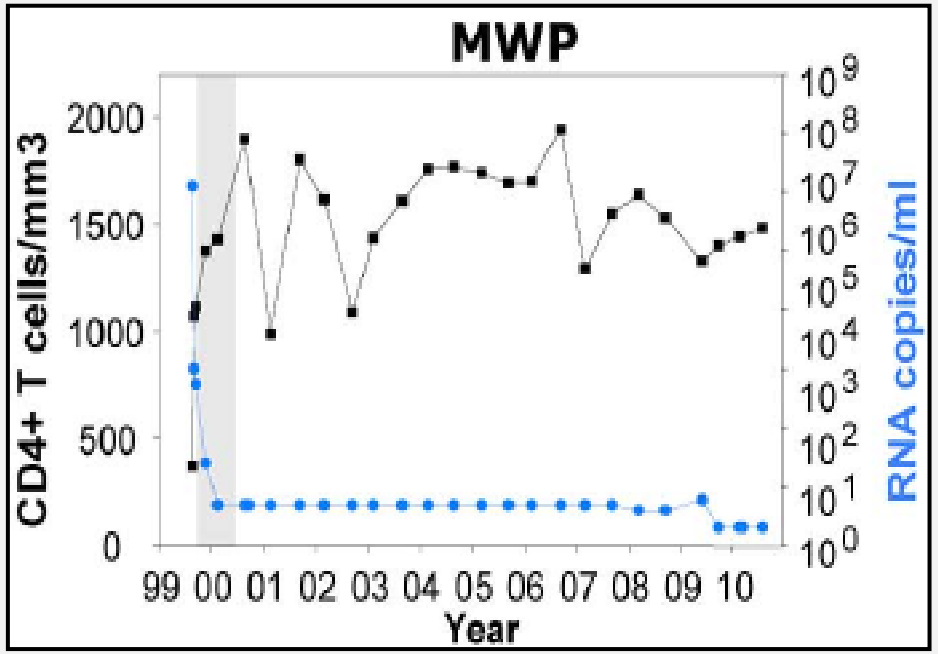
i) replicação viral residual;
ii) diversidade e reservatórios virais
iii) imunidade inata e de células T e B
iv) restauração imune

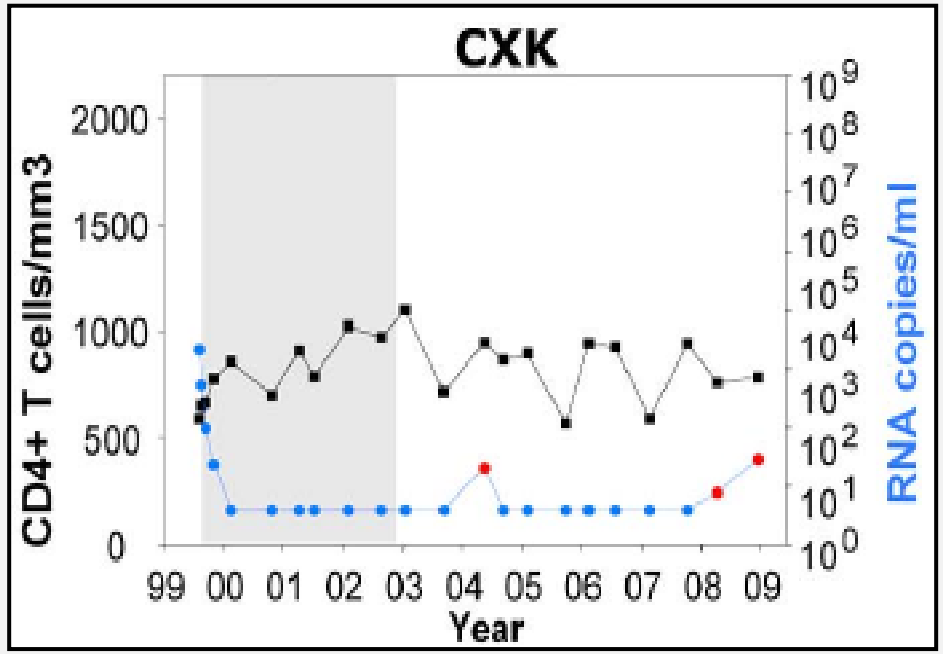
as contagens de células CD4 são mais elevadas e o *rebound* viral ocorre mais tarde (e em nível inferior) após a descontinuação do tratamento, quando iniciado durante a infecção primária

Sáez-Cirión A et al.: PLOS Pathogens March 2013

"*Estudo VISCONTI*" ...cura funcional

HIV infecção 1ª: TARV precoce (<6m.) => prolongado controle da viremia e CD4 estável em 14 pacientes após interrupção

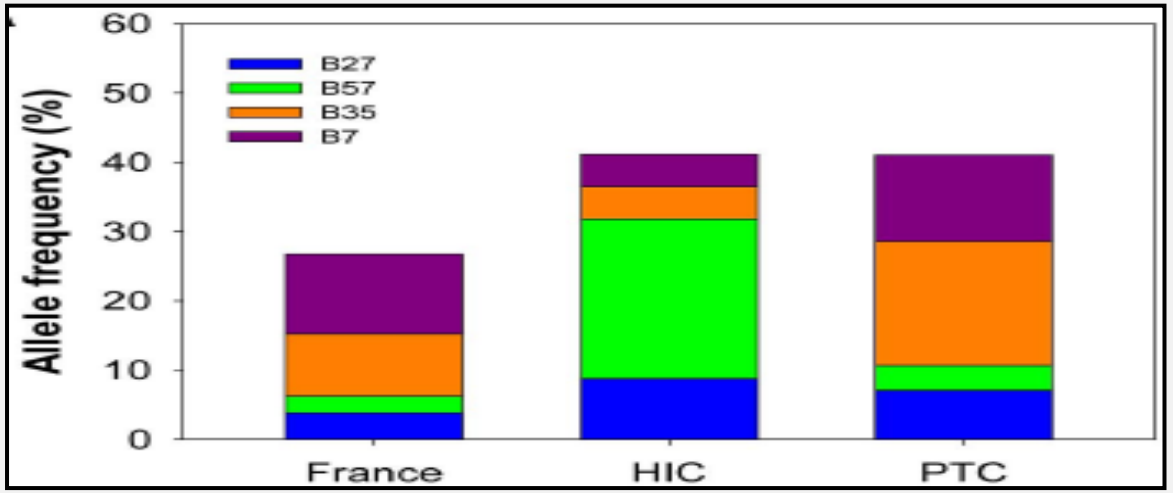




Long-term control of viremia and stable CD4+ T cell counts in patients after interruption of antiretroviral treatment initiated in primary HIV-1 infection

Sáez-Cirión A & The ANRS Visconti Study Group: PLOS Path 2013

“functional cure”: similar to “HIV controllers” - to achieve viral remission: HIV remains at low levels controlled by the host in the absence of cART - status for 5–15% of patients treated very early during primary HIV infection for long periods who experience treatment interruption afterwards - known as “PTC = post-treatment controllers”



“HIV controllers” or “elite controllers”: small % of HIV patients that can naturally control viral replication below the levels of detection with standard clinical assays - important model to understand the mechanisms underlying control of infection in the absence of treatment

PTC=post-treatment controllers differ from **HIV controllers** in terms of HLA class I profile

The frequencies of the protective alleles HLA-B*27 and B*57 and the risk alleles HLA-B*07 and B*35 in the general French population (n = 6094), HICs (n = 148) and PTCs (n = 28)

VISCONTI: 3 years **cART duration after primary infection; after interruption: PTCs presented a median sustained control for 7years. Acute phase: PTCs had higher viremia & lower CD4 counts than patients who naturally control infection afterwards. In addition: PTCs different genetic background**

"Estudo VISCONTI"

Viro-Immunological Sustained CONtrol after Treatment Interruption

Post-Treatment HIV-1 Controllers with a Long-Term Virological Remission after the Interruption of Early Initiated Antiretroviral Therapy ANRS VISCONTI Study

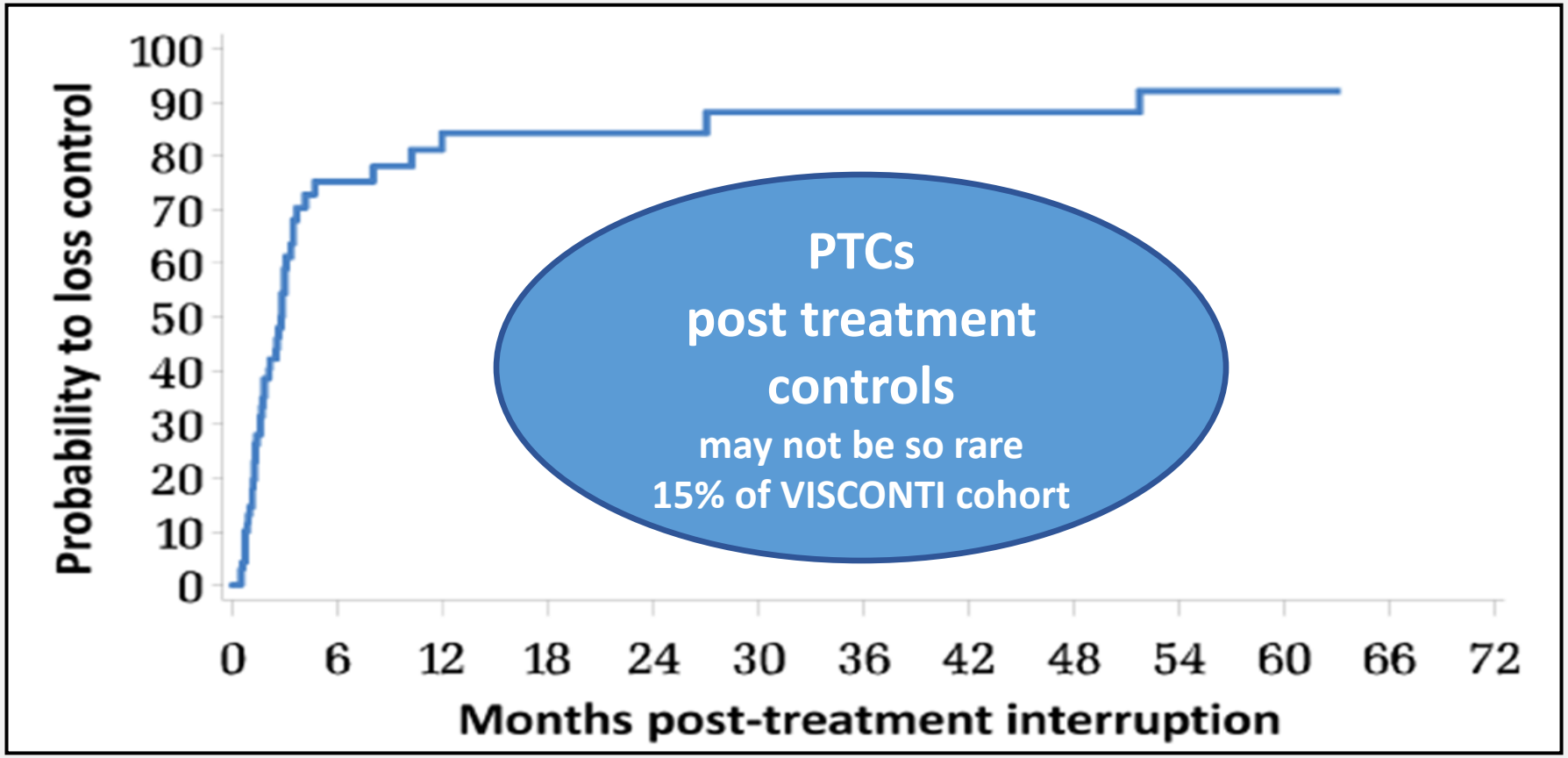
Combination antiretroviral therapy reduces HIV-associated morbidities and mortalities but cannot cure the infection. Given the difficulty of eradicating HIV-1, a functional cure for HIV-infected patients appears to be a more reachable short term goal. **We identified 14 HIV patients (post-treatment controllers) whose viremia remained controlled for several years after the interruption of prolonged cART initiated during the primary infection.** Most PTCs lacked the protective HLA alleles that are overrepresented in spontaneous HIV controllers. Accordingly, the PTCs had poorer CD8+ T cell responses and more severe primary infections than the HICs did. Off therapy, the PTCs were able to maintain and, some cases, further reduce an extremely low viral reservoir. We found that long-lived HIV-infected CD4+ T cells contributed poorly to the total resting HIV reservoir in the PTCs because of a low rate of infection of naïve T cells and a skewed distribution of resting memory CD4+ T cell subsets.

