TESIS DOCTORAL

2025

New therapeutic strategies based on tyrosine kinase inhibitors for the elimination of the latent reservoir of HIV-1

Mario Manzanares Torrijos

Programa de Doctorado en Ciencias Biomédicas y Salud Pública

Directora de tesis: María Teresa Coiras López

Este trabajo está financiado por el Programa Intramural 2020 del Instituto de Salud Carlos III a través del contrato ISCIII-PFIS FI20CIII/00021 y los proyectos:

- Ensayo clínico Ponazero (NCT040443676) de la Fundación Teófilo Hernando.
- Proyecto R01AI143567 del National Institutes of Health (NIH)
- Proyecto PID2019-110275RB-I00 del Ministerio de Ciencia e Innovación de España.

A mi tío Carlos

A mi familia

“Sólo sé que no sé nada”

Sócrates

“Lo único que podemos decidir es qué hacer con el tiempo que se nos ha dado”

Gandalf-El Señor de los Anillos. Obra de J. R. R. Tolkien

Agradecimientos

Aquí llega una de las partes que más esperaba, y es el poder agradecer a toda la gente que me ha acompañado estos 4 años todo su apoyo, ayuda, consejo, risas, y, como no, que me hayan soportado con mis bromas. Si con algo me quedo de este tiempo, es el haber tenido la gran suerte de haber estado rodeado y haber conocido a personas que han estado ahí, fueran momentos complicados o no, y que han hecho toda la tesis mucho más amena y gratificante, además de haberse convertido (si no lo eran ya antes) en una parte esencial de mi vida.

Debo empezar agradeciendo a mi directora de tesis, Mayte Coiras, el gran apoyo y todo lo que me ha enseñado en estos años. Nunca voy a olvidar aquella llamada telefónica un 31 de diciembre de 2020 para ofrecerme la posibilidad de hacer la tesis, que para entonces yo llevaba buscando casi 1 año. Desde entonces, siempre me has aportado una gran enseñanza como científico, siempre disponible para todo el grupo y para dar tu ayuda y consejo. Sin duda, en cada reunión o charla que hemos tenido, me has transmitido una gran curiosidad por saber más y aprender más, sin temer a hacerlo, dado que ese aprendizaje y esa curiosidad, aunque conduzcan al fracaso o a no obtener resultados, siempre nos hacen crecer. Por supuesto, debo agradecerte todas las oportunidades que nos ofreces para seguir adelante en nuestra recién comenzada carrera científica, y que siempre estés luchando por todo el grupo. Muchísimas gracias por todo.

Durante estos 4 años, el grupo de equipo reservorio ha ido creciendo mucho y ha sido una suerte haber conocido a compañeros de trabajo con los que compartir el día a día es un gusto. Con ellos, he tenido la suerte de compartir los Congresos a los que hemos asistido durante estos años y en los que me los he pasado genial gracias a su compañía. Quiero comenzar agradeciendo a Guiomar, la reciente Dra. Casado-Fernández, que ha estado ahí desde el principio, su gran apoyo y enseñanzas en mis inicios, y durante el resto de la tesis. Sin duda, una de esas grandes personas que siempre ofrece su ayuda de forma desinteresada, se preocupa por el resto del grupo, y de la que su forma de trabajar y pensar han sido un ejemplo a seguir para mí. También debo agradecerle a Guiomar nuestro primer Congreso en Miami de 2022, donde era mi primera vez cruzando el charco y su seguridad y organización resultaron ser un gran respiro en aquel primer viaje y Congreso que tan de los nervios nos tenían. Gracias a Clara, que también has estado ahí desde los inicios, y que me enseñaste a hacer mi primer marcaje de citometría (para luego acabar haciendo 104^{18} marcajes más), y con la que he tenido la suerte de compartir laboratorio, conciertos, alguna

cervecilla, risas, y de la que admiro su voluntad de trabajo y su estómago infinito. Gracias a Elena, la cual se me hace hasta complicado agradecer en unas pocas líneas todo lo que hace por el equipo y el gran apoyo moral que significa tener a alguien tan genial en el grupo. Debo agradecerte todas tus enseñanzas en estos años, tanto en el laboratorio, como en la forma de trabajar, esa actitud en la vida de dar importancia a las cosas que realmente las tienen, que siempre estés disponible para ayudarnos y para escucharnos, aunque sea una de mis bromas (es probable que hayas sido mi mejor público en estos 4 años). Has estado siempre ahí en los momentos más complicados y siempre disponible para ayudarnos en decisiones complicadas con una forma de ver las cosas que me encanta. Gracias también por ser un apoyo para la idea de que un incendio suele ser la solución a gran parte de los problemas en la vida. Gracias a Alicia, por ser una gran compañera de laboratorio y un gran apoyo para seguir creciendo en ciencia. Me ha encantado encontrarme con alguien con quien compartir esa visión más escéptica de la ciencia y la vida, y con la que siempre nos echamos unas risas, sobre todo cuando toca poner un parte porque cualquier cosa funciona mal en el CNM. A Oli, la persona que ha tenido la grandísisisima suerte de compartir mi mismo lado de despacho y así poder disfrutar de mi persona a diario. Ahora en serio, gracias por ser también un gran apoyo. siempre dispuesta a ayudar, escuchar y echarnos unas risas, sobre todo cuando nos traes alguna de tus nuevas modas para comer, beber o pestañear mejor o cuando inicias otro máster más. Siempre agradezco los días que te toca venir por el CNM porque haces el día mucho mejor. Gracias a Montse, por ser también guía en esos primeros meses en el grupo y por ser un gran pilar de apoyo y organización para todo el equipo. Gracias a Laura, con la que he compartido buenos ratos, charlas y algún que otro día de estrés en el labo. A Luis, gracias por tu apoyo en el labo, al que te adaptaste tan rápido, y por las risas que nos echamos contigo. A Valentina, gracias por tu compañerismo, lo bien que me lo he pasado contigo y por ser una gran alumna en el espectral desde tus inicios. A María José, a la que agradezco su gran compresión conmigo cuando le tocó empezar a hacer toda la parafernalia con las NKs, y con la que se ha convertido también en una suerte compartir grupo. Y gracias a María por convertirte tan rápido en una más del equipo y compartir tantos ratos buenos en los desayunos y comidas.

