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VERS UNE THERAPIE CURATIVE POUR LE VIH/SIDA : PANORAMA DES STRATEGIES ETUDIEES ET APPORTS DES MODELES ANIMAUX

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« *Nec ratione alia mortales esse videmur*
Inter nos, nisi quod morbis ægrescimus isdem
Atque illi quos a vita natura removit. »

« *Nous-mêmes, comment nous reconnaissons-nous tous mortels,*
Si ce n'est parce que nous sommes sujets aux mêmes maladies
Qui ont retranché nos semblables du nombre des vivants ? »

- Lucrèce, De rerum natura, La Nature des choses

« *La connaissance des maladies infectieuses enseigne aux hommes qu'ils sont frères et solidaires. Nous sommes frères parce que le même danger nous menace, solidaires parce que la contagion nous vient le plus souvent de nos semblables*. »

- Charles Nicolle, Destin des maladies infectieuses, 1933

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Avant-propos - Résumé en langue française

Il y a bientôt quarante ans, le monde était confronté à l'émergence d'une nouvelle épidémie dévastatrice, qui s'est rapidement propagé à travers le monde, pressant la communauté scientifique et médicale à réagir promptement et à déterminer quelles étaient les origines et les causes de cette maladie mortelle. En effet, en 1981, des formes rares de pneumonies à *Pneumocystis carinii*, des candidoses buccales extensives, des infections virales multiples, et des cas de sarcome de Kaposi étaient décrits à Los Angeles et San Francisco[1–3], initialement chez des hommes ayant des rapports sexuels avec des hommes (HSH), puis chez des usagers de drogues injectables, chez des personnes ayant reçu des transfusions sanguines, et enfin au sein de la population générale[4,5]. Cet état d'immunodéficience acquise donnant lieu à des maladies opportunistes a été par la suite défini en tant qu'entité nosologique : le *syndrome d'immunodéficience acquise* (SIDA)[6–9]. L'étiologie du SIDA a été déterminée en 1983, lorsqu'un rétrovirus – qui serait plus tard nommé « *virus de l'immunodéficience humaine* » (VIH) – a été isolé et proposé comme agent causal de la maladie[10].

L'émergence du VIH/SIDA est survenue alors que les progrès de la médecine moderne et de la recherche, consécutivement à l'avènement de l'antibiothérapie et de la vaccination, laissaient imaginer que l'époque des « pestes » était révolue et que les pandémies catastrophiques liées à des maladies infectieuses (la dernière en date étant alors la « grippe espagnole » de 1918-1920) appartenaient au passé[11]. Depuis, c'est pourtant une accélération de l'apparition de « nouvelles maladies » chez l'homme qui a été observée au cours des quarante dernières années[12–17], avec l'émergence notable du SARS-CoV-2 en 2019, responsable de la Covid-19 et qualifiée de pandémie le 11 mars 2020 par l'OMS. Ces multiples émergences d'agents infectieux pathogènes et les épidémies ou pandémies qui en découlent ravivent un sentiment de vulnérabilité à l'égard de nouvelles pandémies mondiales et d'un retour vers les temps où l'humanité était incapable de contrôler les épidémies[18]. « *Ce qui est nouveau, ce n'est pas la survenue d'une maladie antérieurement inconnue, c'est cette survenue au sein d'un monde qui se croyait définitivement aseptisé, protégé et tranquille.*»[19] (Charles Nicolle, *Naissance, vie et mort des maladies infectieuses*, 1930).

Dans ce travail, on se propose de fournir dans un premier temps un panorama des raisons pour lesquelles une intervention thérapeutique curative (souvent désignée sous le terme « HIV Cure » en Anglais) est toujours nécessaire pour le VIH. Dans un second temps, les étapes nécessaires vers le développement d'un tel « HIV cure » seront évoquées. Dans un troisième temps, on exposera quelles sont les difficultés majeures et les principaux obstacles à surmonter. Le rôle prégnant revêtu par les modèles animaux dans la recherche sur le VIH, ainsi que les contributions et limites qui leur sont associées seront ensuite étudiées. Dans un cinquième temps, on considèrera les principales stratégies et approches de « HIV cure » définies et étudiées au cours des dernières années. Enfin, on exposera certains concepts moins conventionnels, novateurs ou moins étudiés qui pourraient avoir des implications bénéfiques pour la recherche d'intervention thérapeutique curative du VIH/SIDA.

I. De la nécessité d'une intervention thérapeutique curative pour le VIH

On estime aujourd'hui à 38 millions le nombre de personnes vivant avec le VIH (PVVIH) à travers le monde (ONUSIDA)[20], parmi lesquels seulement 81% connaissent leur statut et seulement 67% ont accès à un traitement. Le nombre de nouvelles infections annuelles s'élèverait encore à 1,7 millions[20].

L'avènement de thérapies antirétrovirales, avec l'azidothymidine en 1987[21,22], puis les thérapies combinant plusieurs antirétroviraux pour limiter l'apparition de résistances en 1996[23–25], ont constitué des progrès majeurs dans la gestion de la pandémie de VIH/SIDA et ont permis de réduire la mortalité de manière marquée. Ces antirétrovirothérapies (ART), associées à des politiques de santé publique et à des progrès des approches préventives, ont en effet permis de réduire le nombre de morts liés au SIDA de plus de 60% depuis un pic atteint en 2004[20,26].

Toutefois, il semble que les ART actuelles ne soient pas suffisantes pour mettre un terme à la pandémie. En effet, si les antirétroviraux permettent de supprimer de manière durable la réplication virale, ils ne sont pas curatifs, et ne permettent pas d'éviter l'état d'activation immunitaire chronique associée au VIH. A cause de difficultés et d'obstacles divers, toutes les personnes vivant avec le VIH et ayant un accès théorique à des thérapies antivirales ne parviennent pas à maintenir une charge virale indétectable sur le long terme. Les PVVIH sous ART présentent fréquemment des comorbidités, souvent en lien avec l'état inflammatoire résiduel associé avec le VIH, parmi lesquelles des maladies cardiovasculaires, des désordres métaboliques, des troubles neurologiques, des dommages rénaux, des pathologies hépatiques, et une plus forte prévalence de cancers non définissants du VIH que chez des individus non infectés[27–30]. Il est à noter que les PVVIH constituent une population globalement vieillissante, d'une part grâce aux ART qui permettent de prolonger l'espérance de vie, d'autre part en suivant les tendances démographiques mondiales vers un vieillissement de la population.[31,32] Ceci est associé à de nouvelles problématiques concernant la prise en charge clinique des PVVIH âgés, les comorbidités non-directement liées au VIH mais associées à l'âge, ainsi que la polypharmacie (prise durable par le même patient de quatre à six médicaments ou plus) et de l'anticipation de possibles interactions médicamenteuses[33–35].

A l'échelle des populations, le recours aux ART est entravé par les difficultés matérielles

d'approvisionnement des PVVIH en médicaments, en particulier dans les pays à faible revenu et à revenu intermédiaire. Malgré la démocratisation du traitement contre le VIH au cours des dernières années, 15 millions de personnes séropositives n'ont pas accès à la thérapie antirétrovirale[26]. Le risque de résistance aux médicaments antirétroviraux constitue également une menace pour l'efficacité des traitements du VIH-1[36]. En outre, la pandémie liée à la Covid-19 a entrainé des bouleversements dans les systèmes de santé de nombreux pays, ainsi qu'au niveau de l'aide internationale et de l'approvisionnement en ART des pays à faible revenu et revenu intermédiaire, ce qui pourrait avoir des conséquences délétères sur l'évolution de la pandémie de VIH/SIDA en 2020 et dans les années à venir[37–41].

Il convient également de mentionner que mettre un terme à la pandémie de VIH/SIDA, serait bénéfique sur les plans économiques et financiers. L'amélioration des conditions de santé et des durées de vie des PVVIH pourrait en effet être significativement bénéfique pour l'économie des pays les plus affectés[42,43]. Toutefois, fournir des traitements antirétroviraux quotidiennement à 38 millions de PVVIH – quoique largement bénéfique sur le long terme d'un point de vue économique – présente un certain nombre de challenges opérationnels, logistiques, et financiers, ce qui représente un obstacle à la résolution de la pandémie sur le court terme[44,45]. Dans ce contexte, un traitement curatif – en particulier s'il ne requiert d'un nombre restreint d'interventions – pourrait représenter une contribution soutenable, viable et réaliste vers une maîtrise de la pandémie de VIH/SIDA[46].

II. Des étapes progressives vers le développement de telles thérapies

A ce jour, aucune intervention thérapeutique curative qui permette d'éliminer complètement tout virus du VIH capable de se répliquer au sein d'un individu (« éradication ») ou bien supprimer la réplication virale et garantir une absence de pathologie pendant une période prolongée sans recourir à l'ART (« rémission ») de manière peu risquée, efficace, et durable n'est disponible pour le VIH/SIDA. Une thérapie curative permettant une « rémission » apparaît comme plus réaliste et atteignable à court ou moyen terme[5,45–47].

Le fait de définir en amont le cahier des charges d'un tel « HIV Cure » apparaît comme un élément clé dans le développement d'intervention(s) thérapeutique(s) qui puisse(nt) être mis(es) en place de manière efficace auprès des PVVIH. C'est dans cette optique qu'à été développé le concept de « Target Product Profile » (« profil de produit cible »), qui consiste à définir les caractéristiques minimales et optimales requises en concertation avec les différentes parties prenantes (bénéficiaires finaux, communauté scientifique et médicale, industrie, décideurs politiques, …)[46].

De nombreuses questions doivent être soulevées, pour lesquelles des réponses simples n'émergent pas aisément : Peut-on se satisfaire d'une intervention thérapeutique à l'efficacité comparable à l'ART, ou qui ne permette pas de contrôler tous les virus capables de se répliquer ? Dans quelle mesure peut-on contrôler le risque de rebond de la charge virale, et surveiller les risques de contamination qui pourront se poser alors ? Peut-on accepter d'avoir recours à une thérapie curative efficace mais dont la sureté et l'innocuité est moindre (ou moins bien définie) que dans le cas de l'ART ?[45,46,48]. « Rêver de remèdes absolus c'est souvent rêver de remèdes pires que le mal. » (Georges Canguilhem, le Normal et le Pathologique, 1966)[49]. Il apparaît que les prérequis pourraient être définis de façon minimale dans un premier temps avant d'accroître le niveau d'exigence pour de nouvelles thérapies curatives, dans un processus progressif vers un traitement idéal.

Des problématiques d'importance sont alors d'être techniquement et logistiquement capable de déterminer si les ARN viraux capables de se répliquer ont bien tous été éliminés[50–53], et de surveiller efficacement la survenue d'éventuels rebonds avec présence de virus infectieux[46].

III. Des difficultés à surmonter dans la recherche d'un « HIV Cure »

En 1988, la journaliste américaine Susan Sontag vulgarisait dans *Le SIDA et ses métaphores* : « Ce qui rend l'attaque virale si terrifiante, c'est que la contamination, donc la vulnérabilité est considérée comme permanente. Même si l'individu infecté ne devait jamais manifester le moindre symptôme – c'est-à-dire si l'infection demeurait inactive, ou pouvait être rendue telle par une intervention médicale –, l'ennemi viral serait à jamais à l'intérieur. […] Le sida est une maladie progressive, une maladie du temps. »[11]

D'un point de vue physiopathologique, un certain nombre d'obstacles représentent en effet des difficultés pour la mise en place de thérapies curatives. En particulier :

(i) La persistance du VIH sous forme latente au sein de réservoirs cellulaires. Il existe une grande variété de cellules infectées par le VIH, parfois mal définies. Les stratégies d' « éradication » doivent cibler toutes les cellules qui abritent du VIH capable de se répliquer. Il est généralement difficile ou impossible de différencier les cellules infectées de manière latente des cellules non infectées. Les stratégies « de rémission » qui visent à diminuer la taille du réservoir doivent être spécifiques et éviter trop de dommages en éliminant des cellules non-infectées

(ii) La persistance du VIH au sein de « sanctuaires » (réservoirs profonds) protégés du système immunitaire et des traitements antiviraux actuels. Le virus latent persiste en effet au sein de nombreux tissus, parfois très peu accessibles. Les stratégies curatives doivent pouvoir les atteindre malgré des barrières qui sont parfois à traverser (comme la barrière hémato-encéphalique), et des tissus de propriétés différentes pour être efficace au niveau des cellules infectées.

(iii) Les dommages tissulaires et l'altération de l'immunité. Le fonctionnement du système immunitaire et de ses parties-prenantes est modifié lors de l'infection par le VIH, et des dégâts tissulaires sont également engendrés dès les premières heures de l'infection, comme par exemple au niveau de la muqueuse intestinale et de l'architecture des nœuds lymphatiques. Une thérapie curative devra donc contourner ces perturbations de l'état physiologique de l'immunité et de certains organes.

(iv) L'inflammation persistante et l'état d'activation immunitaire chronique associée au VIH. L'activation immunitaire chronique aberrante est reconnue comme

facteur majeur de progression vers l'immunodéficience, favorise l'Immunosénescence et est également liée au développement de nombreuses comorbidités, y compris chez des personnes recevant l'ART. La seule inhibition de la réplication virale, comme dans le cas de l'ART actuelle, ne suffit pas à la restauration d'un système immunitaire fonctionnel et à prévenir son vieillissement prématuré. Il est donc possible que des thérapies curatives puissent être avantageusement complémentées par des thérapies immunomodulatrices.

IV. Du recours aux modèles animaux dans la recherche sur le VIH

Il n'existe pas de modèle animal idéal qui mime parfaitement l'infection par le VIH chez l'homme. Néanmoins, l'étude de différents virus et de certaines espèces animales ainsi que leur utilisation comme modèle a contribué de manière incontestable à l'amélioration des connaissances sur le VIH/SIDA et à de nombreux progrès thérapeutiques[54]. Ces modèles animaux comprennent notamment les souris humanisées (généralement infectées par le VIH-1) ainsi que les modèles de primates non humains (PNH) infectés par des virus d'immunodéficience simienne[55].

Parmi ces derniers, des hôtes non-naturels de lentivirus (*Macaca mulatta*, macaques rhésus, d'origine indienne et chinoise ; *Macaca nemestrina*, macaques à queue de cochon ; *Macaca fascicularis*, macaques crabier) infectés par différentes souches de SIVmac (virus issu d'un franchissement de barrière d'espèce récent en captivité depuis un lentivirus SIVsmm de mangabey enfumé, *Cercocebus atys*)[56,57] sont souvent utilisés comme modèle d'infection pathogène par les lentivirus.

D'autre part, des hôtes naturels de lentivirus (*Chlorocebus sabaeus*, singes verts d'Afrique ; *Cercocebus atys*, mangabeys enfumés ; *Mandrillus sphinx, mandrills*) sont étudiés dans le contexte de l'infection par les lentivirus avec lesquels ils ont co-évolués pendant des millions d'années (SIVagm, SIVsmm, et SIVmnd-1 & -2, respectivement), et utilisés comme modèles d'infection non pathogène par les lentivirus[55,58].

Chaque modèle (espèce hôte + lentivirus) présente des caractéristiques qui lui sont propres, comprenant des avantages et des inconvénients. Il convient de les étudier en connaissant leurs limites. En effet, le choix d'un modèle pertinent en fonction de la question scientifique posée revêt une importance capitale.

Il est à noter qu'à plusieurs occasions dans le domaine de la recherche thérapeutique et préventive sur le VIH, des résultats très prometteurs observés dans des modèles de primates non humains n'ont pas pu être réitérés lors d'essais cliniques chez l'homme. Une des raisons est en particulier le niveau d'exigence plus rigoureux chez l'homme en termes de contrôle d'éventuels rebonds de la charge virale : Après une intervention thérapeutique et/ou l'interruption du traitement antirétroviral, on peut par exemple accepter d'observer un rebond transitoire de la virémie dans les modèles PNH avant un retour de la charge virale à

un niveau indétectable, ce qui n'est généralement pas toléré lors d'essais cliniques chez l'homme[59].

Le recours aux animaux pour la recherche doit se faire avec parcimonie et précaution, en pleine conscience des implications éthiques associées et en respectant le principe des 3Rs (Réduire, Raffiner, Remplacer)[55,58,60]. Ils représentent néanmoins un potentiel unique en termes de découvertes scientifiques pouvant bénéficier à la recherche de traitement curatif pour le VIH, ainsi qu'à la caractérisation des interactions virus-hôte. Leur utilisation est complémentaire à d'autres outils précieux développés pour la recherche préclinique, comme les explants de tissus ou les organoïdes[55,58,60–62].

V. Des approches thérapeutiques curatives définies au cours des dernières années

Au cours des dernières années, différentes stratégies et concepts thérapeutiques ont été développés vers un « HIV Cure », via « éradication » du réservoir viral, ou dans une perspective peut-être plus réaliste via une « rémission » [46,52,53,63–71]. La plupart de ces stratégies impliquent un contrôle, une réduction, ou une élimination du réservoir viral et ciblent le virus (et les cellules infectées) ou le système immunitaire.

Une des principales stratégies conceptualisées est celle du « Shock and Kill », qui consiste à utiliser des agents révertants la latence du VIH (inhibiteurs d'histone déacétylases, agonistes de certains TLRs, activateurs de la voie NF-κB, …) pour induire une réversion de la latence des provirus et une expression virale (gènes, ARNs, protéines) au sein des cellules infectées. Ces cellules pourraient ensuite être « tuées » du fait des effets cytopathiques du virus ou bien devenir des cibles identifiables par le système immunitaire (Lymphocytes T cytotoxiques, Natural Killers, …) ou des thérapies immunitaires (Anticorps neutralisants à large spectre, vaccins thérapeutiques, …)[72–74].

Une autre stratégie, conceptuellement contraire, celle du « Block and Lock », a pour but de renforcer la latence et de maintenir les provirus dans un état inactif, silencieux, et durable en l'absence d'ART[75–77].

En dehors de ces deux stratégies paradigmatiques, d'autres approches et agents ont été étudiés et pourraient également être utilisés seuls ou en combinaison.

C'est le cas en particulier d'approches d'édition génomique, par exemple à partir de l'utilisation de *CRISPRCas9* ou de *zinc-finger nucleases* (ZFN), pour cibler divers gènes viraux ou éventuellement de l'hôte.

De nombreuses et diverses immunothérapies sont également étudiées, comprenant les vaccins thérapeutiques, les anticorps monoclonaux et anticorps neutralisants à large spectre, le transfert adoptif de cellules et en particulier de lymphocytes T exprimant des récepteurs antigéniques chimériques, ainsi que les inhibiteurs de point de contrôle immunitaire[46,47,64,67].

VI. De certains concepts innovants, non-conventionnels, ou moins étudiés

En plus – ou en complément - de celles précédemment mentionnées, d'autres approches vers une thérapie curative pour le VIH suscitent un intérêt croissant ou pourraient s'avérer intéressantes dans les années à venir.

Elles comprennent en particulier des approches visant à exploiter le métabolisme des cellules immunitaires et des cellules cibles du VIH à l'encontre du virus[78–81]; ou des approches exploitant les voies de mort cellulaire (en particulier de l'apoptose) pour tuer les cellules infectées[63].

D'autres approches ont pour but de limiter les dommages tissulaires associés aux VIH, de restaurer les dommages immuns causés par l'infection, et de réduire l'inflammation chronique et l'activation immunitaire excessive. Des immunothérapies avec l'administration de cytokine IL-21 pourraient favoriser la préservation de l'immunité intestinale et la maintenance de cellules Th17 fonctionnelles subsets[82–85]. Des thérapies modérant les effets de la cascade interféron pourraient également atténuer l'inflammation chronique[86].

Certaines thérapies ciblant et potentialisant des cellules immunitaires non conventionnelles ou de l'immunité innée, comme les Natural Killer pourraient également s'avérer déterminantes vers une meilleure maitrise de l'infection et une régulation de l'état dysimmunitaire associé au VIH[87].

Il apparait également crucial de développer de nouvelles stratégies pour parvenir aux cellules infectées au sein des réservoirs profonds, traverser des barrières biologiques ou présenter des épitopes immunogènes au sein des follicules B des nœuds lymphatiques[88].

La recherche de traitements curatifs pour le VIH/SIDA a bénéficié d'efforts et de progrès ininterrompus depuis bientôt quarante ans. Il s'agit d'un long cheminement, avec plusieurs obstacles à surmonter où contourner, et où la progression se fait pas à pas avec parfois des bonds en avant. Les modèles animaux, et en particulier l'étude des primates non-humains, quoique présentant certaines limites, a apporté des contributions majeures dans ce domaine[55]. La recherche curative pour le VIH bénéficie également des progrès réalisés dans les autres domaines qui ont trait au VIH/SIDA (prévention et prophylaxie, recherche vaccinale, fondamentale et clinique, …)[69] ainsi qu'aux autres maladies infectieuses. De très nombreuses connections existent aussi avec le domaine de l'oncologie, où les difficultés thérapeutiques rencontrées sont parfois comparables et dont certaines approches thérapeutiques sont transférées dans le domaine du VIH/SIDA et où certains progrès ont été permis par la recherche sur le VIH[89–93].

Dans les années à venir, les stratégies les plus prometteuses devraient probablement consister en des combinaisons de plusieurs agents ciblant des mécanismes différents ou des approches complémentaires. Les prérequis en termes d'efficacité seront peut-être minimaux dans un premier temps, et une association possible avec un maintien initial de l'ART pourra permettre de bénéficier d'éventuels effets synergiques. De nouveaux progrès techniques restent néanmoins à entreprendre, notamment dans le suivi thérapeutique et la surveillance des patients après interruption du traitement antirétroviral. De tels progrès, ainsi que de nouvelles avancées technologiques devraient permettre de poursuivre avec un niveau d'exigence croissant la recherche de traitements curatifs pour le VIH/SIDA. Déjà, de nombreux concepts prometteurs et des résultats encourageants (bien qu'encore épars), en particulier dans les modèles animaux, permettent de jeter un œil optimiste vers les avancées thérapeutiques à venir.

HIV Cure research: Strategies in pipeline and contribution of animal models

GAUTHIER TERRADE

Introduction

Thirty-nine years after the first clinical observations of what would later be called “Acute Immunodeficiency Syndrome” (AIDS) and thirty-seven years after the identification of HIV as the etiologic cause of AIDS[10], uninterrupted research works have been conducted, leading to major scientific and therapeutic breakthroughs. Nonetheless, a cure is still missing.

According to the Joint United Nations Programme on HIV and AIDS (UNAIDS), It is estimated that, globally, 38.0 million [31.6 million–44.5 million] people were living with HIV in 2019. About 7.1 million people do not know that they are living with HIV, since only 81% of the 38 million people living with HIV (PLWH) have been diagnosed. Only 67% [54–79%] of all PLWH have access to treatment, and another 1.7 million people are newly infected each year[20].

A safe, effective and durable way of completely eliminating HIV infection (eradication) or suppressing viremia in the absence of antiretroviral therapy (remission), referred to as “HIV cure”, will likely play a critical role in in the control of the AIDS epidemic and its termination[46].

In this work we aim at providing an overview of why an HIV cure is needed and raise the following questions; what are the major steps required to develop an acceptable target product profile for a cure, and the major hurdles to be overcome; and why animal models are needed in HIV science and what are the contributions of non-human primate models. We also present several novel, unconventional, and scarcely explored concepts aimed at finding a cure. Here, we focus on fundamental and translational research, the social sciences and implementation issues already being discussed and reviewed elsewhere.

I. Why do we need a HIV cure?

I.1. ART has reached major achievements

Over the past four decades the clinical outcome of HIV infection has been revolutionized by the progress made in the therapeutic options available, hence transforming HIV infection from a fatal illness to a manageable chronic disease that has limited impact on life expectancy.

At the time of the first reports of AIDS, in the early 1980s, the lack of comprehensive understanding of the etiology and pathophysiology of the disease compelled clinicians to treat only the opportunistic infections associated with the disease, with limited success. The identification of HIV as the virus responsible for the disease, in 1983[10], and the subsequent identification of the CD4 cell surface molecule as its main receptor were the first steps to understand the tropism of HIV and its life cycle, which enabled the medical and scientific communities to start investigating antiretroviral approaches.

In 1987, a clinical trial showing that azidothymidine (AZT; also known as zidovudine) decreased mortality and opportunistic infections in patients with AIDS paved the way to HIV therapy[21]. AZT had been originally synthesized as an anticancer treatment and was found to also block the reverse transcription step of the HIV-1 life cycle[22]. Nonetheless, a subsequent emergence of viral resistance quickly developed, spurring the quest to develop new drugs, generally on the basis of insights into the HIV replication cycle and how to target it[5].

A major breakthrough was made in 1996 with the advent of a therapy that combined several drugs to limit the development of resistance. The introduction of a protease inhibitor alongside two nucleoside reverse transcriptase inhibitors (NRTIs) in combination antiretroviral therapy (ART) was shown to markedly reduce morbidity and mortality[23–25]. It changed the course of the pandemic, contributing to save a large number of lives and to improve the quality of life of PLWH

The success of life-long ART is now clear on an individual basis, and a study performed in 2013 showed that adult life expectancy in South Africa increased by 11 years over the period between 2003 (just before ART became available in the public healthcare system) and 2011, highlighting its global health benefit and cost-effectiveness[94].

ART is life-saving. According to the latest UNAIDS estimates, AIDS-related deaths have been reduced by more than 60% since the peak reached in 2004. In 2019, around 690 000 [500 000–970 000] people died from AIDS-related illnesses worldwide, compared to 1.7 million [1.2 million–2.4 million] in 2004 and 1.1 million [830 000 –1.6 million] in 2010. AIDS-related mortality has declined by 39% since 2010[20].

In high-income countries, most PLWH now have access to highly active antiretroviral therapy (HAART) soon after they are diagnosed with HIV, thus their viral loads are rapidly controlled. In low- and middle-income countries (LMICs), the proportion of treated-PLWH is increasing[44].

I.2. Yet Current ART is not sufficient for ending the pandemic

ART can durably suppress HIV replication. However, even today, only 67% of PLWH have access to ART. For multiple reasons, it will be most likely impossible to achieve > 90% of virologically suppressed PLWH at the global level. Moreover, ART it is not curative, must be taken for life and does not target HIV-associated chronic immune activation. The majority of PLWH are diagnosed only several years after their infection. As a consequence, they start treatment late and metabolic disorders can be aggravated. PLWH can develop non AIDS-related co-morbidities and serious non-AIDS events (SNAEs) that underlie much of the morbidity in ART-treated PLWH[78,95]. In a 2016 systematic review of life expectancy estimates, it was assessed that life expectancy of PLWH on ART is still below the corresponding HIV-negative life expectancy. Indeed, estimated life expectancy at age 20 years in HIV-positive people on ART ranges from 60% of HIV-negative life expectancy in Rwanda to 90% in Canada[96].

Because of various challenges, many people are unable to achieve long-term viral suppression, even if provided theoretical access to ART. Lifelong adherence to treatment is challenging to many, and in particular to adolescents, and not everyone with access to ART does well[45,46]. Adherence to ART thus remains a major challenge. Patients on ART face multiple barriers to adherence. Although interventions exist that may address many of the most common patient-reported barriers to adherence, no single intervention currently

seems likely to be sufficient to ensure that high levels of adherence to treatment and virological suppression are sustained[97].

I.3. The need for a cure at an individual perspective

It is important to note that, at least in high-income countries, PLWH are aging. The aging of the world's population is nowadays one of the most significant demographic trends, and according to UNAIDS, there is a growing number of people aged 50 and older living with HIV in the world today, that accounts for approximately 13% of the global population of PLWH, and up to 31% PLWH in high-income countries[31,44]. The increasing number of people living with HIV aged 50 and older can be explained by three factors. First, antiretroviral therapy has been successful in prolonging the lives of people living with HIV in high-income countries. The life expectancy of a person living with HIV who achieves and maintains viral suppression on antiretroviral therapy now tends to be close to that of a person who has not acquired HIV. Finally, the trend of decreasing HIV incidence among younger adults is shifting the proportion of disease burden to older age groups[44]. This is associated with an increased prevalence of age-related comorbidities, including metabolic disorders. Older people also tend to be more prone to immunosenescence. Associated co-morbidities, due both to age and to HIV infection itself, can be only partially prevented with ART[32,95,98].

The chronic immune activation is the driving force of CD4+ T-cell depletion in untreated PLWH[99] (*Figure 1.*). This systemic chronic inflammation might result in part by the disruption of the intestinal barrier which is responsible for microbial translocation and the entering of some microbial components into circulatory system, increasing inflammation in a vicious circle[27]. The chronic status of immune activation and inflammation associated with HIV persistence leads to an increased production of pro-inflammatory cytokines and tissue damage, such as fibrosis in lymph nodes.

In ART-treated PLWH, the levels of systemic immune activation and inflammation are lower but still often higher than in HIV-uninfected individuals despite undetectable viral load and a significant factor responsible for higher than normal risk for developing noninfectious comorbidities, including cardiovascular and renal disease[100]. Moreover, HIV infection and long-term ART have been associated with the development of metabolic syndrome (MetS)[101,102]. MetS is a combination of various metabolic disorders, namely hypertension, hyperglycemia, changes in fat distribution, increase of cholesterol low-density lipoprotein

(LDL) and triglycerides, a and reduced level of cholesterol high-density lipoprotein (HDL), which can additionally lead to cardiovascular diseases (CVDs) such as heart disease, stroke, and diabetes[27].

First-generation ART, that PLWH received prior to 2000, including nucleoside analogue reverse transcriptase inhibitors (NRTIs: thymidine analogues, stavudine, zidovudine, didanosine) and protease inhibitors (PI: indinavir, ritonavir, nelfinavir) was responsible for marked adverse events on lipid and glucose metabolism and on adipose tissue, such as altered fat repartition related with stavudine and zidovudine[95,103,104]. Newer molecules then replaced these antiretrovirals, presenting a markedly lower metabolic toxicity. However, prior exposure to first-generation NRTIs, even years after treatment discontinuation may have long-lasting detrimental effects on adipose tissue function, consequently, on cardiometabolic health in PLWH. Indeed, low adipose tissue density and impaired adiponectin production have been shown to be associated with prior exposure to thymidine analogues and didanosine[105].

On the other hand, some newer antiretrovirals, such as an integrase strand transfer inhibitor (INSTI, dolutegravir), supposedly metabolic-friendly, were recently associated with weight gain and obesity. Therefore, the effect of ART on metabolism and adipose tissue in PLWH still constitutes a significant concern[95,106,107].

PLWH also still display a significantly increased risk to develop some non-AIDS-defining cancers (NADCs) such as non-Hodgkin and Hodgkin lymphomas as well as cervical anal lung, liver, and oral cavity/pharyngeal cancers[28,29]. This higher cancer rate is apparent even among long-term ART-treated PLWH[32]. HIV-associated chronic immune activation and dysregulation have been suspected to be closely implicated in the multiple myeloma development in PLWH by accelerating the carcinogenesis events in a complex aging process[30].

Furthermore, drug toxicities and complex drug–drug interactions (polypharmacy) have substantial health consequence and have emerged as new concerns. Indeed, polypharmacy is more frequent among PLWH across all age groups (except the elderly) than in individuals without HIV. Potential concerns associated with polypharmacy include increased pill burden, decreased medication adherence, increased risk for drug–drug interactions (DDIs), rare

adverse drug reactions including organ system injury, hospitalization, death, and rising treatment-related costs.[33–35]

It is to emphasize HIV-related stigma still remains widespread[108,109]. Stigma experienced can be relative to society, family, profession, health care workers, or self-inflicted[110], with repercussions for both PLHIV and HIV-negative individuals. It now recognized as a major factor that affects health and well-being[111–113]. It could be a factor co-responsible for a delay in HIV diagnosis and delayed treatment initiation. The possibility of a HIV cure or remission of HIV would considerably reduce stigma and discrimination.

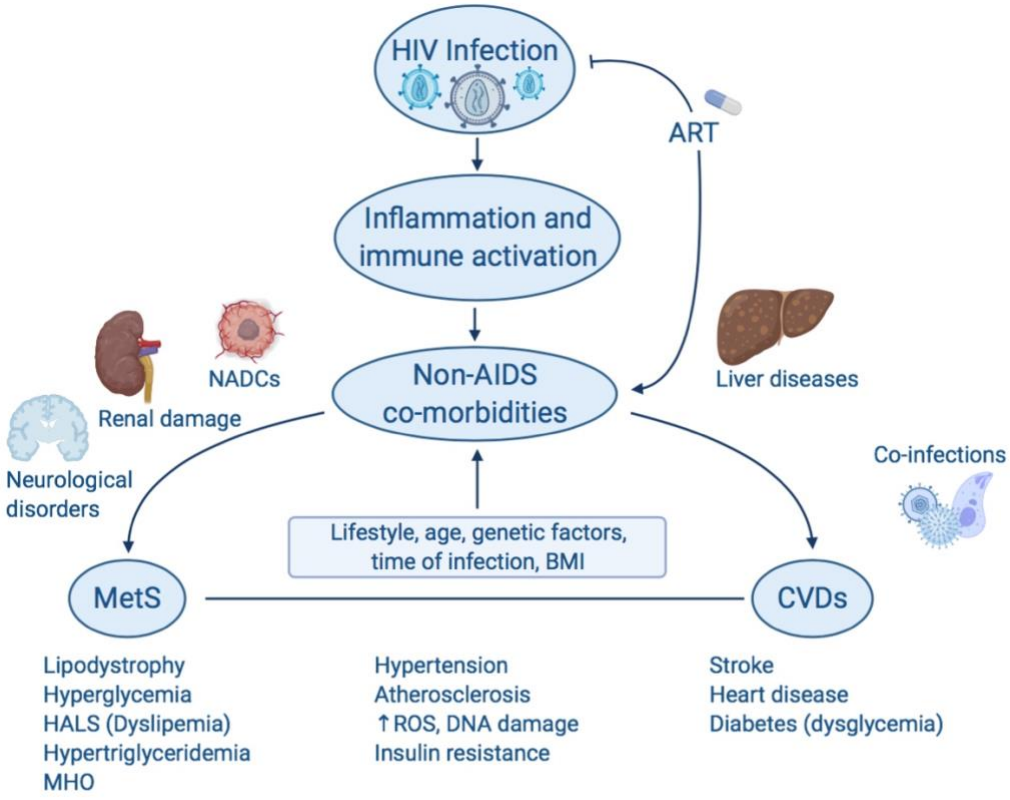


Figure 1. HIV-induced chronic immune activation and its implications on pathophysiology.
Chronic immune activation persists in people living with HIV (PLWH) over the years and despite effective ART. This even residual inflammation is associated with the development of non-AIDS co-morbidities in PLWH. These are most often, cardiovascular diseases (CVDs), metabolic syndrome (MetS) and non-AIDS-defining cancers (NADC). HALS: HIV associated lipodystrophy syndrome; MHO: metabolically healthy obesity; ROS: reactive oxygen species; BMI: body mass index.
Adapted from Zicari, S. et al. Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. Viruses 11, 200 (2019).