Our results show that early and prolonged cART may allow some individuals with a rather unfavorable background to achieve long-term infection control and may have important implications in the search for a functional HIV cure

Sáez-Cirión A & The ANRS Visconti Study Group: PLOS Path 2013

"*Estudo VISCONTI*" 74 pacientes

Perda do controle da viremia após interrupção da TARV iniciada dentro de 6 meses da aquisição do HIV



Sáez-Cirión A & The ANRS Visconti Study Group: PLOS Path March 2013

OPTIPRIM-ANRS TRIAL: Intensive five-drug ART regimen *vs* standard triple-drug therapy during primary HIV-1 infection

randomised, open-label, phase 3 trial

Early cART initiation at the time of primary HIV-1 infection could restrict the establishment of HIV reservoirs. Aim: effect on HIV-DNA load of a intensive regimen (with RAL and MVC) compared with standard triple-drug cART

Inclusion criteria: primary HIV infection (an incomplete HIV western blot and detectable plasma HIV-RNA), with either symptoms or a CD4+ cell count below 500 cells/µL

Random (1:1) : 5-drug intensive regimen (RAL, MVC *plus* TDF/FDC, DRV/r) or standard 3-drug cART regimen (TDF/FDC, DRV/r)

Primary endpoint: median number of HIV-DNA cps/10^6 PBMC at month 24
(analysis: modified ITT population)

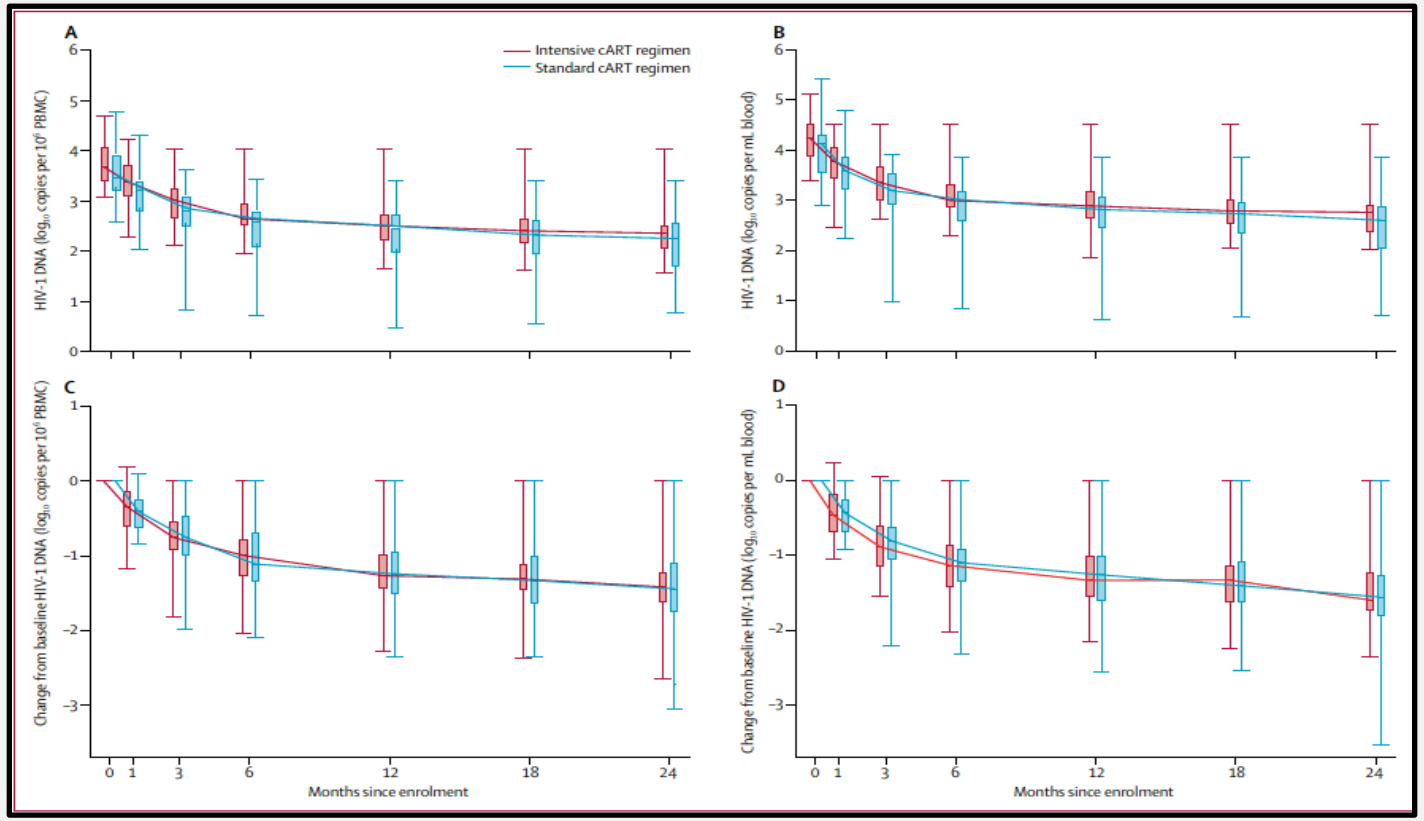
France (April 26 2010 – July 13 2011): **92 randomised and 90 started treatment**
(45 in each treatment group)

Chéret A et al. (for the OPTIPRIM ANRS Study Group): Lancet Infect Dis 2015; 15:387–96

OPTIPRIM-ANRS TRIAL

Median HIV-DNA load and change from baseline (24 mo.)

modified intention-to-treat population

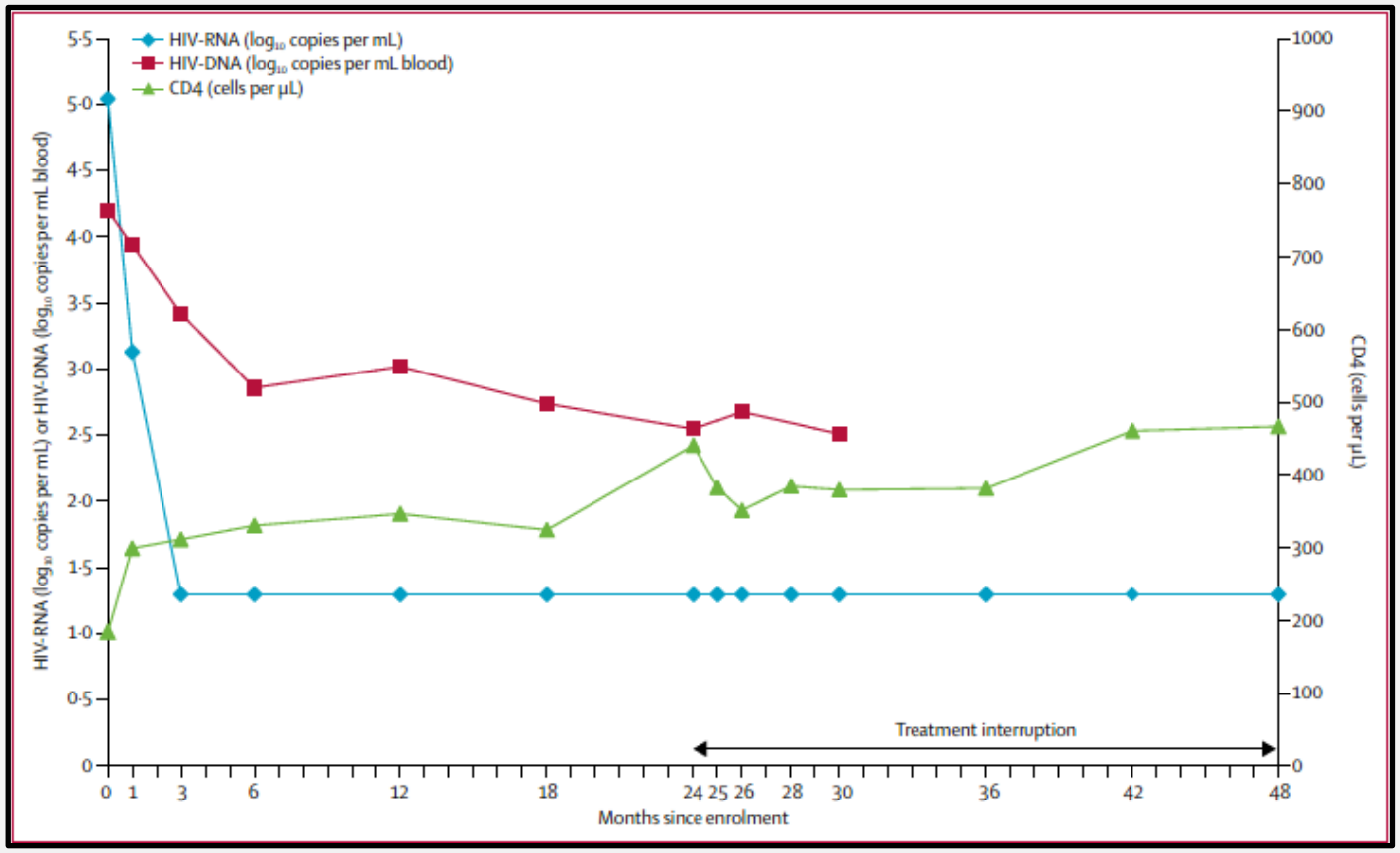


Boxes show IQR and bars show range. cART=combination antiretroviral therapy. PBMC=peripheral blood mononuclear cells