Me gustaría mencionar también a aquellas personas que han pasado por nuestro grupo y con las que he tenido el placer de compartir muy buenos momentos. A Lorena, que estuviste también desde el principio haciendo de nuestra guía y aguantaste todas mis preguntas de citometría cuando los gráficos tenían forma de churro. A Fer, mi gran compañero en el Ponazero, con el que he podido compartir grandes risas, sustos, algún

grito y estrés en mis primeros años, y que fue también un gran apoyo en esos inicios, y con el que aprendí muchísimo. Gracias a Lucía, que aunque estuvo solo un año con nosotros como ”Pokemon salvaje”, fue también parte del grupo y me encantó poder compartir algunas risas y dolores contigo, y con la que es siempre un gran placer volver a encontrarse.

Igualmente, me gustaría agradecer a mis compañeros de despacho todo su apoyo en estos años. A Marcos, que, aunque recientemente te has marchado del CNM, ha sido un placer poder compartir buenos y malos momentos contigo, anécdotas de la vendimia, alguna cerveza y la pasión por la divulgación. Te deseo mucha suerte en lo poco que te queda para completar la tesis y en la nueva etapa que estás iniciando. A Clara y Carmen, por los buenos ratos en las comidas y en el despacho. A Juan, por todas las risas y buenos momentos en el despacho o cuando toca desahogarse un rato haciendo el tonto. A Almu, la mejor profesora de andaluz y ganadora Best Outfit 2024, y camino a serlo en 2025 de momento. Es una suerte haber coincidido contigo, por todo lo que me haces reír, tu actitud en la vida (escuchamos y juzgamos, siempre), por ser una persona tan amable y que se integra tan bien. También gracias a esos Tik Toks tan buenos que me mandas siempre que me alegran las semanas. Ah, y gracias a tu *strong andalusi accent* que es de lo mejor del CNM. Gracias a Cris Millán, que aunque te marchaste hace unos pocos meses, siempre que has vuelto a hacernos una visita me ha hecho una gran ilusión verte. Te convertiste en poco tiempo en una más del despacho y lo bien que me lo he pasado contigo no tiene precio. Gracias por todo tu apoyo en los momentos más duros, tanto personales como de la tesis, y por ser una gran persona en todos los aspectos, sobre todo en destrozar gimnasios con tremendos biceps. Y a Cris Mínguez (Cristina Mínguez Anchuelo), que en poco tiempo te convertiste en mi vecina de sitio en el despacho y descubrí lo gran persona que eras y el gran apoyo que me dabas siempre. Gracias por esos buenos momentos para elegir ropa de Mango, por ayudarme a aprenderme las palabras Uniqlo y Outlander, muy importantes en la vida de cualquier ser humano, y por soportar las bromas a la hora de comer, que siempre te caía todo el bullying. Aunque ya no estés por el CNM, recuerda, el CNM son los amigos que hacemos en el camino, no el lugar.

Quiero agradecer también al resto de gente de otros grupos del CNM con la que he podido pasar buenos ratos y reírnos un rato. A la gentecilla del despacho de Hepatitis con la que he tenido la suerte de compartir buenos ratos en los Congresos, comidas o en los pasillos: a Violeta y Sonia, gracias por las charlas en los pasillos y por ese gran viaje a San Francisco en el que casi morimos congelados (pero el vino y un poco de pala lo salva todo). A Rafa, Manu, Ana V. y Sergio, gracias por todos los buenos ratos, consejos y las fiestas en los

Congresos. Me gustaría también agradecer al equipo de Inmunopatología del Sida, Javi, Fran, Almu, Manoli, Paloma, Mercedes y Luismi, por las conversaciones de pasillo, laboratorio y ofrecerme vuestra ayuda siempre que la he pedido. También dar las gracias a Eloísa, por su sus buenas charlas y su ayuda en estos años. No me quiero dejar atrás a Pepe, que, aunque ya se ha marchado del CNM, siempre ha sido un gusto poder hablar contigo, encontrarnos en los Congresos y poder compartir vivencias y preocupaciones. Me gustaría aprovechar para dar mil gracias a la Unidad de Citometría de Flujo, Mario, MariCarmen e Irene, por toda vuestra ayuda y consejo en estos años, sin la cual no podría haber aprendido tanto en tan poco tiempo.

Aunque vaya a dedicarles un párrafo aparte, mi tiempo en el CNM también me ha permitido conocer a varias de las personas a las que más valoro en mi vida, y es que sin ellos este camino habría sido mucho más complicado. A Ana G. (Dra. Moños), millones de gracias por tu apoyo diario en los peores momentos (me salvaste 2023, para que mentir) y también los mejores, tus cafecitos los días que duermo 5 horas y todas las risas y buenas charlas intentando arreglar el mundo. Gracias Rubén, por tu gran acogida en mis primeros días en el CNM, darme una gran perspectiva de pensamiento como persona y como científico, y por ser un gran consejero y mejor amigo. A Ana "Dounouso", mil gracias por toda tu ayuda en estos años al enfrentarnos a toda la burocracia del ISCIII, o del programa de doctorado, o haciendo las dichosas comisiones de servicio para los Congresos. Gracias también por todo tu apoyo durante la tesis, las buenas charlas para arreglar la ciencia del país o para resistir a los que nos desmotivaban para no seguir adelante. Nunca olvidaré ese gran viaje a Seattle y Vancouver (infarto incluido en el alquiler del coche) donde lo pasé en grande y disfrutamos del verdadero countryside de Estados Unidos.

No quiero tampoco olvidarme de Anäis, Jessica, Ilde e Ilya, que brindan de una especial ilusión a los Congresos por esos rencuentros, con alguna cervecita y muy buenos ratos. Gracias chicos.