I.4. The need for an HIV cure at the population level

Current ART also entails several limits at the population level, from small communities to the world scale in the current globalized era.

Resistance to antiretroviral-drugs constitutes a threat to the efficacy of HIV-1 treatment. It can be a consequence of a lack of adherence to treatment. The combination of a high mutation rate, together with frequent recombination events and the tremendous capacity of the virus to tolerate a high number of mutations leads to the apparition of many viral variants in each infected individual at levels much higher than for Influenza viruses. These variants, usually described as “quasispecies”, enable HIV to escape very quickly leading to the emergence and selection of drug-resistant variants in the case of non-efficient adherence[36].

HIVDR can be either acquired HIV drug resistance (ADR), which develops because of viral replication in the presence of ARV drugs, or transmitted HIV drug resistance (TDR), which is detected among ARV drug–naive people with no history of ARV drug exposure. Pretreatment HIV drug resistance (PDR) refers to resistance detected among ARV drug–naive people initiating ART, and can be either TDR, ADR or both. TDR occurs when previously uninfected individuals are infected with a virus that has drug resistance mutations. The prevalence of TDR mutation is stable in Europe, currently approximately 10%. It is generally lower in areas where ART has been introduced more recently, but tends to be increasing over time[114,115].

According to World Health Organisation estimates, in 12 of 18 countries reporting survey data to WHO between 2014 and 2018, levels of pretreatment HIVDR (PDR) by efavirenz (EFV) and/or nevirapine (NVP) among adults initiating first-line ART exceeded 10%. The prevalence of PDR among children younger than 18 months diagnosed with HIV is alarmingly high. Based on surveys conducted in nine countries in sub-Saharan Africa between 2012 and 2018, over half of the infants newly diagnosed with HIV carry a virus resistant to efavirenz and/or nevirapine. Levels of PDR to nucleoside reverse-transcriptase inhibitors (NRTIs) also exceeded 10% in some low- and middle-income countries[114]. Although HIV drug resistance is generally less prevalent in high-income countries[116], some cases of multidrug resistance have been recently described, such as a HIV-1 subtype-B strains that have been recently described in two patient in France with resistance mutations to all available NRTIs, non-NRTIs, and protease inhibitors[117].

In 2014, UNAIDS had set targets to be reached by 2020. These Fast-Track Targets included achieving a "90-90-90" goal (90% of PLWH knowing their HIV status; 90% of people who know their HIV-positive status on treatment; and 90% of people on treatment with suppressed viral loads), alongside reducing the annual number of new HIV infections by more than 75%, to 500 000 in 2020, and achieving zero discrimination (*Figure 2*). Fast-Track Targets were implemented as a move toward the achievement of the longer-term goal to end the AIDS epidemic as a global health threat by 2030. Back in 2014, Michel Sidibé, Executive Director of UNAIDS, stated that "We have bent the trajectory of the epidemic. […] Now we have five years to break it for good or risk the epidemic rebounding out of control."[42,118]. Despite huge efforts and achievements over the past few years, the Fast-Track Targets were not reached in early 2020. Available estimates indicate that only about 79% of PLWH know their HIV status, 78% of whom are on treatment, while 86% of people on treatment have suppressed viral loads. According to estimates from the Institute for Health Metrics and Evaluation (IHME), HIV/AIDS is responsible for the largest number of lost disability-adjusted life years (DALYs) and the second largest number of deaths globally (behind only tuberculosis) of any single communicable disease, excluding the recent outbreak of Covid-19 (http://ghdx.healthdata.org/gbd-results-tool).

Of note, even if the goal will be achieved, this would mean that 73% at maximum of PLWH will be virologically suppressed.

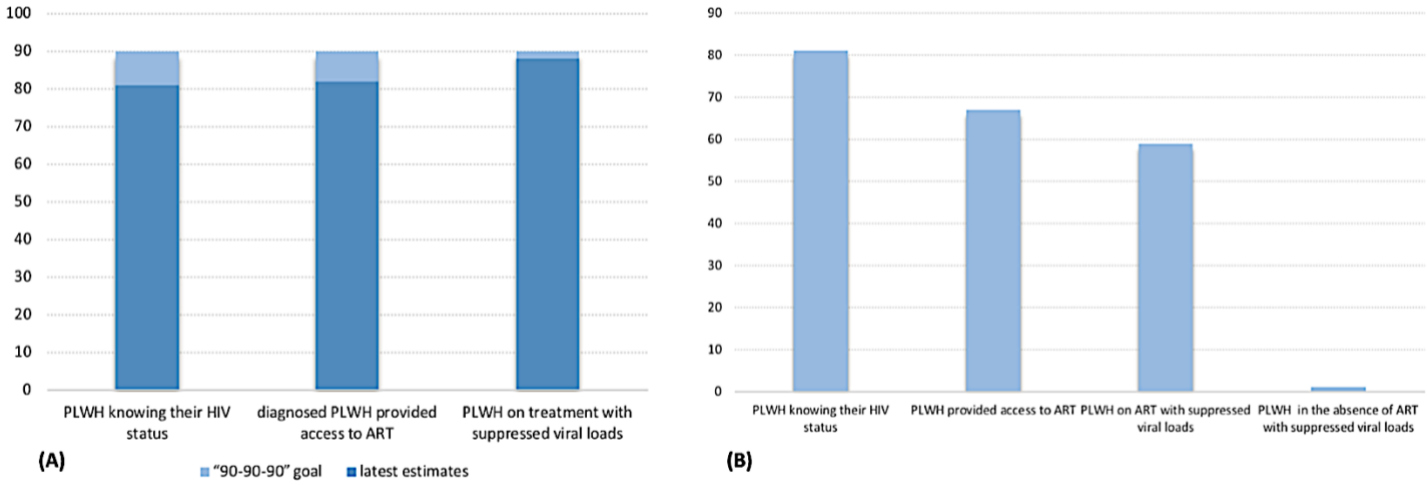


Figure 2. Representation of HIV treatment and control estimates in PLWH.
(A)*The most recent UNAIDS estimates expressed in percentage compared to the "90-90-90" goal (90% of PLWH knowing their HIV status; 90% of people who know their HIV-positive status on treatment; and 90% of people on treatment with suppressed viral loads). In 2019 (last data available), 81% [68–95%] of people living with HIV knew their status. Among people who knew their status, 82% [66–97%] were accessing treatment. And among people accessing treatment, 88% [71–100%] were virally suppressed.*
(B) *Of all people living with HIV in 2019, 81% [68–95%] knew their status, 67% [54–79%] were accessing treatment and 59% [49–69%] had undetectable viral loads. It is estimated that about 1% PLWH are able to spontaneously keeping their plasma HIV-1-RNA load below a quantifiable concentration for at least several years in the absence of therapy ('elite' control and rarely 'post-treatment control')*
(Data from https://www.unaids.org/en/resources/fact-sheet).

In early 2020, an unprecedented upheaval arose with the Covid-19 Pandemic. The pandemic has caused serious disruptions to health systems, especially in countries facing shortages of protective equipment and/or large numbers of hospitalizations. These significant repercussions on health systems will likely have consequences in many areas, among which HIV/AIDS[37]. Some studies were recently undertaken by different teams using mathematical models to analyze the effects of various possible disruptions to HIV testing, prevention and treatment services caused by COVID-19. They considered the potential impact of three-to-six months treatment disruptions on AIDS mortality and HIV incidence, focusing in sub-Saharan African. In the six-month disruption scenario, estimates of excess AIDS-related deaths in one year ranged from 471 000 to 673 000, making it inevitable that the world will miss the global 2020 target of fewer than 500 000 AIDS-related deaths worldwide, and more strikingly setting the clock on AIDS-related deaths back to its 2008 level. At least for the next five years, people would continue to die as a consequence of ART-disruption imputable to the Covid-19 crisis, with annual excess deaths putatively reaching up to 40% over the next half a decade in Low- and Middle-Income Countries. Even more sporadic interruptions of ART

supply would affect adherence to treatment, potentially leading to the spread of HIV drug resistance, with long-term consequences for future treatment success in the region [37–41].

On another note, it must be emphasized that ending the AIDS epidemic as a public health threat would lead to substantial economic benefits. There are many operational and logistical challenges involved in delivering lifelong treatment. Among other factors, the economic costs of providing ART to the more than 37.9 million PLWH, albeit potentially sustainable in a long-term perspective, constitutes a major short-term hurdle[44,45]. Due to the need for lifelong ART and the increasing life expectancy of those living with HIV, global treatment costs are unlikely to decline, even as funding for HIV programs has stagnated in recent years. Although a continued increase in global HIV prevalence, funding for HIV programs has stagnated, falling to just over USD$ 19,0 billion in 2018[119]. This is more than USD$7 billion, or ~37 percent, short of the UNAIDS estimated US$ 26.2 billion that would have been required to reach the UNAIDS global goals by 2020 and the 73% of virologically suppressed PLWH worldwide[26,119].

Importantly, the health improvement and extension of life expectancy of PLWH enabled by access to HIV prevention and treatment would enhance countries' economic potential, averting future HIV-related productivity losses, health-care expenditure on people who will not become infected, and reducing the social resources required. UNAIDS estimates indicated that fast-tracking the AIDS response prior to 2030 would yield economic returns of US$ 15 per dollar invested, based on the total economic benefits of improved health from increased access to life-saving treatment and from infections averted[42,43]. In this respect, an HIV cure could represent a sustainable contribution for containing the epidemic, freeing up global health resources for other pressing issues, such as emerging infectious diseases or the increasing burden of non-communicable diseases in low- and middle-income countries. An accessible HIV cure could maintain and strengthen progress achieved thus far in the field of HIV[46].

All the points aforementioned highlight the urgency of identifying an effective means of controlling the virus in the absence of ART, or finding a cure. The search for a curative strategy for HIV is together with the search for a vaccine a current key target for the HIV community[5,44]. Although the road to an HIV cure is long and difficult to forecast, different

strategies are being studied, from bench to bedside and encouraging results have already been reported.

II. The steps toward achieving a cure for HIV

II.1. Definition of "HIV cure"

In 2016, the International AIDS Society stated that the optimal outcome for a cure would be the "complete eradication within an individual of all replication-competent HIV"[45]. This type of cure is sometimes referred to as "sterilizing cure"[5,45,46], and requires the complete elimination of replication-competent HIV from a PLWH's body. It would likely involve getting rid of all infected cells. This approach appears to be challenging to achieve while limiting the harm to the patient. Moreover, it is currently not technically possible to prove that all cells containing replication-competent HIV have been eliminated, including in deep reservoirs, and that not a single virus particle (that could restart a systemic infection) persists. This difficulty to definitively prove the infection has been completely eliminated is a shared feature with the cancer field, where it is virtually impossible to assess the elimination of all cancer cells[89,90,120]. Borrowing from oncology, the HIV field also considers a more realistic goal of a cure, toward "remission" rather than "eradication"[121]. Remission can be defined as the absence of viral replication and disease for an extended period off therapy. Such a permanent and stringent control of HIV infection without the need for medication would constitute a ''functional cure''[5,45–47].

II.1.1 Requirements of a HIV cure therapy

A key element for the development of a potential cure is to determine upstream what it needs to achieve. For this purpose, the concept of target product profile (TPP) might be useful. According to WHO, TTP can be used as planning tools to align funding agencies, industry, community, implementers, regulators and end-users. TPP should provide an overview of the minimal and optional characteristics of an effective therapy.

Indeed, many issues and questions need to be debated and no simple answer can be provided:

Can we be satisfied with a cure whose efficacy is comparable to ART? With a functional cure that does not suppress all replication-competent virus? What residual viral load threshold would be adequate? Would a cure that prevent disease progression to AIDS and co morbidity but not productive viral infection, and thus not HIV transmission, be an acceptable outcome? What about a cure that would lead to undetectable viral loads but with the threat

of a potential rebound, that would require a close and heavy follow-up to mitigate the risk of contamination it would represent for others? Can we be satisfied with a cure that – for any reason – is not accessible to all PLWH, that is not scalable to the global population, or that can't be applied to a significative number of PLWH? Can we accept a cure whose safety is lower than – or not as well defined as – the safety of ART? The benefit/risk ratio is indeed crucial to consider and constitutes the limitation to several curative strategies that would be potentially too harmful to be beneficial. Hence, higher tolerance of a less effective strategy would make it more suitable. However, it must be emphasized that a therapeutic strategy that initially presents some adverse effects or potential harms can be refined and improved to mitigate these risks; every therapeutic mechanism that presents some toxicity in the first place should not be abandoned too soon. Can we be satisfied with a cure that leaves an individual susceptible to reinfection? If a cured patient is left vulnerable upon exposure to re-infection, would it be effective from a public-health perspective? To which extent a curative strategy could also be used as a protective, prophylactic tool to hinder the HIV/AIDS pandemic?[45,46,48]

The development of a therapeutic intervention to cure HIV is a progressive and multi-step process. In the first instance, the initial requirements could be minimal. On the one hand, an efficient cure but with risks entailed, or a procedure that is too complex and aggressive to be implemented at a large scale, would still provide a proof of concept of the efficiency of a given mechanism. On the other hand, a safe and cost-effective therapy displaying low efficiency could still be useful to analyze acceptance and applicability of a curative intervention at the population level. Therapies that would not achieve a functional cure in absence of ART, but that could effectively delay viral rebound upon ART interruption, hinder the establishment and maintenance of latent HIV reservoirs, or reduce the burden of HIV-associated chronic immune activation and tissue damage would already represent milestones in the road towards an ideal HIV cure.

II.1.2 The methods to evaluate efficacy of a HIV cure strategy and quantification of the impact on viral reservoirs

For the time being, the effect of most interventions can only be clearly assessed by interrupting ART. In order to address the risks it might represent, in particular for

transmission to partners, some methods and practices have been developed. Nonetheless, there is no consensus about when to initiate again ART. Biomarkers of control are still missing. In these respects, continued efforts need to be undertaken to identify circulating biomarkers that can predict the rebound of infectious virus as early as possible[46].

Another issue is to prove that every replication competent HIV RNA has been eliminated from a person's body. Various techniques can be used to detect and evaluate the amount of persistent virus, but all these entail some limitations (*Table 1*). While quantifying persistent virus in an individual, the ability to detect an infected cell depends on the total number of cells tested, which has an upper limit, and on the number of tissues that can be sampled[50–53]. Also, assays that measure the free virus in bodily fluids (plasma and cerebrospinal fluid) are good markers of viral production but do not evaluate the latent reservoir.

One assay for measuring persistent replication-competent virus is the quantitative viral outgrowth assay (QVOA). It consists in limiting dilutions of patient CD4+ T cells that are maximally stimulated with a T-cell-activating mitogen, usually phytohaemagglutinin (PHA), incubated with HIV-1 negative donor cells, and outgrowth measured by p24 ELISA, viral RNA or reverse transcriptase activity[51–53,122,123]. However QVOA invariably underestimates the size of the replication competent reservoir and thus lacks sensitivity[51–53,124].

Several variations and modifications to the original QVOA methods have been developed in the last ten years, that can be more informative and allows for more rapid throughput. One of these noteworthy variations is murine viral outgrowth assay (MVOA)[125]. Potentially infected T cells or PBMCs are injected to a mouse host where they become activated and release virus into the mouse circulation. HIV-specific RT-PCR is then used to detect virus in the murine plasma. If, for now, MVOA is not used as a quantitative method but more of a proof-of-infection assay, some modifications could enable to quantify the virus in murine plasma to infer the size of the reservoir and amount of persisting virus of the donor. Such quantitative assay requires multiple animals to be injected with the serial dilutions of patient cells. The high number of animals required, the long duration of this protocol, and volume of patient material for ensuring a sufficient number of cells needed thus appears as limiting factors to the use of MVOA.

In contrast, some other assays that quantify or sequence specific genomic regions of HIV tends to overestimate the size of the replication-competent reservoir due to their inability to distinguish between intact and defective virus. However, single-region PCR-based methods are still valuable for their ability to quickly and relatively cheaply quantify HIV DNA using real-time PCR or digital droplet PCR read-outs. These methods can be used to measure cell-associated DNA to quantify the number of cells that are infected with either intact or defective virus.

Other methods that consists in full genome sequencing of the integrated genome (or near-full-length individual proviral sequencing) provide quantitative measurement of levels of intact proviruses and can better distinguish replication-competent provirus from defective proviruses, thus limiting the overestimation of viral reservoir, but they are expensive, labor-intensive and time-consuming[126,127].

At last, some assays afford the advantage of indicating replication-competency, namely: TILDA and single cell analysis. The Tat-/Rev-induced limiting dilution assay (TILDA) consists in the maximal stimulation of resting CD4+ T cells with PHA and ionomycin in order to induce provirus expression. These cells are then serially diluted and nested PCR performed using primers specific for the tat/rev region is performed on the resulting provirus[128]. Drawbacks of the TILDA assay include the high amount of cell input required and the fact that measured transcripts may arise from defective proviral genomes.

By now, assays at the single cell level can also be performed to evaluate the inducible virus. For example, in the Flow-FISH method, CD4+ T cells are reactivated with PHA and ionomycin, before fluorescent in situ hybridization (FISH) staining is used to detect gag-pol mRNA together with gag protein using flow cytometry and/or confocal microscopy[129].

Along similar lines to assays of inducible provirus (QVOA, MVOA), these assays of translation-competent provirus may underestimate the size of viral reservoir.

The development of novel, scalable assays to accurately quantify the viral reservoir and its depletion, and potentially the combination of these assays, will be critical to the development and evaluation of future curative strategies for HIV[50–53]. Some tools developed in parallel will be helpful for this purpose. For example, the recently developed ultrasensitive digital ELISA assay (SIMOA) allows to identify viral proteins produced by a single infected cell[130] and is complementary to the previously mentioned assays.

After a therapeutic intervention to potentially cure HIV, a close clinical monitoring and follow-up is required, since a complex matter is to determine how soon it can be known that the patient has been cured of HIV. If the contingency of a viral rebound will presumably become less and less likely over time, the question of the reduction of the frequency of testing arises, as well as the perspective of the monitoring eventually being stopped one day. Once again, some similarities can be pointed out with the field of oncology: in the follow up of many malignant diseases, it is customary to reduce the frequency of monitoring for disease relapse over time. Though, to determine when follow-up can be reduced or stopped, a deep understanding of the natural history of the specific malignant disease is required, which implies large cohorts of cured and relapsing patients. Such cohorts are not currently available in the case of HIV, which makes the likelihood of a late viral rebound impossible to assess for now[50].

Assay	Sample used	Cell input requirement (relative)	Does the assay indicate replication competency?	Does the assay over- or underestimate reservoir size?	Other comments
Assays that measure the free virus					
Sampling of free virus	Plasma, cerebrospinal fluid	–	Yes	–	- Good markers of viral production - No evaluation of latent reservoir
Assays of cell-associated HIV					
DNA	CD4+ T cells	Low	No	Over	
RNA	CD4+ T cells	Low	No	–	- Generally indicates a cell that is productively infected - More sensitivity for replication competent provirus - Cannot distinguish transcripts that arise from replication competent cells and defective cells
Assays of the inducible provirus					
QVOA	Resting (HLA-DR−) peripheral blood mononuclear cells or CD4+ T cells	High	Yes	Under	- Most specific assay - Only measures replication competent provirus -Time consuming –Requires large volumes of patient material - Low sensitivity and underestima te size of the reservoir
MVOA	Resting (HLA-DR−) peripheral blood mononuclear cells or CD4+ T cells	High	Yes	Under	- High number of animals required and long duration
Assays of translation-competent provirus					
TILDA	CD4+ T cells	High	Indicates translational competency	Under (?)	- High sensitivity for replication competent provirus - Faster and cheaper than VOA - Measured transcripts may arise from defective proviral genomes and not represent replication-competent virus
Single cell analysis	CD4+ T cells	Low	Indicates translational competency	Under	- Expensive
Sequencing of the virus					
Single-region sequencing assays	CD4+ T cells	Low	No	Over	
Full genome sequencing	CD4+ T cells	Depends on methodology	Yes	Unknown; likely a small overestimate	- Expensive and time consuming

Table 1. General characteristics of the assays used to measure HIV persistence in individuals
Inspired of and adapted from: Horsburgh, B. A. & Palmer, S. Measuring HIV Persistence on Antiretroviral Therapy. in HIV Vaccines and Cure (eds. Zhang, L. & Lewin, S. R.) vol. 1075 265–284 (Springer Singapore, 2018);
Pitman, M. C., Lau, J. S. Y., McMahon, J. H. & Lewin, S. R. Barriers and strategies to achieve a cure for HIV. Lancet HIV 5, e317–e328 (2018);
and Thomas, J., Ruggiero, A., Paxton, W. A. & Pollakis, G. Measuring the Success of HIV-1 Cure Strategies. Front. Cell. Infect. Microbiol. 10, 134 (2020).

II.2. Identified cases of cured patients or people in remission of HIV

II.2.1. Cases of individuals cured of HIV

The identification of the two main co-receptors for HIV, (CXC-chemokine receptor 4 (CXCR4) and CC-chemokine receptor 5 (CCR5)), in the year 1996, after more than 10 years of intensive research, constituted an important milestone[131,132]. Indeed, the expression on the cell surface of the main HIV-1 receptor CD4 and at least one additional co-receptor, mainly CCR5 or CXCR4, determines HIV-1 cell tropism[133]. The main targets of HIV infection are CD4+ T cells, and HIV-1 replication occurs substantially in activated CD4+ T cells. The majority of the viruses after infection are R5-tropic (HIV using CCR5), while X4-tropic HIV (which use CXCR4) arise only later on during infection in about half of the patients (in absence of treatment). R5 viruses thus seem to be those and solely variants capable of establishing a persistent infection.

As a result of this discovery, genetic screens were performed in the CXCR4 and CCR5 gene. Mutations in CXCR4 gene are lethal for the foetus which explains the lack of polymorphism. In contrast, CCR5 is a highly polymorphic gene. A homozygous CCR5 deletion was identified in the coding region that was frequent in European descendants (while absent in Africa and Asia). This specific mutation, observed in approximately 1% of the population, consisted in a 32-base-pair deletion in the CCR5 gene that renders the host CCR5-expressing cells resistant to R5-tropic viruses. The CCR5Δ32 mutation was pointed as a mechanism able to confer resistance to HIV infection[134–137].

The discovery of cells resistant to HIV had a major impact for HIV cure. “The Berlin Patient” is the first patient cured of HIV. Until 2019, it was the only patient cured of HIV. This 40-year-old man, Timothy Ray Brown (which decided to publicly disclose his name), lived with HIV-1 infection for about 10 years and was on ART regimen for 4 years (efavirenz, emtricitabine, tenofovir) when he developed acute leukemia (unrelated to HIV) requiring a first myeloablative allogeneic hematopoietic stem cell transplant in February 2007. He was given HSCT with $CD34^+$ peripheral-blood stem cell from an HLA-identical donor who was screened for homozygous CCR5Δ32 mutation. Because of a relapse of his acute myeloid leukemia Timothy Brown received a second HSCT from the same donor and successfully recovered. Timothy brown was himself CCR5Δ32 heterozygous (CCR5Δ32/ CCR5wt), underwent total body irradiation and strong conditioning with each HSCT, and discontinued combined ART

immediately during the first HSCT [138,139]. He remained off ART for his remaining life, for more than 12 years, without any sign of HIV.

The case of Timothy Brown is often considered as the first documented example of a sterilizing HIV cure after combined ART cessation, as not any evidence for residual replication-competent virus could be found so far despite an extensive search during more than ten years. Nonetheless, these negative tests cannot completely exclude the presence of replication-competent virus everywhere in the patient's body. What's more, the complexity of the interventions, make it difficult to assess which parameters were ultimately responsible for the elimination of the virus and the resolution of HIV infection[140]. In addition, other cases of allogeneneic stem cell transplantation (ie Boston patient) did not result in a stable state of HIV cure as the virus rebounded after a while.

In order to better assess the mechanism underlying the success in the Berlin patient, thousands of potential bone marrow donors were screened. 22,000 potential donors homozygous for CCR5Δ32 mutation were identified and a large cohort of PLWH who have undergone allo-HSCT to treat diverse hematologic disorders was assembled through the ICISTEM consortium (www.icistem.org). 39 people living with HIV and cancer that received HSCT from matched CCR5Δ32 or "wild-type" donors to cure the cancer, have been included in the cohort (among which 26 are still alive).

Among these, the "London Patient" became the second person to achieve HIV remission for more than a year after having received an allogenic HSCT from a donor with cells resistant to CCR5-tropic HIV. This adult male individual had been living with HIV since 2003 when he underwent allo-HSCT in London for Hodgkin's lymphoma, using cells from a CCR5Δ32/Δ32 donor. CCR5-tropic, but not CXCR4-tropic viruses were identified in HIV-1 DNA prior to transplant. No viral rebound has been observed 30 months after treatment interruption[141,142]. In contrast to the Berlin patient, the London patient received only one HSCT, did not undergo whole body irradiation, interrupted cART only 16 months after HSCT and even though his genotype was CCR5wt/wt, full chimerism with CCR5Δ32/Δ32 cells was achieved after transplant (maintained at 99% in peripheral T cells), which together indicated that a multiple HSCT as in the Berlin patient were not necessary to induce HIV cure in this context[141–143].

Another patient in Düsseldorf also received allo-HSCT from a CCR5Δ32/Δ32 donor and

represents a third possibility of HIV remission. The fifty-year old male individual was diagnosed with HIV in 2010, and with acute myeloid leukemia in 2011. He received unmodified HSCT from an HLA-identical CCR5Δ32/Δ32 donor in February 2013 and remained on ART with undetectable viral load in plasma until November 2018. No viral rebound have been observed for 3 months after cART interruption [66,140,144]. It still appears premature to conclude that these two patients have been cured.

Nonetheless, based on these 2 additional cases, it seems that the CCR5Δ32 mutation, ie the fact to transplant HIV resistant cells is a key factor even if not the only factor that lead to HIV cure in these rare individuals. Indeed, the ICISTEM study has shown that allo-HSCT is associated with a drastic reduction in the HIV reservoir, independently of engraftment with CCR5Δ32 or wild-type cells. Indeed, in most cases allo-HSCT in presence of cART was followed by drop of all virological markers below detectable limits, possibly related to graft-versus-HIV-reservoir like effects[66,140,142,145]. It is important to note that allo-HSCT does not systematically leads to HIV remission, even when full donor chimerism is achieved. In the cases of the Boston patients A and B, as in other individuals who received allo-HSCT with cells from CCR5 wild-type donors, undetectable viremia was maintained for 3 to 9 months following treatment interruption but the virus eventually reappeared[146,147]. Even when HSCT is performed with cells from a CCR5Δ32/Δ32 donor, viral rebound is still a possible contingency. It notably occurred in a case known as the “Essen patient” where a PLWH that had developed anaplastic large-cell lymphoma underwent HSCT from a homozygous CCR5Δ32 donor: a viral rebound occurred 3 weeks after HSCT, and the subsequent genotypic analyses of HIV-1 variants in this patient showed a shift from a dominantly CCR5-tropic HIV before HSCT toward a CXCR4-tropic HIV after transplantation[148].

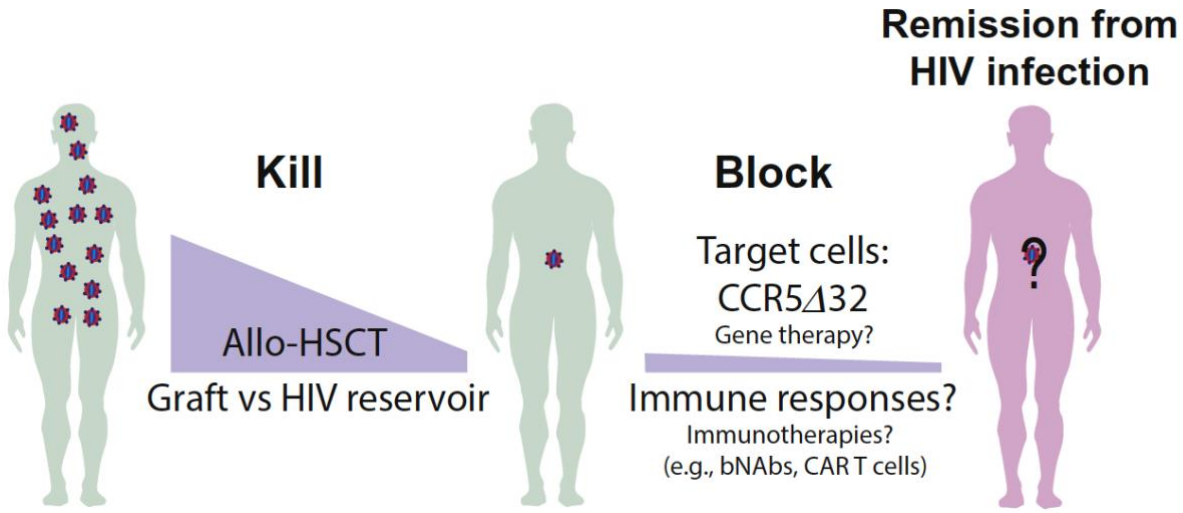


Figure 3. Allogenic hematopoietic stem cell transplantation (allo-HSCT) that replace host immune cells with donor cells in PLWH is associated with a dramatic decrease of HIV reservoirs*. In most cases, allo-HSCT in presence of cART is followed by drop of all virological markers below detectable limits, possibly related to graft-versus-HIV-reservoir like effects ('kill' axis). It is nonetheless important to note that allo-HSCT does not systematically leads to HIV remission, even when full donor chimerism is achieved. Viral rebound is still a possible contingency even when HSCT is performed with cells from a CCR5Δ32/Δ32 donor. To achieve durable remission of HIV infection, additional interventions to block the virus may be needed ('block' axis). If no (CRR5Δ32/ Δ32) donors are identified, alternative additional interventions could include modification of target cells through gene therapy or Immunotherapies to boost immune responses and control the remaining infected cells (including therapies based on vaccines, on broadly neutralizing antibodies (bNAbs) against HIV, on T cells adoptive therapy e.g. CAR T cells) or on other strategies.*
Adapted from Saez-Cirion, A. & Müller-Trutwin, M. The Yellow Brick Road towards HIV Eradication. Trends Immunol. 40, 465–467 (2019).

To date, allo-HSCT with cells from CCR5Δ32/Δ32 donor is the only curative intervention that have led to a potentially complete HIV remission (*Figure 3*). Nonetheless, it remains a very complex an aggressive intervention and cannot represents a scalable therapy for the 38 million people living with HIV.

II.2.2. Cases of HIV remission

More recently, it has been shown that early initiation of ART in hyperacute or primary HIV infections is beneficial as it decreases the viral reservoir size, limits viral diversity, protects immune responses, reduces HIV-associated chronic immune activation, preserves the function of immune cells in both blood and gut, promote partial restoration of HIV-associated GALT damage, and improve T-cell functionality in terms of cytolytic activity and cytokine production[149–156]. In most cases, though, it is not sufficient to cure a patient, as shown also the well-known case of the 'Mississippi baby': this infant, which had been

infected in utero by HIV and was born with a viral load of approximately 20,000 copies of RNA per ml, was immediately given ART within 30 h after birth. ART was interrupted when the baby was 18 months old and the child experienced 30 months of undetectable viral load before rebound eventually occurred[157]. Other similar cases were studied, such as the "Canadian" and the "Milan" babies, and a drastic viral rebound occurred upon ART cessation in these cases[158]. Studies in the nonhuman primate model (macaques infected by SIVmac) showed that ART initiated at 3 days post-infection is not sufficient to avoid the viral rebound after ART interruption[159].

In 2013, in the ANRS VISCONTI cohort, 14 PLWH who interrupted prolonged ART that was initiated shortly after primary HIV-1 infection (PHI) were shown to durably control viremia[160]. These PLWH were diagnosed with PHI in the late 1990s or early 2000s and received standard combined ART available at the time, for at least 3 years, and their viral load became undetectable within a median of 3 months after treatment initiation. Following the interruption of ART, viral control persisted for a median of 89 months, and their CD4+ T cell counts remained stable. Eight individuals had viral loads below the detection limit in all available samples after treatment interruption, whereas occasional blips (low levels of detectable virus in blood) were recorded for the other six patients[160]. The existence of individuals in whom the viral load remains undetectable for several years after the interruption of prolonged therapy that was initiated very early after infection was already reported in 2010, and these individuals were called "post-treatment controllers (PTCs)"[161]. PTCs were identified also in other studies, being estimated to account for approximately 5–15% of those treated early during the course of disease, and their ability to control infection is seemingly not achieved spontaneously but rather favored by the early initiation of therapy[62,160,162].