Chéret A et al. (for the OPTIPRIM ANRS Study Group): Lancet Infect Dis 2015; 15:387–96

OPTIPRIM-ANRS TRIAL

HIV-RNA, HIV-DNA, and CD4: patient in the standard cART group who achieved PTC = *post-treatment controller* status



viral load < 50 cps/ml for at least 24 months after treatment interruption

Chéret A et al. (for the OPTIPRIM ANRS Study Group): Lancet Infect Dis 2015; 15:387–96

OPTIPRIM-ANRS TRIAL: interpretation

- Largest study: no difference in HIV-DNA load between intensified ART & standard ART
- HIV-DNA declined substantially in both groups
- More than 90% of patients < 50 HIV-RNA cps/ml
- Intensive regimen: particularly effective at reducing the HIV-RNA load in the first 3 months; significant proportion of patients: low but persistent viral replication until month 18 (paradoxical result: transient effect of MVC on immune cell trafficking through CCR5 blockade)
- First time: standardised interruption of an early cART regimen can lead to posttreatment controller status

Suggestion: continuous decrease of HIV-DNA until month 24 suggests that more than 2 years of treatment initiated at primary HIV-1 infection would increase the effect of combination ART on HIV in patients with primary HIV-1 infection.

Together, these results reinforce the recommendation that treatment started at the time of primary infection is essential, and the findings should contribute to the design of trials aiming to decrease the HIV reservoir and achieve lifelong HIV remission in patients with primary HIV-1 infection

HIV: estratégias cura funcional & *esterilizante*

observações & intervenções...

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- **Transplante de "*stem cell*"**
- Manipulação gênica – "*knockout*" do coreceptor CCR5
- Reversão da latência viral – "*purging*"
  "*kick & kill*"
- "*Enhancement*" da resposta imune - vacinas terapêuticas
- etc.

"*The Berlin patient*"

Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation

Infection with the human immunodeficiency virus type 1 (HIV-1) requires the presence of a CD4 receptor and a chemokine receptor, principally chemokine receptor 5 (CCR5). Homozygosity for a 32-bp deletion in the CCR5 allele provides resistance against HIV-1 acquisition.

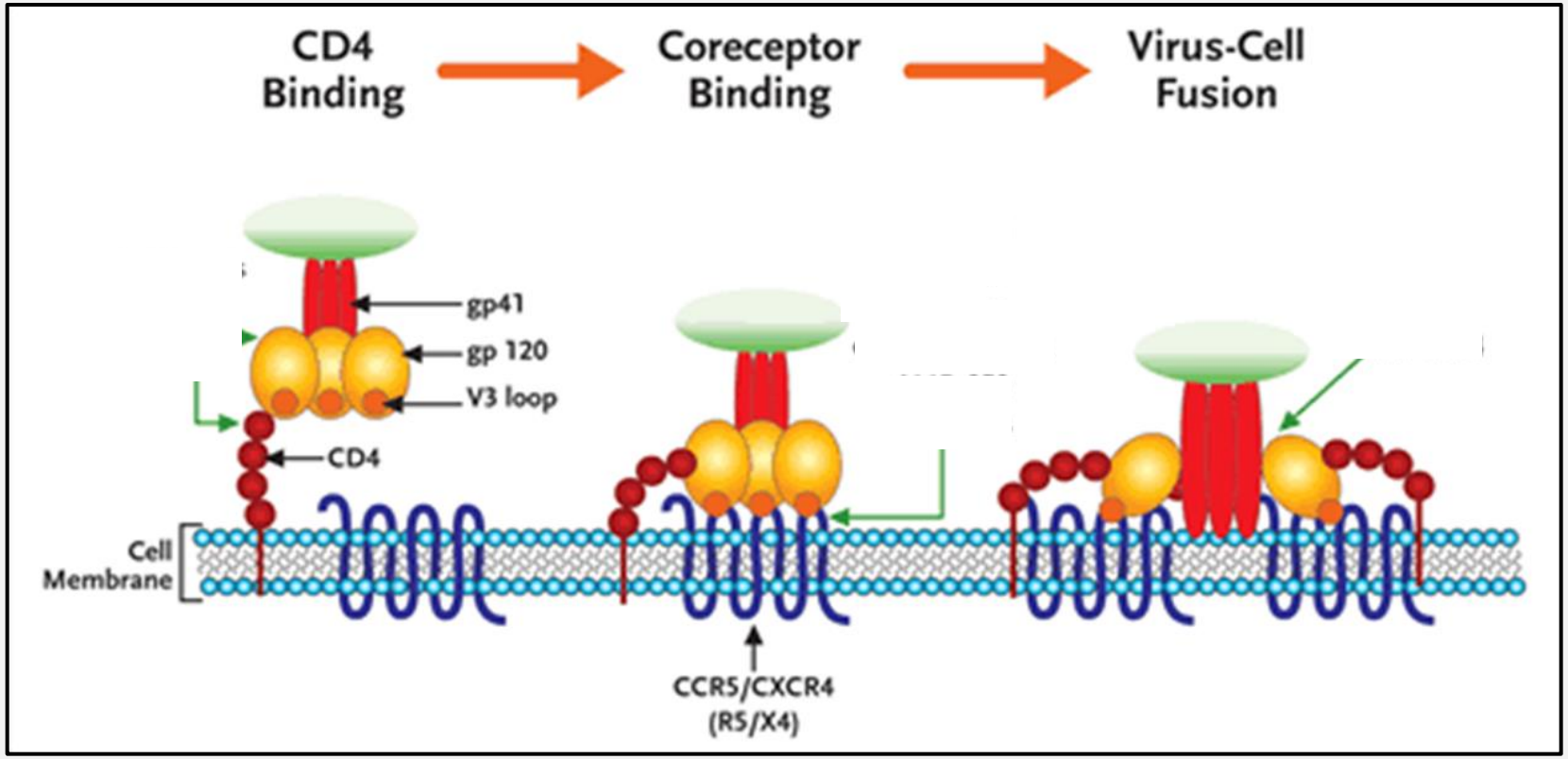
We transplanted stem cells from a donor who was homozygous for CCR5 delta32 in a patient with acute myeloid leukemia and HIV-1 infection. The patient remained without viral rebound 20 months after transplantation and discontinuation of antiretroviral therapy.

This outcome demonstrates the critical role CCR5 plays in maintaining HIV-1 infection.

Hütter G et al.: N Engl J Med 2009

HIV & CCR5 Delta32/Delta32

(homozygozity: 32-bp deletion)