Now, I would like to thank my colleagues at the Institut Pasteur who made my stay there so pleasant and fun. Their support not only made my time there pleasant but also allowed me to gain new scientific perspectives. I would like to start by thanking Asier for allowing me to stay in his group and for everything I learned with him and his team during those months. Thanks to Mäel, for being a great lab partner, his teachings and the laughs when I tried to learn something in French or he in Spanish. Thanks to Luis, for being a great guide for me during my stay, for transmitting me so much about science and giving me so many

teachings. To Mariane, from whom I still have in my office the Badge I stole from her at the Pasteur Virology conference, and with whom I had so much fun. I will always remember those days at P3 with the music and the slippery floors that made it seem like we were dancing. Thanks also to Grégoire, for his great help in the first weeks of my stay and for making my life so much easier by taking care of all the bureaucracy at Pasteur. I would also like to thank the HIV, Inflammation and Viral Persistence team, who also made me feel like one of the group and were so supportive. To Michaela, thank you for those good talks and your motivation to keep going. To Emma, Aurelio, Caroline and Faustine who gave me so much support and welcomed me so well from the beginning. I also don't want to forget Merlin, who I had such a hard time learning to pronounce his name well, and who turned out to be such a great person and who welcomed me so well. Thanks to all of you for those Fridays at StaPa and L'arttiste (Luis' Bar, best place in Paris) where I had such a good time and which soon became one of my favorite moments of the week.

En París también tuve la oportunidad de alojarme en el Colesp, donde tuve la grandísima suerte de conocer a mis Tiparracos. Con ellos compartí grandes viajes por Francia, en los que nada salía mal porque somos un grupo brutal. Gracias a mi hermana de Colesp, Cris, de la que siempre recuerdo la primera vez que hoy tu voz a mi espalda, y que tanto me apoyó en mis primeros días por París. Por fortuna pude conocer a una persona con la que tanto me rio, es tan interesante tener cualquier tipo de charla y siempre muestra una cara agradable y una cálida sonrisa. A Tania, mi compañera de cervezas, que sus desconexiones mentales tanto nos han hecho reír, y con la que he podido compartir hasta mi último día en Paris. Gracias por esos viajes y buenos momentos, y por esa fiesta en la Féte de la Cité en la que tan bien me lo pasé contigo. Gracias a Fran, mi gallego favorito, una gran persona y un gran apoyo durante toda mi estancia, y cuya dedicación y perspectiva profesional son un ejemplo a seguir. Gracias también por ese acentazo gallego que tanto se me pegaba y por enseñarnos a adorar la Illa de Arousa. Gracias a Inés, y gracias a la suerte que tuvimos de que entrara a formar parte de nuestro grupo y pudiera llegar a conocerla. Gracias por ser una persona tan increíble y una caja de sorpresas, por esas charlas de cine y música tan entretenidas y que siempre estoy deseando tener contigo. A la Yola, a la que tan pronto comenzamos a echar de menos, gracias por las risas y los grandes momentos contigo, y por esa risa única de ti que tanto nos gustaba. A Jaime, que tan bien se integró en el grupo y que nunca faltaba a las cenas, tardes de cervezas y viajes. Gracias por tu apoyo durante estos meses y toda la suerte del mundo con el final de la tesis. Gracias Miki, que siempre recordaré tus caras cuando Fran, Cris o yo soltábamos alguna barbaridad. Y gracias a

Claudia, cuyo acento canario adoraba, y con la que tan bien me lo pasé en las fiestas del Colesp. Fue una alegría tenerte por París.

A mis amigas Celia, Silvia y Paula, con las que unos (malditos) cursos de bioinformática nos unieron y a los que hoy doy gracias de haber decidido hacer con ellas hace ya casi 6 años. Gracias Celia, a la que conozco desde la Universidad, y que desde entonces hemos seguido el mismo camino hasta hace poco. Siempre he agradecido tu amistad por tu gran apoyo, en todas las fases de mi vida en las que has estado, con la que tanto he aprendido y mejorado como persona y con la que tanto me he divertido. Siempre recordaré esos saludos por los pasillos del CNM llenos de "cariño", los bailes en los Congresos y los piezas con los que nos encontrábamos en los viajes. A Silvia, con la que tuve además la suerte de compartir piso durante un año, y que tantísimo me apoyo en los peores momentos y tan geniales hace los buenos momentos. Gracias por ser un referente como científica y como persona, y por unirte a los gritos por la casa. Gracias a Paula, por todo tu apoyo durante estos años, tus ánimos para avanzar y motivarme en la ciencia, y por esos buenos viajes a Copenhague, Disneyland o Tenerife. También me gustaría incluir en estos agradecimientos a Irene, que aunque se marchó a EEUU hace más de dos años, siempre me ha dado su apoyo y me ha motivado para seguir adelante, explorar nuevas ideas y perspectivas en la vida.

También quiero mencionar mis amigas María y Laura, que tanto me han ayudado en este proceso. A María puedo agradecerle medio mundo, por tu simpatía, empatía y por ser una persona increíble que tanto siempre me ha aportado, sobre todo para conseguir ser una mejor persona. Gracias por darme tanta alegría y buenos ratos desde la Universidad, por escuchar mis consejos o mis quejas sobre el mundo, y por aguantar mis bromas y mi continuo bullying. A Laura, mil gracias por transmitir esa calma y bondad que tanto te caracterizan, y por tu apoyo y paciencia en estos años (sobre todo para aguantar a María, que telita), y todos mis ánimos en esta nueva etapa que has comenzado para hacer el doctorado.

A mis profesores del Instituto, a los que agradezco sus enseñanzas y comenzar a cultivar en mí la curiosidad y las ganas de hacer algo diferente. Me gustaría agradecer a Rosa y José Manuel, mis profes de Biología, que tanto y me enseñaron y tanto disfrutaba sus clases. También a Arancha, profe de Física y Química, que tanto nos inculcó la curiosidad y las posibilidades de la ciencia como herramienta de aprendizaje y progreso. Y gracias a Félix, profe de Historia, por esas clases que a mí tanto me gustaban de la que es ahora una de mis aficiones favoritas.