Such post-treatment controllers (PTCs) are suspected to achieved control of infection through different mechanisms than those observed in elite controllers (PLWH highly efficiently controlling HIV replication efficiently for long periods, also termed "HIV controllers" (HICs)). Indeed, PTCs tends to display more severe primary infections than HICs (*Figure 4*). In the VISCONTI cohort, PTCs lacked protective HLA B alleles (B*27 and B*57) and instead carried risk-associated HLA alleles (i.e. HLA*B35) that were largely absent among the

HICs[62,160]. The CD8+ T cell responses displayed by PTCs were also weaker than those of HICs. The levels of T cell activation were also lower in PTCs than in HICs. These differences likely indicate that the mechanisms responsible of virus control are not the same in PTCs and HICs. The underlying mechanisms of HIV remission need to be identified.

Some of the HIC or PTC show a progressive decrease in seropositivity and decreased viral reservoirs (*Figure 4*). On the other hand, for some cured patients, it is unclear if there is still persisting virus that might be under control. There might thus be a continuum between individuals in remission and cured patients.

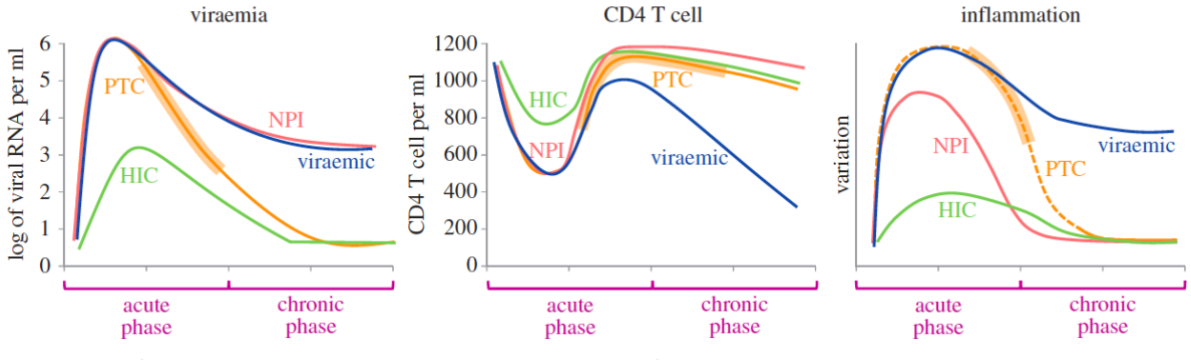


Figure 4. Schematic representation of the hypothetical variation in viraemia, CD4+ T cell counts and inflammation in post-treatment controllers (PTC, orange) compared with HIV controllers (HIC, green), non-pathogenic infections such as simian immunodeficiency virus (SIV) infection of natural hosts (NPI, pink) and viraemic progressors (blue). *The orange box on the PTC curves indicates the period of cART initiation, and the dashed line indicates hypothetical levels. Adapted from Saez-Cirion, A., Jacquelin, B., Barré-Sinoussi, F. & Müller-Trutwin, M. Immune responses during spontaneous control of HIV and AIDS: what is the hope for a cure? Philos. Trans. R. Soc. B Biol. Sci. 369, 20130436 (2014).*

III. What are the major hurdles to overcome in the quest for a cure?

Many curative interventions are currently being explored addressing both virologic and immunological factors. There are several important issues that must be acknowledged, addressed and more thoroughly understood. These issues are (1) the challenges raised by HIV Latency and persistence in cellular reservoirs, (2) HIV persistence in sanctuary sites relatively protected from ART and/or the immune system, (3) Tissue damage and alteration of local immunity, and (4) Persistent residual inflammation.

The term "HIV reservoir", for its part, refers to the cells and tissues that continue to harbor HIV under optimum therapy[64]. According to some definitions, it can comprise all proviruses that participate in HIV pathogenesis whether or not they are replication competent[163]. Other definitions are more restrictive, including only cell types or anatomical sites in which replication-competent forms of HIV can persist on a timescale of years in PLWH on optimal ART[164,165], and that can be reawakened to begin actively reproducing HIV virions, causing viral rebound if ART is stopped[166]. Here, closer to the latter definition, we refer to cellular and tissue reservoirs as environments where a viral rebound can occur from[64].

The reservoir can be considered at different scales: (i) the molecular reservoir, namely the HIV genomes persisting in infected cells that can participate in pathogenesis (ii) the cellular reservoir that are the infected cells, and (iii) the anatomical reservoir, composed of the tissues and organs that harbor (demonstrated or potentially) replication-competent HIV[47,64].

Although these matters have been widely addressed and discussed in the literature, we try to provide here a synthetic overview of what needs to be kept in mind for the conception and implementation of HIV cure strategies.

III.1. HIV Latency and persistence in cellular reservoirs

HIV genome integrates into the host genome alike all retroviruses, and persists in infected cells all along the lifetime of these[167–169]. The current ART effectively suppresses HIV replication, but is not able to eliminate infected cells. In ART-treated PLWH, one of the major hurdle that hinders HIV eradication is the establishment of long-lived and proliferating latently infected cells[170–172]. The study of HIV latency and persistence in cellular reservoirs is fundamental in the search for an HIV cure[45,173–175].

HIV latency is defined as the persistence of integrated HIV DNA that is transcriptionally silent but replication competent[63,168,176–178]. In other words, latency is a reversibly nonproductive state of infection of individual cells[164]. Latently infected cells can escape immune recognition and clearance due to the low or inexistent expression of viral proteins (*Figure 5*). **Latently infected cells are so far undistinguishable from uninfected cells** and, hence being all the more difficult to target with therapies. Intensive research is being conducted to find markers for identifying latently infected cells. Some cellular markers which are more frequent on infected cells have recently been proposed as HIV reservoir markers, including immune checkpoint molecules – such as programmed cell death 1 (PD-1) – as well as CD30 and CD32a[179,180].

The state of latency of HIV in the reservoir cells can most likely be influenced by the responsiveness of these cells to external signals (such as inflammatory cytokines or contact with antigen-presenting cells) or their localization and capacity to circulate. CD4+ T cells carrying latent provirus persist despite multiple decades of treatment thanks to the survival of long-lived cells and homeostatic proliferation [173,181]. It has been suggested that latently infected cells may also have a potential survival advantage, but no such mechanism has been identified yet[63].

The molecular reservoir *sensus lato* comprises several types of HIV nucleic acids (Figure 5). The more persistent one is the integrated form, the provirus, which permits the production of virions after the reactivation of quiescent infected cells. In this latent reservoir, the virus is transcriptionally silent and must be reactivated in order to produce new virions. Low-level viral replication leads to virions, able to infect new cells and maintain the HIV reservoirs. The provirus form persists during cell proliferation, also participating in the maintenance and amplification of reservoirs. Unless they revert to the productive state, latent proviruses are not affected by ART, and cannot be detected by the host immune system due to absent expression of virus protein and/or RNA. Since the maintenance of quiescence, persistence and latency require some cellular determinants and molecular mechanisms, proviral latency appears to be an unstable state, hence foreshadowing potential therapeutic targets[47].

III.1.1. Molecular reservoirs

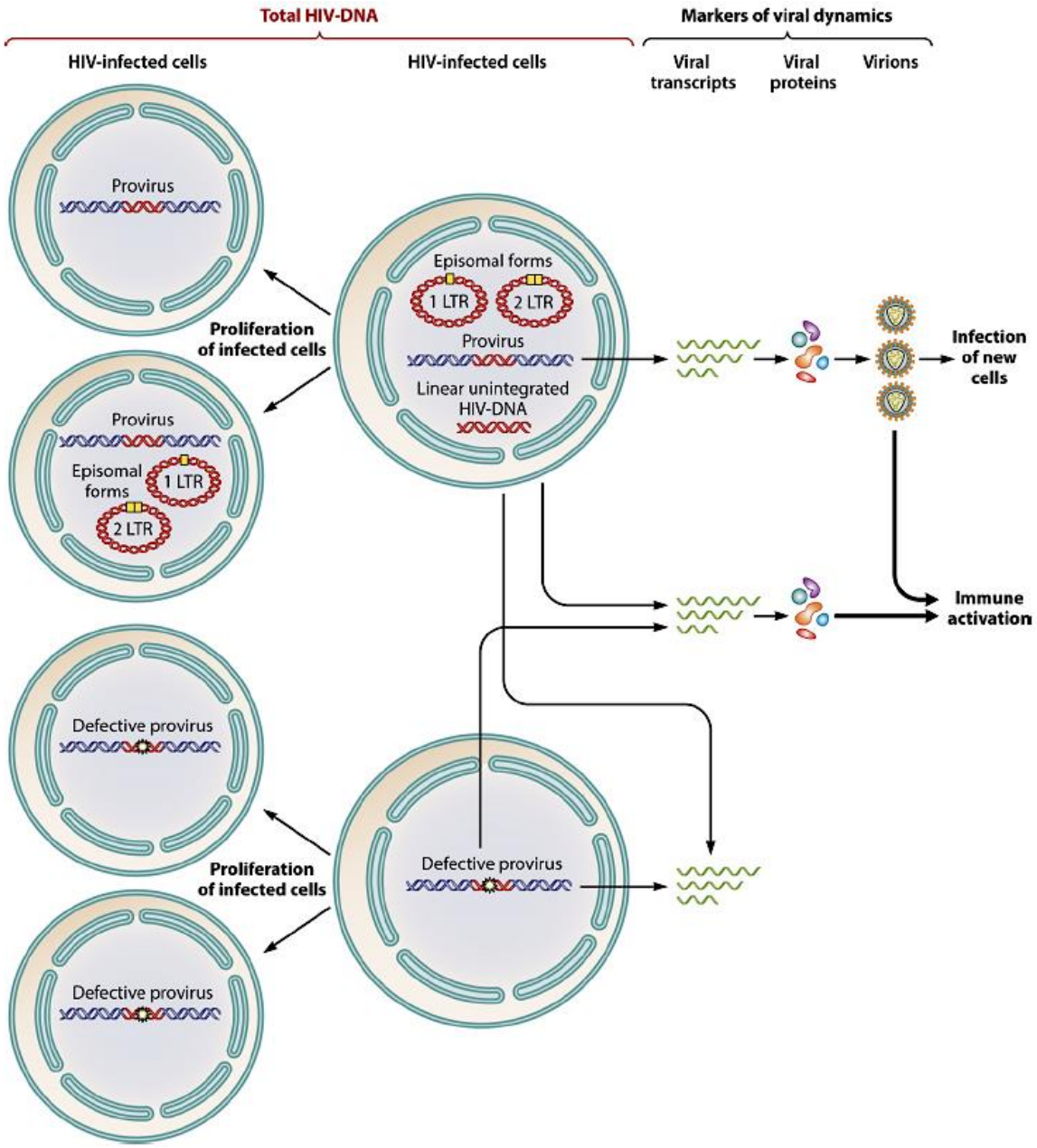


Figure 5. Molecular reservoirs and total HIV DNA.
Several forms of HIV DNA compose the total HIV DNA and participate in HIV pathogenesis. The integrated form, the provirus, is the more persistent form and permits production of virions when quiescent infected cells are reactivated. Virions can infect new cells and propagate infection and the HIV reservoir. The provirus form persists in all cells during cell proliferation. Episomal forms with 1-LTR or 2-LTR persist and are diluted in some daughter cells during cell proliferation. Linear unintegrated HIV DNA is the more labile form and is essentially present when the virus is replicating. Defective provirus, with a deletion, nonsense mutation, or hypermutation, cannot produce new virions but can produce transcripts and viral proteins which could activate the immune system and participate in HIV pathogenesis.
Adapted from Avettand-Fènoël, V. et al. Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications. Clin. Microbiol. Rev. 29, 859–880 (2016).

HIV DNA can also stay within the cell nucleus in the form of circularized episomal forms with one or two long terminal repeats (LTRs). These episomal forms also persist and are diluted in some daughter cells following cell proliferation.

Unintegrated HIV DNA in the linear, more labile, form can be present, essentially during viral replication.

Finally, defective provirus, with a deletion, nonsense mutation, or hypermutation, are HIV nucleic acids that cannot produce new virions. Defective virus represents the majority of viral genomes measured.[126,182,183]. Even if defective provirus is generally impotent to cause viral rebound, thus not properly included in the term "reservoir", it produces transcripts and viral proteins which could activate the immune system and participate in HIV pathogenesis[163,184].

III.1.2 Cellular reservoirs

At the cellular scale, it is critical to understand the nature and characteristics of the cells that can harbor HIV. **HIV-infected cells are diverse and the HIV reservoir cells still not sufficiently well defined.**

All the cell populations that are susceptible to HIV infection do not contribute equally to HIV persistence[174] . HIV-1 cellular reservoirs are established and maintained according to protean and miscellaneous processes, especially depending on (i) the relative cell susceptibility to HIV infection, (ii) the ability of the infected cell to escape immune surveillance and resist HIV-induced apoptosis, and (iii) the infected cell's life span and turnover potential (*Figure 6*). These processes are determined by each cell type's features and regulated by tissue location, activation, and differentiation state of the cells in response to environmental conditions and stress signals[173].

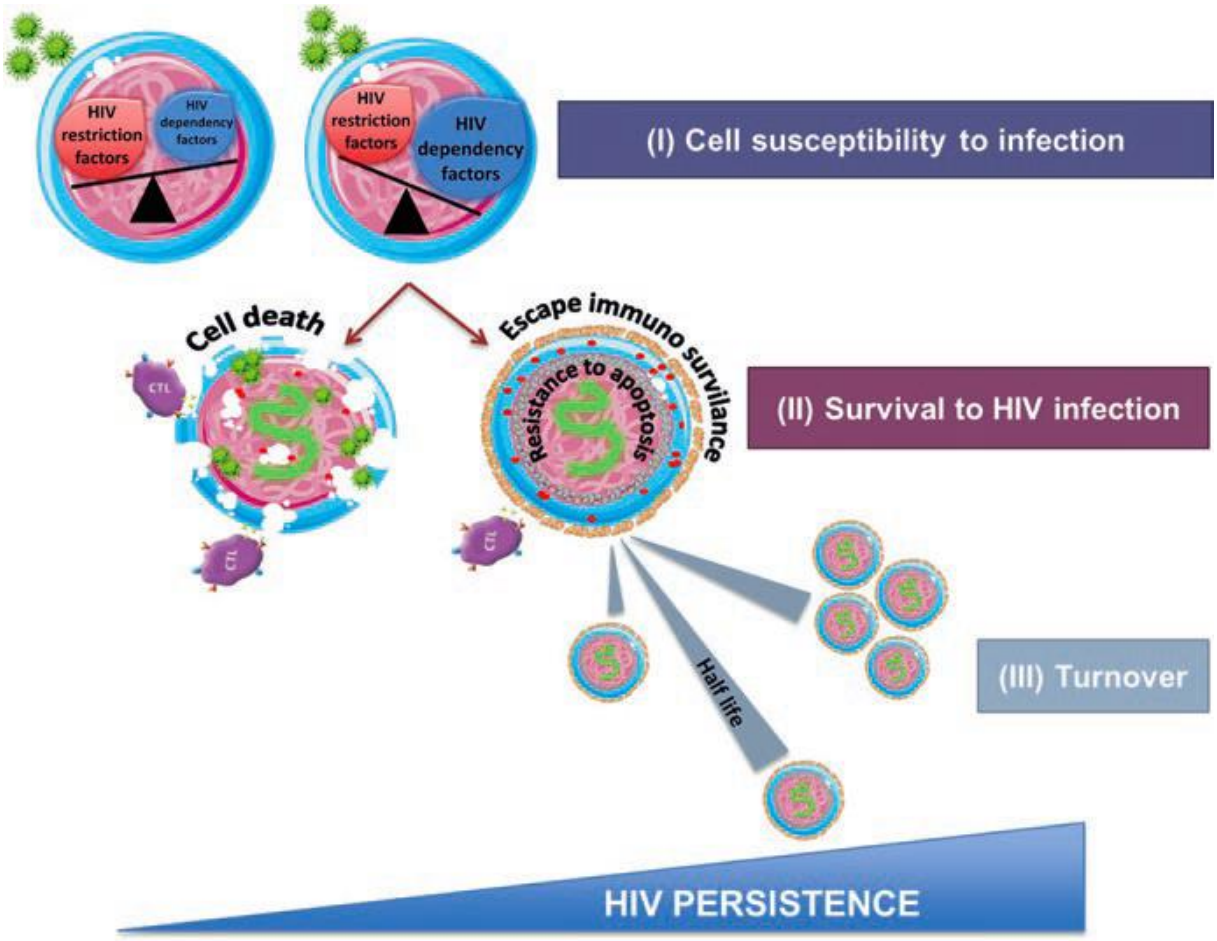


Figure 6. Cellular determinants for the establishment and persistence of HIV on ART.
HIV persistence is first determined by the susceptibility of different cells to infection (i), which is regulated by the balance of HIV host dependency factors and viral restriction factors present in the cells. In order to persist, infected cells need to resist apoptotic signals induced by viral infection and avoid immune surveillance (ii). These resistant infected cells will persist for variable periods of time depending on their specific life span and capacity to proliferate without enhancing HIV-dependent cell death signals (iii).
Adapted from Mikhailova, A., Valle-Casuso, J. C. & Sáez-Cirión, A. Cellular Determinants of HIV Persistence on Antiretroviral Therapy. in HIV Vaccines and Cure (eds. Zhang, L. & Lewin, S. R.) vol. 1075 213–239 (Springer Singapore, 2018).

In PLWH under ART, HIV-1 persists mainly in the memory subsets of CD4+ T cells, in blood these correspond in particular to the central memory and transitional memory $CD4^+$ T cells, which displays very low decay, even in patients on ART[47,166,181]. Moreover, differences in HIV-1 susceptibility were found to be associated with the metabolic activity of the cells. HIV-1 thus requires a metabolically rich cellular environment to establish both productive and latent HIV-1 infection in CD4+ T cell subsets[81].

Other cells can also be targets for HIV [185–187]. These include cells from the myeloid lineage (macrophages and immature dendritic cells (DC) or plasmacytoid DC. HIV-infected macrophages upregulate bcl2 and protect themselves from cell death. They are long-lived cells and might be able to survive for many years[188,189] . Infected macrophages are found in

many tissues and in particular in the brain[190,191]. Whether myeloid cells can support latent HIV infection has been very controversial and is still debated[192,193]. In a study performed on humanized myeloid-only mice (MoM), systemically reconstituted with human myeloid and B cells but with no human T cells, infected with HIV and suppressed with ART, viral rebound occurred 7 weeks after ART interruption in 3/9 (33%) mice[194]. These results support the existence of a myeloid reservoir for HIV. A role for macrophages as a viral reservoir is also supported by several other studies performed in macaques and humanized mice[187,195] and humans[196,197]. In a recent study, it was shown that macrophages isolated from the urethras of three individuals on suppressive ART for at least three years harbored not only replication-competent integrated proviruses but also HIV RNA, proteins, and viral particles, that were able to produce replication-competent virus when stimulated with lipopolysaccharide[197]. This corroborates the growing evidences that HIV-infected cells can persist in the genital tract during ART[198–201].

Additionally, some cells, such as myeloid dendritic cells can internalize free virions in non-cytolytic vesicles and transfer them at high concentrations to CD4 T cells upon interaction.

HIV-1 can thus reside in multiple cell subsets and the main mechanisms for persistence are diverse. Their comprehensive understanding is necessary to define and target all infected cells, as well as specifically identify and distinguish latently infected cells from uninfected cells.

III.2. HIV persistence in sanctuary sites protected from ART and/or the immune system

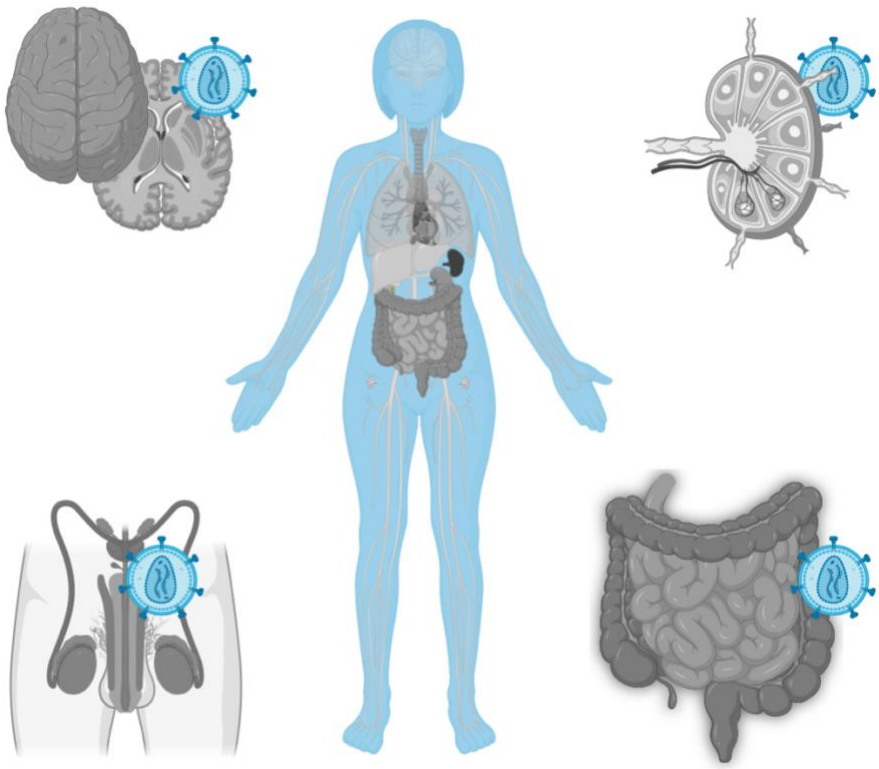


Figure 7. Anatomical reservoirs and sanctuary sites.
The main sites harboring replication-competent virus in PLWH under ART are the GALT and secondary lymphoid organs. Other tissues non-exhaustibly comprise: MALT, reproductive tract, peripheral blood, thymus, bone marrow, central nervous system and cerebrospinal fluid, adipose tissue liver, and kidneys. Privileged sites and unique body compartments that can represent "sanctuary sites" for HIV replication have been represented at the four corners: central nervous system (CNS), lymph nodes, B follicles of the MALT, especially in the gut and the reproductive tract, and macrophages from the urethra.

The establishment of tissue reservoirs (*Figure 7*) occurs very early in the pathophysiology of HIV infection and is seemingly impossible to prevent even by very early ART[47,64,149].

HIV infection is principally related to lymphoid tissue. Indeed, CD4+ T lymphocytes, which are the main target cell of HIV, primarily resides within organs of the immune system. Secondary lymphoid tissues, in particular lymph nodes (LNs) and mucosa-associated lymphoid tissue (MALT) are the principal HIV/SIV replication sites. As shown in the macaque model, under ART, SIV is detected in nearly every organ, except the heart[202]. Estes et al. illustrated that the primary reservoir sites were indeed lymphoid tissues, which accounted for approximately 98.4% of the tissues where cells producing viral RNA (vRNA+ cells) are found (gut, LNs, and spleen). When the lung, a tissue, with abundant MALT, was added, it

increased their estimate of the frequency of vRNA+ cells residing in LTs to circa 99,6%[202]. Notably, the gut contains a significant proportion of the lymphoid tissue (up to 85%) and lymphocytes (up to 90%) in the body, in line with other data showing that the GALT is a major HIV reservoir[203–205]. After prolonged ART, the gut can comprise up to 98% of the measurable vRNA+ cells, making it the most quantitatively significant anatomical site of infected cells in treated PLWH[202]. However, LN where the sites with the most frequent signs of residual replication (Ref Estes). In addition, other tissues, such as the lungs, skin, and adipose tissue, are also likely to contain cells harboring reservoir cells[206]. It has been shown that when ART is interrupted the virus rebounds from multiple sites[207].

Thus, HIV persistence occurs in multiple tissues. Each of the aforementioned tissues contains unique, diverse and variously differentiated cell types that contribute to the persistence of HIV during effective ART[174]. The localization and environment and activation status of each tissue also influence various parameters of persistence, including activation of infected cells and susceptibility to cell death[173].

"Sanctuary sites" are locations where infected cells are relatively protected from ART and from the immune system, and where persistent HIV replication can consequently occur. These tissue reservoirs complicate the efforts to induce HIV remission and cure HIV infection. Their understanding is important in order to find ways for therapeutic interventions to reach reservoir cells in these sites[208,209].

The CNS could be such a sanctuary site. This can possibly be linked to suboptimal ART in the CNS levels due to impedance of penetration by the blood–brain barrier (BBB). Since an intact BBB also limits the passage of immune cells and antibodies, it may raise the concern of a reduced effectiveness of potential immune interventions to eliminate HIV reservoir cells in the brain[209–211]. Yet, the size of this reservoir and the diversity of cells types that could serve as HIV reservoir (macrophages, microglia, astrocytes, CD4+ T cells) still remains to be better known [47,212–214].

B cell follicles in secondary lymphoid organs have also been identified as sanctuary sites. It has been shown that ART levels in lymph nodes are lower than in peripheral blood[215,216] and that ongoing viral evolution happens in lymph nodes despite undetectable HIV RNA in the peripheral blood[216]. Hence, ART concentration in the lymph node may not be sufficient to completely suppress viral replication. Other studies have shown that CD8+ T cells are

excluded from B cell follicles where there is active viral replication[217,218]. Notably, using a non-human primate model of HIV "elite control", Fukasawa et al. demonstrated that CD8+ T cell–mediated control of SIV infection is linked to restriction in the anatomic location of productive Infection[219]. Furthermore, Banga et al. demonstrated that even in individuals under long-term cART, infectious virus persists in CD4 T cells within lymph nodes[220]. Under ART, PD-1+ and CTLA-4+ CD4+ T cells in the T zone and Tfh cells in B cell follicles of lymph nodes seem to be the major reservoir cells for HIV-1 and SIVmac infections[220,221].

In addition, follicular dendritic cells (FDC), a non-migratory population of the immune system of mesenchymal origin (unlike dendritic cells) present in B cell follicles, have been shown to trap and retain infective HIV-1 particles for extended periods of time.[173,222,223]. Indeed HIV-Immune complexes accumulate on the surface of FDCs. FDC-bound HIV virions remain infectious even in the presence of neutralizing antibodies and can persist for months or even years. Years or decades of ART may be necessary to clear HIV-ICs[88].

The several aforementioned features make B cell follicles a critical sanctuary for HIV replication and persistence.

These findings raise concerns regarding the ability to eliminate HIV from privileged sites and unique body compartments such as the central nervous system (CNS), B cell follicles or the genital tract that can represent "sanctuary sites" for HIV replication. As a consequence, it seems determinant to figure out how to facilitate the entry of HIV-1 specific CD8+ T cells or other efficient immune stakeholders into anatomical sanctuaries of viral replication[224,225].

III.3. Tissue damage and alteration of immunity

HIV infection and the immune responses that it triggers are responsible for significant damage of several tissues and specific organs. These damages can disrupt the cellular players of innate and adaptive immunity and alter the equilibrium and the potency of the immune system. Some of these damages occur early and can in most cased not be totally repaired by ART as explained below.

III.3.1. B cell follicles disruption and lymph node stromal cell network damage and fibrosis

HIV induces damage of mucosal B cells and B cell follicles. Dysregulations of the B-cell compartment has been described very early, since the first years of the HIV/AIDS epidemic. It involves lymphadenopathy, activation-induced cell death, polyclonal B Cell differentiation and hypergammaglobulinemia, increased turnover of B cells due to the altered expression of homing receptors on their surface, loss of memory B-cell responses and loss of gastrointestinal tract germinal centers. These damages occur as soon as the earliest stages of HIV-1 Infection. The loss of normal B-cell follicle architecture in lymph nodes and MALT and the Dysregulations of the B-cell compartment have numerous pathological consequences[150,226–231]. HIV replication in the B cell follicle also likely mediates defects in humoral immunity[88].

Lymph node stromal cells, notably fibroblastic reticular cells (FRCs) and follicular dendritic cells (FDCs) networks, as the underpinning element of secondary lymphoid organs, constitutes the architectural scaffolding of lymphoid tissue. They are also involved in the production and storage of cytokines required for T-cell survival. The interconnected relationship between CD4+ T lymphocytes and the FRC stromal network is disrupted during HIV persistent infection, which causes profound modifications of lymphoid tissues. These modifications include progressive collagen deposition and fibrosis, which damages and disrupts the FRC network, predominantly as consequence of persistent chronic immune activation and inflammation[232].

Uncontrolled inflammation leads to collagen deposition and fibrosis in lymph nodes. In particular, irreversible fibrosis might be linked to persistent IFN-related inflammation, TGF-β produced by regulatory T cells (Treg) leading to collagen deposition, and other yet unknown possible factors[233].

III.3.2. Intestinal epithelial damage and gut disruption

As soon as the first weeks of HIV infection, the virus offensively spreads over the gut, which causes a significant depletion of immune cells, in particular memory CD4+ T cells. This substantial depletion is followed by disruption of the tight junctions in the intestinal epithelium, which may not be fully restored even with early ART initiation (within the six months following infection)[234]. It has been shown that the integrity of mucosal epithelial barrier, can be directly breached upon exposure to HIV-1 allowing translocation of virus and bacteria. the gut microbiota itself is impacted and the altered bacterial communities possibly play significant roles in the pathogenesis of chronic HIV infection. ART alone does not provide an effective control of microbial translocation through the gastrointestinal tract, nor leads to a complete restauration of the disturbed bacterial communities.
Additionally, it was suggested that following HIV infection, dysfunction in the switching of B cells could cause a loss of IgA and IgG in the gut, and a negative correlation between IgA titer in plasma and bacterial translocation could be observed[226,235]. A correlation was also reported between the loss of IgA in plasma and intestine during HIV infection and the level of inflammation markers[235].

Hence, the disruption of the gut leads to both an imbalance of the intestinal microbiota composition (dysbiosis) and to the release of bacterial products in the circulation (microbial translocation) that induce chronic immune activation and inflammation, and the initial integrity and immune competence of the gut cannot currently be restored[27,236,237].

The depletion of intestinal CD4+ T cells caused by HIV/SIV in primary infection particularly impacts Th17 cells. This lineage of CD4+ T cells is characterized by the production of the two interleukins IL-17 and IL-22. These cytokines promote epithelial regeneration, production of tight junction proteins, production of antibacterial defensins, and recruitment of neutrophils to areas of bacterial infection. Therefore, Th17 cells are thought to be crucial for mucosal

immunity, and their specific loss may impair the ability of PLWH to maintain the physical and immunological integrity of the mucosal barrier[99].

ILC3s are the innate lymphoid cell (ILC) that mirror Th17 cells, and share many functions with them. Both cells combat extracellular microbes, such as bacteria and fungi. A lack of ILC3s in the intestine can lead to a loss of control over the symbiotic microbiota[238]. As in the case of Th17 cells, there is an early and sustained abrogation of ILC function and numbers during HIV infection, most notably ILC3 in the gastrointestinal tract [239–241]. Since ILC don't express CD4 on their surface, their depletion is most likely due to a number of yet unknown indirect effects. The exact degree to which gut ILC3 loss contributes to HIV/SIV-associated intestinal pathology is unclear. Also, other particular or newly described IL-17 producing cell types, such as γδ cells and MAIT cells are also impacted in the gastrointestinal tract during HIV/SIV infections [241].

Early ART restores numbers of Th17 cells but not their function[152]. Other studies also showed that while early ART restores Th17 cells it does not restore the normal levels of Th22 cells in the gut[153].

III.4. Persistent inflammation and chronic immune activation of treated HIV infection.

Chronic immune activation is recognized as the major driver of progressive CD4+ T cell depletion and the immunosuppressive state associated with untreated HIV infection. Chronic immune activation established during pathogenic HIV and SIV infections impairs the integrity and functionality of the host immune system and causes tissue damage by diverse and interconnected mechanisms[99]. Whether it is associated with the extent of the reservoir size is debated. Several mechanisms could link chronic inflammation and HIV reservoirs. One of these is that generalized immune activation provides available targets for HIV replication. Indeed, activation, proliferation, and differentiation of naïve and memory CD4+ T cells lead to increased CCR5 expression that renders these cells more susceptible to infection. Loss of specific CD4+ T-cell subsets might result in increased proliferation of some cells due to homeostatic balance. In addition, interferon-stimulated chemokines in tissues

can attract novel target cells to the sites of inflammation and HIV production triggering a vicious cycle.

Chronic immune activation can also contribute to the HIV-associated immunosuppressive state by inducing regulatory molecules (ie PD-1) and regulatory cells (ie Treg). These would lead to a suppressive effects on the anti-viral iimmune responses, leading to less efficient viral control[99,227,242].

Moreover, the proliferation and activation of immune cells (such as monocyte and macrophages) together with increased levels and a prolonged presence of many proteins (IL-6, sCD14, C-reactive protein, D-dimer, TGF-b1 etc.) can induce damage in the vascular system, as well as lipid abnormalities, and result in cardiovascular diseases. They also facilitate the proliferation of premalignant cells and malignant cells, thus predicting development of non-AIDS-associated cancers[99,243,244]. Thus, chronic immune activation contributes to premature aging and non-AIDS-related events.

Many of the previously mentioned elements and features of the pathophysiology of HIV infection are responsible for a state of chronic immune activation. This latter can be induced as a consequence of several, non-mutually exclusive mechanisms [99]:

(i) The direct innate and adaptive immune responses against the virus and its antigens. Nonetheless, even if immune activation and inflammation are significantly lower in PLWH who control viral replication spontaneously or under ART, high levels of HIV replication are neither sufficient nor necessary to induce pathological levels of immune activation. The Type I interferons and pro-inflammatory mediators produced leads to the activation/maturation of innate immune cells that can then produce other inflammatory molecules[27,99]. IFN-I which is produced at detectable levels in the blood when the viremia levels are high, such as in primary infection, can be responsible for the elevated expression of proteins encoded by interferon-stimulated genes (ISG). In the chronic phase of infection, ISGs might rather be induced by IFN-g[245].

(ii) Viral proteins, such as Tat or Nef, that have been suggested to be responsible for making infected CD4+ T cells highly sensitive to re-stimulation through the T-cell receptor.