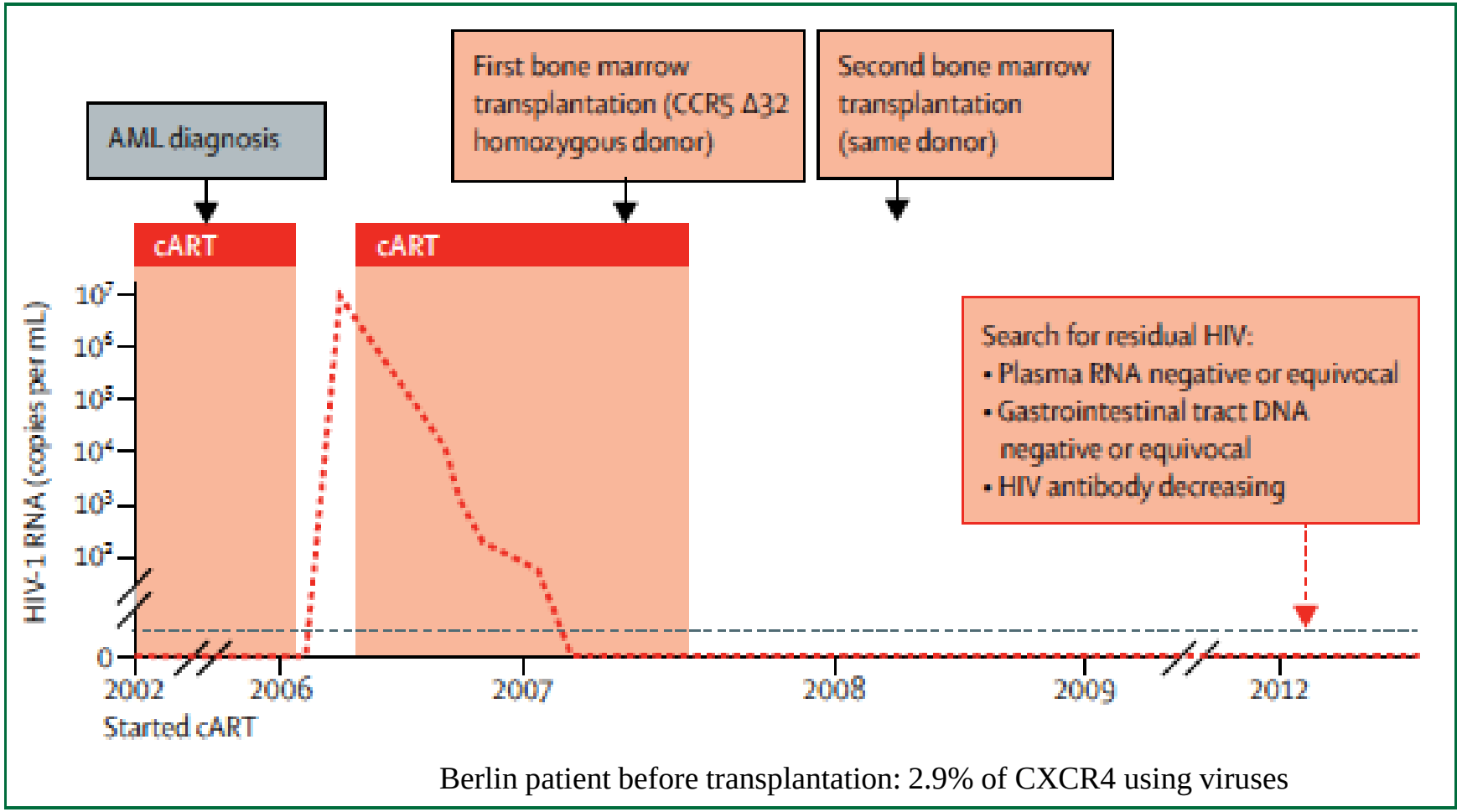


Δ32 Homozygous

Δ32/Δ32

2 Δ32 alelos (1% prevalence) → **Resistant to HIV**

“Paciente Berlim”: *único caso de cura virológica*



limite de detecção: 1 cp/ml nos testes pós transplante
AML=leucemia mielóide aguda cART= TAR combinada

The "Boston patients" TMO com "GvH"

Boston patients: ambos transplantados (TMO) para tratamento de linfoma em 2008 e 2010. ARVs mantidos após o TMO

Oito meses após TMO: *"not detect any sign of HIV in the blood"*

Início de 2013: decisão de interrupção dos ARVs em ambos
A seguir: *"they appeared to remain HIV-free"*

Julho 2013: *"they may have been cured"*

Rebound: em agosto & novembro de 2013

Ambos pacientes: seguem em *"good health and back on ARV therapy"*

- ***"underlying how inginious HIV can be in finding places in the body to evade attack efforts by the immune system"***
- ***"the HIV reservoir is deeper and more persistent than previously known"***
- ***"the current standards of probing for HIV may not be sufficient"***

Henrich T & Kuritzkes D: Brigham & Women's Hospital – Boston / Harvard

HIV cure research:

hematopoietic stem cell transplantation

Study Reference	Patients			Diagnosis	Graft type	Strategy against HIV infection	Effect on HIV persistence	Clinical Outcome
	N	Gender	Age					
Holland et al. (1989)	1	M	41	NHL	Allogeneic (bone marrow)	High-dose zidovudine for 2 weeks before transplantation. Lower maintenance dose zidovudine after transplantation.	No detectable HIV RNA and DNA at day 32 after transplantation and at autopsy.	Death 47 days after transplantation.
Contu et al. (1993)	1	F	25	NA	Allogeneic (bone marrow)	Zidovudine, IFN-alpha 2 and anti-HIV-1-specific T cell clones.	No detectable HIV RNA at day 30 after transplantation and at autopsy.	Death 10 months after transplantation.
Sora et al. (2002)	1	F	33	AML	Allogeneic (bone marrow)	cART before and after transplantation, with interruptions due to side-effects.	No detectable HIV RNA on cART from day 210 after transplantation.	Alive after 42 months of follow up.
Gabarre et al. (2004)	14	M/F	27–53	BL, HL, NHL	Autologous	cART before and after transplantation.	No detectable HIV RNA on cART in three patients who survived.	Five patients were alive.
Resino et al. (2007)	4	NA	31–58	BL, HL, NHL	Autologous	cART before and after transplantation.	On cART No detectable HIV RNA after transplantation (two patients), viral load rebound (two patients). Detectable HIV DNA at month 12 after transplantation for all patients.	The four patients were alive after 12 months of follow up.
Avettand-Fenoel et al. (2007)	1	M	17	BL,AML	Allogeneic (bone marrow)	cART before and after transplantation, with interruptions due to side-effects.	Undetectable RNA and DNA on cART. Detectable HIV RNA and DNA at day 16 after TI.	Death 191 days after transplantation.
Hutter et al. (2009)	1	M	40	AML	Allogeneic (bone marrow)	Donor homozygous for CCR5 Δ32.	No cART. No trace of HIV after 6 years of follow-up.	Alive after 6 years of follow up. Considered the first case of AIDS cure.
Simonelli et al. (2010)	24	M/F	<45 ($n=15$) ≥ 45 ($n=9$)	HL, NHL	Autologous	cART before and after transplantation, with interruptions due to side-effects ($n=8$).	On cART Detectable HIV RNA and DNA after transplantation. HIV DNA significantly lower at month 24 than those at baseline.	Alive, immunologic characteristics comparable to HIV negative patients.
Cillo et al. (2013)	10	M	24–60	BL, HL, NHL	Autologous	cART before and after transplantation, with interruptions due to side-effects ($n=3$).	On cART. Detectable HIV RNA (9/10 patients) and DNA (10/10 patients) after transplantation.	Alive with undetectable VL by conventional methods, but with detectable proviral DNA.
Henrich et al. (2013)	2	M	NI	HL	Allogeneic (bone marrow)	cART before and after transplantation.	Undetectable HIV RNA on cART, detectable after TI. Detectable HIV DNA early after transplantation and undetectable in long term follow-up.	Alive 5 and 3 years after transplantation, but viremia rebounded after TI.
University of Minnesota (not published)	1	M	12	ALL	Allogeneic (cord blood)	Donor homozygous for CCR5 Δ32 deletion.	No detectable HIV after treatment discontinuation.	Died 2 months after transplantation by a severe graft-versus-host disease.

ALL: acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BL: Burkitt lymphoma; BM: bone marrow; F: Female; HL: Hodgkin lymphoma; M: male; NHL: non-Hodgkin lymphoma; NA: not available; PBMC: Peripheral blood mononuclear cells; PCR: polymerase chain reaction; TI: treatment interruption; VL: viral loa

Passaesa CP & Sáez-Cirion A: Virology 2014

HIV cure research:

hematopoietic stem cell transplantation (HSC)

Study Reference	Patients			Diagnosis	Graft type	Strategy against HIV infection	Effect on HIV persistence	Clinical Outcome
	N	Gender	Age					
Gabarre et al. (2004)	14	M/F	27–53	BL, HL, NHL	Autologous	cART before and after transplantation.	No detectable HIV RNA on cART in three patients who survived.	Five patients were alive.
Simonelli et al. (2010)	24	M/F	<45 ($n=15$) ≥ 45 ($n=9$)	HL, NHL	Autologous	cART before and after transplantation, with interruptions due to side-effects ($n=8$).	On cART Detectable HIV RNA and DNA after transplantation. HIV DNA significantly lower at month 24 than those at baseline.	Alive, immunologic characteristics comparable to HIV negative patients.
Cillo et al. (2013)	10	M	24–60	BL, HL, NHL	Autologous	cART before and after transplantation, with interruptions due to side-effects (n=3).	On cART. Detectable HIV RNA (9/10 patients) and DNA (10/10 patients) after transplantation.	Alive with undetectable VL by conventional methods, but with detectable proviral DNA.

Several studies: autologous or allogeneic HSC transplantation in association with ARV therapy as a strategy to eradicate HIV in seropositive patients diagnosed with leukemia and/or lymphoma

HIV detection after transplantation: either following therapy withdrawal or because the therapeutic regimen was not able to completely eliminate the viral reservoirs. In addition, in several cases, the patients died after transplantation

HSC transplantation to eradicate the virus: successful only in the Berlin patient

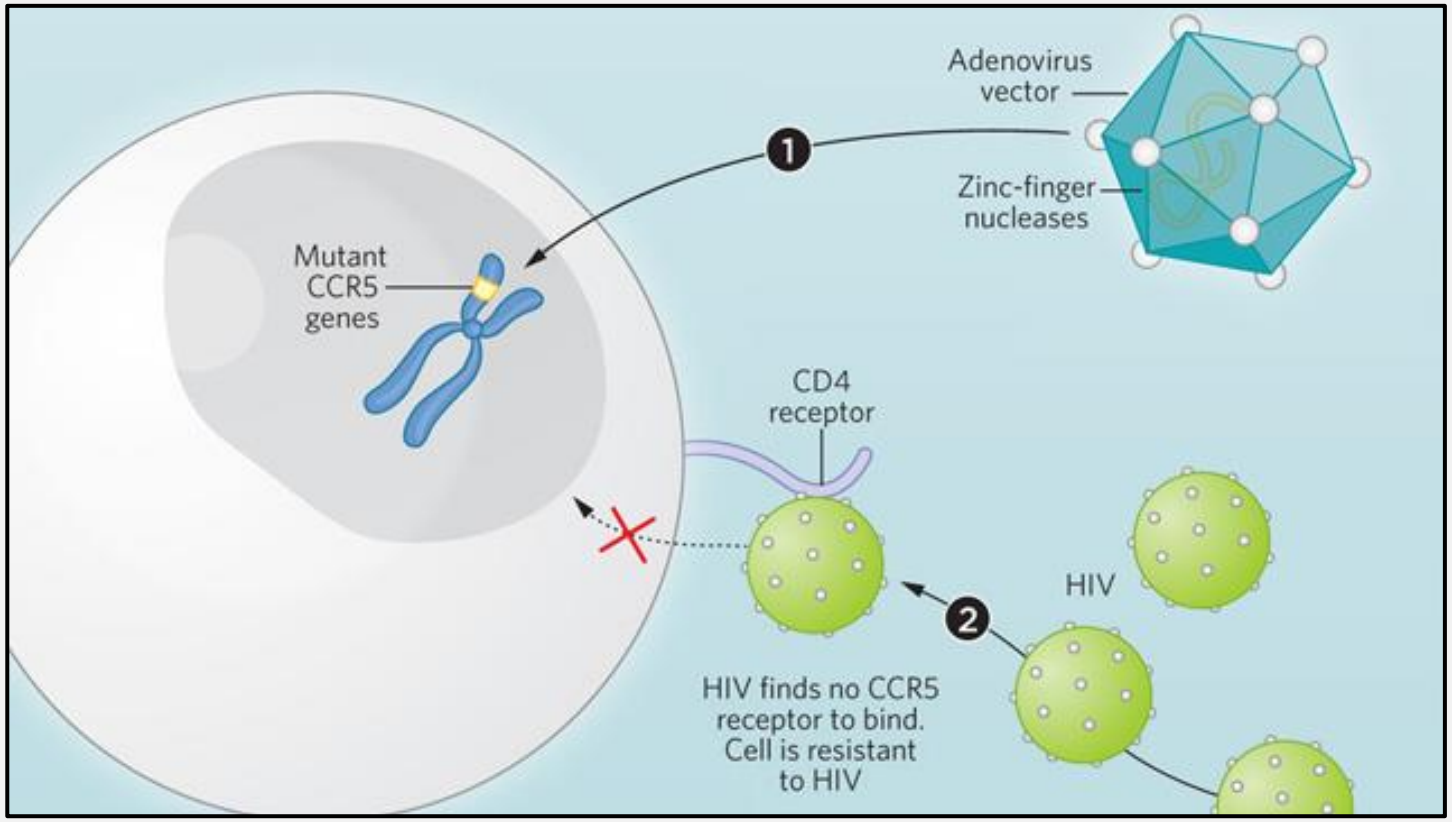
Passaesa CP & Sáez-Cirion A: Virology 2014

HIV: estratégias cura funcional & *esterilizante*

observações & intervenções...