Y ahora viene la traca final, utilizando términos del pueblo, en la que quiero comenzar agradeciendo a mis amigos de toda la vida, que llevan acompañándome desde que prácticamente tengo conocimiento, por darme siempre fuerza pasando buenos ratos con ellos y por esos desahogos en las Fiestas del pueblo que son uno de los mejores momentos de todo el año. A mis amigos de La Fiebre: Ángel, Berto, Parri, Isma, Javito, Javi Xurre, Jaime, Rosco, Mario Caba y Tomás. Gracias por estar siempre ahí y aguantar la matraca de petardos e incendios en las Fiestas. Es un placer poder disfrutar de amigos tan buenos, con sus toques de locura, que hacen que a mi pueblo pueda llamarlo hogar. Siento no haber podido descifrar durante mi tesis toda nuestra problemática con los números de Mason o lo que ocurrió en Bahía de Cochinos. Gracias a mis amigas de PaQ+: María Mora, María Jota, Cris, Mirian, Irene, Irene Vallecas, Arancha, Fátima, Esme y Andrea. Gracias por vuestra sensatez, ofrecer vuestro hombro siempre que lo he necesitado, y esos grandes momentos en todas las fiestas. Gracias a ambos grupos de amigos por cada minuto que he pasado con ellos desde mi infancia y que su compañía y crecimiento junto a ellos me han hecho, sin lugar a dudas, mejor persona.

Gracias a mi familia Torrijos: Chacha Feli, Antonio, Piter, Teresa, Álvaro, Cache Pedro, Tía Ana, Víctor y Pedro José. Gracias por estar siempre presentes para dar vuestro apoyo incondicional, por sacarme una sonrisa siempre que bajo al pueblo y nos juntamos y por esas cenas de Navidad que son siempre de lo mejor del año y que tanto me han levantado el ánimo y me han hecho comenzar el año dando las gracias por teneros cerca. También debo agradecer a mi abuela Ramona su apoyo y sus sonrisa y alegría siempre que nos encontramos, y a mi abuelo Pedro por su entereza y la fuerza que transmite. A mi familia Manzanares: Luis, Paz, David, José Luis, Gabina, Ana, MariCarmen, Carlos, Paz, Sara y Nerea. Gracias por estar siempre ahí, por vuestro apoyo durante estos años y por ese gran sentido de humor de los Manzanares que animan hasta la peor de las ocasiones.

Quiero agradecer a la persona a la que he dedicado esta tesis, mi tío Carlos, todo su apoyo, cariño y por hacer del mundo de todas las personas que tuvimos la suerte de estar a su lado, un lugar mejor. Gracias por esa fuerza, paciencia, infinita bondad y optimismo que siempre transmitiste, y que siempre han sido un gran ejemplo a seguir. Mil gracias de corazón porque he tenido la suerte de ser una de las pocas personas, entre los más de 8 mil millones que somos, de haber conocido y haber tenido como Tío a alguien tan especial y que era capaz de alegrarle el día a cualquiera. Me quedo con esos grandes momentos que disfrutamos a tu lado y con esas ganas de continuar haciendo ciencia que siempre me motivaste a seguir.

También quiero agradecer a mi abuela Alejandra, todo su apoyo, por creer en mí y por sus ánimos para seguir estudiando y explorar nuevos caminos. Espero que estés donde estés, sigas disfrutando de ver a tus mocetones crecer. Y a mi abuelo Claudio, que aunque no ha podido llegar a ver esta tesis concluida, también le dedico mi más profundo agradecimiento por esa bondad y paciencia infinitas que tanto espero quedarme en mi vida. Os quiero mucho, abuelos.

Por último, me gustaría agradecer a mis padres, sin los cuales no habría llegado a este final del principio. Gracias Papá por transmitirse esa bondad, paciencia y constancia tan tuyas, enseñarme a aprender y a poner interés y curiosidad en el mundo que nos rodea. Gracias también a tu lado bromista que también comparto y que siempre me hace reír esté donde esté. A mi madre, gracias por transmitirme ese afán lector y enseñarme a cuidar y mostrar cariño a los que tenemos alrededor. Gracias a ambos por estar siempre orgullosos de vuestros hijos. Gracias a también a mi hermano Juan Luis por su incasable apoyo y la fuerza que transmite para continuar adelante y seguir creciendo. Gracias a María, que también ha sido un gran apoyo y una fuerza de comprensión durante este tiempo y que tanto me ha animado a continuar. Y gracias a Mía, que es una más de la familia, por sus locuras que nos alegran todos los días.

INDEX

ABREVIATIONS

2-DG	2-Deoxyglucose
ABL1	Abelson murine leukemia gene
ADAs	Anti-drug antibodies
ADCC	Antibody-dependent cell mediated cytotoxicity
AIDS	Acquired immunodeficiency syndrome
APCs	Antigen-presenting cells
APOBEC3G	Apolipoprotein B mRNA editing catalytic polypeptide-like
ARS	Acute retroviral syndrome
ART	Antiretroviral treatment
BCR	Breakpoint cluster region
BCR::ABL1	Breakpoint cluster region Abelson murine leukemia viral oncogene homolog 1
bNAbs	Broadly neutralizing antibodies
CA	Capsid protein p24 (HIV)
CCR5	C-C chemokine receptor 5
CCR5Δ32/Δ32	Homozygosity for the deletion of 32 amino acids in CCR5 allele
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CRF	Circulating recombinant forms
CXCR4	C-X-C chemokine receptor 4
DCC	Direct cellular cytotoxicity
DCs	Dendritic cells
ddPCR	Droplet digital PCR
DMR	Deep molecular response
dNTPs	Triphosphate nucleotides
dsDNA	double-stranded DNA
DSI	DNA shearing index
ECAR	Extracellular acidification rate
ECs	Elite controllers
Env	Envelope (HIV)
ESCRT	Endosomal sorting complexes required for transport
FBS	Fetal bovine serum
FCCP	Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone
FCS	Fetal calf serum
fDCs	Follicular dendritic cells
Gag	Group-specific antigen (HIV)
GALT	Gut-associated lymphoid tissue
GM-CSF	Granulocyte macrophage colony-stimulating factor
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
IFN	Interferon
ILC	Innate lymphoid cells