(iii) Loss of mucosal integrity of the gastrointestinal tract leading to microbial translocation. Among translocated bacterial and fungal products, peptidoglycan, lipoteichoic acid, LPS,

flagellin, ribosomal DNA, and unmethylated CpG-containing DNA may induce the production of pro-inflammatory cytokines, such as TNF-a, IL-6, IL-1b, and type I interferons, in various immune cell types, such as macrophages.

(iv) Loss of specific CD4+ T-cell subsets. This might result in increased proliferation of some cells due to homeostatic balance. Moreover, the ratio of Regulatory T cells (Tregs) and Th17 cells in the gut is reduced[246–248]. Tregs have been reported to be increased in the gut and LN[249–254]. HIV-induced pro-inflammatory molecules sustain an abnormal development of regulatory T cells (Tregs) in the thymus [255]. Tregs appears to act like a double-edged sword in the context of HIV infection. Their effects on disease outcome depend on the equilibrium between attenuating HIV-induced immune hyperactivation and mounting an appropriate immune response against HIV-1 and opportunistic pathogens[254].

(v) immune response to reactivated infection, notably in the absence of treatment, such as highly prevalent latent viruses, including cytomegalovirus (CMV), Epstein–Barr virus (EBV), and or other Herpesviridae.

Effective inhibition of viral replication alone, as successfully provided by ART, is not sufficient to restore a fully functional immune system and to prevent its premature aging[152,153,256–258]. Even with long-term ART, HIV-associated chronic immune activation is rarely resolved. Early ART initiation is associated with stronger decreases in immune activation[152,259]. In most cases, however, ART is initiated in chronic infection (in France, 3 years after estimated time of infection). Some abnormalities are though not always restored as mentioned above[152,153,256–258]. The residual immune activation is considered a major player for the increased 'non-AIDS' morbidity and mortality observed in treated HIV-infected individuals. Searching for therapeutic interventions with immunomodulatory aims would be highly beneficial for the clinical management of HIV-infected individuals. For this purpose, a better identification of the underlying mechanisms to HIV-associated chronic immune activation, and their understanding at the molecular, cellular, and pathophysiological levels, as well as their interactions is crucial to better target immune activation in PLWH in a specific and effective way. Curative therapies will need to be complemented by combined immunomodulatory approaches (*Figure 8*). Even in the absence of a successful eradication or substantial reduction of the HIV reservoirs, novel therapeutic interventions improving the

recovery of a competent immune system and reducing activation/inflammation-induced cardiovascular disease, neurocognitive dysfunction, and non-AIDS defining cancers, among others, would already be highly beneficial[27,99].

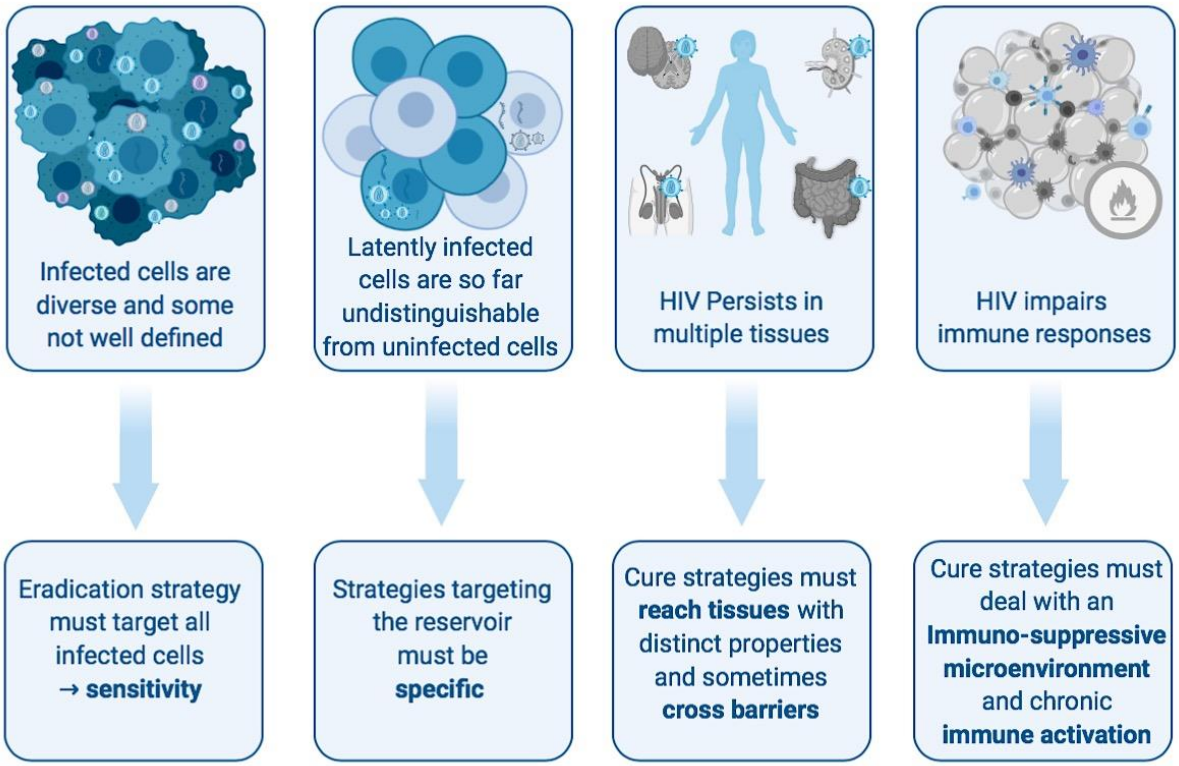


Figure 8. Overview of the main challenges to cure HIV and their implications.
There is a large variety of HIV infected cells, that are not all well-defined. Strategies aimed at eradication must target all cells harboring replication competent virus. Latently infected cells generally undistinguishable from uninfected cells. Strategies aimed at reducing the size of the reservoir must be specific and avoid excessive damage through elimination of non-infected cells while successfully targeting preferentially the HIV-infected cells. HIV persistence occurs in multiple tissues. Cure strategies must reach tissues with distinct properties and sometimes cross barriers to attain HIV-infected cells to the right place. Immune responses are impaired in the course of HIV Infection. HIV infection is associated with residual chronic inflammation that induces exhaustion and immune aging. Cure strategies must consequently deal with an immuno-suppressive microenvironment in tissues.
Adapted from slides by Nicolas Chomont (IAS 2015) and Michaela Müller-Trutwin (HIV DART and Emerging Viruses 2018).

IV. Why do we need animal models? What are they?

IV.1. General considerations on the use of animals in research

Animals have been involved in biological research and medicine as a long-established practice. The use of animal models enables to investigate a variety of mechanisms that are relevant for human diseases and provide means to assess novel therapies before they are applied to humans[54].

It should be stressed however that not all results obtained on animals can be directly translated to humans. Indeed, results are not necessarily confirmed in further human studies because of various reasons. First, one must keep in mind that there are always significant genetic and physiological differences between a given animal species and humans, despite their numerous similarities. These differences have to be thoroughly described and understood in order to be taken into account in experimental designs and interpretation of observations. If they can represent challenges, they can also eventually lead to the discovery of novel mechanisms and enable to imagine innovative therapies. A second reason that could explain the difficulty to translate the results obtained on animals to humans is the highly homogeneous genetic composition of the animals used in many animal models, which in those cases does not reflect the genetic and physiological variations within each species. There does not exist an animal model that is able to totally mimic a given human disease, which is itself polymorphic between patients. Yet the differences that characterize animal species and strains can represent remarkable opportunities to understand differential host response, disease development and diverse host-pathogens interaction (particularly in the case of infectious diseases), which can actually benefit the research for new cures[54].

The legitimacy of using animals to benefit human purposes, with the possibility that animals are harmed, has been increasingly debated while the status of the animals in our modern societies is at the heart of many ethical and political debates. This aspect, alongside with the above-mentioned fact that not all results obtained on animals can be directly translated to humans, often mixed in confusing arguments, are often used to refute any value to animal research. The issues raised by the use of animals for scientific purposes are not always clearly and properly addressed. Hence, it is an important matter to conciliate the perspectives, needs and concerns of the animal-protection community and the animal-

research community committed to the scientific value and moral acceptability of laboratory animal research[54,260].

In this perspective, some guidelines have been established to figure out the best approaches to permit high-quality science while ensuring the highest standard of ethical consideration is applied in regulating the use of animals in scientific procedures. The most influential framework has been provided by 3 directives, often referred to as "the 3Rs", first introduced by zoologist and psychologist William M. S. Russell and microbiologist Rex L. Burch in 1959 in *Principles of Humane Experimental Technique*[261]. Russell and Burch's 3Rs principles call for replacing sentient animals with other models where possible, reducing the number of animal subjects to what is needed for statistical adequacy, and refining techniques to reduce animal pain and distress. After a gathering that occurred in Basel (Switzerland), in 2010, a group of academic researchers representing various research fields that depend on research involving animals adopted the Basel Declaration as a call for more trust, transparency and open communication on animal research. They publicly committed to the 3Rs and to respect the highest ethical and animal welfare standards in carrying out research using animals[262]. Nowadays, some additional principles are also used, such as the exigency that the experiments performed on animal models should represent a prospect of making substantial and otherwise unattainable social benefit permissible, or the requirement that harming of animals in research is limited by identifiable considerations of animal welfare[260].

It is worthy to note that animal research has contributed to undisputable medical advances. Of the 106 Nobel Prizes awarded for Physiology or Medicine, 94 were dependent on research using animals, including every single prize awarded for the past 30 years[263]. All chemotherapy and radiotherapy treatments against cancer, as well as most cancer detection methods, or organ transplantations are the results of animal research, generally in mice and rats, but also NHP (i.e. heart transplantation). Other examples include immunizations against Polio, Diphtheria, and Rabies, that have been developed thanks to animal models, including large animals, and have then saved countless human lives. What's more, preclinical testing of new drugs is still highly reliant on animal procedures, and only an estimated 10% of new drugs successfully pass to acceptance for clinical use. It seems impossible in this context to currently replace animal testing in the absence of alternatives[54,264].

At last, it should be mentioned that animals also benefit from animal research, whether

directly through veterinary medicine, with 90% of the veterinary drugs used to treat animals being identical or highly similar to those used to treat humans[54], or through a better comprehension of diseases that affect animals, which can be used for conservation purposes in the wild. For example, it has been shown that chimpanzees (*Pan troglodytes troglodytes*) infected with SIVcpz, the ancestor of HIV-1, also suffer from decreased lifespan and AIDS-similar immunopathologies. This discovery, which can contribute to understand the population dynamics stakes of this endangered species, has been permitted by noninvasive samplings and observations in the wild but also benefited from the general knowledge on lentiviruses that was gained using animal models[265–268]. These important bioethics considerations are widely discussed elsewhere[260,264,269,270].

IV.2. Animal species used for research on HIV cure

IV.2.1. Introduction to the use of animal models in HIV research

The only animal species susceptible to HIV viruses are non-human primates (NHP). That is the major reason why NHP are the most widely used animal models in HIV science. Among NHP, the main used animal model corresponds to Asian monkeys (macaques) infected with viruses close to HIV-2 (SIVmac). The inoculation of SIV to the latter leads to persistent infection and progression to an acquired immunodeficiency syndrome, generally happening in a similar way as in PLWH. They therefore provide an important model for the study of lentivirus-associated pathogenesis[58,271] Almost all of the pathophysiological features of HIV-1 pathogenesis in humans are recapitulated in this model, including viral targeting of CD4+CCR5+ T cells, very early depletion of CD4+ T cells at mucosal sites, massive CD4+ T cell depletion in the gut in the very early phase of infection, and associated damage to the gut epithelium, translocation of microbial products from the gut lumen into the host, chronically elevated levels of immune activation, progressive depletion of CD4+ T cells in the periphery, and the eventual development of opportunistic infections and malignancies[58,272].

The implementation of an animal model for a searching an HIV-1 vaccine still represents a significant difficulty. This difficulty is due to the fact that HIV-1 tropism is strictly limited to humans and chimpanzees. Hence, the first animal models developed and use in the fight against HIV involved chimpanzees, a model today abandoned for ethical reasons[61,273].

Historically, feline models using cats infected with immunodeficiency virus (FIV) also served as a model of naturally occurring immunodeficiency. As in HIV/SIV infection, FIV infection is responsible for a CD4+ T cell depletion and the establishment of a chronic inflammatory state. Hence, the FIV model has been used in the past to test some antiretroviral drugs[274]. However, FIV infection does not reproduce all characteristics of HIV infection. FIV mutation rate differ from HIV and that it evolves more slowly at the intra-individual level. Moreover, FIV-infected cats establish different viral reservoirs than HIV in humans, since FIV can use CD134 as a receptor and is consequently able to infect CD8+ T cells and B cells in addition to CD4+ T cells and macrophages[58,275]. Since some important features of the infection are clearly different in cats and in humans, this model is hardly ever used anymore.

Mice, rats or rabbits have been initially proposed, but they couldn't be successfully infected with HIV. Attempts to overcome this species-specificity issue by using mouse and rats engineered to express the human viral receptor on the cell surface were unsuccessful. Indeed, HIV interactions with its receptor CD4 and co-receptors CXCR4 or CCR5 on host cells is not the only parameter responsible for its species-specificity. Additional factors limit the ability of HIV to replicate in cells from other species, including viral restriction factors like TRIM5α and APOBEC3[58,276,277].

More recently, humanized mice models have been developed that can be infected by HIV and used to answer specific questions[278–281].

IV.2.2 The use of macaques as a model of HIV infection

Macaques, once infected with SIVmac, can reproduce all the distinct outcomes of infection observed in humans: Some animals can progress rapidly (in a few months) towards an AIDS-like disease, while the majority will progress toward disease after a period of several months/years of chronic infection, and at last, a minority of macaques can spontaneously control HIV infection, similarly to what is observed in human “elite controllers” or ”HIV controllers”[55,219,282]. Such viral control is often associated with specific MHC alleles.

Two species of macaques are principally used as models in HIV science: rhesus macaque (*Macaca mulatta*) and cynomolgus macaque or crab-eating macaque (*Macaca fascicularis*). The pigtailed macaque (*Macaca nemestrina*) is used less frequently (*Figure 9*) . Each species displays a distinct susceptibility to SIVs and disease progression profile, which also varies according to the geographic origin of the animal. The most frequently used rhesus macaques (RM) are of Indian and Chinese origin. Compared to Indian RM, Chinese RM, when infected by SIVmac, have lower plasmatic viral loads. The dynamics of their virological and immune markers is more close to the one observed in humans[55,60,283].

Cynomolgus macaques (*Macaca fascicularis*) display variable levels of viremia and disease progression, depending on the host, the viral load and route of infection. When infected intrarectally with a high viral load of SIVmac, the viremia levels and disease progression profiles are more similar to that observed in Chinese rhesus macaques or humans[284]. Spontaneous control is though more often observed than in rhesus macaques and humans. Cynomolgus macaques used in experimental reaearch often are from the island of Mauritius, Mauritius contains a population of cynomolgus macaques that descended from a small number of founder animals that since their establishment have been geographically isolated from other cynomolgus macaques. As a result, this population of animals has limited MHC class I and II diversity and has become an intriguing tool for studies seeking to more feasibly research on MHC haplotypes than might be possible for other, more outbred NHP populations[55,272,285–288].

Pigtailed macaque (Macaca nemestrina), when infected by SIVmac can represent a model of rapid progression to AIDS, the progression to disease generally occurring in three months. In the absence of SIV infection, pigtailed macaques have been shown to have higher levels of baseline immune activation, in association with greater intestinal epithelial permeability

compared with rhesus macaques[289]. It is possible that residual immune activation levels are higher in pigtailed macaques than in SIV-infected RMs or HIV-infected humans in the setting of combined ART[272]. Notably, the pigtailed macaque model has been used to study the central nervous system diseases and reservoir[290].

However, in macaques, and in monkeys in general, the diversity of MHC sub-combinations, as well as other immune components, such as their various Fc receptors, KIR genotypes and interferon alpha subtypes remain poorly characterized for the time being and need further investigation.

Thus, the chosen model can vary according to the species and origins of the macaque, as well as the virus strain used (which will be developed in part IV.3.2.) and the experimentally controlled parameters of infection (viral load, route, time, …). In that respect, what can be inferred from a macaque model is highly dependent on the parameters employed, and those must be wisely set up according to the question addressed.

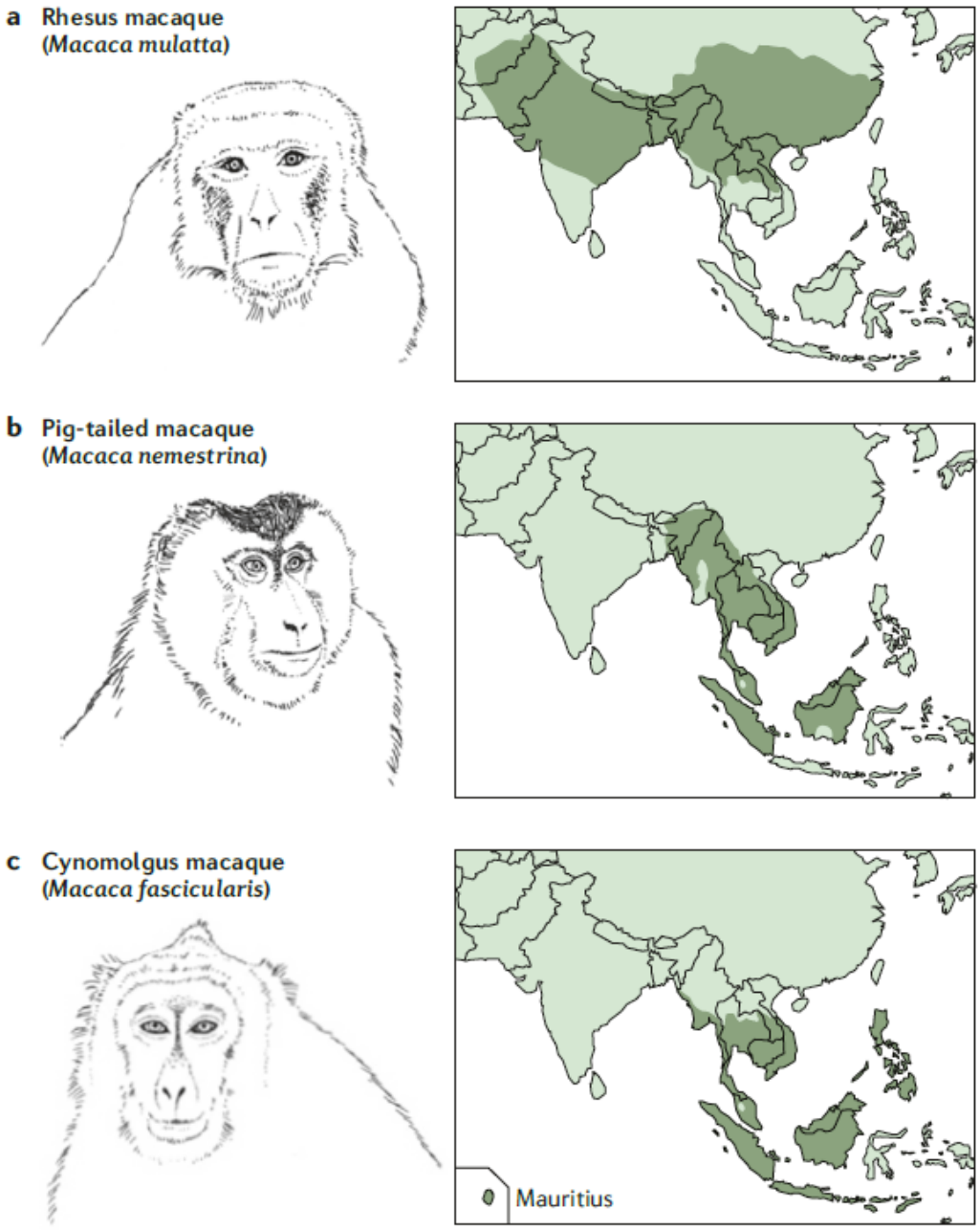


Figure 9. Macaque species commonly used in AIDS research, and their geographical ranges.
a. *The geographical range of the rhesus macaque (Macaca mulatta) exceeds that of all other primate species except humans, extending from western India and Pakistan across China. Captive-breeding programmes in the United States were initially established using animals imported from India, contributing to the widespread use of Indian-origin rhesus macaques in AIDS research. However, owing to the increasing demand for rhesus macaques and an embargo on the exportation of these animals from India since 1978, there has been a substantial decline in their availability and a sharp increase in their cost. This has led to greater dependence on rhesus macaques imported from China and Burma.*
b. *The pig-tailed macaque (Macaca nemestrina) is native to Southeast Asia, Malaysia and Indonesia, and last shared a common ancestor with rhesus macaques approximately 3.5 million years ago.*
c. *The cynomolgus macaque (Macaca fascicularis), also known as the long-tailed or crab-eating macaque, is native to regions of Indochina, Malaysia, Indonesia and the Philippines. Genetic evidence suggests that cynomolgus and rhesus macaques diverged from a common ancestor approximately 1.9 million years ago. Of note, the breeding center for the cynomolgus macaques used in research is located on Mauritius islands. The animals there display a genetic bottleneck Adapted from Hatziioannou, T. & Evans, D. T. Animal models for HIV/AIDS research. Nat. Rev. Microbiol. 10, 852–867 (2012).*

IV.2.3 The use of Humanized mouse models in HIV research

With the advent of humanized mice, it became possible to infect these animals with HIV-1 and use humanized mice models to answer some specific research questions. These models are produced via transplantation of CD34+ stem cells and/or implantation of human tissue into immunodeficient mice[291]. The unique capacity of human hematopoietic stem cells to engraft, expand, and repopulate immunodeficient mice with virtually all different types of human immune cells has been used to conceive different humanized mice models. Systemic or local reconstitution can be obtained in mice with human hematopoietic cells, depending on the strain of mouse and on the use of whether tissue or CD34+ cells. The various humanized mouse models are repopulated with different human immune cell populations that can include B cells, monocytes/macrophages, dendritic cells, NK cells and T cells. Nonetheless, as far as T cells are concerned, they can only be generated in certain mouse strains, where they are produced in the mouse thymus and thus supposedly educated in the context of mouse major histocompatibility complex (MHC)[292]. To more accurately obtain human T cells that are educated in the context of human MHC (HLA molecules), human thymus tissue is generally implanted under the mouse kidney capsule. In order to avoid thymic tissue involution and disappearance, a piece of autologous human liver can be co-implanted. This way, a functional human thymus 'facsimile' is obtained, lifelong persisting in the mouse and continuously producing human thymocytes. In this case, T cells can grow and expand in the context of HLA class I and II restriction. Examples of such humanized mice models used in HIV research comprise SCID-hu thy/liv mice, T cell-only mice (ToM), and bone marrow/liver/thymus (BLT) mice. By contrast with SCID-hu thy/liv mice and ToM, BLT mice also receive an autologous human bone marrow transplant after the implantation of human liver and thymus tissue. Hence, BLT mice are virtually reconstituted with all types of human hematopoietic cells, including T cell progenitors[276,277,291]. (*Figure 10*)

More recently, a humanized mouse model expressing human HLA-A2 and -DR2 transgenes have been developed to improve the research on antigen-specific T and B cells in the context of human pathogen infections[293].

Importantly, HIV-infected humanized mice can be treated with the same antiretroviral drugs that are used in humans and ART treatment results in systemic recovery of CD4 T cells. And the animals can be infected by the same natural routes as in humans[58,61,276–279,291,292].

However, the use of humanized mice models faces some significant limitations. Their development requires a source of human hematopoietic stem cells as well as complex and invasive surgical engineering techniques, sometimes considered as ethically debatable. A long waiting period is needed until full immune reconstitution is observed: the timeframe needed for mouse production can last up to 7 months, and they need to be generated de novo for each experiment. The cost of their establishment and maintenance is high[55,58,60,276,278,294]. They cannot be bred and have a limited lifespan, all the more so as graft-versus-host disease may develop and restrict timescale of experiments. The small size of these animals limits the study of many tissues and implies that only a relatively small volume of peripheral blood plasma can be obtained from the same animal. There remain significant anatomical differences with humans, such as the lymphoid organs that are not fully developed and do not display the full physiopathology of HIV infection. Murine pharmacokinetic characteristics and drug metabolism also differ from humans. Finally, rodents display an innate immune system that display many differences with regard to human innate immune cells[55,58,60,276,278,294].

In conclusion, humanized mice models seem to be an appropriate and useful model for the study of antivirals and several viral parameters. With regard to the analysis of the physiopathology and immune responses against the virus, the model still needs major improvements.

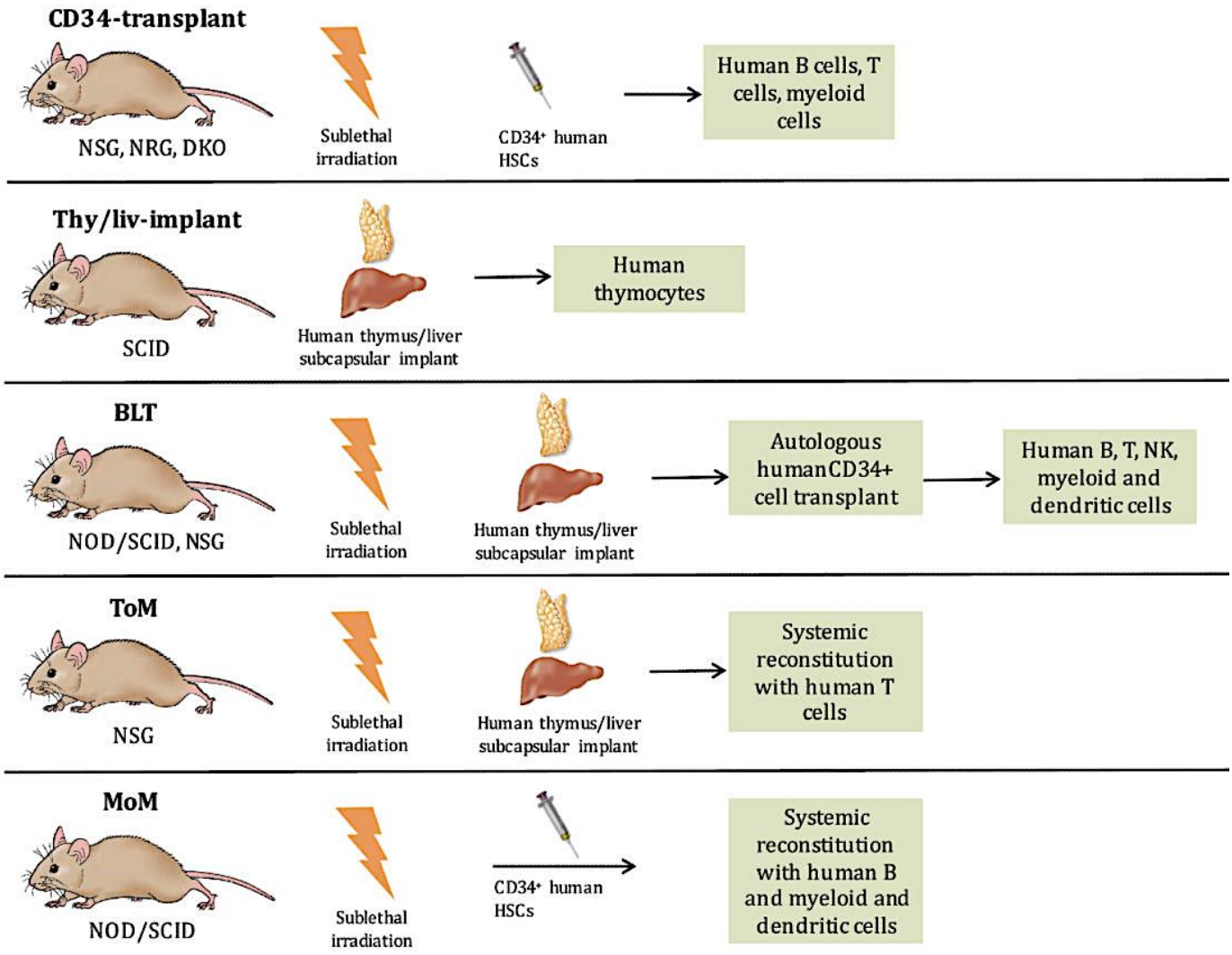


Figure 10. Five humanized mice models used in HIV research.
Illustrated are five different humanized mouse models that have been extensively used for the study of HIV in vivo. Note that each model has a different type and/or distribution of cells. For example, ToM have systemic repopulation with human T cells whereas MoM do not have any T cells but have a full complement of antigen presenting cells. BLT mice have both all different types of T cells in addition of a full complement of antigen presenting cells. Adapted from Victor Garcia, J. Humanized mice for HIV and AIDS research. Curr. Opin. Virol. 19, 56–64 (2016).

IV.3. Viruses used to infect animal models of HIV

IV.3.1 Isolation of SIVs and origin of HIV

The first known simian lentivirus was isolated in 1985 in rhesus macaques (RM, Macaca mulatta) of Indian origin kept in captivity, and later named SIVmac (Simian Immunodeficiency Virus, macaque)[56,295]. These monkeys presented clinical symptoms similar to AIDS in humans. Nonetheless, SIV infection has not been observed in wild macaques, nor in other species of Asian monkeys in their natural habitat. Only African primates carry lentiviruses in the wild. The first African primate species identified as a natural SIV carrier was the sooty mangabey (*Cercocebus atys*), which can carry the virus SIVsmm[296]. It has been subsequently showed that SIV infection in captive macaques resulted from experimental tissue transmission from sooty mangabeys that happened to be infected by SIVsmm and held in captivity in the same primate research centers[56].

Simian lentiviruses (grouped under the denomination *simian immunodeficiency viruses*, SIVs) have been identified in more than 40 African non-human primate species in the wild. Hence, these primates constitute a vast reservoir of lentivirus that can potentially cross species barriers[57,297] . The HIV viruses present in humans are the result of at least 12 zoonotic transmission events of African primate lentiviruses to humans[298,299] (*Figure 11*). The most frequent HIV and main causative agent of AIDS worldwide is HIV type 1 group M (HIV-1 M).

In the present state of knowledge, three species – namely chimpanzees (*Pan troglodytes troglodytes*), gorillas (*Gorilla gorilla gorilla*) and sooty mangabeys (*Cercocebus atys*) transmitted their viruses to humans[300–303] (*Figure 11*). HIV-1 group M and group N (more rare) originated from SIVs infecting chimpanzees (*Pan troglodytes troglodytes*)[300]. In contrast, strains more close to HIV-1 groups O and P have been found in gorillas (Gorilla gorilla gorilla) who might have themselves acquired HIV from Chimpanzees[57,302]. The second human immunodeficiency virus, HIV-2, for its part, originated from sooty mangabeys (SM, *Cercocebus atys*)[303]. SIVsmm was transmitted at least in 9 independent occasions, resulting in HIV-2 group A to I, among which only HIV-2 A and B (as well as a recombining form of these two) significantly spread in humans[304].

The seroprevalance varies depending on the species, sub-species and age of the animals. It is very high (45-50%) in adult sooty mangabeys, African green monkeys (AGM, Chlorocebus

sabaeus) and mandrils (Mandrillus sphinx). The natural habitat of sooty mangabeys is West-Africa and of mandrills Central Africa. AGMs are present in all subsaharian Africa except in tropic forests and constitute the largest reservoir of SIVs[305]. Mandrills (*Mandrillus sphinx*) are infected with two types of SIV: SIVmnd type 1 (SIVmnd-1) and SIVmnd type 2 (SIVmnd-2)[306–309]. Humans with antibodies cross-reacting with SIVmnd antigens have been reported, but there were no human blood samples available to analyse the sequence of the virus. SIVmnd-infected mandrills share a number of features with SIV-infected AGMs and SMs and generally do not progress to disease[309].

Sooty mangabeys, AGMs and mandrills display high viremia but do not develop any immunodeficiency despite high replication rates[310]. These three species are commonly called natural hosts of SIV[311]. They therefore represent models for nonpathogenic infection. In contrast, macaques – which are not SIV-carriers in their natural habitat – will often develop an AIDS-like syndrome after SIVmac infection[58]. Hence, they are frequently used as model for pathogenic infection.