- HAART: intensificação - tratamento precoce
- Transplante de "*stem cell*"
- **Manipulação gênica – "*knockout*" do coreceptor CCR5**
- Reversão da latência viral – "*purging*"
  "*kick & kill*"
- "*Enhancement*" da resposta imune - vacinas terapêuticas
- etc.

"knockout" do CCR5: terapia gênica



Introduction of a mutant copy of the gene for the HIV co-receptor CCR5 into the T cells of HIV patients. This prevents the expression of CCR5 on the cell's surface, and thus inhibits HIV from infecting cells **Sangamo Biosciences**

HIV penetra nas células por meio receptores (CD4, CCR5…). *Zinc fingers nucleases* (ZFN) expressas por adenovirus recombinantes podem bloquear a expressão do gene CCR5, tornando a célula desprovida de CCR5 e resistente ao HIV

Kent SJ et al.: Lancet ID 13 July 2013

CCR5D32 mutation and HIV infection: basis for curative HIV therapy

Clinical trials of CCR5 gene editing-based cell therapy for the treatment of HIV-infected persons

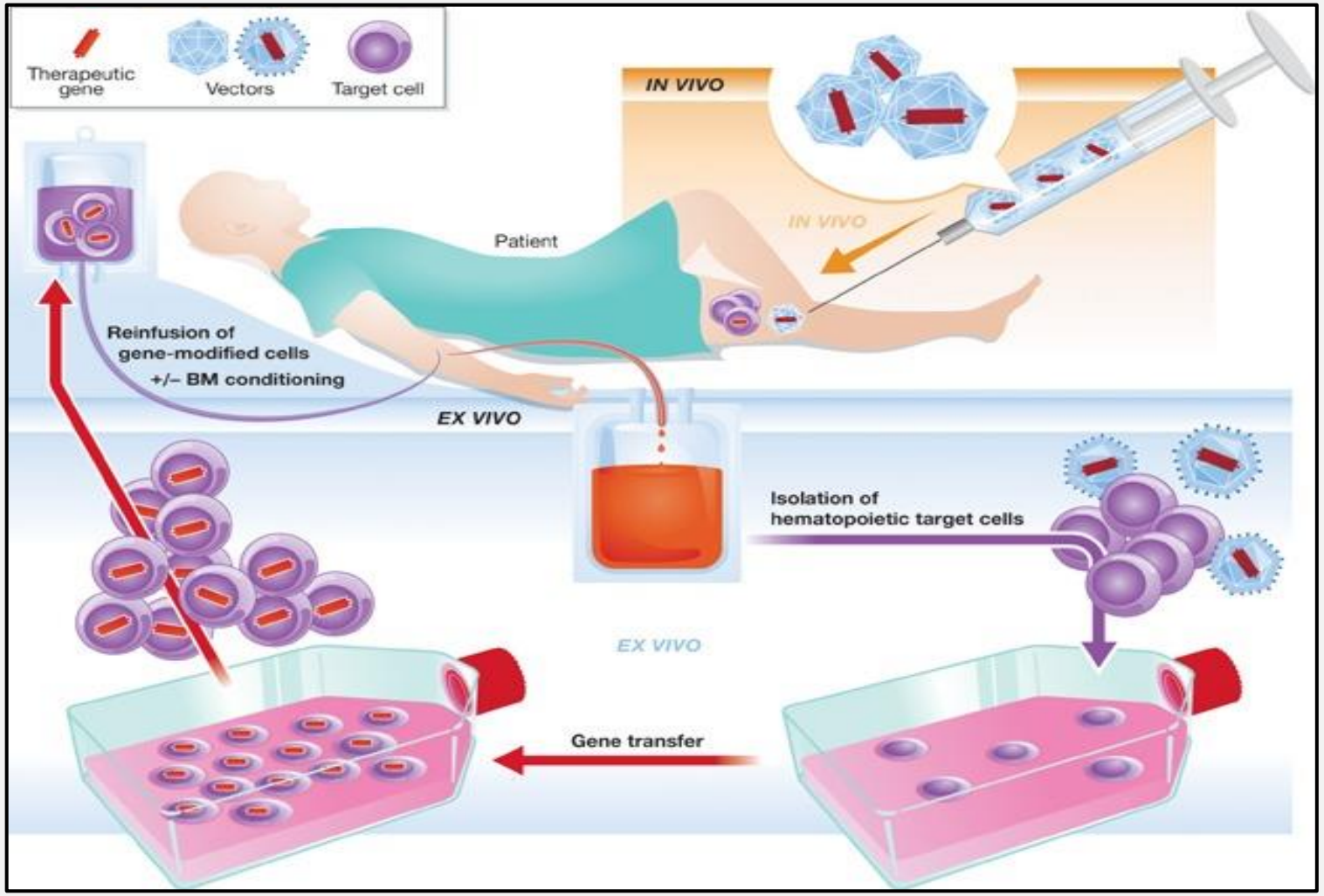
Intervention	Phase	Recruited subjects (*n*)	Outcome measures	Status (ClinicalTrials.gov identifier)	Institution/ Company
Single dose of ZNF-modified autologous CD4$^+$ T cells	I	Patients on cART with or without treatment failure (12)	PR: Safety, Side-effect profile SRY: Effect on viral load and T cells	Published [31**] (NCT00842634)	University of Pennsylvania, Albert Einstein College of Medicine, Sangamo Biosciences
Escalating doses of ZNF-modified autologous CD4$^+$ T cells	I	Patients on cART with or without heterozygosity for CCR5Δ32 mutation (19)	PR: Safety SRY: Long-term persistence and activity of modified cells	Completed (NCT01044654)	Sangamo Biosciences
Single dose of ZFN-modified autologous CD4$^+$ T cells	I/II	Untreated viremic patients (21)	PR: Safety and tolerability SRY: Persistence of modified cells, Effect on HIV and CD4$^+$ T cells	Completed (NCT01252641)	Sangamo Biosciences
Single dose of ZFN-modified autologous CD4$^+$ T cells with and without cyclophosphamide conditioning/pretreatment	I	Aviremic patients on cART with or without CCR5Δ32 mutation (15)	PR: Safety	Recruiting (NCT02388594)	University of Pennsylvania, National Institute of Allergy and Infectious Diseases
Escalating doses of cyclophosphamide administered before single dose infusion of ZFN-modified autologous CD4$^+$ T cells	I/II	Aviremic patients on cART (26)	PR: Safety SRY: Engraftment of modified cells, Effect on HIV and CD4$^+$ T cells	Recruiting (NCT01543152)	Sangamo Biosciences

PR: primary outcome; SRY: secondary outcome

Allers C & Schhneider T: Curr Opinion Virol 2015; 14:24–29

Gene therapy on the move:

In vivo and *ex vivo* gene therapy concepts



Kaufmann KB et al.: Mol Med 2013; 5:1642-61

Bone Marrow Gene Therapy for HIV/AIDS (review)

Bone marrow gene therapy remains an attractive option for treating AIDS caused by HIV. This technology combines the differentiation and expansion capacity of hematopoietic stem cells (HSCs) with long-term expression of therapeutic transgenes using integrating vectors

Clinical trial of HIV gene therapy based on modified HSC transplantation

Gene Therapy Mechanism	Phase
Rev-responsive element decoy (Rev protein)	Pilot
Trans-dominant Rev (Rev protein)	I–II
Ribozyme (Tat/Rev mRNA)	II
	II
Combinatorial trans-dominant Rev (Rev protein) and antisense (Pol mRNA)	I–II
Combinatorial strategy: fusion inhibitor C46 (Env protein) and shRNA (CCR5)	I–II
Combinatorial strategy: shRNA (Tat/Rev mRNA), TAR decoy (Tat protein) and ribozyme (CCR5)	Pilot