IN	Integrase (HIV)
IPDA	Intact proviral DNA assay
ISGs	Interferon-stimulated genes
IVs	Integrase inhibitors
KRAB	Krüppel-associated box domain
LGLs	Large granular lymphocytes
LPAs	Latency promoting agents
LRAs	Latency reversal agents
LTRs	Long terminal repeats
MA	Matrix protein p17 (HIV)
MCP-1	Monocyte chemotactic protein 1
MDA-SGS	Multiple-displacement-amplification single-genome sequencing
MHC-I	Major histocompatibility complex class I
MIP-seq	Matched integration-site and proviral sequencing
MSM	Men who have sex with men
NC	Nucleocapsid protein p7 (HIV)
Nef	Negative effector (HIV)
NF-κB	Nuclear factor-kappa B
NK	Natural killer
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NPC	Nuclear pore complex
NRTIs	Nucleoside reverse transcriptase inhibitors
OCR	Oxygen Consumption rate
OXPHOS	Oxidative phosphorylation
p6	Late assembly protein (HIV)
PAMP	Pathogen-associated molecular pattern
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
pDCs	Plasmacytoid dendritic cells
PEP	Post-exposure prophylaxis
PER	Proton efflux rate
PFA	Paraformaldehyde
Ph	Philadelphia chromosome
PHA	Phytohemagglutinin
PIs	Protease inhibitors
PKCθ	Protein kinase C theta
PMA	Phorbol 12-myristate 13-acetate
Pol	Polymerase (HIV)
PR	Protease (HIV)
PrEP	Pre-exposure prophylaxis
PRR	Pathogen-recognition receptor
PTCs	Post-treatment controllers
P-TEFb	Positive transcription elongaton factor b kinase
PWH	People with HIV
qPCR	Quantitative PCR

QVOA	Quantitative viral outgrowth assay
Rev	Regulator of viral gene expression (HIV)
RNApol II	RNA polymerase II
RT	Reverse transcriptase (HIV)
RT-qPCR	Real time reverse transcription PCR
SAMHD1	S-adenosylelmethionine-dependent demethylase 1
SEM	Standard error of the mean
SFKs	Src family of tyrosine kinases
SIMOA	Digital p24 molecule assay technology
SIV	Simian immunodeficiency virus
sRNA	Single-stranded RNA
STAT5	Signal transducer and activator of transcription 5
SU	Surface protein gp120 (HIV)
T538	Threonine 538
T592	Threonine 592
Tat	Transcriptional activator (HIV)
TCA	Tricarboxylic acid
T_{CM}	Central memory T cells
T_{EM}	Effector memory T cells
T_{EMRA}	Terminally differentiated effector memory T cells
Tfh	Follicular helper T cells
TFR	Treatment free relapse
Th	Helper T cells
TILDA	Tat/Rev induced limiting dilution assay
TKIs	Tyrosine kinase inhibitors
TLR	Toll-like receptor
TM	Transmembrane protein gp41 (HIV)
T_N	Näive T cells
Treg	Regulatory T cells
TRIM5α	Tripartite motif-containing protein 5 alpha
T_{SCM}	Stem cell memory T cells
T_{TM}	Transitional memory T cells
γδT	Gamma delta T cells
UNAIDS	The Joined United Nations Programo n HIV/AIDS
VCs	Viremic controllers
Vif	Viral infectivity factor (HIV)
VIP-SPOT	Viral protein spot assay
Vpr	Viral protein R (HIV)
Vpu	Viral protein U (HIV)
vRNA	Viral genome RNA
Y90	Tyrosine 90
ZNF	Zinc finger genes

INDEX OF TABLES AND FIGURES

List of tables

List of Figures

Figure 31. Dasatinib does not interfere with mitochondrial metabolism and permits low-level activation of glycolytic metabolism in NK cells.

RESUMEN

El mantenimiento del reservorio latente del virus de la inmunodeficiencia humana de tipo 1 (VIH-1) en los linfocitos T CD4+ y otros tipos celulares constituye el principal problema para conseguir la eliminación del virus en las personas con VIH (PCV). El tratamiento antirretroviral (TAR) de por vida no consigue eliminar el reservorio viral, y su interrupción da lugar al incremento de la viremia en pocas semanas. Los inhibidores de tirosina kinasa (ITK), utilizados en la práctica clínica para el tratamiento de la leucemia mieloide crónica (LMC), se plantean como un posible tratamiento conjunto al TAR que permita una reducción notable del tamaño del reservorio viral. Entre los ITKs, destacan por su potencial actividad antiviral dasatinib y ponatinib, cuya capacidad para interferir con la proliferación homeostática y la activación mediada por el TCR de los linfocitos T CD4+ podría disminuir notablemente el mantenimiento y proliferación del reservorio viral de VIH-1. Este proceso de inhibición parece centrado en dos mecanismos principales: i) el mantenimiento de la actividad antiviral del factor SAMHD1 (SAM domain HD domain-containing protein 1); y ii) la inducción de la proliferación de poblaciones inmunes citotóxicas, como las células Natural Killer (NK), los linfocitos T CD8+ y los linfocitos γδT, que son cruciales en el mantenimiento de la respuesta antileucémica en individuos con LMC que discontinúan el tratamiento cuando los clones leucémicos se vuelven indetectables después de varios años de terapia.