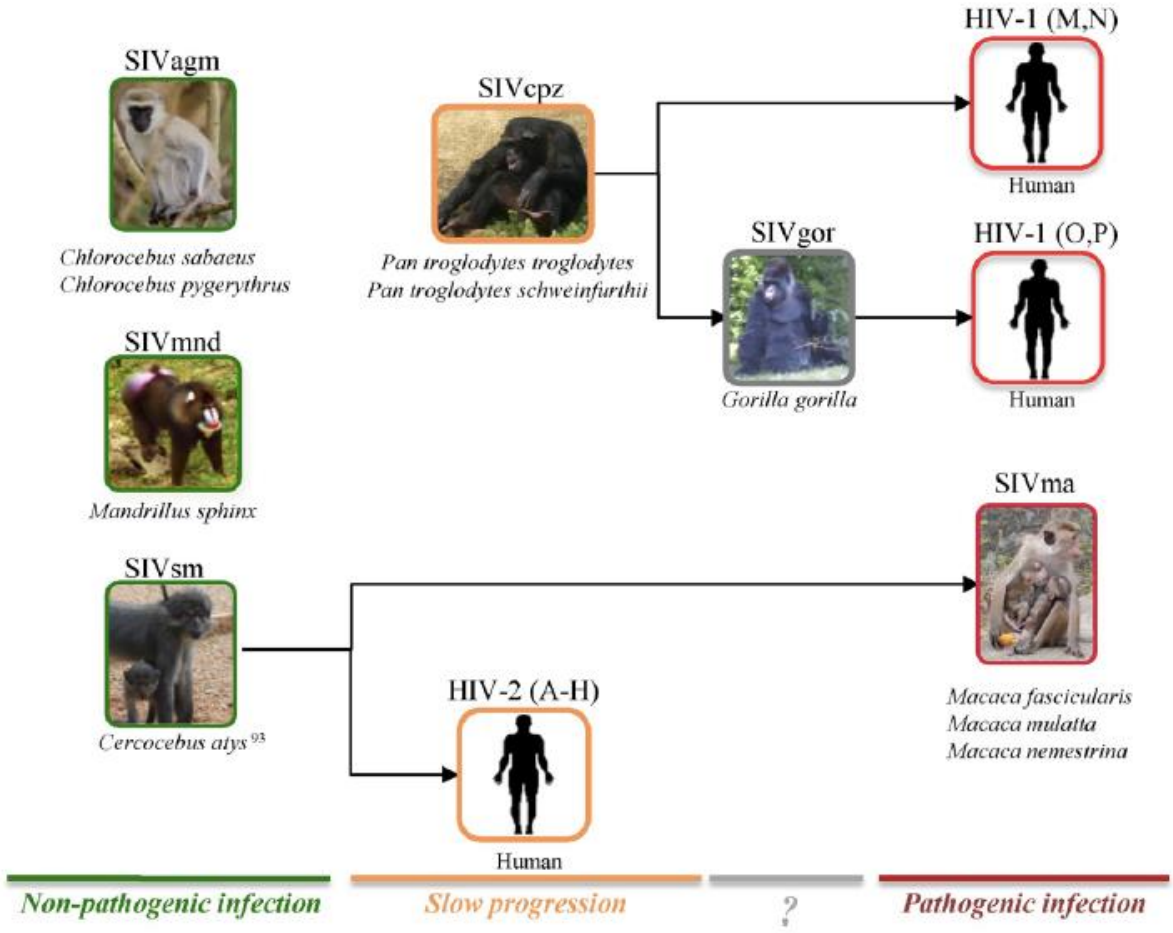


Figure 11. Simplified representation of primate lentiviruses od interest, their natural and non-natural hosts, and their major transmission events.

Non-human primates can be divided into pathogenic (macaques) and non-pathogenic (sootymangabeys,mandrills and African Green Monkeys) hosts of SIV. The figure depicts the relationship between the HIV and SIV viruses and the type of infection caused by these viruses.

Adapted from Garcia-Tellez, T. et al. Non-human primates in HIV research: Achievements, limits and alternatives. Infect. Genet. Evol. 46, 324–332 (2016).

IV.3.2. Lentiviruses used to infect animal models

Several lentiviruses can be used to infect animal models, with distinct degrees of virulence. These notably include SIVmac239, SIVmac 251 and SHIV for NHP models. Humanized mice models are generally infected with HIV-1.

Since rhesus macaques of Indian origin were the first recognized non-natural hosts of pathogenic SIV infection, the initial development of SIV for animal models involved serially passaging virus from animal to animal within Indian-origin rhesus macaques, with selection of virus swarms and molecular cloning of infectious viral genomes associated with rapid or attenuated disease profile. In this respect, the most widely used and best characterized pathogenic SIVs are exquisitely well adapted to Indian-origin rhesus monkeys. The SIVs thus obtained were called SIVmac lineage[272]. Most of Indian-origin rhesus macaques develop high peak viral loads (generally exceeding 10^7 viral RNA copies per mL of plasma) and sustained high chronic viral loads when infected either with the uncloned viral isolate SIVmac251 or the infectious molecular clone SIVmac239[312]. A more rapid disease progression is generally observed with SIVmac239. Some other (rarely used) SIVmac strains display lower virulence, including SIVmacΔnef and SIVmac1A11. The latter lead to an attenuated infection in rhesus macaques[58,313,314]. (*Figure 12*)

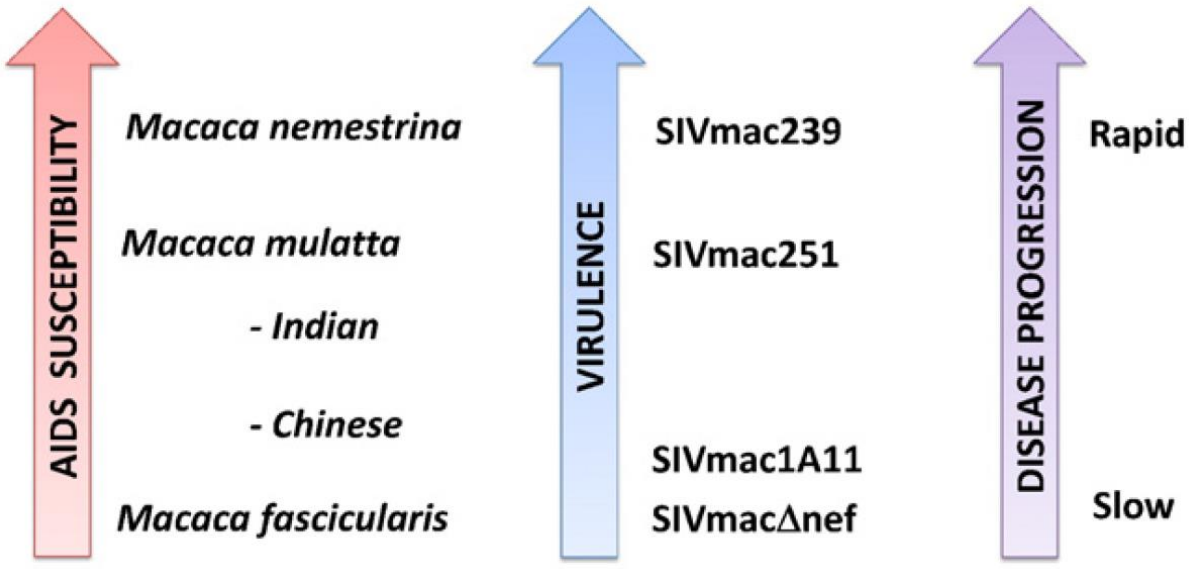


Figure 12. Host and viral determinants of disease progression rate.
The progression of the disease depends on the intrinsic susceptibility of each species to develop AIDS and on the virulence of the SIV strain used.
Adapted from Garcia-Tellez, T. et al. Non-human primates in HIV research: Achievements, limits and alternatives. Infect. Genet. Evol. 46, 324–332 (2016).

SIVsmm and SIVagm viruses are generally non-pathogenic in their respective natural hosts, but rhesus and pigtailed macaques, when infected with certain SIVsmm, or SIVagm strains progress towards AIDS-like disease. This is the case of rhesus macaques infected with SIVsmm9 and pigtailed macaques infected with SIVagm.VER90 or SIVagm.sab92018. However, rhesus macaques infected with SIVagm.VER90 and pigtailed macaque infected with SIVagm.VER155 remain asymptomatic. SIVagm.sab92018 infection in rhesus macaques results in something similar as what is observed with HIV infected "elite-controllers" in humans; and consequently, SIVagm-infected RMs have been suggested as an animal model for Elite-Control of HIV infection[315].

The outcome of SIV infection in NHP is therefore determined by the combination and complex interactions between viral and host determinants that are still not sufficiently well understood[58,60,315–320].

Vaccine studies are hampered by the fact that macaques are not susceptible to HIV-1 infection. In particular the study on the efficacy of neutralizing antibodies is dependent on the possibility to challenge animals with viruses coding for the HIV-1 ENV gene. To address these limitations, SIVmac strains have been engineered where chimeric simian-human immunodeficiency viruses (SHIVs) were produced where SIVmac ENV has been replaced by HIV-1 ENV. Similarly, to overcome the resistance to some drugs (NNRTIs) by SIVmac for use in RMs and pigtailed macaques, SHIVs have been engineered coding for the HIV-1 reverse transcriptase (RT) gene. Two RT chimeras have been produced: RT-SHIVmac239 and RT-SHIVmne. RT-SHIVs still have their limitations, including difficulty in suppressing virus replication with the same triple ART treatments used in humans (i.e., tenofovir/emtricitabine/ efavirenz)[60]. In addition, SHIVs in general have limitations as they do not fully reproduce the physiopathology of HIV-1 and SIVmac infection. Thus, the infection is more often attenuated and the CD4+ T cell subset dynamics different.

Primate species (origin)	SIV species/strain	Spontaneous control of infection	Rapid progression towards AIDS-like disease	Controlled viremia on ART	Chronic immune activation
 Rhesus macaque (Indian)	SIVmac251/239	YES depending on genotype	YES 20-30%	YES	YES
	SIVsmm	YES depending on TRIM5α	NO	YES	YES
	SIVagm	YES 100%	NO	no information available	NO
 Rhesus macaque (Chinese)	SIVmac239	YES depending on genotype	YES 20-30%	YES	YES
	SIVmac251	no information available	YES (rare)	no information available	yes
 Pigtailed macaque	SIVmac251/239	YES (rare)	YES 30-40%	YES	YES
	SIVsmm	NO	YES >75%	YES	YES
	SIVagm	NO	YES 30-40%	no information available	YES
 Cynomolgus macaque	SIVmac239	no information available	YES (rare)	no information available	YES
	SIVmac251	YES depending on genotype	NO	YES	YES
 African Green Monkey	SIVagm	NO	NO	no information available	NO
 Sooty Mangabey	SIVsmm	NO	NO	YES	NO

Table 2. Main models of SIV infection in NHP studied in HIV research.
This chart provides a non-exhaustive overview of non-human primates most often studied or used as models in HIV research. The principal features of each species/strain are indicated in the table. The viral load and other pathophysiological features vary depending on various parameters such as species and genetic background, age, viral species or strain, laboratory and sampling techniques, to mention just a few. Studies performed in Chinese rhesus macaque and in cynomolgus macaque are less frequent than those performed in Indian rhesus macaques. The amount of available data regarding these animals is therefore limited.
Inspired by Huot, N., Rascle, P. & Müller-Trutwin, M. Apport des mod.les animaux dans la recherche sur le VIH. Virologie 23, 229–240 (2019).

IV.4. The study of natural SIV hosts and some insights it provides

IV.4.1 Characteristics of SIV infection in natural hosts

Natural hosts of SIVs share some common features that delineate their specific pattern of infection.

(i) First, Natural hosts display high viremia levels similar to those seen in pathogenic SIV infection of RMs and HIV infection of humans[309,321–325]. During the chronic phase, the virus continues replicating to high levels, in most cases at around 10^3-10^6 SIV RNA copies/ml of plasma, but AGMs do not progress to AIDS.[323] These characteristics are similar to the 'viremic non-progressors', very rare human individuals who display elevated viremia but maintain CD4 T+cell counts and avoid disease progression for years[326].

(ii) Natural hosts avoid chronic immune activation, which is the driving force of CD4+ T cell depletion and progression to AIDS in humans[245,322,327–330]. SIVagm and SIVsm trigger a potent type I-interferon (IFN) production, but this response is rapidly controlled in AGM[331]. After the acute phase, the immune activation is controlled and returns close to pre-infection levels[327,331].

(iii) Natural hosts display strong control of infection and immune activation in secondary lymphoid tissue (SLT). AGM display a strong control of viral replication in LN (both T zone and B cell follicles) shortly after peak viremia, that lasts throughout infection. The viral control is mediated by NK cells. The latter express CXCR5 in SLT during SIVagm infection and are able to migrate into follicles and contribute to this efficient control of viral replication in LN follicles[233,332,333]. This constitutes a striking difference to HIV in humans and SIVmac in RMs where virus persist in lymph node "sanctuary sites". The immune activation, including of IFN-stimulated gene (ISGs) expression, is particularly rapidly controlled in SLT. LNs do not display lymphadenopathy nor fibrosis. The FDC network in LNs remains intact in contrast to HIV-1 and SIVmac infections[233].

(iv) Natural hosts can preserve CD4+ T cell homeostasis. Expendable T cell subsets are relatively spared from infection. Thus, central memory CD4+ T-cells (T_{CM}) have been reported to be infected at a lower frequency than in non-natural hosts. Based on this observation, it has been suggested that long-lived T_{CM} cells are relatively resistant to SIV infection. Indeed, it has been shown that SM central memory CD4+ T cells exhibit low levels

of SIV co-receptor CCR5 expression and are less infected in vivo and in vitro (compared with SM effector memory CD4+ T cells and RM central memory CD4+ T cells)[334]. However, SIVsm does not need CCR5 to infect CD4+ T cells, but can efficiently use other receptors. Thus, the underlying mechanism could be a different one. For instance, the fact that SIVsm infection is strongly controlled in SLT but not in the intestine could contribute to this, since the frequency of TCM is higher in SLT than in the intestine. Whatever the mechanism is, the preservation of long-lived cells in lymphoid tissues in natural hosts can contribute to the reduced pathogenicity[321,334–336].

(v) In natural hosts, the CD3-TCR receptor is downregulated by the action of the SIV-Nef protein[337]. This might limit the levels of activation and apoptosis in CD4+ T cells[337,338]. This function of the Nef gene was lost in SIVs infecting chimpanzees and humans.

(vi) Infected natural hosts preserve their gut mucosal immune system and the integrity of their gut mucosa. Thereby they efficiently prevent the translocation of microbial products from the intestinal lumen to the systemic circulation[339,340]. In addition, no early preferential depletion of CD4+ Th17 cells is observed during SIV infection of natural hosts[246,341,342]. Th17 maintnance in the gut could positively contribute to maintain the intestinal barrier integrity[341]. A recent study[343] by Raehtz et al. documenting early SIV infection of AGMs showed that despite a strong, but transient, interferon-based inflammatory response the levels of plasma markers linked to enteropathy did not increase. They did not document any significant increase in apoptosis of either mucosal enterocytes or lymphocytes, nor any damage to the mucosal epithelium[343].

(vii) Stronger or more efficient tissue repair mechanisms might act in natural hosts of SIV. A recent study by Barrenas et al. demonstrated that monocytes from AGMs rapidly activate and maintain evolutionarily conserved regenerative wound healing mechanisms in mucosal tissues , eventually via fibronectin production and TGF-beta signature[344,345].

(viii) Of note, SIV-specific adaptive immune responses do not seem to be exacerbated in natural hosts. SIVagm-infected AGMs do not exhibit strong suppressive CD8+T cell capacities nor significant infiltrations of CD8+T cells into LN follicles[346–348]. Besides, in vitro neutralization assays showed that in SIVagm infection there are only few or no detectable neutralizing antibodies[349,350]. Thus, these stakeholders of the adaptive immune response appear to be weakly induced in SIVagm-infected AGMs, similarly as in HIV-1 and SIVmac

infections. These may still contribute to the control of viral load in SIVagm infection, but it is unlikely that they play a major role in this model. Nonetheless, some B cell responses seem to be different between pathogenic and non-pathogenic infections. AGMs do not demonstrate hypergammaglobulinemia. They develop more antibodies directed against the surface part of the ENV encoded proteins[351]. The strong gp120-specific, functional antibody responses in the milk of SIV-infected AGMs may contribute, together with a lower rate of target cells, to the rarity of postnatal transmission observed in natural SIV hosts[352,353].

Allover, SIV infection in natural hosts is characterized by a rapid resolution of inflammation and lack of tissue damage, despite high viremia. Virus and host are thus in an equilibrium where the host stay healthy while the virus is highly transmissible horizontally (between adult animals). The absence of progression towards disease in SIV-infected natural hosts may be related to synergistic or simultaneous mechanisms, and not all of these are fully understood yet.

IV.4.2 Objectives of studies in natural hosts

Because of the high viremia levels characterizing SIV infection in natural hosts, it is often considered that they could not constitute a model contributing to the search for a cure of HIV. Indeed, a similar control of disease with absence of progression but such high viremia levels can not be tolerated in humans because of the high risk of transmission. Nonetheless, we propose that natural hosts can represent excellent models to decipher the strong viral control in SLTs (in contrast with PLWH in which SLTs constitute important HIV reservoirs) and the mechanisms of protection against tissue damage, microbial translocation, and undue immune activation.

A better understanding of the mechanisms involved in the pattern of SIV-infection in natural hosts could allow the development of novel strategies to control HIV associated non-AIDS comorbidities and mortality in PLWH under ART, and guide the development of new agents to treat the HIV-associated chronic immune activation. It might prove highly useful as well for a better understanding of the regulation and impact of the early anti-viral innate immune responses. In addition, uncovering the mechanisms of TH17 preservation during natural SIV infection could enable the identification of new therapeutic targets to improve TH17 cell homeostasis in PLWH, thereby promoting the immunologic restoration of the intestinal

mucosal barrier[321]. Furthermore, the observation that the pattern of infected cells may be critical in dictating the progression to AIDS in HIV/SIV infections encourages investigations on the susceptibility and importance of the different CD4+ T cell subsets (such as T_{CM}) in natural history of infection and the development of therapeutic interventions selectively protecting the more relevant subsets[81,321].

Thus, the comprehension of the mechanisms responsible for this absence AIDS-like disease and the study of mutually beneficial coadaptations between primate lentiviruses and their natural hosts, that evolved jointly during thousands of years, might prove to be very insightful for HIV cure research[55,305,321].

IV.5. Essential insights on HIV infection obtained with the help of animal models

Some of the major past and recent advances in the field of HIV could not have been obtained without animal models[224]. Here we list some of the major contributions of animal models to HIV/AIDS research.

Important proof of concepts for preventive or curative therapeutic approaches were provided by studies performed on animal models, such as the ability of neutralizing antibodies (Nab) to protect against infection[354]; the ability of cytomegalovirus-vectored vaccines to induce wide CD8+ T cell responses against SIV/HIV[355–360]; or other curative strategies that will be developed in part V. and VI.

The cellular targets of HIV and their dynamics in blood and tissues were also better understood thanks to NHP models, including: The fact that resting memory T cells are a major target of the virus in lymphoid tissues[361,362], the fact that loss of central memory T cells is associated with disease progression[361,363], a better understanding of Trafficking of Treg, PDC, and NK cells to the gut[364–366] and the rapid and dramatic depletion of CD4+ T cells in gut[341,367]. Humanized mice models also proved helpful to study the ability for myeloid cells to support latent HIV infection and supported the possibility of a myeloid reservoir for HIV[187,194,195].

NHP models contributed to the understanding that adaptive immune responses arrive too late at the site of early viral replication. Studies in the macaques have shown that when infection takes place at mucosal sites, the virus crosses the epithelial barrier using several

distinct mechanism, rapidly establishes initial founder populations of infected cells (foci) and disseminates thereafter in draining lymph nodes (LNs)[368].

The concepts that CD8+ T cell responses arrive “too little” and “too late” into mucosa to control viral dissemination and that the “window of opportunity” to prevent infection is very short thus raised from studies performed on macaque models[369–371].

The impact of CD8+ T cells on viral set-point and the association of HLA/MHC-I genotypes with rapid or slow AIDS progression was also elucidated with the help of animal studies, notably through studying CD8+ T cell responses during the first days after infection, something that was not possible to do in humans for a long time[372].

Animal models still remain essential for the understanding of the events in tissues (the major site of viral replication) as well as for the development of curative and preventive approaches to end the HIV epidemic.[373–376].

Novel techniques are being developed, such as in vivo imaging of large animals, now models developed (ie humanized mice) and new human cohorts are being created (to allow the analyses of the early events in tissues) in order to replace, reduce and refine research with NHP[377,378].

IV.6. Could studies in humans and in vitro models be sufficient for HIV cure research?

The study of new therapeutic strategies to cure HIV have involved, inter alia, the longstanding contributions of animal models. These models enable pharmacological and pre-clinical trials to assess the pertinence of a curative strategy in vivo. Yet, with the improvements of in vitro models, some might consider that the use of animal models is no longer needed in the HIV research field. Indeed, other valuable tools are used in HIV science, such as model cell lines, primary cells from healthy individuals, primary cells from HIV-infected suppressed patients, and even tissue explants and organoids that enable to reflect some pathophysiological mechanisms closer to the in vivo condition. Nonetheless, the complexity of an entire in vivo model can only be provided by the use of animal models.

Studies in humans and in vitro models alone enable to address numerous issues. Yet, the major barriers in HIV cure research are unlikely to be overcome without additional contributions of animal research. These challenges include : (i) The large variety of HIV infected cells, that are not all well-defined, and are dependent of complex anatomical and immunological (micro)environments. (ii) The difficulty to selectively target and distinguish latently infected from uninfected cells. (iii) The occurrence of HIV persistence in multiple tissues, sometimes hardly accessible (deep reservoirs). (iv) the impairment of immune responses in the course of HIV Infection and HIV-asociated chronic immune activation inducing and immune aging.

The use of animal models to better understand and overcome these barriers provides some advantages that are not mirrored in clinical trials and in vitro models.
They allow to control the time, dose, inoculation route, host genetic factors, and virulence and genetic diversity of the infecting virus. This provide a high degree of experimental control, and thus less unforeseen/ unknown confounding variables than is typically achievable in human clinical studies. They provide a critical tool to understand the complex virus–host interactions and immune responses, which cannot be accurately modeled in cell culture or other more reductive systems[272,379].

Another key advantage of studies performed on animal models is the possibility to perform longitudinal analyses, with baseline situation being accessible before infection, as the different studied parameters can be measured before inoculation is performed[272].

Animal models enable to study the very first hours of infection, including the ability to initiate ART very early post-infection. Moreover, with proper animal welfare oversight and appropriate veterinary support and expertise, they enable to routinely and longitudinally collect fluids and biopsies with greater frequency than what is feasible in human trials (including blood, cerebrospinal fluid, bronchoalveolar lavages, and bone marrow aspirates). They also provide a means to sample tissues that are difficult or impossible to collect in humans for logistic and ethical reasons, but could represent key sites for the understanding of viral replication and pathogenesis (for instance vaginal and rectal mucosae, lymph nodes, spleen, liver, lungs and brain)[60,272,380].

The implementation of tools for a better understanding of non-human primate (NHP) genome and immune responses, the development of better murine models, and the advent of new recombinant viruses have led to an enhanced relevance of available animal models. While none of these models exactly match HIV infection in humans, each model can be valuable to address the key specific questions of HIV cure research. It always remains essential to define the benefits and limits of each proposed project, as well as continuing the use of the 3Rs principles (Replacement, Reduction and Refinement) while performing animal research[55,58,60]. Nonetheless, their use shouldn't be opposed to other precious tools used in basic, translational and preclinical research, such as tissue explants and organoids, which needs to be further developed in order to be used in a complementary manner and to replace the use of animals when possible, even though the use of some animal models will likely still be necessary to make the connection with clinical research and address specific issues. Recent advances in technology, for instance in new imaging techniques, are also likely to improve research while enabling to reduce the required number of animals[55,58,60–62].

V. Well-defined approaches and strategies toward a cure

V.1. Several strategies have been conceptualized to achieve an eradicative cure or remission

Over the years, different therapeutic concepts have been well-defined as strategies towards an HIV cure. These strategies are aimed either at eliminating every persistent replication-competent virus – for an "eradication" – or, in a more achievable perspective, to **silence** and/or **reduce the size of** the reservoir and use the host immune system to control the remaining infected cells and impede a viral rebound.[46,52,53,63–71].

Nearly all strategies involve eliminating or reducing the reservoir and target either the virus or the immune system. There is a range of strategies specifically aimed at eliminating the virus-infected cells and a range of strategies that boost immune function.

One of the main strategies is often referred to as "shock and kill" (sometimes also termed "kick-and-kill"[74]). It consists in the use of latency reversing agents (LRAs) with the goal of reversing transcriptional silencing of the virus and, thus, inducing viral gene expression and productive infection in latently infected cells. Accordingly, these cells expressing viral RNA and proteins would either be exposed to viral cytopathic effects or become recognizable targets for the immune system or host-directed therapies. In the best-case scenario, this strategy could conceptually lead to HIV eradication (*Figure 13*). ART would likely have to be continued during the LRA treatment[73].

An alternative to "shock-and-kill" has been proposed, which consists – after an identical step of latency reversal – in blocking the release of virions and inducing apoptosis of the infected cells. Such a strategy termed "Lock in and apoptosis" has been proposed in a paper by Tateishi et al., which suggests for instance to block virus budding from the cell, leading to apoptosis of the infected cell[72,381].

Contrary to "shock and kill", another strategy is termed "Block and Lock" and aims to reinforce viral latency in order to maintain the provirus in an inactivate state in the absence of ART[75–77] (*Figure 13*). Transcriptional gene silencing could conceptually be reached with a range of compounds that can disrupt the regulation of chromatin structure, thus maintaining the epigenetic mechanisms responsible for HIV-1 latency.

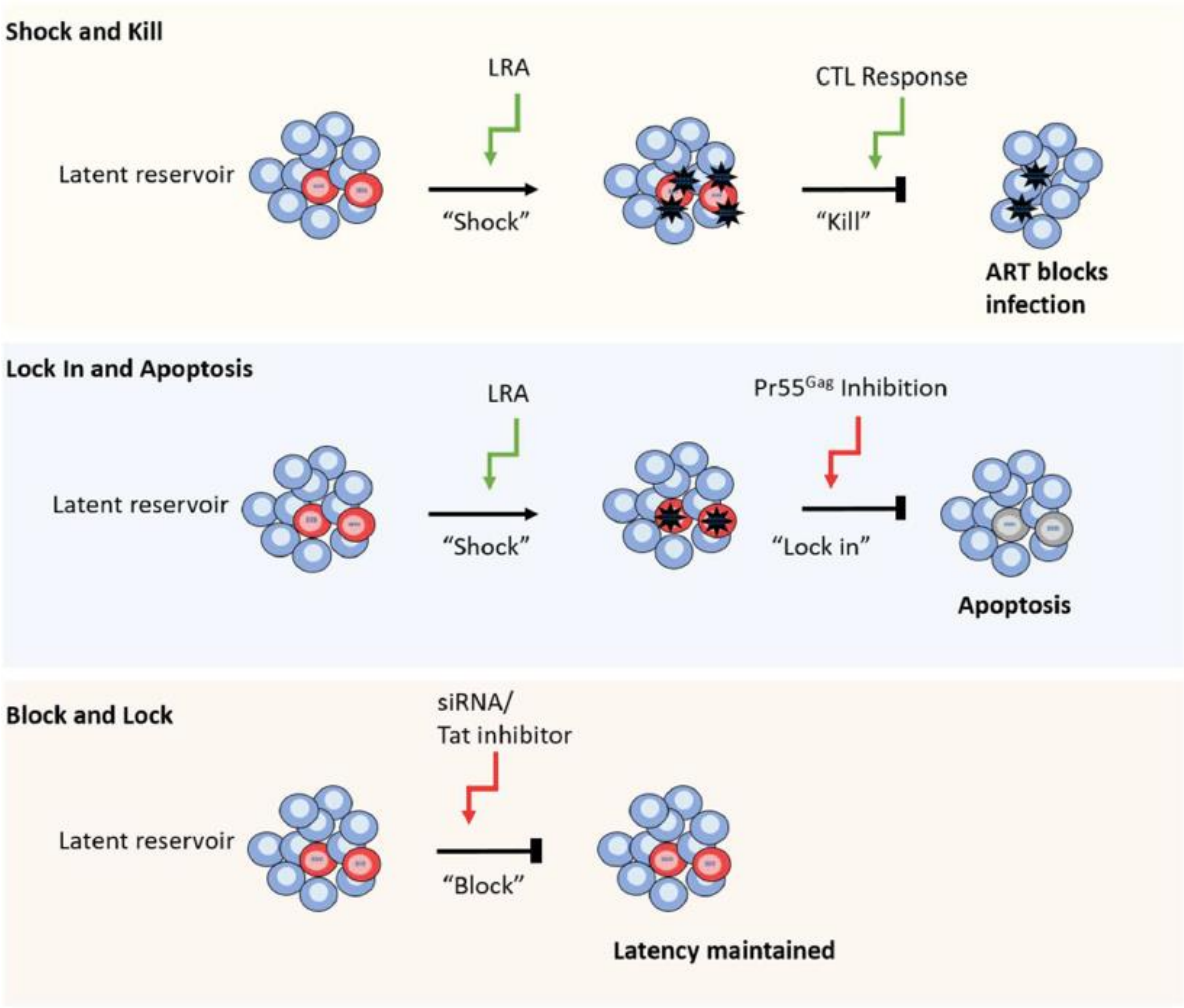


Figure 13. Different strategies towards a HIV cure.
"shock and kill" consists in the reversal of latency of the reservoir with LRAs followed by immune-mediated cell clearance. "Lock in and apoptosis" is similar but relies on an enhancement of mechanisms of apoptosis for the "kill", for instance using $Pr55^{Gag}$ inhibition. In contrast, "Block and Lock" aims at reinforcing and maintaining viral latency, for instance using Tat inhibitors or siRNAs. (red cells represent HIV-1 latently infected cells).
Adapted from Thomas, J., Ruggiero, A., Paxton, W. A. & Pollakis, G. Measuring the Success of HIV-1 Cure Strategies. Front. Cell. Infect. Microbiol. 10, 134 (2020).

Outside these paradigmatic strategies, other approaches and agents are also foreseen and could be used, either alone or in combination (*Figure 14*).

Gene editing approaches consist in the use of gene editing tools such as CRISPRCas9 and zinc-finger nucleases (ZFN) to target various host or viral genes. They mirror the strong interest in developing in vivo gene-editing strategies in other areas of medicine[382].

A range of immune therapies have also been extensively studied, and continuous progress are still being made. They notably comprise therapeutic vaccines, the use of broadly

neutralizing antibodies, cell-based therapies such as chimeric antigen receptor (CAR)-T cells, Immune checkpoint blockade, and other non-specific therapies influencing innate or adaptive immunity, immune dysfunction and the chronic inflammatory state

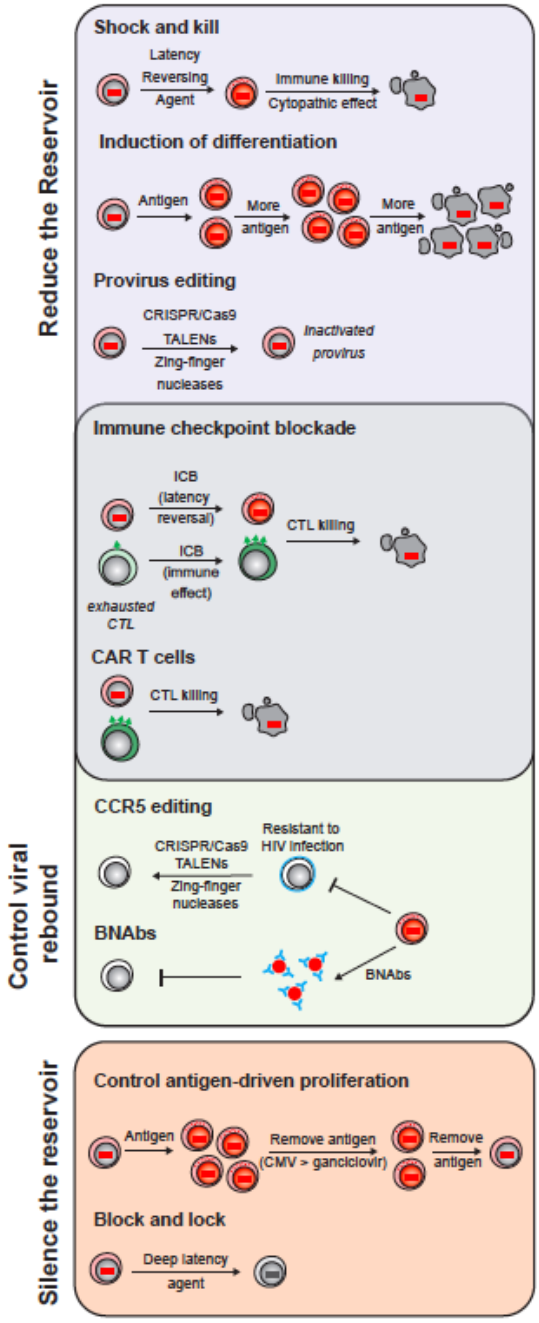


Figure 14. Approaches towards an (ultimately strerilizing) cure to HIV.
Various strategies ("shock and kill", "Block and Lock") and gene- or immune- therapy approaches aimed at reducing the size of the reservoir, controlling viral rebound, and/or silencing the reservoir.
Adapted from Cohn, L. B., Chomont, N. & Deeks, S. G. The Biology of the HIV-1 Latent Reservoir and Implications for Cure Strategies. Cell Host Microbe 27, 519–530 (2020).

In the next parts we will focus in some categories of agents that could possibly be used, either alone or in combination in HIV cure approaches.

V.2. Overview of the various latency reversal approaches for "shock and kill"

Reversible silencing of viral expression (i.e. latency) in purified resting CD4+ T cells was demonstrated throughout the first studies on latent HIV infection in the 1990s[47,167,168]. In the late 1997, phytohemagglutinin, a mitogen and TCR agonist (still used in viral outgrowth assays), IL-2, and anti-CD3 and anti-CD28 antibodies were used to reverse latency in resting central memory CD4+ T cells *in vitro*, targeting the activation of immune signaling pathways as a mean to induce responsiveness from the integrated provirus[169]. This led to the attempt, in 1999, to use immune signaling pathways and T cell activation in the presence of potent antiretroviral therapy to reduce the latent reservoir *in vivo*. For this purpose, a clinical trial studied the effects of an anti-CD3 antibody and IL-2 in PLWH on ART[383]. Unfortunately, it led to excess global immune activation, T cell depletion and an undue increase in inflammatory cytokines, causing severe toxicities, and the IL-2 dosage was decreased to safer levels. Viral replication was successfully induced but did not result in a reduction of the viral reservoir[384]. Although the treatment was a disastrous failure from a clinical perspective, it constituted one of the first attempts to use agents to reverse latency and paved the way to the "shock and kill" approach[72].

Since then, numerous mechanisms have been considered for the purposes of reversing latency of the replication-competent proviruses, which constitutes the necessary first step of "shock and kill" and "Lock in and apoptosis" strategies.

The latency of the provirus being closely linked to host cellular programming, most LRA target host processes, sharing common features and similar pathways with some agents and mechanisms used in oncology[47]. The approaches to inducing Latency reversal are constantly evolving and numerous demonstrated or potential latency reversing agents have been studied in the past 20 years.