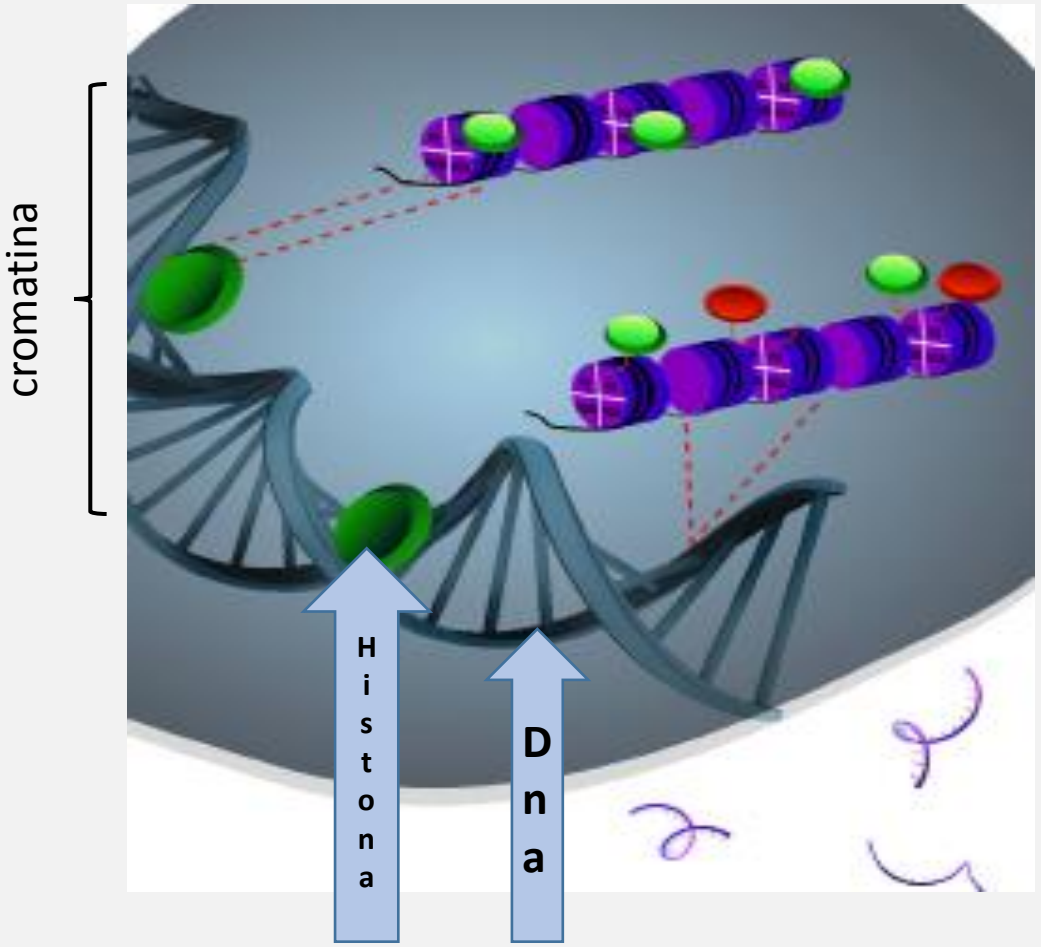
Herrera-Carrillo E & Berkhout B: Viruses 2015; 7:3910-6

HIV: estratégias cura funcional & *esterilizante*

observações & intervenções...

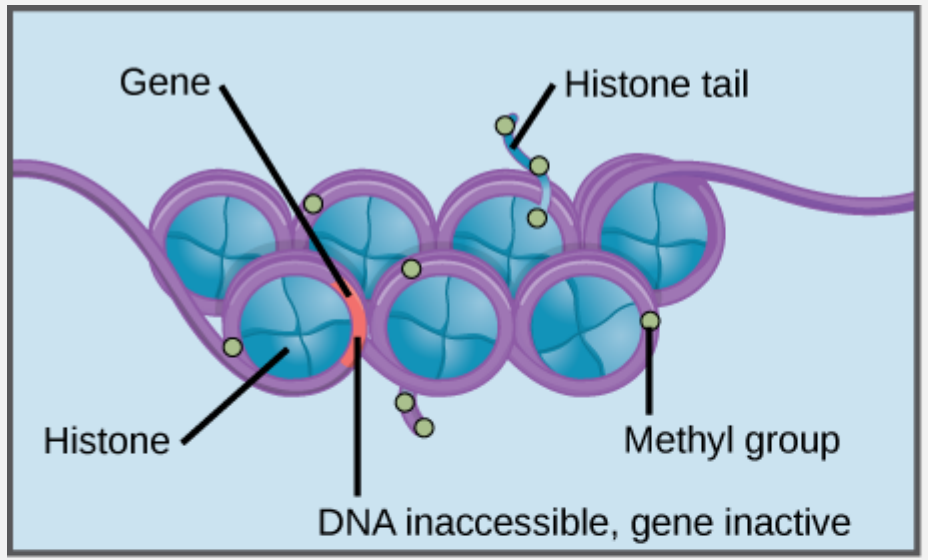
- HAART: intensificação - tratamento precoce
- Transplante de "*stem cell*"
- Manipulação gênica – "*knockout*" do coreceptor CCR5
- **Reversão da latência viral – "*purging*"**
  "kick & kill"
- "*Enhancement*" da resposta imune - vacinas terapêuticas
- etc.

Mecanismos epigenéticos que regulam a expressão dos genes

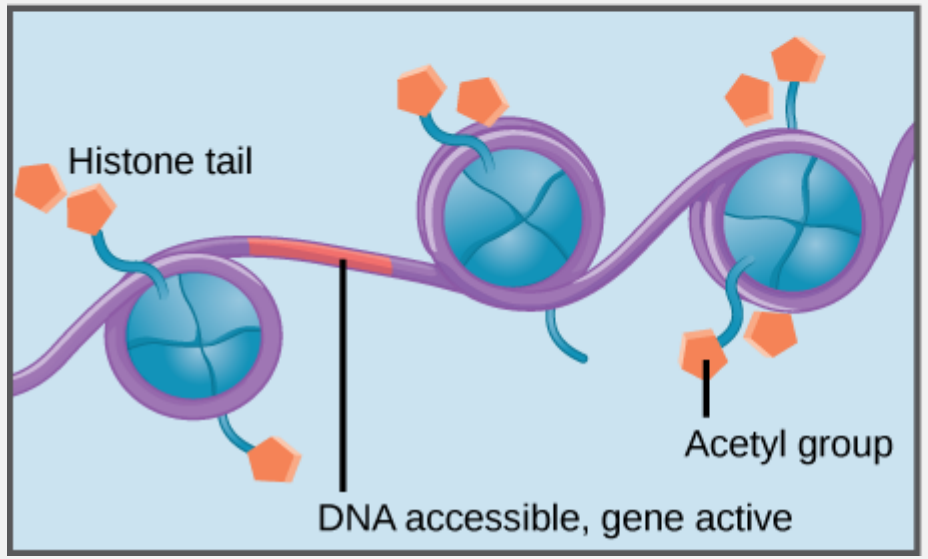


O genoma das células dos mamíferos está acondicionado no núcleo via empacotamento do DNA ao redor de histonas (estrutura conhecida como cromatina). O estado *on* e *off* da expressão do gene é regulada por: *DNA methylation, post-translational modifications of various residues within histone tails, and non-coding RNAs*

Zeybel M et al.: J Hepatol 2013; 59:1349-53



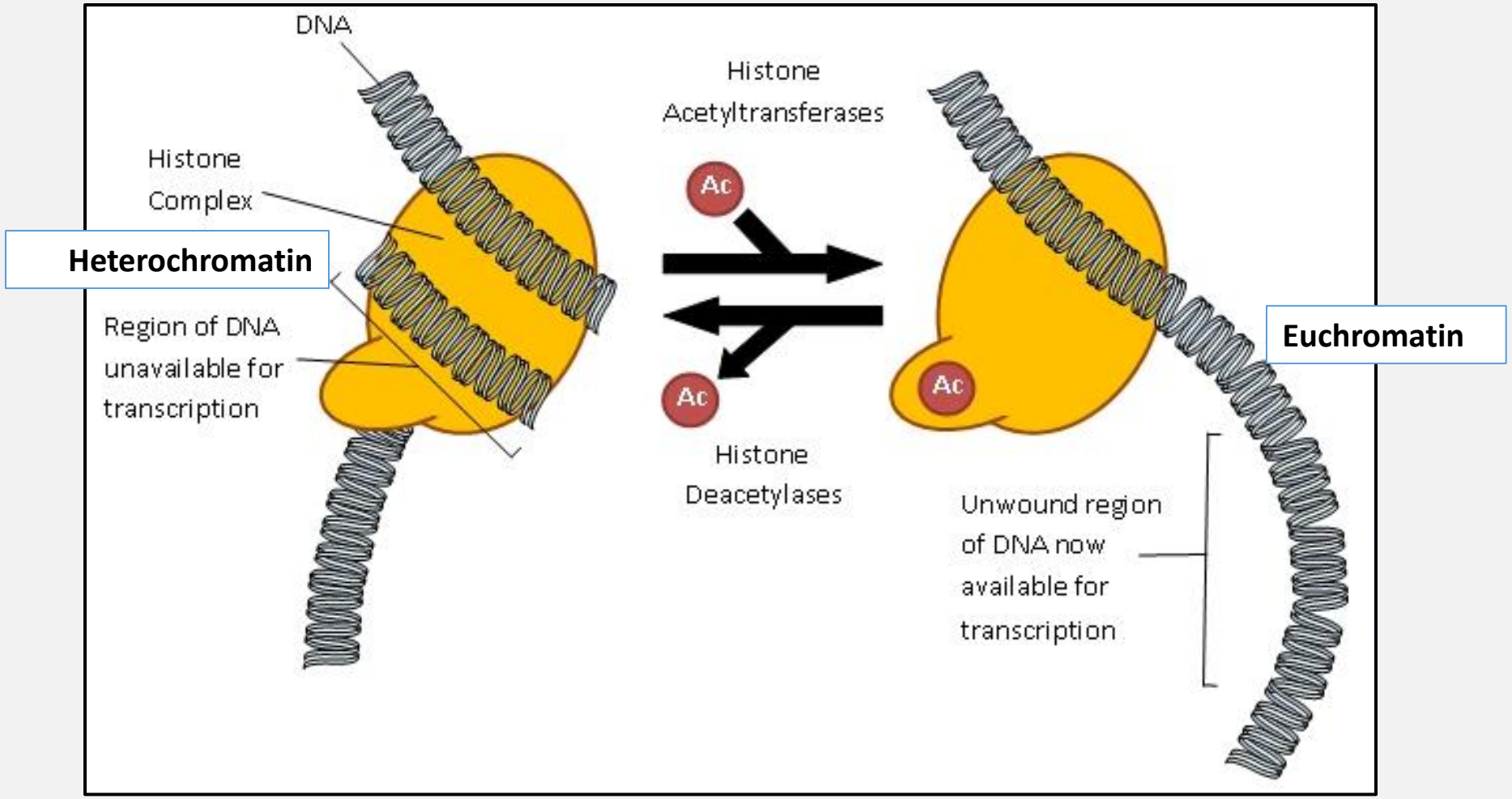
Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.



Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

Inibidores das deacetilases histônicas

HDACs inhibitors for “purging” HIV-1 from the latent reservoir



Matalon S, Rasmussen TA & Dinarello CA: Mol Med 2011; 17:466-72

HIV latency reversal agents
various phases of HIV therapeutic development

Latency reversal agent	Class of agent	Agent tested on	Mechanism of action	Stage of therapeutic development
Vorinostat (SAHA)	HDAC inhibitor	J89 cells and Resting CD4$^+$ T cells	Induce acetylation of histone H3K4, H4K4 resulting in remodeling of nuc-1	In vitro, ex vivo and tested in a clinical trial
Valproic acid	HDAC inhibitor	J-Lat cell lines and U1 cells, patient derived cells	Formation of euchromatin at HIV-1 5'LTR and reactivation of HIV-1 transcription	In vitro, ex vivo, and tested in a clinical trial
Panobinostat	HDAC inhibitor	CD4$^+$ T cells	Formation of euchromatin at HIV-1 5'LTR and reactivation of HIV-1 transcription	Phase 1/2 clinical trial
Romidepsin	HDAC inhibitor	CD4$^+$ T cells	Formation of euchromatin at HIV-1 5'LTR and reactivation of HIV-1 transcription	Ex vivo
Entinostat	HDAC inhibitor	CD4$^+$ T cells, ACH2, and J-lat cell lines	Formation of euchromatin at HIV-1 5'LTR and reactivation of HIV-1 transcription	In vitro, ex vivo
M344	HDAC inhibitor	J-Lat clones (A7)	Increases histone acetylation and activation of NF-kappaB	In vitro
Sodium butyrate	HDAC inhibitor	CD4$^+$ T cells, J-Lat cell lines, ACH2 and U1 cells	Increases histone acetylation resulting in transcriptional activation of HIV-1 promoter	In vitro
Trichostatin A	HDAC inhibitor	CD4$^+$ T cells, ACH2, and J49 cells	Increases histone acetylation resulting in transcriptional activation of HIV-1 promoter	In vitro, ex vivo
Oxamflatin	HDAC inhibitor	J89GFP and A7 cell	Increases the acetylation level of histone H3 and histone H4 at the nucleosome 1(nuc-1) site	In vitro
Scriptaid	HDAC inhibitor	J89GFP and A7 cells	Promotes hyperacetylation of histone	In vitro
Givinostat (ITF2357)	HDAC inhibitor	J89GFP, ACH2 and U1 cells	Induces hyperacetylation of histone	In vitro
CG05/CG06	HDAC inhibitor	ACH2 cells	Induces hyperacetylation of histone	In vitro

Kumar A et al.: Clinical Epigenetics 2015
DOI 10.1186/s13148-015-0137-6

HIV latency reversal agents
various phases of HIV therapeutic development

Chaetocin	HMT inhibitor	Resting CD4⁺ T cells isolated from HIV infected patients, ACH-2, OM10.1 cells, infected Jurkat-tat cells	A Suv39H1 inhibitor, induces loss of H3K9me3	In vitro, ex vivo
BIX-01294	HMT inhibitor	ACH-2 and OM10.1 cells	A G9a inhibitor, promotes repressive H3K9me2	Ex vivo
3-deazaneplanocin A	HMT inhibitor	Latently infected Jurkat E4 and G4 cells	An inhibitor of EZH2, Induces loss of H3K27me3	In vitro
5-aza-2' deoxycytidine	DNMTI	ACH-2 cells, U1 cells, and J-Lat cell lines	Inhibits of cytosine methylation and prevent the recruitment of MBD2 and HDAC2 to the 5'LTR	In vitro
Prostratin	PKC agonist	Patient derived CD4 + T cells, J-Lat cell lines	Activates NF-KB	Ex vivo
Phorbolmyristate acetate (PMA)	PKC agonist	J-Lat cell lines	Activates NF-KB	Ex vivo
Diterpene ester ingenol-3-angelate	PKC agonist	U1 cells	Activates NF-KB	In vitro
Bryostatin-2	PKC agonist	CD4⁺ T-cells, J-Lat cell lines, U1 and OM10.1 cells	Activates NF-KB	In vitro, ex vivo
JQ1	Unclassified agents	CD4⁺ T cells derived from patient, J-Lat cell lines, U1, ACH2, and OM10.1 cells	Releases BRD4 from the 5'LTR and allows Tat-mediated recruitment of P-TEFb to the 5'LTR.	In vitro and ex vivo
I-Bet, I-Bet151 and MS417	Unclassified agents	J-Lat cell lines, primary CD4⁺ T cells	Releases BRD4 from the 5'LTR and allows Tat-mediated recruitment of P-TEFb to the 5'LTR.	In vitro
Disulfiram	Unclassified agents	CD4⁺ T cells	Reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog.	Ex vivo, clinical trial

Kumar A et al.: Clinical Epigenetics 2015
DOI 10.1186/s13148-015-0137-6

Epigenetic control of HIV-1 post integration latency

implications for therapy

- **Complete cure of HIV-1 infection is difficult to achieve without the elimination of latent reservoirs established during early infection have long life span.**
- **Several epigenetic and non-epigenetic mechanisms have been implicated in the regulation of viral latency.**
- **Epigenetic mechanisms such as histone post translational modifications (e.g., acetylation and methylation) and DNA methylation of the proviral DNA and microRNAs are involved in the establishment of HIV-1 latency.**

Several latency-reversing agents (LRA) have been found effective in reactivating HIV-1 reservoirs in vitro, ex vivo, and in vivo. These therapeutic approaches aimed at achieving a sterilizing cure (elimination of HIV-1 from the human body).

Kumar A et al.: Clinical Epigenetics 2015
DOI 10.1186/s13148-015-0137-6

Epigenetic control of HIV post integration latency
implications for therapy

Targeting latent HIV-1 reservoirs.

- **Latent reservoirs: key issue to the complete eradication of HIV**
- **"kick and kill" strategy: virus can be activated in reservoirs using latency reversing agents including: HDACis, HMTis, DNMTis, PKC agonists and several other small molecules**
- **Impact of LRAs: well studied in CD4+ T cells and to lesser extent in the cells of monocyte/macrophage lineage.**
- **Upon reactivation: latent virus undergoes robust replication resulting in production of enormous amount of virus which:**
  - **can induce the lysis of target cells or**
  - **infected cells can be recognized by the cellular immune clearance machinery**

Kumar A et al.: Clinical Epigenetics 2015
DOI 10.1186/s13148-015-0137-6

HIV: estratégias cura funcional & *esterilizante*

observações & intervenções...

- **HAART: intensificação - tratamento precoce**
- **Transplante de "*stem cell*"**
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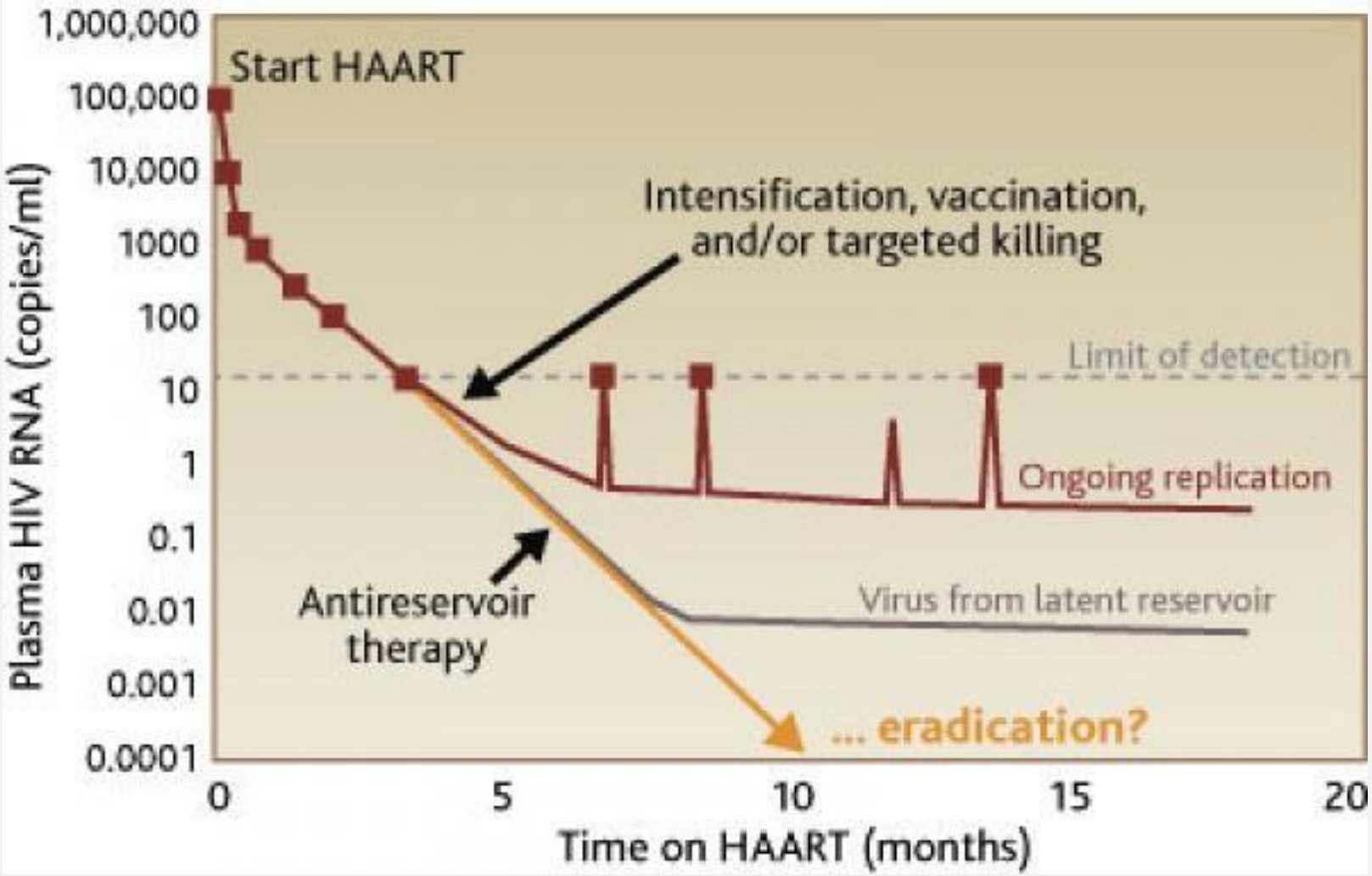
- **Reversão da latência viral – "*purging*"**
  "*kick & kill*"
- **"*Enhancement*" da resposta imune - vacinas terapêuticas**
- **etc.**