En este trabajo se describen tres estudios relacionados con el efecto de los ITKs en el reservorio del VIH-1. En primer lugar, hemos reportado que los linfocitos T CD4+ de personas con LMC que recibieron un tratamiento de intensificación con ponatinib durante un año eran resistentes a la infección por VIH-1 *in vitro* gracias al mantenimiento del efecto antiviral de SAMHD1 en estas células. Además, los linfocitos T CD4+ de estos pacientes permanecieron resistentes a la infección por VIH-1 un año después de que discontinuaran el tratamiento con ponatinib, gracias a la proliferación y reprogramación de las células NK y γδT CD8-. En segundo lugar, hemos estudiado los cambios producidos en el tamaño del reservorio viral, su composición y competencia para ser reactivado en dos PCV con LMC que se encontraban en tratamiento con dasatinib durante 4 años. Pudimos observar que el inicio del tratamiento con dasatinib en ambos individuos supuso un descenso drástico en el tamaño del reservorio viral, siendo los provirus intactos indetectables durante la mayor parte del tratamiento con dasatinib de ambos individuos. La capacidad de reactivación del reservorio era menor que la de PCV que sólo tomaban TAR. La inhibición de la fosforilación de SAMHD1, junto con el bloqueo de la proliferación y activación de los linfocitos T CD4+,

parecían ser los mecanismos implicados en estos efectos. Esta es la primera descripción de un tratamiento farmacológico oral *in vivo* que reduce de manera significativa el tamaño del reservorio. Por último, en tercer lugar, se describe la capacidad de dasatinib y ponatinib para modificar el metabolismo celular de los linfocitos T CD4+, CD8+ y las células NK. Ambos ITKs mostraron ejercer un fuerte efecto citostático sobre linfocitos T CD4+ y CD8+, lo que conferiría un potente efecto protector a los linfocitos T CD4+, pero podría reducir la respuesta citotóxica de los CD8+. Sin embargo, en las células NK, el tratamiento con dasatinib no impidió la activación mediada por citocinas, lo cual permitiría el desarrollo de su capacidad citotóxica.

En conclusión, los datos presentados en esta tesis demuestran que el tratamiento con los ITKs dasatinib y ponatinib produce un potente efecto antiviral que interfiere eficientemente con la infección por VIH-1 y el mantenimiento del reservorio viral. Entre los mecanismos subyacentes a este efecto antiviral que permiten su mantenimiento tras la interrupción del tratamiento se encuentran el potente efecto citostático de los linfocitos T CD4+ y la mejora de la capacidad citotóxica de algunas poblaciones celulares. El fin último de estos estudios es trasladar estos hallazgos a la clínica para poder evaluar si el tratamiento a corto plazo con ITKs como adyuvantes del TAR en PCV podría interferir con la dinámica del reservorio viral y contribuir a una posible cura funcional.

ABSTRACT

The persistence of the latent human immunodeficiency virus type 1 (HIV-1) reservoir in CD4+ T cells and other cell types remains the primary barrier to viral eradication in people with HIV (PWH). Life-long antiretroviral therapy (ART) fails to eliminate the viral reservoir, and treatment interruption results in increased viremia within weeks. Tyrosine kinase inhibitors (TKIs), clinically used for the treatment of chronic myeloid leukemia (CML), are proposed as a possible treatment in conjunction with ART that may significantly reduce the viral reservoir. Among TKIs, dasatinib and ponatinib stand out for their potential antiviral activity by interfering with homeostatic proliferation and TCR-mediated activation of CD4+ T cells, which are key mechanisms for HIV-1 reservoir maintenance. This inhibition process seems to be focused on two mechanisms: i) the maintenance of the antiviral activity of the antiviral factor SAM domain HD domain-containing protein 1 (SAMHD1); and ii) the expansion of cytotoxic cells, such as Natural Killer (NK) cells, CD8+ T cells, and γδT cells, which are crucial for the maintenance of the antileukemic response in people with CML who discontinue treatment when leukemic clones become undetectable after several years of therapy.

In this thesis, we described three studies related to the effects of TKIs on HIV-1 reservoir. First, we have reported that CD4+ T cells from CML individuals who received one-year ponatinib intensification treatment, were resistant to HIV-1 infection *in vitro* due to the maintenance of the antiviral effect of SAMHD1 on these cells. Moreover, CD4+ T cells from these individuals remained resistant to HIV-1 infection one year after they discontinued ponatinib treatment, due to the proliferation and reprogramming of NK cells and CD8- γδT cells. In the second study, we analyzed changes in viral reservoir size, composition and competence to be reactivated in two PWH and CML who were on treatment with dasatinib for 4 years. We observed that the initiation of dasatinib in both individuals resulted in a drastic reduction in reservoir size, with intact proviruses being undetectable for most of the treatment. Reservoir reactivation was decreased when compared to PWH only on ART. Inhibition of SAMHD1 phosphorylation, coupled with blockade of CD4+ T cell proliferation and activation, were suggested as the main mechanisms for these effects. This is the first description of an *in vivo* oral drug treatment that significantly reduces reservoir size. Finally, third study describes the ability of dasatinib and ponatinib to modify the metabolism of CD4+ T cells, CD8+ T cells and NK cells. Both TKIs were shown to exhibit a potent cytostatic effect on CD4+ and CD8+ T cells, protecting CD4+ T cells against HIV-1 infection but potentially modifying the cytotoxic response of CD8+ T cells. In NK cells, dasatinib did not

impair cytokine-driven activation, which would allow the development of their cytotoxic capacity.

In conclusion, the data presented in this thesis demonstrate treatment with the TKIs dasatinib and ponatinib produces potent antiviral effects that effectively interfere with HIV-1 infection and reservoir maintenance. Among the mechanisms underlying this antiviral effect that allow its maintenance after treatment discontinuation are the potent cytostatic effect on CD4+ T cells and the enhanced proliferation and cytotoxic capacity of some cell populations. The goal of these studies is to translate these findings to the clinic for the evaluation of short-term adjunct therapy with TKIs in PWH as adjuvants of ART could interfere with the dynamics of the viral reservoir and contribute to a possible functional cure.

INTRODUCTION

1. More than 40 years of HIV pandemic.

The human immunodeficiency virus (HIV) pandemic emerged in the early 1980s as a mysterious and devastating illness that initially confused the medical and scientific communities. The first cases were reported in 1981 in the cities of Los Angeles and New York. There, five previously healthy young men were diagnosed with unusual conditions such as pneumonia produced by *Pneumocystis jirovecii*, or Kaposi's sarcoma, diseases typically associated with severe immune suppression (1). These early cases, primarily among men who have sex with men (MSM), signaled the onset of a global health crisis that would soon spread to other populations, including people who use injected drugs, hemophiliacs, and heterosexual individuals. This was the beginning of what later would be known as acquired immunodeficiency syndrome (AIDS). By 1983, the causative agent, HIV, was identified by the team of Françoise Barré-Sinoussi, and Luc Montaigner (2,3).