Many of the potential LRA that were tested proved to be efficient in inducing viral RNA or Protein expression from latent proviruses *in vitro* and a few *in vivo.* Nonetheless, only a minor proportion of the latent reservoir was depleted in clinical trials of LRAs thus far and virologic remission after treatment interruption following this approach was never observed in humans for now[47,385]. Moreover, although being very diverse, many LRAs share the commonality of presenting off-target toxicities. This represents a challenge which should lead to the search for more HIV-specific LRA that selectively induce HIV expression from

infected cell, the attenuation of the toxicity of known LRAs and the careful examination of all the potential effects of these agents before their use in clinical trials.[47,53,63]

The variety of LRAs have already been extensively reviewed[47,63,71,385–387] and several classification of these agents have been proposed according to their mechanisms of action. In an extensive inventory of published LRA, Abner and Jordan categorized them into six groups as follows: “histone post-translational modification modulators”, “non-histone chromatin modulators”, “NF-κB stimulators”, “TLR agonists”, “extracellular stimulators”, and “miscellaneous uncommon compounds”[386].

Another – not that different – categorization was suggested by Kim et al.[63] and Zerbato et al.[71], with seven (possibly overlapping) classes of LRAs, that can be sorted between transcription *activating LRAs* (comprising “epigenetic modifiers”, “NF-κB agonists”, “protein Kinase C agonists”, and “activators of the PI3K/Akt pathway”), and *immunomodulatory LRAs* (comprising “TLR agonists”, “TCR activators and Immune Checkpoint Blockers”, and “cytokines”). In their recent review, Margolis et al.[47] nearly consider the same classes of LRA but sort them between “epigenetic LRAs” and “signal agonists LRAs”.

We do not propose a proper classification of LRAs but a non-exhaustive overview of some latency reversing agents of interest (*Figure 15*). .

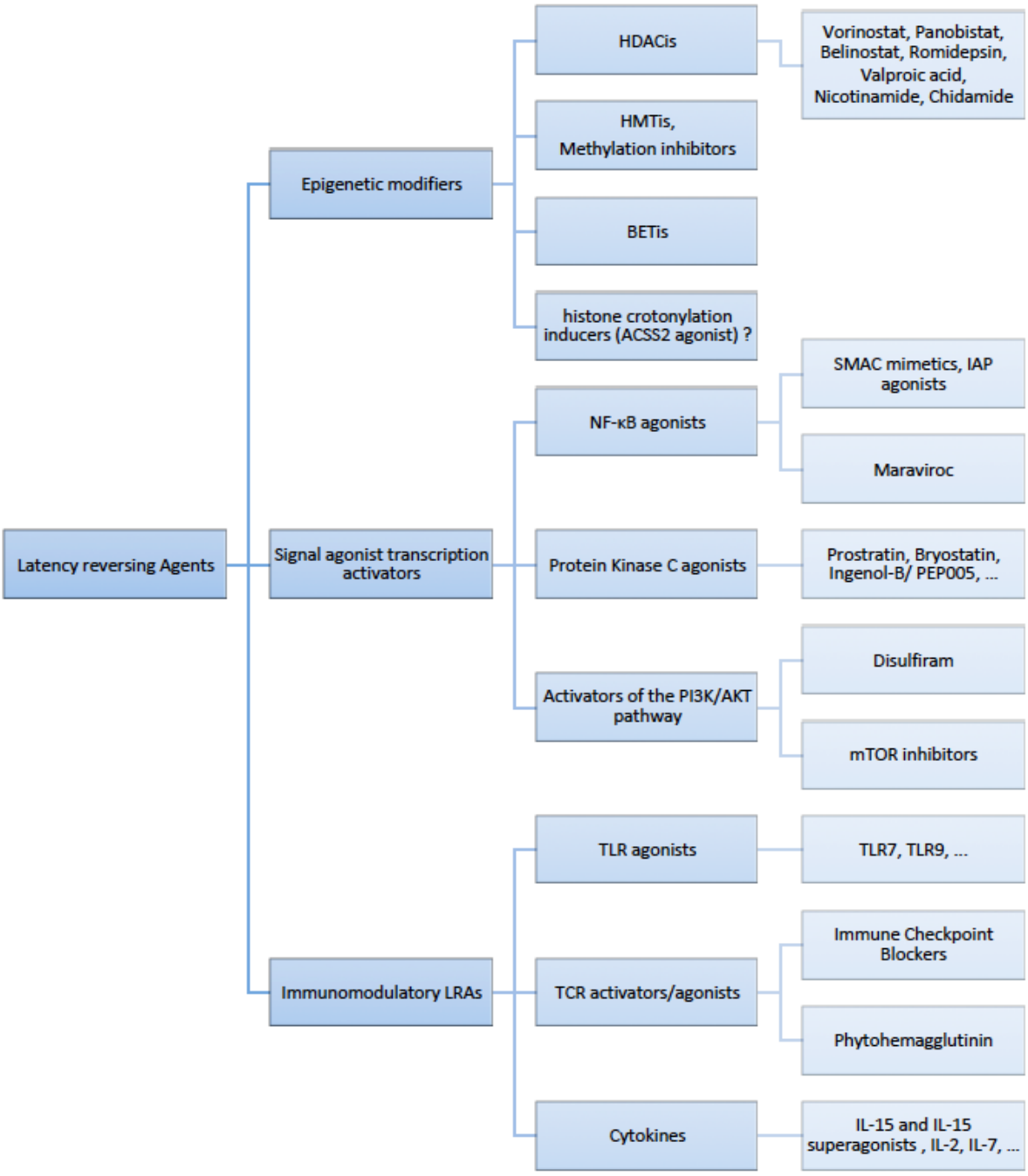


Figure 15. Overview of some latency reversing agents and their classes,
according to Kim et al.[63] and Zerbato et al.[71], Abner and Jordan[386] and Margolis et al.[47]

V.2.1. Epigenetic LRAs

Amongst the most studied LRAs are epigenetic modifiers, that target the chromatin restrictions maintaining HIV latency (*Figure 16*). They notably include histone deacetylases inhibitors (HDACis), histone methyltransferase inhibitors (HMTis), methylation inhibitors, bromodomain and extra-terminal domain inhibitors (BETis), and histone crotonylation inducers (ACSS2 agonist).

HIV expression is restricted by several mechanisms upholding proviral latency in resting CD4+ T cells, including DNA methylation and histone deacetylation[385,388,389]. Histone deacetylases (HDACs) induce chromatin condensation and transcriptional repression, which favors HIV latency. The effects of the histone acetylation/deacetylation equilibrium on chromatin condensation can be altered by several drugs, some of which are borrowed to the field of oncology, where they were developed – and some of them approved – *inter alia* for the treatment of T cell lymphomas[47,385]. Such chromatin modulators can exert an effect on viral gene expression by reversing epigenetic silencing while avoiding global cellular activation.

Some HDACis are currently being (or about to be) tested in clinical trials[64,71]. They notably include vorinostat[390], panobistat[68,391], romidepsin[392], Valproic acid[393], Nicotinamide[394], and Chidamide[395]. Some result from early clinical trials demonstrated only small increase in HIV RNA, not a significant reduction of the reservoir size, and no delay in time to viral rebound[71,387]. Their inability to induce a strong latency reversal and clear infected cells when used alone might be linked to several reasons, including their possible inhibition of CD8+ T cell and natural killer (NK) cell function[396–398] and the persistence of chronic immune activation and T cell exhaustion that hinder T cell-mediated clearance of infected cells. For these reasons, HDACis seem likely to be more efficient while used in combination with other LRAs and/or additional agents to “kill” the infected cells[63].

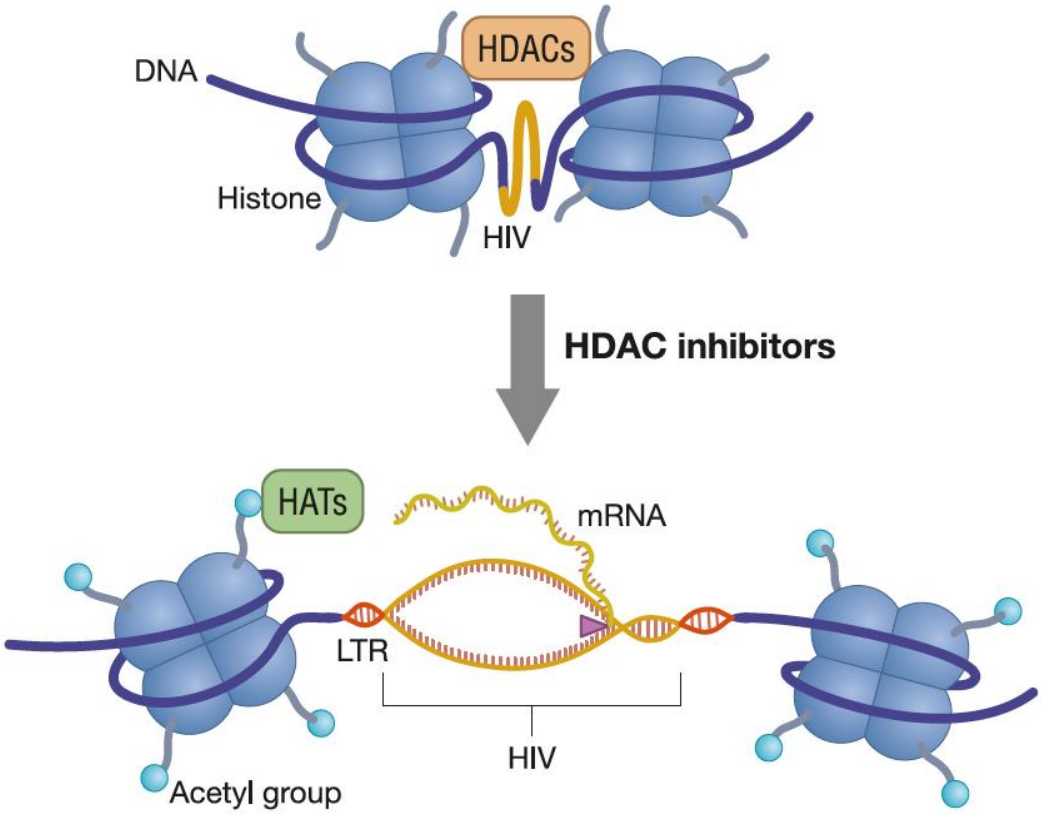


Figure 16. Disruption of HIV latency by HDAC inhibitors
"The activity of HDACs suppresses HIV expression by promoting deacetylation of lysine residues on histone tails and keeping the chromatin in a compacted state. Inhibition of HDACs by HDACi promotes histone acetylation by HATs, which leads to chromatin relaxation and initiation of transcription. HDACs histone deacetylases, HDACi histone deacetylase inhibitors, HATs histone acetyl transferases, LTR long terminal repeat."
Adapted from Rasmussen, T. A. & Søgaard, O. S. Clinical Interventions in HIV Cure Research. in HIV Vaccines and Cure (eds. Zhang, L. & Lewin, S. R.) vol. 1075 285–318 (Springer Singapore, 2018).

Interestingly, some other epigenetic modulation mechanisms are being investigated. For instance, it was recently shown that histone crotonylation is a regulator of HIV latency that could be targeted for therapeutic purposes. Indeed, in a NHP model of HIV/AIDS (SIV-infected rhesus macaques), reactivation of latent HIV was observed after an increase in the expression of the fatty acid metabolic enzyme ACSS2 (crotonyl-CoA–producing enzyme acyl-CoA synthetase short-chain family member 2), which induced histone lysine crotonylation[399].

V.2.2. Signal agonist LRAs

Epigenetic modulators alone did not succeed so far in inducing strong latency reversal in vivo. Continued efforts are being undertaken to develop and investigate other agents that could be used to reverse latency, notably through the use of cellular signaling pathways. These agents include protein kinase C (PKC) and activators of the NF-κB pathway such as CCR5 agonist Maraviroc and SMAC mimetics, activators of the PI3K/AKT pathway. The use of Toll-like receptor agonists and some cytokines such as IL-15 have also been explored, and resulted in mixed results for latency reversal purpose so far at least when used alone. TCR activators, such as Immune Checkpoint Blockers have recently raised an important interest, as potent LRAs and beyond.

Protein kinase C (PKC) agonists target signaling pathways that are usually induced by activation of the TCR. Hence, they can induce non-specific T cell activation and alter gene expression levels. They comprise diverse compounds, like small-molecule PKC agonists (PKCas), such as Phorbol esters and Diterpenes, Prostratin, Bryostatin-1 (BRY-1), and Ingenol derivates such as ingenol-3-angelate (PEP005) and Ingenol- B, purified from the Euphorbia family.

Bryostatin-1 is currently the only PKC agonist that has been tested in a clinical trial, and did not induce latency reversal which may be related to the low concentrations administrated[400]. Another clinical trial (NCT02531295) has been started to assess the latency reversing potential of *Euphorbia kansui*, an ingenol-containing plant used in traditional Chinese medicine[401] in a dose escalation study.

Some NF-κB pathway activators also represent potential LRAs. Indeed, NF-kB likely plays a critical role in the induction of HIV expression[402], and several compounds can intervene in its modulation pathways.

CCR5-antagonist Maraviroc, known to inhibit HIV entry, has been shown to induce latency reversal *in vitro.* Maraviroc may activate provirus transcription through the activation of NF-κB as a result of binding CCR5, and thus appeared as a potential LRA[403,404]. It has been tested in a clinical trial (NCT02475915) in 2015, in combination with Vorinostat and hydroxychloroquine. Despite a transient increase in HIV plasmatic viral load, no reduction in the reservoir size nor delay in time to viral rebound was observed[405]. Furthermore, conflicting data emerged from a study in the NHP rhesus macaque model where Maraviroc was administrated with the PKC activator ingenol-3-angelate (PEP005): it was suggested that Maraviroc treatment could in fact attenuate the reactivation of latently infected cells after PEP005 stimulation[406].

Another example of LRA linked to the NF-κB pathway is provided by increasingly studied activators of the non-canonical (nc) NF-κB pathway.

The ncNF-κB pathway is activated by the second mitochondrial-derived activator of caspases (SMAC), which inhibits the inhibitor of apoptosis proteins (IAPs). IAPs (for example, X chromosome-linked IAP (XIAP), cellular IAP1 (cIAP1), and cellular IAP2 (cIAP2)) represses the ncNF-κB pathway by constantly degrading the NF-κB-inducing kinase[407,408]. It has been shown *in vitro* that SMAC mimetics, such as AZD5582, could relieve this repression in CD4+ T cells. SMAC mimetics thus appeared as agents that could potentially reactivate the latent reservoir[409]. Using an ART-suppressed HIV-infected humanized BLT mice model, Nixon et al. showed that AZD5582 treatment could induce systemic HIV RNA production *in vivo*. They completed their results in mice model with the use of a NHP model, in which AZD5582 induced SIV RNA expression in the plasma and lymph nodes of ART-suppressed SIV-infected rhesus macaques. Their experiments demonstrated that HIV or SIV reactivation could be robustly induced by SMAC mimetics, although no consistent reduction in the size of replication-competent reservoirs were observed[408]. Nonetheless, in a recent study by Dashti et al. that also used a NHP model to assess the efficacy of the same SMAC mimetic AZD5582 in combination with an Ab-derived DART molecule, AZD5582 apparently did not induce a sufficient latency reversal[410]. A potential correlation between the latency reversing activity of AZD5582 and the viral reservoir size before intervention has been hypothesized to explain

this disappointing latency reversal in the last study, but this also highlights the fact that the ability of SMAC mimetics to reproducibly revert latency *in vivo* has to be better characterized and further investigated.

V.1.3. Immunomodulatory LRAs

Toll-like receptors (TLRs) and their therapeutic stimulation may also be targeted as mechanisms to revert HIV latency. TLRs are pathogen recognition receptors that recognize a multitude of molecules present in virus, bacteria, fungi or protozoa. Various agonists of the TLRs have been studied in vitro and in vivo as possible LRAs. Outside this potential use, they can also present other potential benefits for immunotherapies, as TLRs can promote and augment antiviral responses, promote dendritic cell maturation and antigen presentation, activate natural killer cells, and enhance immune responses[411–413]. The ability of several agonists of TLR2, TLR3, TLR7, and TLR9 to reverse HIV latency have been explored.

Notably, agonists of TLR7 have led to interesting results while tested (alone or in combination with other immunotherapies) in NHP models. In 2016 Borducchi et al. observed that TLR7 agonist GS-986, in combination with therapeutic vaccination, could led to improved virologic control, increased SIV-specific immune responses, and delayed viral rebound following ART discontinuation in SIV-infected rhesus macaques that began ART during acute infection[414]. In 2018, they also observed a delayed viral rebound in SHIV-infected macaques after administration of TLR7 agonist GS-9620 (vesatolimod) alone or in combination with an Env-specific broadly neutralizing antibody (bnAb) PGT121[415,416]. (for bNabs, see also chapter V.4). In the group of macaques that received both GS-9620 and the bnAb PGT121, for 5 out of 11 animals, the virus did not even rebound after ART interruption. In these two studies, it is possible that the interesting effects of the two TLR7 agonists GS-986 and GS-9620 (vesatolimod) were not – or not only – imputable to a latency revering effect, as transient increases in plasma virus were not clearly observed, and other beneficial properties, such as immune enhancement and NK cell activation also possibly came into play[71,415]. A third study by Lim et al. studied the ability of GS-986 and GS-9620 to induce transient viremia in SIV-infected rhesus macaques. Plasma viremia was successfully induced and their results were consistent with a reduction of the viral reservoir, in addition to the

activation of multiple innate and adaptive immune cell populations[417]. Phase 1 clinical trials (NCT02858401 and NCT03060447) have been undertaken to study the effects of Vesatolimod alone and in combination with other agents.

Other TLR agonists have been tested with mixed results in preclinical studies and clinical trials, such as TLR9 agonists[418]. In particular Lefitolimod (MGN1703) was studied in a single arm study NCT02443935, and did not result in a clear latency reversal, nor detectable changes in the size of the latent HIV-1 reservoir. Nonetheless, it interestingly increased the activation of plasmocytoid dendritic cells, enhanced the activation of cytotoxic NK cells and effector CD8+ T cells, upregulated the levels of cytokines and increased the levels of IFN-α2 leading to a significant upregulation of numerous interferon-stimulated genes.

Cytokines have also been studied as potential immunomodulatory LRA, notably IL-2, IL-7 and IL-15, but generally led to disappointing or mixed results in terms of latency reversal[47,71]. IL-15 and IL-15 superagonists have increasingly raised interests over the past few years. Initially considered for their latency reversing potential, which is still debated, they might provide other benefits as immune therapies, thanks to their immunomodulatory properties and potential abilities to augment the antiviral immune response and improve clearance of persistent infection. Different studies of IL-15 superagonist N-803 have been recently conducted in NHP models. Webb et al. did not observe any evidence of any latency reversal from N-803 subcutaneous administration in SHIV-infected macaques, although disclosing other very interesting effects of this IL-15 superagonist[419]. Congruently with this work, another study on N-803 in SIV-infected ART-treated macaques also suggested that this IL-15 superagonist is not sufficient to exert an in vivo LRA effect when used alone[420,421]. They suggested that CD8+ T cells could play a substantial role in suppressing the latency-reversal effect of N-803 by promoting the maintenance of viral latency, and the same team subsequently demonstrated that the combination of CD8β depletion and N-803 could induce virus reactivation in SHIV-infected ART-treated rhesus macaques[422].

Lastly, agents that blocks immune checkpoints have been increasingly studied, following parallel advances in cancer research[423]. Emerging data indicate that these immune checkpoint blockers (ICB) could be exploited to reverse latency, by promoting cell cycling and increasing viral transcription. For instance, antibodies against PD-1 and CTLA-4 may be

able to enhance latency reversal[424,425]. It was recently reported that the CTLA-4 and PD-1 combined blockade could induce SIV reactivation in SIV-infected long-term ART-treated rhesus macaques, although no delay in viral rebound was observed after ART interruption[426]. Further investigation of ICB is needed, all the more as they could present other benefits for therapeutic approaches.

The great variety of potential LRAs that emerged in the last years and the encouraging data from studies in animal models still make "Shock and Kill" one of the most promising approach for curing HIV. However, LRAs in "Shock and Kill" strategies are generally unable to manage chronic immune activation, immune exhaustion, and undue inflammatory state, and can even worsen those by activating immune cells and triggering the production of cytokines and other pro-inflammatory compounds. Each LRA comes with potential toxicities, all the more so as the structure of the genome and cell circle mechanisms are involved, and these need to be assessed (including long-term effects), reduced, and managed. Some potential LRAs that displayed only modest or haphazard effects *in vivo* would be interesting to study in combination with others. Furthermore, it is unlikely that use of LRAs alone will enable a significant elimination of infected-cells, as initially envisioned in "shock and kill"[72,73], and additional intervention is likely to be needed for the "kill", whether complementing or potentiating the clearance by the immune system[63].

V.3. Transcriptional silencing for "block and lock"

In contrast to "shock and kill" approaches, where the provirus shall be induced, "Block and Lock" has been more recently proposed as an opposite strategy in which the provirus would be permanently silenced, and maintained in an inactivate state in the absence of ART[75–77].

The achievability of such an approach is supported by several observation, as precedents exists for deep and irreversible latency of retroviruses[67]. Indeed, a substantial proportion of the human genome is constituted of human endogenous retroviruses (HERVs). The ancient DNA of these retroviruses integrated our genome and was permanently silenced throughout evolution. Except under exceptional circumstances, it is never transcribed and is silenced through epigenetic mechanisms such as DNA methylation[427]. Interestingly, recent studies

suggest that HIV is subjected to similar regulation mechanisms as HERVs, leading to selection of less expressed intact proviruses with features of deeper viral latency during prolonged antiretroviral therapy[428]. Agents and strategies that can bolster these mechanisms could therefore be of the utmost interest for therapeutic purposes.

The viral Tat protein is necessary to the trans-activation of integrated HIV DNA transcription by recruiting P-TEFb as well as other processivity factors that constitute the super elongation complex (SEC). The kinase activity of P-TEFb is required for switching to processive elongation. The RNA Pol II complex remains paused in absence of sufficient amount of the Tat protein[47]. Hence, agents that inhibit Tat appeared as some of the first latency-promoting agents (LPAs) to study[75]. Didehydro-cortistatin A (dCA), a Tat inhibitor, was studied in a humanized BLT mouse model, and some modest effects could be observed. The activation of the reservoir seemed to be reduced after its administration, since there was a decrease in mRNA in tissues. Viral rebound was delayed upon treatment interruption and their levels were reduced in dCA-treated mice[76].

Multiple mechanisms and molecules have been proposed as potential latency promoting agents, such as small interfering RNAs (siRNAs) that could disrupt chromatin structure regulation and maintain the epigenetic mechanisms enforcing latency[77]; but also other molecules that inhibit diverse transcription factors such as P-TEFb complex, mTOR complex[429,430], HSP90 inhibitors suppressing the HSP90-NF-κB-axis[431], NFAT (CsA), and Transcription Factor II H (TFIIH), as well as other drugs like complex-targeting curaxin CBL0100[432], levosimendan[433], and spironolactone[434] (*Figure 17*). Some of these molecules have already been studied for therapeutic purposes in other areas of medicine and could be advantageously repurposed as LPAs[435,436].

In particular, the mammalian target of Rapamycin (mTOR) has been shown to be related to the control of HIV Latency[429,430]. mTOR complex inhibitors (such as Rapamycin) lead to Tat protein degradation through autophagy, and were therefore identified as promising candidates for block and lock strategies[430,437], amongst other possible beneficial uses in HIV cure strategies such as immunomodulatory effects and control of cytokine-associated toxicity[438].

Other potential LPAs of interest are Jak-STAT inhibitors such as Tofacitinib and Ruxolitinib[89,386,439,440]. Downregulation of the Jak-STAT pathway with extracellular JAK-

inhibitors can lower the STAT5-dependent transcription[386]. These compounds are considered to be clinically well tolerated and present other potentially beneficial features, as their ability to hinder pro-inflammatory signaling and to inhibit HIV reservoir seeding[440]. Jak-STAT inhibitors could be beneficial to cope with chronic inflammation or immune dysfunction in ART-treated PLWH[89].

The strengths of "block and lock" approach over the "shock and kill" strategies include the likelihood that agents used as LPAs will display less adverse effects than LRAs in the clinic and the unlikely ability of "shock and kill" to completely eradicate the provirus from all the reservoirs. In addition, some LPAs, including those involved in jak-STAT, mTOR-, PKC- or HSP90- NF-κB pathways, might have immunomodulatory properties that could beneficially enable to diminish undue inflammation but would have to be monitored and carefully evaluated to avoid worsening preexisting immunocompromization or causing deleterious effect to the immune system.

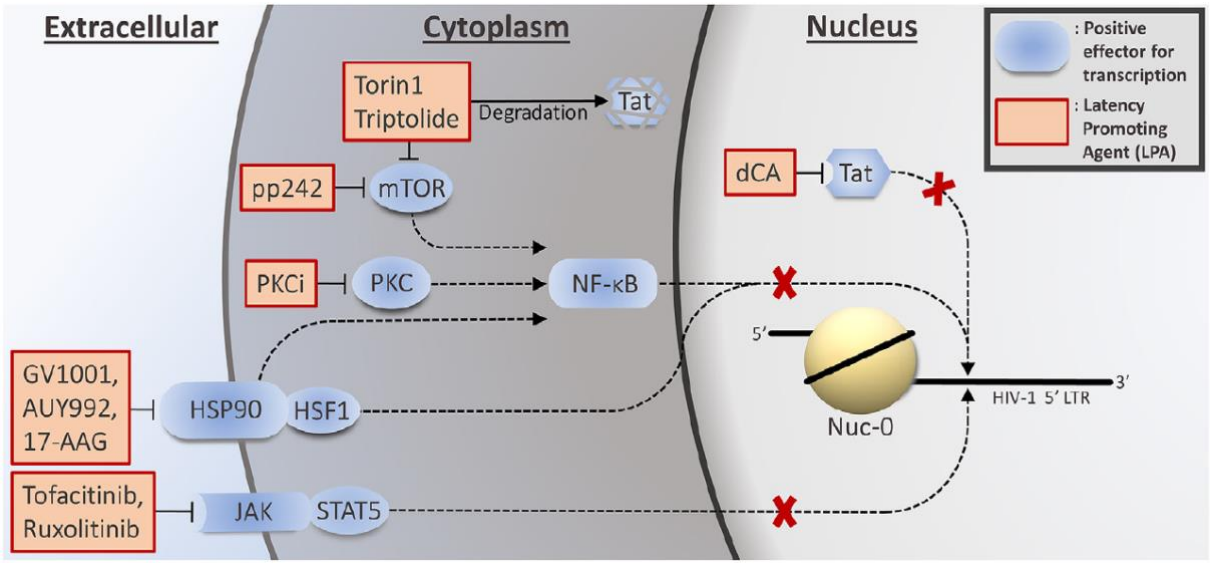


Figure 17. Potential pathways and mechanisms to target with Latency Promoting Agents (LPAs) for "block and lock" therapy.

Potential LPAs include agents that inhibit eiter the HIV Tat protein or cell signals involved in mTOR, jak-STAT, or HSP90-NF-κB pathways. Dashed arrows represent inhibited pathways. mTOR, Mammalian target of rapamycin; PKC, Protein kinase C; HSP90, Heat-shock protein 90; HSF1, Heat-shock factor 1; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; FACT, Facilitates chromatin transcription; LTR, Long terminal repeat; Nuc, nucleosome; Tat, trans-activator of transcription.

Adapted from Abner, E. & Jordan, A. HIV "shock and kill" therapy: In need of revision. Antiviral Res. 166, 19–34 (2019).

V.4.Immunotherapies to elicit and strengthen potent immune responses

V.4.1. Antibodies : Broadly Neutralizing Antibodies and beyond...

Antibodies have been the subjects of longstanding interests in HIV cure (and vaccine) research, and often played a major role in the most successful immunotherapeutic studies. Antiviral humoral responses usually generate two types of antibodies: antibodies that bind to the envelope and block infection (neutralizing antibodies) and antibodies that bind to viral proteins but do not impede viral infection (non-neutralizing antibodies)[47]. Both neutralizeing and non-neutralizing antibodies can contribute with distinct efficacies to eliminate infected cells expressing viral proteins through have antibody-dependent cellular cytotoxicity (ADCC). HIV is capable to rapidly escape recognition by antibodies. However, some antibodies are able to neutralize multiple viral strains and were termed Broadly neutralizing antibodies (bNAbs)[441,442]. These antibodies arise late (often after only 2 years of infection) and only in very few individuals.

These bNabs can be administered passively and when used in combination have a potent durable antiviral effect if maintained to sufficiently high levels[46,415,443–446] In addition to their neutralizing mechanism, bNAbs could stimulate ADCC and other Fc-receptor dependent cytotoxic effects. They could also enhance potent HIV-specific immune responses. Indeed, Niessl et al. demonstrated that passive administration of the bNabs 3BNC117 and 10-1074 was associated with enhanced HIV-1-specific T cell responses [446]. However, bNAbs are unable to clear latently infected-cells. It has been suggested, that antigen-presenting DCs that express activating Fc receptors could bind to immune complexes and efficiently process antigens for presentation and cross-presentation to CD4+ and CD8+ T cells, sustaining a T cell effect similar to a “vaccinal effect”[46,444].

Three complementary studies studied the effects of two bNabs (3BNC117 and 10-1074). Using a NHP model with SHIV-infected macaques, Nishimura et al. first showed that early administration of these two bNAbs could reduce the levels of persistent viremia, establish T-cell immunity and result in long-term infection control[444]. The combination of these two bNAbs was subsequently studied in a phase 1b clinical trial (NCT02825797), and Mendoza et al. reported that in these PLWH, the administration of 3BNC117 and 10-1074 immediately

after interruption of ART could maintain long-term viral suppression in the absence of ART in individuals with antibody-sensitive viral reservoirs[445]..

Targeted engineering of new antibodies (or antibody-based molecules) allows to improve the pharmacokinetics, potentiate the effector functions (for example with modifications of the antibodies Fc portion), and design new multi-specific antibodies which bind to two or three sites on the viral envelope[447,448], which could enable to enhance the possible vaccinal-effect of antibodies and eliminate HIV-infected cells[46,435]20/12/2020 16:53:00. Such engineered trispecific bNAb successfully conferred potent immunity in a SHIV-infected macaque model[447].

Antibodies targeting cellular epitopes of target cells might also been used in multiple possible therapeutic pathways. The monoclonal antibody-mediated blockade of the host-expressed α4β7 integrin have raised some interests over its ability to enhance virologic control in NHP models[449,450]. Indeed, anti-α4β7 could lead to lymphocytes redistribution throughout the body in NHPs[451], could alter the activation potential of α4β7-expressing cells, and interfere with virus binding to this integrin on target CD4 T cells[452], which together may account for antiviral effects. The data obtained for now are nonetheless conflicting in NHP models[450].

V.4.2. Therapeutic vacines

Therapeutic vaccination approaches could induce strong HIV-1 specific responses, might consequently be harnessed for functional cure strategies, and may prove to be helpful either in “shock and kill” strategy, or in “block and lock”. In most individuals (except PTCs), immune responses are not sufficient to achieve an ART-free HIV remission, and the way to induce such potent immune responses in PLWH remain an unsolved challenge. Used in “shock and kill” approaches, therapeutic vaccines could help to boost the immune system to achieve immune-mediated clearance of reactivated infected cells as soon as they become visible to the immune system. In “block and lock” approaches, therapeutic vaccines could lead to a silencing of the remaining reservoir and an immune control of the infection[53,59,435,453,454].

Traditional therapeutic vaccine approaches are generally aimed at potentiating adaptive immune responses and/or redirecting HIV-1-specific CD8+ T cells. They benefited from recent innovations, notably in delivery modalities, adjuvants, and immunogen design and, an

can be based on a variety of modalities including live-attenuated viral vectors, DNA and RNA vaccines, as well as ex vivo loading of dendritic cell and adoptive T cell therapy[455]. Most of these vaccines target conserved regions of HIV[456].Less conventional therapeutic vaccine approaches include chimeric or biological approaches that harness non-HIV-1-specific CD8+ T cells to kill infected cells[47,53].

In the 2000s, it was demonstrated that T cell vaccines delivering SIV proteins could induce cellular immune responses, reduce plasma viral loads, and preserve memory CD4 T cell counts *in vivo* in SIV-NHP models[457–459]. In a recent study by Nakamura et al., SIV-infected ART-treated rhesus macaques were vaccinated with Sendai virus vectors expressing SIV Gag and Vif. Gag/Vif-specific CD8+ T-cell responses were induced and became predominant. Viral rebound eventually occurred after ART cessation, but these results suggested that Gag-specific CD8+ T- cell induction by therapeutic vaccination could augment anti-virus efficacy of CD8+ cells. If such therapeutic vaccination strategy alone would probably not lead to a functional cure, it may contribute to more durable viral control under ART or in combination with other strategies[460].

In another therapeutic vaccine study in the NHP model, Hansen et al. studied the effects of a SIV protein-expressing rhesus cytomegalovirus (RhCMV/SIV) vector[355,356,358]. They observed that RhCMV/SIV vector-elicited immune responses could control SIVmac239 infection followed by progressive decrease of the viral reservoir and it is likely that the infection was eventually eliminated in some of these animals[356]. They subsequently showed that this replication-deficient CMV vector with SIV inserts could elicit and exacerbate an unconventional MHC-E-restricted CD8+ T cell response of unprecedented breadth[358].