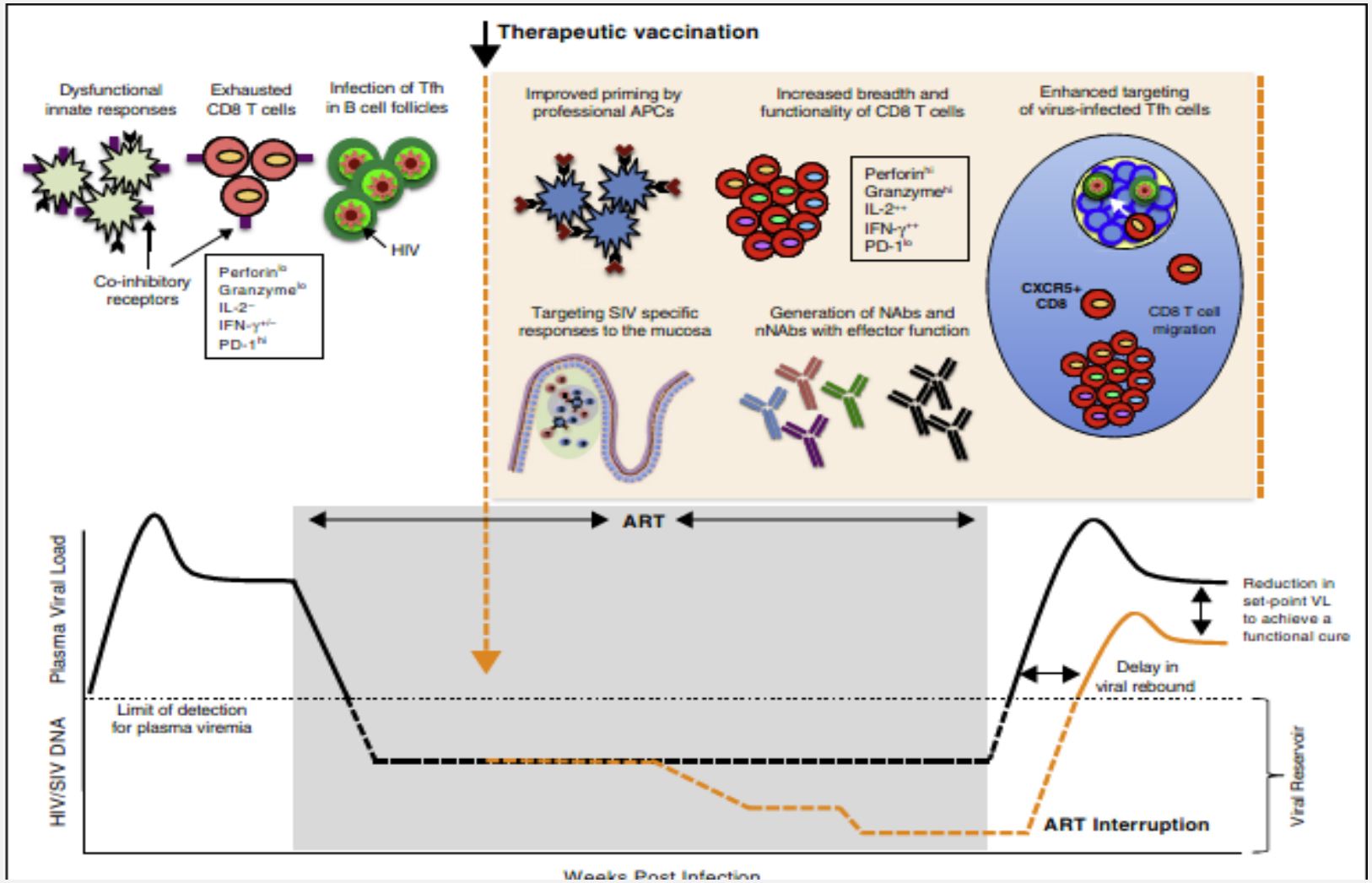
Incremento da imunidade: vacina terapêutica



http://www.sciencemag.org/

HIV therapeutic vaccines

moving towards a functional cure



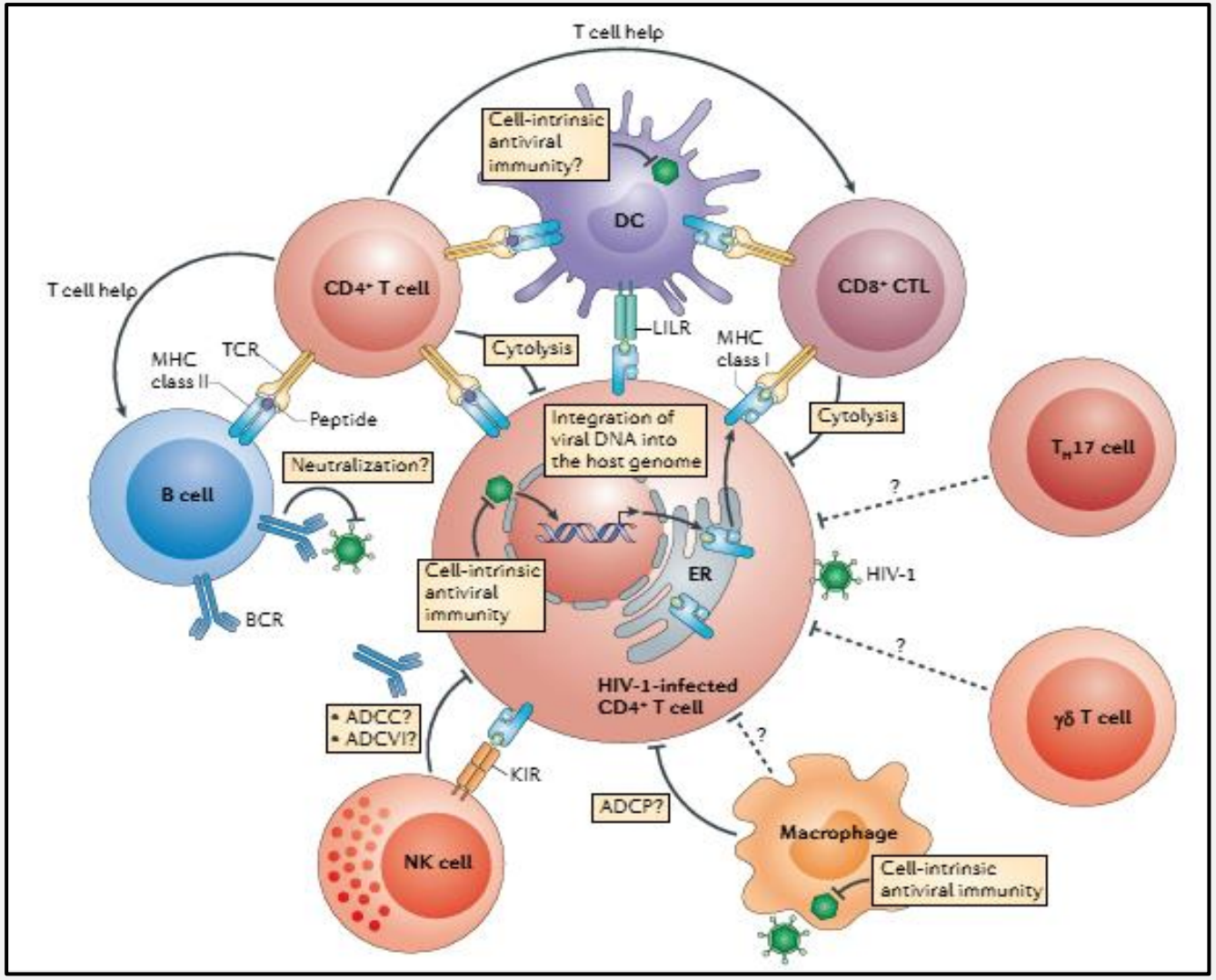
Mylvaganam GH et al.: Curr Opin Immunol 2015; 35:1–8

HIV Cures: Where We Are, Realistically ?

CARI ROMM SEP 29 2014, 12:17 PM ET

http://m.theatlantic.com/health/archive/2014/09/the-berlin-patient-and-the-mysterious-cure-for-hiv/380895

HIV & mecanismos da imunidade inata e adaptiva



Walker BD & Yu XG: Nature Rev Immunol 2013; 13:487-98

Evolução do sistema imune humano ***(filogenia)*****:**

desde o surgimento de vida na Terra com seres unicelulares há mais de 3.5 bilhões de anos, e o bem mais tardio desenvolvimento dos seres multicelulares, até os seres humanos…

International AIDS Society

Stronger Together Against HIV

The IAS Scientific Working Group on HIV Cure

IAS Towards an HIV Cure Symposium: people focused, science driven
18–19 July 2015, Vancouver, Canada

2015 Towards an HIV Cure Symposium Scientific Report

Towards an HIV Cure

Towards an HIV Cure, an initiative of the International AIDS Society (IAS) provides leadership in facilitating more concerted efforts to accelerate global scientific research towards a cure for HIV and in advocating for increased investment in HIV cure research

Mission and Goals

- Facilitate scientific discussion, exchange and collaboration to promote and accelerate research towards a cure for HIV;
- Provide leadership in advocating for increased investment and resource optimization in HIV cure research;
- Provide clear and accurate information and disseminate knowledge in the broader community

anRS

Agence nationale de recherches sur le sida et les hépatites virales

Agence autonome de l'Inserm

BILL & MELINDA GATES foundation

HIV Cure News and Updates: Bill Gates says Vaccine for AIDS Should be Available by 2030

On January 27, 2015

amfAR Launches Initiative Aimed at Finding a Cure for HIV by 2020

About amfAR

The Foundation for AIDS Research is one of the world's leading nonprofit organizations dedicated to the support of AIDS research, HIV prevention, treatment education, and the advocacy of sound AIDS-related public policy. Since 1985, amfAR has invested more than $366 million in its programs and has awarded grants to more than 2,000 research teams worldwide

The Countdown to a Cure for HIV/AIDS

amfAR's "Countdown to a Cure for HIV/AIDS" is a research initiative aimed at finding a **broadly applicable cure** for HIV by 2020.

"Countdown to a Cure" is designed to intensify amfAR's cure-focused HIV research program with plans to strategically invest $100 million in cure research over the next six years.

Obrigado