According to The Joined United Nations Program on HV/AIDS (UNAIDS), there were more than 39.9 million people with HIV (PWH) at the end of 2023, 9.3 million of them living without treatment. From the 88.4 million people that have been infected with HIV since the beginning of the pandemic, 42.3 million people have deceased of AIDS-related causes. To give an end to the pandemics, UNAIDS has proposed the 95-95-95 strategy for 2025 (95% of PWH know their serologic status, of which 95% are on antiretroviral treatment (ART), and 95% of those are with suppressed viral load). By 2023, 86% of PWH knew their serologic status, of which 89% had access to ART, and of which 93% were with suppressed viral load. In the same year, 630,000 PWH died of AIDS-related causes, while in 2005 2.3 million deaths were registered (4). In 2023, more than half (52%) of PWH were living in eastern and southern Africa (Figure 1), and about 1.3 million acquired HIV (4).

Despite the great advances that have been achieved in the past decades, the HIV pandemic is still one of the biggest global health problems. Thus, big efforts are still necessary to develop a safe and effective preventive vaccine, to address against disparities in access to prevention and treatment, to fight the persistence of stigma, and to design immunotherapies and other new strategies that may contribute to achieve a functional cure or to eradicate the infection.

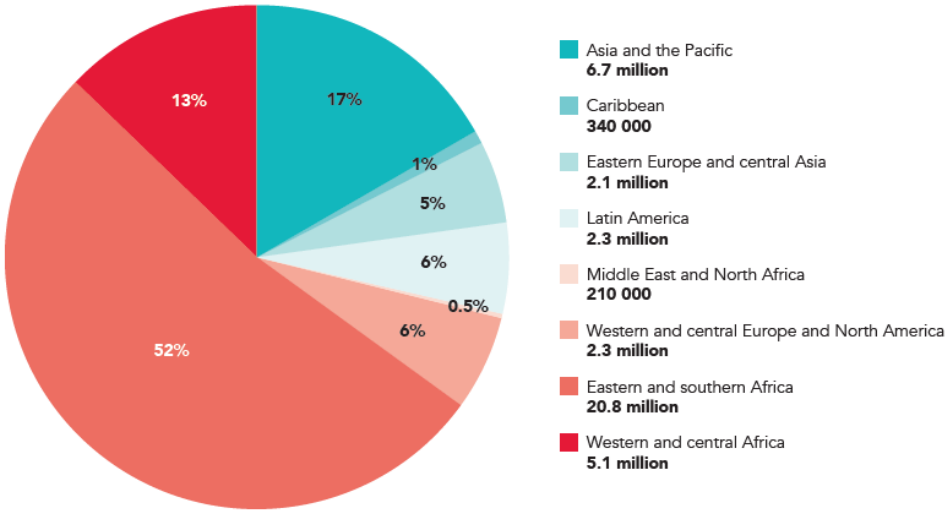


Figure 1. Distribution of PWH by region, 2023. Obtained from (5).

2. Virology of HIV

2.1. HIV origin and classification

HIV is a member of the *Retroviridae* family, *Orthoretroviridae* subfamily and *Lentivirus* genus, which includes enveloped viruses with two copies of single-stranded positive RNA. This RNA is retrotranscribed into double-stranded DNA, which is then integrated into the host cell genome (6).

HIV originated grom multiple zoonotic transmissions of simian immunodeficiency virus (SIV) from nonhuman primates to humans in West and Central Africa. These cross-species transmissions likely occurred through activities such as hunting and butchering primates for bushmeat, as well as the capture, trade, and domestication of monkeys as pets (7). The different lineages of HIV were generated from independent events of zoonotic transmission: HIV type 1 (HIV-1) and type 2 (HIV-2) (Figure 2). Both types are also divided into groups: HIV-1 contains groups M (responsible for the global HIV pandemic), N, O, and P; and HIV-2 contains groups A to H. The incidence of each group is very diverse: more than 39.9 million people are infected with group M, while group O causes thousands of infections in West-Central Africa and group N has been identified in only a few individuals in Cameroon (8–12). HIV-1 group M originated from the SIV of the chimpanzee *Pan troglodytes troglodytes* in West-Central Africa (13,14), and it contains subtypes A-K (except E and I, which are circulating recombinant forms (CRF) (15). HIV-1 group M is also the oldest HIV lineage found

in humans, in which its cross-species transmission is estimated to have taken place between 1853 and the early 1900s (16).

HIV-2 emerged from SIV-infected sooty mangabey (*Cercobeus atys*) monkeys, being the cross-species events with human estimated to be between the 1940-50s in Guinea-Bissau (17,18). HIV-2 is restricted to West Africa, although it has also been identified in North America for the large presence of West African migrants. It is also widespread in other countries like Brazil, India and some European countries like Portugal, France, Spain or Belgium (19–22). HIV-2 is characterized by its lower infectivity, lower pathogenicity, higher CD4+ T cell counts and slower progression to AIDS (23–25,25–27). Although ART is still necessary to control the progress of the infection, it is estimated that only 15-25 % of HIV-2 infections will progress to AIDS (25).