As we already mentioned in the paragraph concerning the use of TLR7 as LRA, Borducchi et al. observed improved virologic control, increased SIV-specific immune responses, and delayed viral rebound following ART discontinuation in SIV-infected rhesus macaques that began ART during acute infection and received a combination of TLR7agonist and Ad26/MVA therapeutic vaccination[414]. Therapeutic vaccination was performed with a recombinant adenovirus serotype 26 (Ad26) prime and a modified vaccinia Ankara (MVA) boost. A therapeutic vaccine involving Ad26 and MVA was recently studied in a clinical trial (NCT02919306). A heterologous vaccine regimen of trivalent Ad26 and MVA that express two multivalent mosaic immunogens from the HIV proteins Gag, Pol and Env was

administrated to PLWH, and generated robust immune responses. However, after ART interruption, the vaccine delayed time to viral rebound by only several days compared to that in placebo recipients, which was statistically non-significant[461]. Still, these results are encouraging for therapeutic vaccines and combination strategies, and, as highlighted by Mothe et Brander, we can wonder whether the lack of control of the virus may be related to to still-insufficient stimulation of immune responses, inadequate response profiles, lack of reservoir mobilization, limited coverage of autologous viruses or the expansion of T cell and B cell responses to irrelevant targets in the virus[59].

Other Immune therapies, including CAR-T cell therapy and the use of immune checkpoint blockers, will be developed in the chapter VI.

V.5. Gene editing

In vivo gene-editing strategies raise strong interests for therapeutic use in other areas of medicine[382], and although most are is in their early stages, they could be beneficial in the field of HIV.

The rise of editing tools such as CRISPRCas9 and zinc-finger nucleases (ZFN) might lead to interventions targeting various host or viral genes. Among other potential uses, these could ultimately allow for the induction of host resistance, or the silencing of integrated provirus and enforcement of viral latency (as part of "block and lock" strategy)[53]. Gene editing tools can be highly specific, by targeting a given gene, which could prevent some global physiological impact. Safety concerns are nonetheless raised since adverse effects have been observed in several studies[462].

Several studies focused on the use of CRISPR-Cas9 in CCR5 or CXCR4 gene editing to induce host cell resistance to HIV-1[463,464]. However, this approach presents safety limitations, as even though individuals who are naturally homozygous for the CCR5Δ32 generally do well, it remains unknown whether such gene modification would be totally harmless. Besides, the use of gene editing tools to directly generate modification in human genome raise some bioethical issues.

On another note, gene editing tools could be used to directly target the provirus. In humanized mice model, CRISPR-Cas9 editing of proviral DNA in combination with sequential long-acting slow-effective release antiviral therapy (LASER-ART) delivery system, eliminated

HIV-1[465]. However, translating such an approach in human would face some difficulties. It would require to reach virtually all proviruses, which imply delivering gene editing tools all across the reservoir sites. Such delivery requires viral vectors or lipid compounds and their ability to attain every sanctuary sites and successfully be expressed wherever would constitute a major challenge[53,382,466].

Since most strategies involving gene editing tools are still at an early stage and major challenges and safety concerns need to be addressed, we do no further develop gene editing strategies. Several of these approaches have been reviewed elsewhere[70,466–468].

VI. Novel, unconventional or less explored concepts

VI.1. Using novel immune therapies to reduce the reservoir and control viral rebound

Following the advances and rapid expands in the field of cancer immunotherapy, and its numerous ongoing clinical trials, new immunotherapies are being in the field of HIV for novel investigations. These immunotherapies notably include chimeric antigen receptor (CAR)-T cell therapy (among other advances in cellular immunotherapy) and immune checkpoint inhibitors[91–93,423,469–471].

VI.1.1. CAR-T-cells

CAR-T cells emerged in oncology, mainly as innovative tools for therapies targeting CD19+ hematologic malignancies. Chimeric antigen receptors (CAR) are hybrid antigen receptors with an extracellular antigen binding domain that are linked to intracellular T cell activation domains, often constituted by the CD3 zeta chain[470]. CAR-T cells are genetically engineered autologous or allogeneic T cells that express an antigen-specific CAR, generally targeting tumor-associated or virus-associated antigen expressed on the neoplastic or infected cell surface. CARs can also be "equipped" with co-stimulatory domains, enabling the activation and function of CAR-T cells, as well as cytokine production, and the proliferation of these cells.[91]. Most CAR-T cells work to some extent in similar ways to bNabs and might lead to reservoir reduction in PLWH during ART or virus-control post-ART.

In a series of pre-clinical works, Herzig et al. studied an adaptable CAR-T cell platform based engineered to bind a variety of broadly neutralizing anti-HIV antibodies. They used a mutant NKG2D receptor that was formatted as a CAR for expression on CD8 T cells. NKG2Dis a receptor expressed on CTL and NK cells that can bind to ligands from the MIC/ULBP family, expressed on stressed cells. They demonstrated that the CAR-T cell could effectively kill HIV-infected primary cells and reduce the inducible reservoir in blood of HIV+ individuals *ex vivo* by 50%–60%. However, as also often observed in studies assessing bNabs, the efficacy of this CAR-T cell was largely dependent of the levels of envelope expression by the targeted cells[472].

In another work, Rust. Et al studied the effects of a virus-specific CD4-based CAR-T cells in SHIV-infected, ART-suppressed rhesus macaques[473]. The macaques were also infused with a

boost of cell-associated HIV-1 envelope (Env). They observed a significant and unprecedented expansion of virus-specific CAR+ T-cells, as well as a significant delay in viral rebounds compared to controls following ART interruption. This constitutes a noteworthy proof-of-concept that CAR-T cells could represent key stakeholders in combination therapies for HIV.

A re-engineered CD4-based CAR inspired by successful cancer targeting CARs was shown to confer promising antiviral activity[474]. This CAR is being tested in a phase 1 clinical trial (NCT03617198) to assess the potential CAR-T cell activity against HIV and to figure out whether it could provide some control of HIV replication or reduction of the viral reservoir[92,475–477].

Like bNAbs, CAR-T cells present the limitation that their epitope expression at the surface of infected cells is required, and the efficacy of CAR-T-cell-based therapies will likely be dependent of such levels of antigen expression, which would thus involve a prior effective latency reversal of the reservoir. Moreover, viral escape mutations could occur, and the ability of CAR-T cell to traffic to Immune-Privileged Sites to reach HIV-infected cells, and to efficiently clear those cells could constitute significant challenges[67,92].

VI.1.2. Immune Checkpoint Blockers

At a crossroads with oncology, immune checkpoint blockade is being increasingly considered for novel therapeutic approaches in infectious diseases[423].

Immune checkpoint molecules are cell surface receptors expressed on immune cells that regulate immune responses. Immune checkpoint proteins can either provide co-stimulatory signals that enhance immune activation, or co-inhibitory signals that negatively regulate the function of immune cells, which has a significant role in the resolution of immune responses and maintenance of self-tolerance[64].

In the particular case of HIV infection, immune checkpoint proteins have been studied as soon as the 2000s, in relation to T cell function, natural history, and complications of HIV infection. The expression of multiple inhibitory immune checkpoint proteins, including PD1, CTLA4, TIM3 and LAG3 have been shown to be upregulated on both CD4+ and CD8+ T cells in absence of ART, and are also enriched (although to a lower extent) on the surface of HIV-infected CD4+ T cells in ART-treated PLWH[64,423,478–480]. These upregulated inhibitory immune checkpoints are associated with immune dysfunction and T cell exhaustion, including loss of

effector functions and failure to proliferate in response to antigen[478–481].

On the basis of these observations and therapeutic attempts in the cancer field, the targeting of immune checkpoint proteins and their blockade has been more and more considered for therapeutic purposes. As previously introduced in the part concerning LRAs (part V.2.3.), blockade of immune checkpoints has been suggested as a new potent mean to reverse latency. The administration of immune checkpoint blockers (ICB), can indeed promote cell cycling and favor the expression of transcription factors, thus increasing viral transcription and potentially leading to latency reversal. In addition, immune checkpoint blockers could partially reverse HIV-associated immune dysfunction, enhance immune effector functions and HIV-specific CD4+ and CD8+ T cell responses in blood and tissues, and could eventually lead to the depletion of infected cells through Fc receptor activation or enhanced T cell killing, especially when used concomittantly with antibodies that activates Fc receptors[71,423,482,483].

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a homolog of the co-stimulatory CD28 molecule. Both costimulatory CD28 and coinhibitory CTLA4 are expressed by activated T cells, and CTLA-4 competes CD28 for CD80 and CD86 (also termed B7) receptor binding, negatively regulating T-cell activation[64,423]. High levels of CTLA4 are often expressed in CD4+ T cells in which HIV preferentially persists[484,485] . HIV also often persists in PD-1+ CD4+ T cells (as mentioned in chapter III.1).

Programmed cell death protein 1 (PD-1) is another major coinhibitory immune checkpoint molecule that induces tolerance and downmodulates antigen-specific immunity while binding to its two ligands PD-L1 and PD-L2[64,423]. Mylvaganam et al. studied the effects of an anti PD-1 antibody in combination with ART in SIV-infected macaques and observed decreased levels of cell-associated replication-competent virus, as well as beneficial effects in Th17 cell reconstitution in the rectal mucosa, expansion of proliferating CXCR5+ effector CD8+ T, enhanced T cell responses, better control of viremia and reduction of the viral reservoir size[486]. This was congruent with an earlier study by Velu et al. which suggested that administration of an anti PD-1 antibody in SIV-infected macaques could enhance proliferation of memory B cells, increase in SIV envelope-specific antibodies and lead to significant reductions in plasma viral load[487].

Studies in NHP models suggested that multiple checkpoint blockade would be necessary to target the largest subset of memory CD4+ T cells harboring replication competent provirus as possible, including both CTLA-4^{+} and PD1^{+} CD4^{+} T cells[221,426]. Indeed, In the NHP model earlier mentioned (in part V.2.3.), Harper et al. demonstrated that the dual blockade of CTLA-4 and PD-1 could induce robust latency reversal, enhance T cell proliferation, and reduce the total levels of integrated virus. Rhesus macaques receiving combined CTLA-4/PD-1 blockade showed a greater number of reactivated viral lineages compared to anti-PD-1 monotherapy. Immune checkpoint blockade were nevertheless insufficient to achieve a strong reduction of the reservoir and a viral control, indicating that combination with other therapeutic strategies could be required[426].

Several anti-PD-1 agents are currently being tested in ongoing phase 1 and 2 clinical trials in PLWH, including Pembrolizumab (NCT03239899, NCT03367754, NCT02595866), Nivolumab (NCT03304093, NCT03316274) and Cemiplimab (NCT03787095). It is also the case of anti-CTLA-4 Ipilimumab (NCT03407105) as well as a combination of Nivolumab and Ipilimumab (NCT02408861).

After their development for the cancer field, some of these checkpoint inhibitors (including pembrolizumab, nivolumab, and ipilimumab) have already been approved by the US Food and Drug Administration (FDA)[488], making conceivable to repurpose these molecules for the HIV field.

Other immune checkpoint molecules that have been less studied thus far in the context of HIV infection, could also potentially be targeted in HIV cure strategies (*Figure 18*). These include TIGIT (T-cell immunoreceptor with Ig and ITIM domains), LAG3 (lymphocyte-activation gene 3), Tim-3 (T-cell immunoglobulin and mucin domain containing-3) CD160, CD244/2B4, BTLA (B- and T-lymphocyte attenuator, also termed CD272), and the costimulatory immune checkpoint GITR (glucocorticoid-induced tumor necrosis factor receptor)[64,423,489–491].

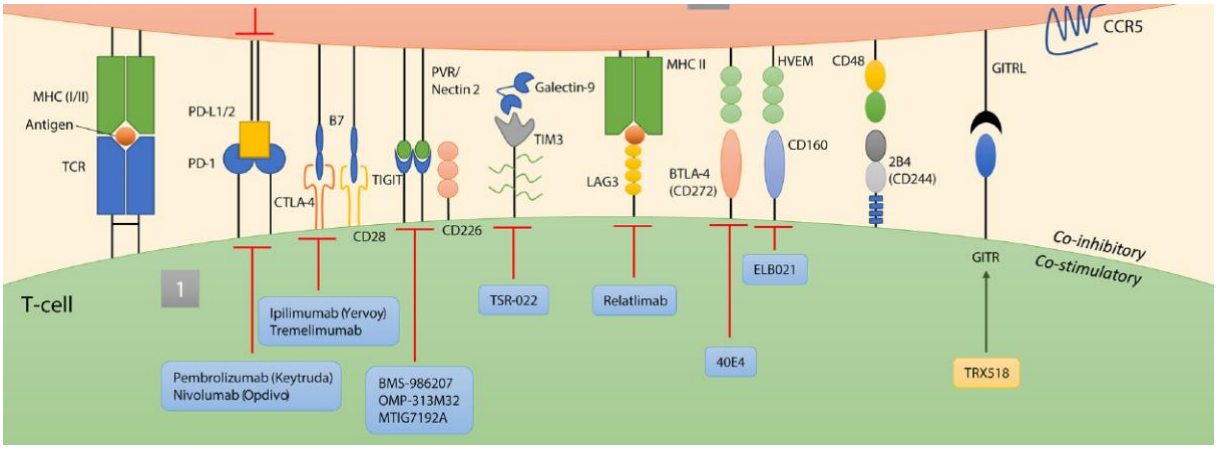


Figure 18. immune checkpoint receptors and their interactions with antigen-presenting cells.
Interactions between Immune checkpoint molecules and their ligands are represented between a T cell (green) and an antigen presenting cell (pink). Their potential inhibitors (or agonists) are represented in blue (or yellow) boxes, although not all of these have been detailed in our synthesis. Co-inhibitory checkpoint molecules such as PD-1, CTLA-4, TIGIT, Tim-3, LAG3, BTLA-4, CD160, and 2B4 are upregulated on T cells during HIV infection and negatively regulate T-cell proliferation and effector function when bound to their cognate ligands. In contrast, the co-stimulatory molecules CD28, CD226, and GITR enhance T-cell activation when ligated in conjunction with TCR-mediated stimulation.
Adapted from Henderson, L. J., Reoma, L. B., Kovacs, J. A. & Nath, A. Advances toward Curing HIV-1 Infection in Tissue Reservoirs. J. Virol. 94, e00375-19, (2019).

VI.2. Targeting metabolic and cell-death pathways

VI.2.1. Exploiting immune cell metabolism against HIV

In the recent years, the study of the relations between immunometabolism and HIV infection have raised as an emerging field of interest, potentially offering new opportunities for cure strategies[78–81].

The susceptibility of CD4+ T cells to HIV infections differs between naive and more differentiated subsets, and depends on various factors. The metabolic activity of the cells was recently found to be associated with differences in HIV-1 susceptibility, independently of their differentiation and activation phenotype. Indeed, CD4+ T cells with high oxidative phosphorylation and glycolysis were selectively infected by HIV-1, suggesting that HIV preferentially infects cells which display high metabolic activity[203].

As also described for other pathogens, HIV induces modifications in the metabolism of infected cells to redirect their resources for its structural and functional requirements and biogenesis[78]. Such metabolic dysregulations influence immune cell responses and cell survival. In order to provide immune response to infection, uninfected immune cells must also divert energy resources and adopt specific metabolic programs, including metabolic shifts in relation with the activation of specific cell functions[79]. It has been suggested that

such metabolic reprogramming and HIV-induced changes in immune cell metabolism and redox state might also contribute to CD4+ T cell depletion, chronic immune activation, and Immunosenescence.[78,79,81,203]

In adult PLWH, a correlation was observed between high glucose metabolic activities and CD4+ T cell depletion, and enhanced glycolytic activity in CD4+ T cells was shown to be associated with T cell activation[78,492,493]. Besides, in PLWH, activated monocytes display increased cell surface expression of Glucose Transporter 1 (Glut1) and increased aerobic glycolysis, which is accompanied by elevated glucose uptake and enhanced lactate production. In monocytes, such high glycolytic metabolism is required to produce pro-inflammatory cytokines, including TNF and IL-6 [79,492]. Upregulation of glycolytic metabolic pathways may dysregulate mitochondrial biogenesis, and result in oxidative stress, causing loss of immune functions[78]. On another note, fatty acid metabolism was also suggested to play an important role in the late steps of viral replication[494].

By taking advantage of this novel knowledge, the inhibition of metabolic activity was recently tested to specificially target HIV-1-infected CD4+ T cells (*Figure 19*). A "starve" approach has been proposed by Palmer et al. and consists in identifying high-priority metabolic activity of reservoir cells and related metabolic needs that are essential for viral infectivity, then creating deficiencies in those needs to limit homeostatic proliferation and eventually cause the death of infected cells[79,495,496]. For instance, 6-diazo-5-oxo-l-norleucine (DON), a glutamine antagonist and 2-deoxy glucose (2-DG), a competitive inhibitor of glycolysis, were shown to reduce HIV-1 infection of CD4+ T cells and block the early steps of its replication, with minimal cell toxicity and low cell death induction *in vitro*[81]. UK5099, a molecule that inhibits the transport of the glycolysis end product pyruvate to the mitochondria also blocked HIV infection, suggesting the importance of glucose oxidation for HIV-1 infection. 2-DG was additionally able to eliminate infected CD4+ T cells and to block HIV-1 amplification.

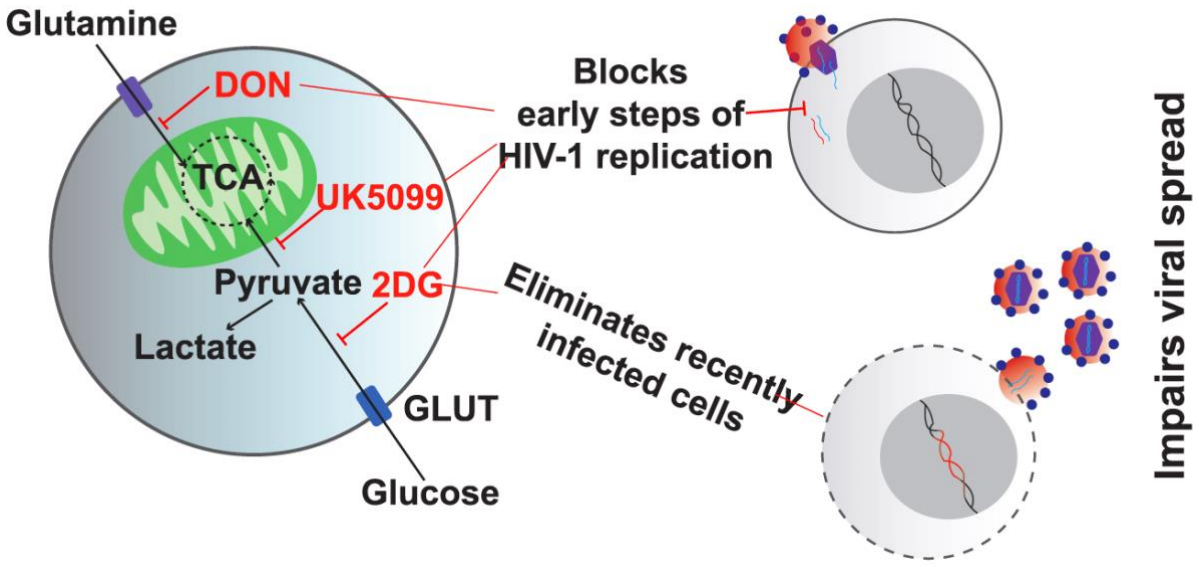


Figure 19. inhibition of metabolic to block HIV-1 replication
Molecules that inhibit metabolic pathways, such as 6-diazo-5-oxo-l-norleucine (DON), a glutamine antagonist; 2-deoxy glucose (2-DG) a competitive inhibitor of glycolysis , and UK5099, a molecule that inhibits the transport of pyruvate to the mitochondria are able to block the early steps of HIV infection in vitro. 2-DG is also able to eliminate recently infected CD4+ T cells and to block HIV-1 amplification.
Adapted from Valle-Casuso, J. C. et al. Cellular Metabolism Is a Major Determinant of HIV-1 Reservoir Seeding in CD4+ T Cells and Offers an Opportunity to Tackle Infection. Cell Metab. 29, 611-626.e5 (2019).

Rapamycin, which targets the (mTOR) pathway, not only raised interests for block and lock strategies[430,437] (as introduced in part V.3.) but is also an important regulator of glucose metabolism and cell growth, energy balance, and exerts effects on immunomodulation and cytokine production[79,438] (*Figure 20*). mTOR pathway inhibitors may thus both have favorable immunomodulatory effects and enhance antiviral responses[79]. Among other mTOR inhibitors that can exert effects on the metabolism is Metformin, an anti-hyperglycemic drug widely used in the diabetes field, that inhibits mTOR signalling independently of the Insulin-signaling pathway. Metformin has been shown to modulate T cell glycolysis by inhibiting mTOR. Improved CD4+ T cell counts were observed in diabetic PLWH initiating ART and receiving Metformin[497,498]. The effects of Metformin have also been studied in non-diabetic ART-treated individuals in a single-arm pilot study (NCT02659306), but no results are yet available[498].

Another molecule that could possibly be used in a “Starve” approach is hydroxyurea. Hydroxyurea is an inhibitor of ribonucleotide reductase that inhibits HIV reverse transcriptase by decreasing deoxynucleoside triphosphate (dNTP) pools[499]. Hydroxyurea has been extensively used in medical practice, mainly for the treatment of leukemia, sickle-cell

anemia and other diseases, because its mechanism targets preferentially highly proliferative cells. Although seemingly a bit forgotten today, hydroxyurea has also been used in the HIV field, mainly in the early 2000s during structured treatment interruptions and indicated that it could lower the rate of viral rebound in PLWH interrupting ART[499–502]. A possible role of hydroxyurea (HU) in reducing the viral reservoir has thus been suggested[500,502]. This well-known molecule thus appears as an interesting candidate to revise and assess in combination with other approaches for a functional cure.

In contrast to the “starve” approach, an opposite strategy (more similar to “shock and kill” and LRAs) could be conceptualized by the use of metabolic modulators to stimulate viral replication in reservoir cells, rendering those cells “unmasked” for the immune system which would turn able to “Purge out” the reservoir. The metabolic programming of immune cells could also be targeted to “redirect” or “boost” the immune response, enhancing their efficiency for functional cures strategies. All these perspectives highlight the need for further investigation concerning the complex questions of how metabolic remodeling of immune cells impacts the course of HIV infection, and how metabolic pathways could be harnessed in curative approaches[79,81].

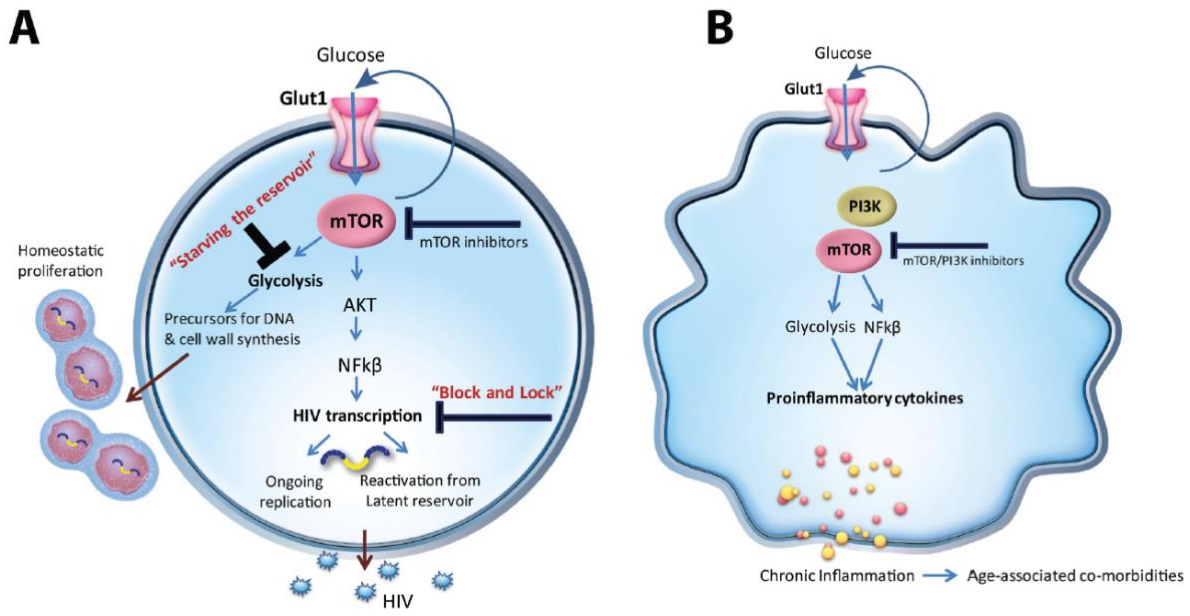


Figure 20. mTOR metabolic pathway and its potential inhibition in an infected CD4+ TCell (A) and in an activated macrophage (B)

HV-infected CD4+ T cells reprogram glucose metabolism toward glycolysis, which is marked by increased cell surface Glut1 and mTOR activation. (A) mTOR regulates HIV transcription, leading to viral reactivation and replication. Glycolysis regulated by mTOR provides precursors for DNA and cell wall synthesis which support homeostatic proliferation of infected CD4+ T cells. (B) Glut1 cell surface expression and glycolytic metabolism is also regulated by mTOR pathway in activated monocyte/macrophage. A metabolic shift toward glycolysis supports pro-inflammatory cytokine production in monocytes/macrophages. Glut1, glucose transporter 1; mTOR, mechanistic target of rapamycin.

Adapted from Palmer, C. S., Palchaudhuri, R., Albargy, H., Abdel-Mohsen, M. & Crowe, S. M. Exploiting immune cell metabolic machinery for functional HIV cure and the prevention of inflammaging. F1000Research 7, 125 (2018).

VI.2.2. Harnessing cell-death pathways to clear infected cells

After numerous studies of “shock-and-kill” strategies, it appears very unlikely that latency reversal alone could lead to eradication of the infected cells. Additional intervention for the ‘‘kill’’ is thus required[63,72]. Hence it was proposed to foster apoptosis with “Lock in and apoptosis” strategy[381] and various means could be used for this purpose. Diverse compounds that elicit cell-death pathways have been developed for the cancer field, and some of these could be used to induce apoptosis of HIV-infected cells (*Figure 21*). Such compounds have been extensively reviewed by Kim et al.[63] and we provide here a quick overview of their main classes.

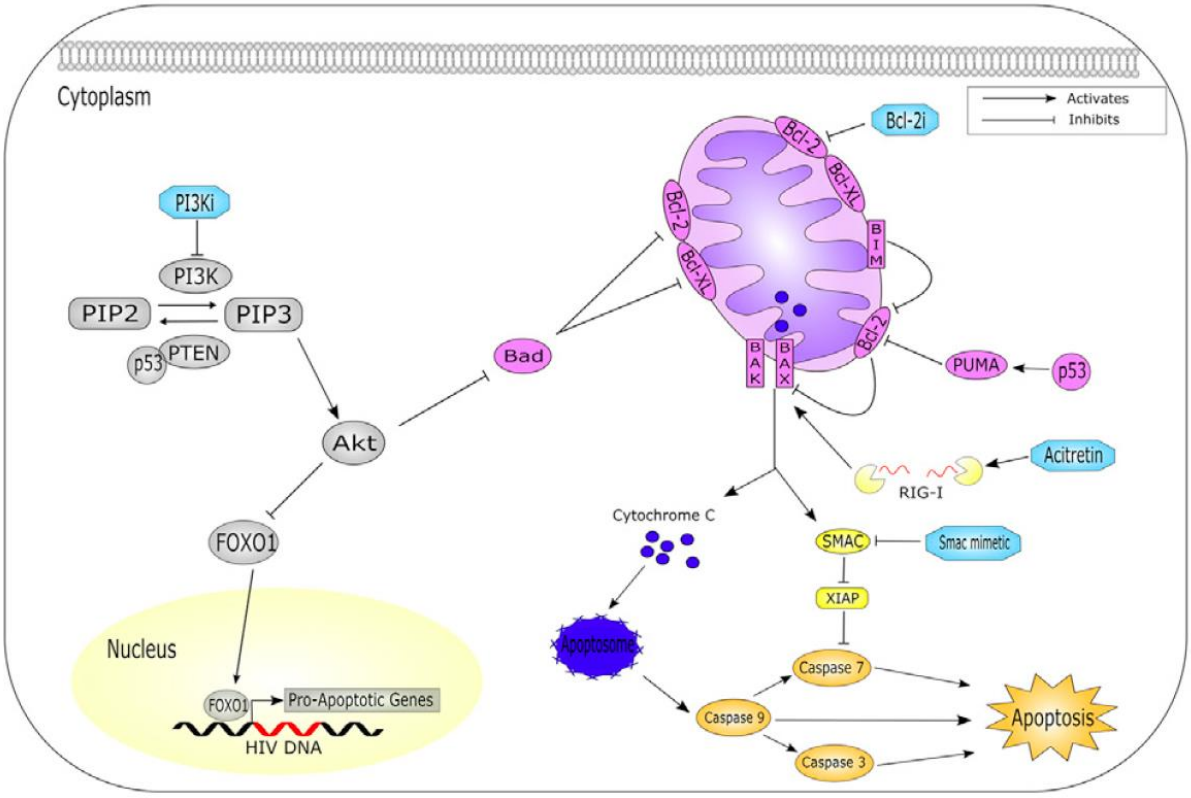


Figure 21. Apoptosis pathways and they modulation using compounds favoring cell-death pathways. *Diverse compounds that elicit cell-death pathways (in light blue) could be used to induce apoptosis of HIV-infected cells Compounds (in light blue). PI3K inhibitors block the conversion of PIP2 to PIP3, decreasing active Akt within a cell to stop Akt from inhibiting apoptosis. Akt inhibitors directly decrease active Akt to prevent Akt from inhibiting apoptosis to ultimately induce apoptosis. Bcl-2 inhibitors such as Venetoclax inhibit anti-apoptotic Bcl-2 to sensitize the cells toward apoptosis. Smac mimetics competitively bind inhibitors of apoptosis proteins like X-linked inhibitor of apoptosis (XIAP) to promote apoptosis. RIG-I detects viral RNA and activates pro-apoptotic Bak/Bax proteins, leading to apoptosis. In addition to viral RNA, RIG-I inducer compounds like the RA derivative acitretin can also trigger apoptosis.*
Adapted from Kim, Y., Anderson, J. L. & Lewin, S. R. Getting the “Kill” into “Shock and Kill”: Strategies to Eliminate Latent HIV. Cell Host Microbe 23, 14–26 (2018).

(i) Bcl-2 antagonists

Bcl-2 is a pro-survival protein that regulates apoptosis[503]. HIV protease generates a pro-apoptotic fragment, Casp8p41, which is sequestrated by Bcl-2. Bcl-2 antagonists, such as Venetoclax and Navitoclax, can Inhibit Bcl-2 leading to the liberation of Casp8p41 and its to promotion of apoptosis. Venetoclax can lead to the selective apoptosis and clearance of infected CD4+ T cells from PLWH on ART *ex vivo*, and is already available in the clinic for the treatment of chronic lymphocytic leukemia[504].

(ii) PI3K/Akt Inhibitors

PI3Ks are lipid kinases that regulates numerous intracellular signaling pathways in leukocytes, and whose activation promotes cell survival. The serine/threonine kinase Akt (also termed protein kinase B, PKB) is the major effector of the PI3K pathway. The two HIV proteins Nef and Tat can activate PI3K/Akt signaling pathway to prevent apoptosis[505]. The Inhibition of PI3K/Akt enables transcription of pro-apoptotic genes. Inhibitors of the PI3K/Akt pathway, such as Edelfosine, Perifosine, and Miltefosine were able to promote cell death in HIV-1 infected macrophages in vitro[505].

(iii)SMAC mimetics and IAP inhibitors :

Proteins of the inhibitor of apoptosis (IAP) family, including X-linked IAP (XIAP) and cIAP, can regulate apoptosis by direct or indirect inhibition of caspase activation[63]. Mimetics of the second mitochondrial-derived activator of caspases (SMAC) have been previously mentioned (in part V.2.2) for their potential as LRAs, but could also bind and block antiapoptotic IAPs to promote apoptosis. Interestingly, Campbell et al. showed that some SMAC mimetics (birinapant, GDC-0152, and embelin) could exploit autophagy and apoptotic machinery to selectively induce killing of HIV-infected long-lived resting memory CD4+ T cells while sparing uninfected cells *in vitro*[506].

(iv) RIG-I inducers

Retinoic acid-inducible gene I RIG-I is an innate immune response pattern recognition receptor that senses viral RNA within the cytoplasm of infected cells and can drive the apoptosis of virus-infected cells[507]. Acitretin, a retinoic acid derivative used in the treatment to psoriasis, was shown to induce the selective apoptosis of HIV latently infected CD4+ T cells from ART-treated PLWH *ex vivo*[508]. These results were not replicated in a similar study which concluded that stimulation of the RIG-I-dependent interferon cascade by Acitretin

may not significantly induce cell-death of HIV-infected cells[509]. Further studies of RIG-I inducers are thus needed to better delineate whether they could be of potential value for HIV cure approaches, including for their potential as innate immune modulators.

VI.3. Aiming at tissue damage protection and repair and reduction of inflammation

As developed in part II., major challenges for HIV therapeutics are raised by HIV-induced tissue damage, disruption of mucosal immunity, immune exhaustion, and chronic inflammation. Tissue damage control and disease tolerance are inherent features of the immune system[510], but are not sufficient to restore tissue and immune integrity and hinder undue inflammation in the case of HIV-infection. Therefore, therapeutic interventions aimed at favoring the protection of tissue and immune cells from the deleterious effects of HIV, repairing some of the induced damage, or reversing the chronic inflammatory state of treated HIV would be highly beneficial. They could improve the quality of life of PLWH by reducing the burden of inflammation-associated co-morbidities and could help to reach functional cure by preserving and enhancing the functionality of the immune system[46,99]. To this extent, various therapeutic approaches are being studied, including modulators of innate immunity, inhibitors of proinflammatory cytokines, probiotic supplementation, and other mechanisms already mentioned, including notably mTOR inhibitors. In this part, we will describe a few examples of molecules and approaches that are currently being tested.

VI.3.1. IL-21 immunotherapy for the preservation of gut immunity

IL-21 is a pleiotropic cytokine that is mainly produced by activated CD4 + T cells[82,83]. IL-21 exerts numerous enhancing and regulatory effects on immunity that include favoring maintenance of functional CD8+ T cell functionality[511], promoting NK cell expansion[82], and inducing and maintaining functional Th17 cells subsets[84]. Reduced plasma levels of IL-21 have been found in PLWH[512,513] and higher levels of IL-21 were found in HIV exposed seronegative individuals (HESN), suggesting that it may confer protective mechanisms[514]. In primates, pathogenic SIV-infection of rhesus macaques is associated with a significant loss of IL-21-producing CD4 + T cells and this is not the case of nonpathogenic SIV infection of sooty mangabeys[321,342,515].

Because of its role in inducing and maintaining functional Th17, IL-21 has been used to boost TH17 cells with the aim that these TH17 cells would foster the intestinal barrier and reduce inflammation. Pallikkuth et al. observed that SIV-infected RMs treated with IL-21 alone displayed transient increases in levels of intestinal Th17 cells associated with reduced levels of intestinal T cell proliferation, microbial translocation and decrease in systemic immune activation and inflammation. When combined with ART, Micci et al demonstrated that IL-21 administration could result reduce residual inflammation and virus persistence in another SIV-infected macaque model[516]. Ortiz et al. subsequently demonstrated that IL-21 combined with probiotic therapy could improve Th17 frequencies while reducing markers of microbial translocation and dysbiosis, resulting in fewer comorbidities compared with controls in a similar NHP model[85]. More recently, the safety of a combination therapy consisting of IL-21 plus anti- α4β7 monoclonal antibody (mAb) has been assessed in uninfected RMs[517]. The combination of IL-21 and anti-α4β7 mAb was well-tolerated and reduction of the gut homing of α4β7+ CD4 T cells was observed along with a decrease in levels of gut immune activation[517].

The promising effects of IL-21 on Th17 cells preservation and reduced immune activation observed in NHP models are encouraging and suggest that immunotherapy with IL-21 could be beneficial for tissue damage control, restoration of intestinal integrity, and immune enhancement in PLWH. In combination with ART, such therapeutics might attenuate some comorbidities in PLWH[85].

VI.3.2. Therapies moderating the effects of the interferon cascade

Interferon-associated therapies have been first considered as a new hope for the treatment of HIV-infection in the late 1980 and were initially aimed at enhancing the production of IFN-stimulated genes for their antiviral properties[86,518]. However, IFN-α2-based strategies were limited by adverse effects and imbalance between antiviral activity and inflammation induced by the IFN pathways[519]. Therapies that enhances interferon pathways might still be beneficial in patients with low immune activation and such approaches are currently evaluated in combination with other treatments to reduce the viral reservoir[86,520].

In a macaque model, IFN-I blockade in primary SIVmac infection (day 0 to 28 post infection) accelerated disease progression, highlighting the importance of the interferon response

during acute infection[521]. In contrast, early IFN-α treatment (day - 7 to 28 post infection) was shown to confer some protection against infection[521]. However, SIV infected macaques treated with IFN-α during acute infection displayed accelerated disease progression. Indeed, IFN-α2 treatment resulted in a in an IFN-desensitized state in these macaques, with decreased antiviral gene expression, increased susceptibility to infection, increased cell-associated virus load and greater CD4 T-cell depletion compared to placebo[521].

HIV infection induces a strong interferon-type 1 response in primary infection, and also IFN-ɣ responses. Chronic HIV infection is associated with a persistent increase in the expression of interferon-stimulated genes. Counteracting IFN pathways to reduce the IFN-associated inflammation was thought to prove helpful for immunological non-responders and PLWH with excessive basal IFN-signature despite viral control on ART[86]. Using a macaque model, it was suggested that blocking IFN-I signaling during chronic SIV infection could suppress IFN-I-related inflammatory pathways without increasing virus replication[522]. Various mechanisms could lead to a decreased IFN pathway, by either limiting the production of endogenous IFN by IFN-producing cells or limiting the signaling transduction in the target cells.

Hydroxychloroquine is an inhibitor of TLR7 and TLR9 signaling, initially developed as an antimalarial drug in the 1950s, that have been extensively studied in autoimmune disorders with chronic IFN-signature, notably in systemic lupus erythematosus[523]. Hydroxychloroquine has been under the spotlight in 2020, and is undoubtedly one of the most discussed drugs of the year, since its use has been proposed for the treatment of COVID-19 in the first months of the pandemic. A positive impact on the clinical outcomes of COVID-19 patients could not be demonstrated[524,525]. A potential benefit of hydroxychloroquine for PLWH had been suggested about ten years ago[86,99,405,526,527]. A reduction of excessive T cell activation by hydroxychloroquine has subsequently been observed in PLWH[528], but was unfortunately not observed in other clinical studies[405,527,529]. The potential of Hydroxychloroquine to limit immune activation in combination with other strategies of HIV cure is unclear.

Anti-INFAR antibodies represent another means of controlling IFN pathways. interferon-α/β receptor (IFNAR) can be targeted to limit the signaling transduction of IFN cascade. The use of anti-IFNAR antibodies antagonizing in humanized mouse models has led to some noteworthy results[530–532]. In those models, IFNAR antagonism led to a decrease in interferon stimulated genes (ISG) expression, that correlated with reduced expression of CD8+T cell

exhaustion and activation markers, restoration of CD8 functionality, and preservation of the CD4+ T cell count. Of note, reductions of the HIV inducible reservoirs were observed after ART interruption in both studies. These results point IFNAR antagonism as a promising means of downmodulating activated T cells towards a more quiescent state and restoring their functionality against HIV-infected cells[86].

Another pathway to hinder the signaling transduction of IFN cascade is represented by the inhibition of the Janus activating kinase signal transducer and activator of transcription (Jak-STAT) pathway. Earlier mentioned as potential latency promoting agents (in part V.3), Jak-STAT inhibitors could present significant benefits in the reduction of HIV-associated chronic immune activation. Indeed, JAK/STAT pathway is activated in chronic HIV infection and is involved upstream in the production of inflammatory cytokines (including IL6 and TNFα) and activation of immune cells, which contributes to the onset of inflammatory-related comorbidities such as cardiovascular disease and gut-immune dysfunction[86,533,534]. Jak inhibitors include ruxolitinib and Baricitinib that are available drugs for the treatment of myelofibrosis or polycytemia vera (Ruxolitinib), and rheumatoid arthritis (baricitinib)[89]. Gavegnano et al. demonstrated that Ruxolitinib could reduce infection of human primary lymphocytes and macrophages by HIV-1 and HIV-2 strains and inhibit reservoir establishment, maintenance and expansion *in vitro* and *ex vivo*[440,535]. The same molecule was also shown to induce reduction in immune activation markers including CCR5, HLA-DR, CD38, CD25, Ki67, and PD-1. A reduction of Bcl-2 expression in primary T cells *ex vivo*, potentially indicating implications in the modulation of cell-death pathways[89,439,440,536,537]. A Phase 2a clinical trial involving Ruxolinib in PLWH have recently been completed (NCT02475655). Besides, in a (SCID) mouse model, baricitinib was shown to be able to reverse HIV-associated neurocognitive disorders[538]. Jak inhibitors thus appear insightful for the management of HIV-associated immune dysfunction and chronic, encouraging further developments.

The downmodulation of interferon pathways by the aforementioned pathways could benefit anti-inflammatory strategies, and might be advantageously combined with other therapeutic interventions in PLWH for reducing HIV comorbidities and relieve the immune system from its chronic inflammatory burden, potentially improving the immune-mediated control of HIV reservoirs towards a functional cure.

Other approaches aimed at tissue protection and repair and reduction of inflammation are being currently tested as well, such as probiotics (to help to repair intestinal damage) and anti-fibrotics (to reduce fibroses in lymph nodes), in diverse combination[85,539].

VI.4. Therapies targeting unconventional and innate immune cells

As developed before, various immunotherapies have been foreseen and assessed in various models and clinical trials. Most of these studies have targeted CD8+ T cells responses, and many factors may account for their mixed results thus far[46]. The regions of the virus that are targeted by initial CD8+ T cell responses are often highly variable and resulting in rapid selection of escape mutants, and the breadth and the magnitude of CD8+ T cell responses is often insufficient for an effective clearance of infected cells[540]. T cells are driven towards a dysfunctional state by sustained exposure to HIV antigens, leading to T cell exhaustion, marked by upregulation of PD-1 and other regulatory pathways[478,479]. These dysfunctional responses that target escaped epitopes are generally immunodominant and rapidly expanded, leading to an overall inefficiency.

On the other hand, as stated in the second part of this work, HIV infection is accompanied by an early and sustained depletion and abrogation of the function and numbers of diverse cell types from innate or adaptive immunity, such as CD4 Th17 cells, γδ T cells, MAIT cells, and Innate lymphoid cells, notably ILC3 in the gastrointestinal tract, leading to disruption of the mucosal barrier and dysregulation of the local immune system[239–241].

Harnessing other cell types than conventional CD8+ T cells, modulating unconventional immune effector cells, or augmenting and potentializing innate immunity could thereby prove insightful to get around these issues.

VI.4.1. Modulating unconventional T cells

In Lymph nodes, CD8+ T cells are generally considered as unable to do migrate into the B cell follicles, which contributes to make follicles a prime sanctuary for HIV replication. However, a small proportion of CD8+ T cells expressing CXCR5+ has been described in both SIVmac and HIV-1 infections[541–543]. These CXCR5+CD8+ T cells in LN were present at higher levels in PLWH compared to uninfected individuals, and were observed in immediate proximity to viral RNA+ cells, probably as soon as the early stages of infection[541,544]. In SIV-

infected rhesus macaques, the viremia and frequency of T follicular helper cells were found to be negatively correlated with the frequency of SIV-specific CXCR5+CD8+ T cells[545]. These CXCR5+CD8+ T cell subsets would need to be better understood and characterized, *inter alia* to determine whether or not they represent a homogeneous population with shared features and potentially beneficial abilities to target.

Other unconventional CD8+ T cells are MHC-E-restricted CD8+ T cells. It was observed in the aforementioned therapeutic vaccine study by Hansen et al., that replication-deficient CMV vector with SIV inserts could elicit and exacerbate an unconventional MHC-E-restricted CD8+ T cell response of unprecedented breadth, contributing (along with other factors) to an efficient control of SIV infection in half of the immunized macaques[358]. MHC-E (or HLA-E in humans) is a nonclassical class I molecule that usually presents peptides derived from cellular proteins, including activation and stress-related proteins, such as Hsp60[546–548]. MHC-E was initially described as the ligand of CD94/NKG2 receptors expressed at the surface of NK cells, but also by some CD8+ T cells. Once complexed with peptides, MHC-E can indeed interact with the αβ T-cell receptor (TCR) expressed on CD8+ T cells[549]. MHC-E-restricted CD8+ T cells are crucial stakeholders for the prevention of autoimmune diseases, the maintenance of self-tolerance, and the prevention of and tissue damage. They are often considered as members of the CD8+ Treg family[550,551]. It has been shown that MHC-E is upregulated during HIV-1 infections[552], possibly in relation with increased circulating levels of inflammation markers such as HSP90 and IFN-ɣ. MHC-E restricted CD8+ T cells have been studied in inflammatory disorders, and have been shown to express tissue homing receptors, including CCR7 and CXCR5, as well as NK cell receptors such as NKG2A[542,549,550,553,554] Although the contributions of conventional CD8+ T cells are well-characterized during HIV/SIV infection, MHC-E restricted CD8+ T cells remain poorly understood. Characterization of their potentially beneficial implication in inflammation and tissue damage control would require further investigations.

Other unconventional immune cell populations, such as γδ T cells, may be worth attention for the HIV field. The γδ T cells is a unique subset of innate-like T lymphocytes with specific features which differ from conventional αβ T cells, which predominate in current cell-based immunotherapies[555,556]. γδ T cells can display anti-viral and cytotoxic activities and have been recently shown to directly inhibit HIV infection and specifically eliminate HIV-infected

cells *in vitro*[555]. They can recognize antigens in a MHC-independent manner and access to certain immune privileged anatomical sites, potentially making them very helpful to circumvent the hurdles faced by CTL-based strategies[556].

Enhancing the beneficial properties of unconventional immune cell subsets could prove to be a valuable approach to potentiate HIV cure strategies, and a deeper understanding of these cells in the context of HIV infection would likely be very insightful for future approaches.

VI.4.2. Harnessing Natural Killer cells

Among innate lymphoid cells, NK cells appear as a cell subtype of particular interest to harness for curative strategies. NK cells play an important role for the recognition and elimination of abnormal cells including stressed or infected cells. They have therefore raised growing interest in the field of oncology[557–560] and in other infectious diseases and viral infections[561–563]. In the case of lentiviruses infections, NK cells play a major role in the control of viral reservoir in lymph nodes.

This has notably been brought to light in natural hosts, such as African green monkeys, in which NK cells migrate into lymph node follicles, and play a major role in viral reservoir control[333]. In contrast, NK cells are not observed in follicles of secondary lymphoid organs in SIVmac-infected macaques[233,333]. SIVagm-infected AGMs display high levels of CXCR5+ NK cells in SLT. Strategies that allow to increase the levels of NK cells in follicles during HIV infection could be useful and are under investigation (*Figure 22*).

It is also likely that in SIVagm infection, Interleukin-15 enhances the survival of NK cells in the follicles. Indeed, in AGM, the increase of NK cell numbers in follicles has been shown to be associated with a high production of IL-15 within follicles. IL-15 is presented there in membrane-bound form by FDC and other antigen-presenting-like cells. IL-15 especially in its membrane-bound form is known to also enhance thee cytotoxic profile of NK cells[564–566]. Accordingly, the use of IL-15 appears as a potential way to target NK cell immunity for HIV clearance strategies. This cytokine has been administered into SIVmac infected macaques and analyzed for its potential to promote NK cell activity and increase their access – but also that of effector CD8+ T cell – to lymph node follicles. IL-15 stimulated NK cells have shown their ability to efficiently clear HIV-1-infected cells ex-vivo[566]. Il-15 and Il-15 superagonists, that mimic the membrane-bound form, such as N-803, were initially considered as potential

LRAs (chapter V.2). However, they could prove to be more beneficial in activating and directing effector CD8+ T and NK cells to the B cell follicle, as suggested by Webb et al. that studied N-803 subcutaneous administration in SHIV-infected macaques[419]. IL-15 mediated activation of NK effector cells could also improve the ability of the vaccine-induced antibodies to recruit effector cells for antibody-dependent cellular cytotoxicity (ADCC)[567].

Outside Il-15 and its agonists, other soluble agents could be used to potentially improve NK cell functionality[87]. Bispecific and Trispecific Killer cell Engagers (BiKEs and TriKEs) are small immunomodulatory molecules that could be used to potentiate NK cell functionality, and that once again are being studied in the cancer field. They are constituted of a single chain variable fragment (scFv) from an antibody connected to one (BiKE) or two (TriKE) variable portions of antibodies specific to an antigen expressed on the surface of target cells, and can induce ADCC via NK-expressed CD16 protein[87,568,569]. Their use could improve specific targeting by NK cells and enhance NK cell activation, survival and expansion and they can be combined with IL-15 administration. Li et al. designed and assessed BiKEs consisting of CD16A binding antibody domains fused to an engineered soluble human CD4 with high affinity to HIV-envelope glycoproteins, and showed that they could mediate killing of chronically and acutely HIV-1 infected T cells by human peripheral blood mononuclear cells[570].

TLR agonists, such as TLR7 agonist Vesatolimod (GS-9620), could also be used to activate NK cells. In the previously mentioned study by Borducchi et al. in which administration of this TLR7 agonist (alone or in combination with bNAb PGT121) led to a delayed viral rebound in SHIV-infected macaques, GS-9620 led to activation of NK cells. They hypothesize that activated NK cells may have had a key role in PGT121-mediated elimination of infected CD4+ T cells[415,416]. In the similar study by Lim et al., it was observed that in SIV-infected rhesus macaques, 20 to 25% of NK cells became activated after treatment with the other TLR7 agonist GS-986[417].

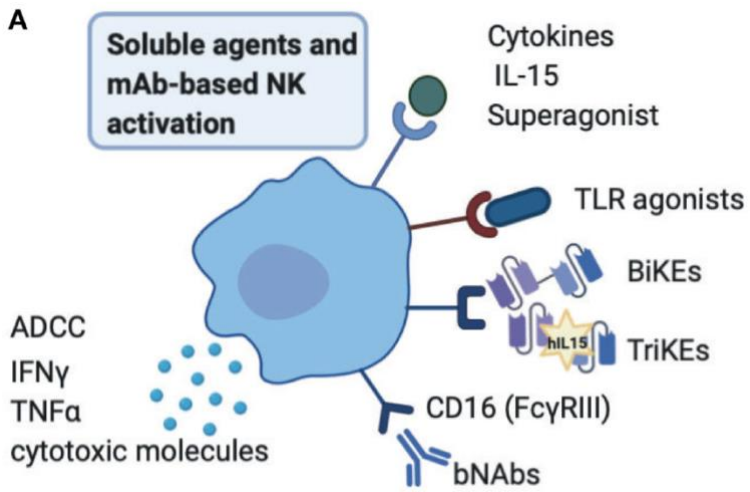


Figure 22. Therapeutic startegies to harness NK cells in HIV infection based on soluble agents and mAB. *The beneficial and potent immune features of NK cells could be harnessed through activation strategies. These include activation of NK cells through broadly neutralizing antibodies (bNAbs), engineered proteins, Bi-specific or Tri-specific Killer engagers (BiKEs or TriKEs), soluble mediators such as cytokines and TLR agonists. The boosted NK effector functions can include cytotoxicity and cytokine production.*
Adapted from Alrubayyi, A., Ogbe, A., Moreno Cubero, E. & Peppa, D. Harnessing Natural Killer Cell Innate and Adaptive Traits in HIV Infection. Front. Cell. Infect. Microbiol. 10, 395 (2020).

Another means of harnessing NK cell immunity includes the use of chimeric antigen receptors, similarly to the aforementioned CAR-T cells therapies. For instance, using a humanized model, Zhen et al. determined that CAR-modified hematopoietic stem/progenitor cell (HSPCs) can differentiate into functional NK cells *in vivo* and suppress HIV replication[475].

Although generally considered as stakeholders of innate immunity, growing data indicates that NK cell responses can also display features similar to adaptive T and B cell responses, such as establishing pools of memory-like cells and expanding antigen-specific NK cell population. “Adaptive” and “memory” NK cells have been reported in SIV/SHIV-infected rhesus macaques[571] and in PLWH[572–574]. The specific features of these “memory” NK cells, including their increased longevity, increased reactivity against immunogenetic epitopes, and enhanced capacity for ADCC, could be harnessed for HIV therapeutic strategies, notably in combination with other immune therapies such as bNAbs[87].

VI.5. Targeting the cells to the right place

HIV persistence in deep reservoirs and anatomic compartments that are not accessible to the immune system or antiretroviral drugs constitutes a major challenge for HIV therapeutic approaches. An increasing number of techniques and strategies are thus envisioned and tested to enable anti-HIV drugs and potent immune cells to traffic to infected cells in tissues, across these barriers and up to the sanctuary sites.

Penetrating the B-cell follicle, in particular, constitutes a significant stake to efficiently tackle HIV[88]. As developed in the previous paragraphs, CXCR5 follicular homing receptor is an important determinant of cell trafficking into the B cell follicle, and CXCR5 expressing NK or CD8+ T cells have been reported. Both immune therapies with bNAbs or multi-specific antibodies and adoptive therapy with *ex vivo* expanded CTL or CAR T cells will likely face significant limitations if they do not penetrate into the B cell follicle. However, Mylvaganam et al. demonstrated the ability of TGF-β to induce CXCR5 expression in CTL[545]. As previously stated, administration of IL-15 superagonist was also shown to promote the migration of CD8+ T cells and NK cells into B cell follicles[419]. On the other hand, using a NHP model, Ayala et al. demonstrated that transduction with CXCR5 performed by T cell engineering could lead to trafficking and localization of CD8 T Cells into Rhesus Macaque B-Cell Follicles[575]. Similarly, Bronniman et al. suggested that CXCR5-expressing lymphocytes could be transduced to secrete multi-specific antibodies or bNAbs in order to localize the effects of antibody-based immune therapy to the follicle[88].

A great variety of works are investigating new means and bioengineering techniques to route drugs to infected cells and/or present immunogens to immune cells in specific compartments that could lead to enhanced efficiency of natural immune responses[576–581]. Martin et al. used a rhesus macaque model to assess two strategies to route HIV immunogens up to B cell follicles: On the one hand, in vivo formation of immune complexes (ICs) with a passively transferred anti-Env mAb; on the other hand, generation of self-assembling protein nanoparticles displaying four copies of stabilized Env trimers[579]. They found that both ICs and nanoparticles led to a concentration of antigens at the periphery of B cell follicles in the draining LNs of NHPs. Nanoparticles were additionally shown to persist on follicular dendritic cells in the germinal centers. These encouraging results suggest that these two techniques could be employed to reach potent effector cells in B cell follicles. In a

similar approach, Francica et al. designed synthetic “star” nanoparticles aimed at trafficking to lymph nodes to bring immunogen proteins on site[580]. They tested these nanoparticles with HIV-1 peptide minimal immunogens in both mice and NHP models. Although no neutralization was observed with the immunogens they used, their study constitutes an encouraging attempt for the use of nanoparticles in immune therapies and vaccine strategies against HIV.

Numerous other nanoparticle-based approaches have been recently developed for the HIV field, and have been extensively reviewed by Bowen et al.[581] Among numerous potential benefits, these approaches could improve HAART delivery and efficacy, potentiate latency reversal and therapeutic vaccine approaches, enhance gene editing approaches, improve the efficacy of adoptive T cell therapy, and boost innate immunity[581]. Although preliminary, encouraging results from preclinical testing indicates that the use of nanoparticles to improve trafficking offers promising perspectives to overcome longstanding hurdles in HIV cure approaches.

The continued progress in bioengineering techniques, along with a better comprehension of the mechanisms responsible for immune cells homing and the establishment of sanctuary sites in HIV infection should hopefully lead to advances for therapeutic interventions in the years to come.

Conclusions & perspectives

Despite continued scientific progress and longstanding efforts from all the stakeholders involved in the attempt to end the HIV/AIDS pandemic, a therapeutic intervention leading to a fully curative “sterilizing cure” or a “functional cure” that ensures a sustained virus control in absence of antiretroviral therapy is still not available. The available effective antiretroviral treatments and prevention strategies that have been developed and implemented over the past decades constituted major achievements. Nonetheless, identifying an effective means of controlling the virus in absence of ART remains urgent from the perspective of individual PLWH as well as to the population level and at a global scale. Target product profiling and comprehensive design, development, and implementation of scalable approach, as well as means to assess a cure are required to search for new therapeutics that would benefit to the highest number of PLWH as possible.

Multiple biological challenges shall be kept in mind for the development of an effective cure approach, including (i) the great variety and diversity of HIV infected cells, (ii) the difficulty to specifically distinguish and target latently infected cells among uninfected cells, (iii) the multiple tissues harboring persistent virus, some of which being extremely difficult to reach (iv) the impairment of Immune responses, HIV-associated chronic immune activation, tissue damage, cell depletion and impaired physiological functions.

The use of animal models such as humanized mouse and non-human primate models – to better understand lentiviral infection and pathogenesis and assess curative approaches in *in vivo* pre-clinical models – as well as the study of nonpathogenic natural SIV hosts – to ascertain the mechanisms they developed throughout their coevolution with their lentiviruses and the ways they escape deleterious effects from SIVs – have provided undisputable contributions and remain very valuable for HIV science.

A wide range of approaches either directly targeting free virus and HIV-infected cells or harnessing immune function and favoring recovery have been studied, some of which led to encouraging and insightful results. These approaches sometimes fit within well-defined strategies such as “shock and kill” or “block and lock”, or sometimes suggest a revision of those paradigms. Future therapeutic prospects could benefit from the ongoing progresses in bioengineering technologies, but also emerge from less explored approaches such as the

modulation of immunometabolism[78,79], the harnessing of innate immunity and unconventional immune cell subtypes[87,555] or the management of HIV-induced damage, inflammation and alterations[85,86,99] following the growing tendency to increasingly enlist the body's own defenses and disease tolerance mechanism[510].

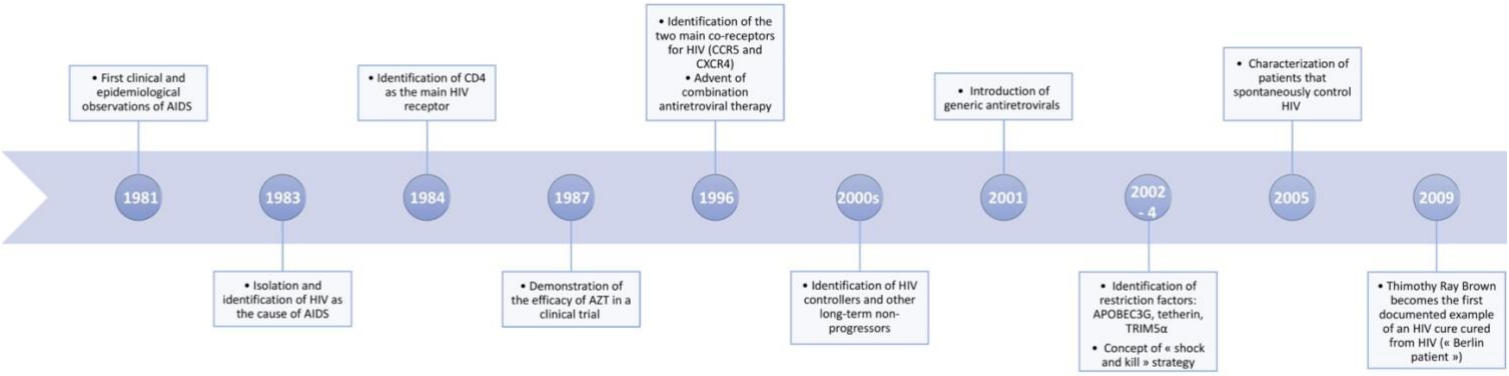
Besides, in the HIV science field and through the approaches studied, connections shall be made and sustained between cure and vaccine research[59,69,455,581,582], as well as with other infectious diseases[423,563,583], and other conditions including inflammatory diseases[203]. Significantly, HIV cure research display numerous parallels and crossroads with oncology[89–92,92,93], facing similar facing similar but also several different challenges.

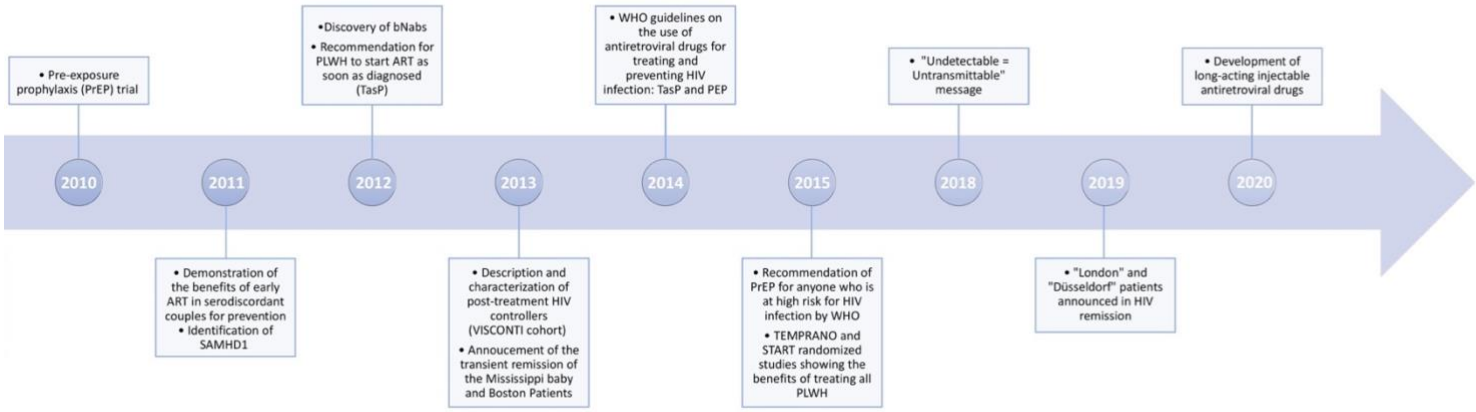
In a near future, the most promising strategies will most likely consist in combining agents targeting different mechanisms or complementary approaches, possibly in association with antiretroviral therapy initiated early after infection in the first instance[414,415,417,422,486].

Although the road to curing HIV is long and difficult to forecast, continuous progress towards an ideal outcome are being made, slowly but steadily, and sudden breakthrough sometimes fast forward the advances. Altogether, a myriad of encouraging results and promising concepts indicates that a HIV cure may be not so far from being attainable. These efforts undertaken might also result, as it has already been the case in the past, in novel concepts and new tools for the fight against other diseases.

Appendix

Figure 23. Timeline presenting key moments in HIV research.
AIDS, acquired immunodeficiency syndrome; HIV, Human Immunodeficiency Virus; CD4, cluster of differentiation 4; AZT, azidothymidine; CCR5, CC-chemokine receptor 5; CXCR4, CXC-chemokine receptor 4; APOBEC3G, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G; TRIM5α, tripartite motif-containing 5α; WHO, world health organization; PrEP, pre-exposure prophylaxis, PEP, post-exposure prophylaxis; TasP, treatment as prevention.




• Pre-exposure prophylaxis (PrEP) trial
2010
2011
• Demonstration of the benefits of early ART in serodiscordant couples for prevention
• Identification of SAMHD1
•Discovery of bNabs
• Recommendation for PLWH to start ART as soon as diagnosed (TasP)
2012
2013
• Description and characterization of post-treatment HIV controllers (VISCONTI cohort)
• Annoucement of the transient remission of the Mississippi baby and Boston Patients
• WHO guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: TasP and PEP
2014
2015
• Recommendation of PrEP for anyone who is at high risk for HIV infection by WHO
• TEMPRANO and START randomized studies showing the benefits of treating all PLWH
• "Undetectable = Untransmittable" message
2018
2019
• "London" and "Düsseldorf" patients announced in HIV remission
• Development of long-acting injectable antiretroviral drugs
2020

AGREMENT SCIENTIFIQUE

En vue de l'obtention du permis d'imprimer de la thèse de doctorat vétérinaire

Je soussigné, Romain VOLMER, Enseignant-chercheur, de l'Ecole Nationale Vétérinaire de Toulouse, directeur de thèse, certifie avoir examiné la thèse de **Gauthier TERRADE** intitulée « **Vers une thérapie curative pour le VIH/SIDA : Panorama des stratégies étudiées et apports des modèles animaux** » et que cette dernière peut être imprimée en vue de sa soutenance.

Fait à Toulouse, le 20/10/2020
Enseignant-chercheur de l'Ecole Nationale Vétérinaire de Toulouse
Docteur Romain VOLMER

Vu :
Le Directeur de l'Ecole Nationale Vétérinaire de Toulouse
M. Pierre SANS

Vu :
Le Président du jury
Professeur Pierre DELOBEL

Vu et autorisation de l'impression :
Le Président de l'Université Paul Sabatier
M. Jean-Marc BROTO

Le Président de l'Université Paul Sabatier,
Par délégation
Le Vice-Président de la CFVU
Fabienne ALARY

M. Gauthier TERRADE
a été admis(e) sur concours en : 2015
a obtenu son diplôme d'études fondamentales vétérinaires le: 09/09/2019
a validé son année d'approfondissement le: 31/08/2020
n'a plus aucun stage, ni enseignement optionnel à valider.

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Gauthier Terrade

Titre :

Vers une thérapie curative pour le VIH/SIDA : Panorama des stratégies étudiées et apports des modèles animaux.

Résumé :

Près de quarante ans après l'émergence du VIH/SIDA, des progrès scientifiques et thérapeutiques majeurs ont été obtenus. Toutefois, aucun traitement curatif n'est actuellement disponible.

On se propose dans ce travail de fournir un panorama : (1) Des raisons pour lesquelles un traitement curatif est nécessaire ; (2) Des prérequis pour développer un traitement curatif ; (3) Des obstacles à surmonter ; (4) De l'utilisation, des contributions et des limites des modèles animaux dans la recherche sur le VIH ; (5) Des approches thérapeutiques curatives définies au cours des dernières années ; (6) De certains concepts innovants, non-conventionnels, ou moins étudiés.

Un ensemble de résultats encourageants et de concepts prometteurs indiquent qu'une approche thérapeutique curative pour le VIH/SIDA demeure atteignable ; et la combinaison de différentes approches et les progrès en cours et à venir rendent tangible l'objectif d'une rémission en l'absence de traitement antirétroviral.

Mots clés : VIH, Thérapie curative, Modèles animaux, Primates non-humains, Rémission

Title:

HIV cure research: strategies in pipeline and contribution of animal models.

Abstract:

Nearly forty years after the emergence of the HIV/AIDS pandemic, research has continued uninterrupted, leading to major scientific and therapeutic breakthroughs, including antiretroviral therapy (ART). Nonetheless, ART is not curative and a cure is still missing.

In this work we aim at providing an overview of: (1) Why an HIV cure is needed; (2) The major steps required to develop a cure; (3) The major hurdles to overcome; (4) Why animal research (and especially non-human primate models) is needed in HIV science and what are their contributions and limitations; (5) Well-defined approaches toward a cure; and (6) a presentation of novel, nonconventional, and scarcely explored concepts.

Whilst a cure for HIV infection and AIDS remains unavailable, a myriad of encouraging results and promising concepts indicate that it is still attainable. Combinations of different approaches and ongoing progress could enable to reach the goal of long-term ART-free viral remission in a not-so-distant future.

Key Words: HIV Cure, Animal models, Non-human primates, HIV, SIV, Remission