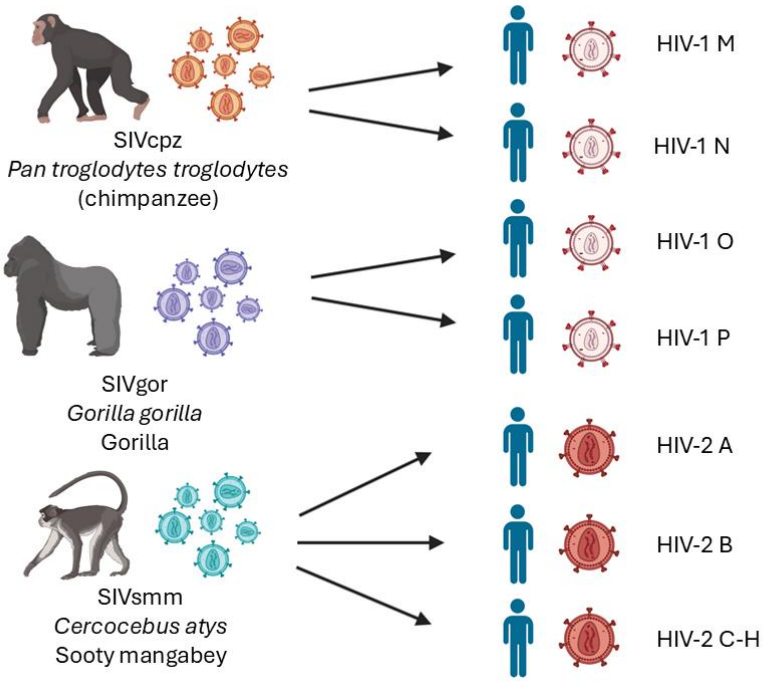


Figure 2. Origin of HIV groups. HIV groups proceed from cross-species transmissions of different SIV strains of nonhuman primates. Created with Biorender.

In this PhD thesis, when HIV-1 is mentioned, it will be assumed that it is referred to group M, responsible for the global pandemic.

2.2. HIV genome and virion structure

The 9.6kb HIV genome contains nine genes that encode the viral proteins (28–30). The genome is flanked by two non-coding sequences, known as long terminal repeats (LTRs), which are essential for integration, transcription and establishment of latency (Figure 3).

The three major genes are group-specific antigen (*gag*), polymerase (*pol*) and envelope (*env*). *Gag* codes for structural proteins: matrix protein p17 (MA), capsid protein p24 (CA), nucleocapsid protein p7 (NC), and late assembly protein (p6). *Pol* encodes enzymes: protease (PR), reverse transcriptase (RT) and integrase (IN). Lastly, envelope (*env*) produces envelope proteins surface gp120 (SU) and transmembrane gp41 (TM). The remaining genes code for regulatory proteins (transcriptional activator [Tat] and regulator of viral gene expression [Rev]) and accessory proteins (viral infectivity factor [Vif], viral protein U [Vpu], viral protein R [Vpr] and negative effector [Nef]) (Figure 3) (31,32).

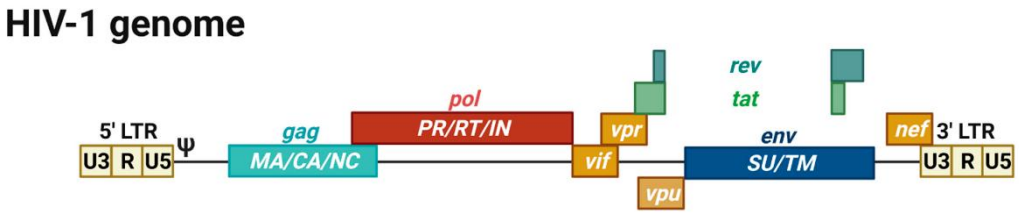


Figure 3. Schematic representation of HIV genome structure. Obtained from van Heuvel et al. (33).

The HIV-1 virion is a spherical enveloped particle of 120nm (Figure 4) (6). Its outer layer is a lipid bilayer derived from the host cell membrane, incorporating Env glycoproteins in the form of trimeric pikes composed of gp120 and gp41, and it also contains host-derived proteins that contribute to immune evasion. The MA protein is located beneath the envelope, anchoring the Env trimers via interactions with gp41 and plays a role in virion assembly and transport. Below the lipid bilayer is the conical-shaped capsid, which forms a fullerene-like structure made of hexameric and pentameric subunits of CA. It encloses the viral genome, composed of two identical positive-sense single-stranded RNA genomes, tightly bound to the NC protein, along with viral enzymes: RT, IN and PR (34,35). The virion contains several accessory proteins, including Vif, Vpr, Nef, and Vpu, which play key roles in regulating viral replication, evading the immune response, and interacting with the host cells (34).

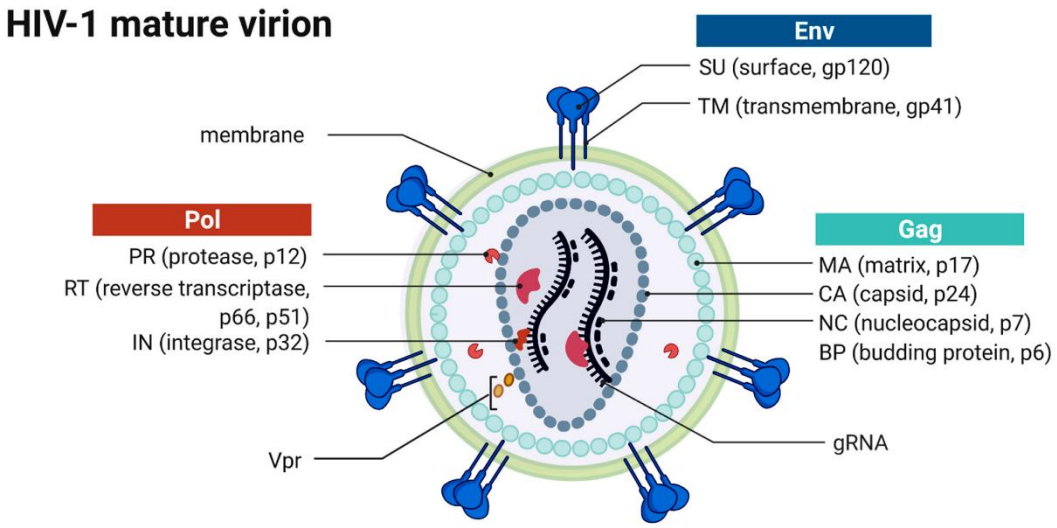


Figure 4. Schematic representation of HIV mature virion structure. Obtained from van Heuvel et al. (33)

2.3. HIV life cycle

Before starting its replication, HIV-1 needs to interact with host cells via two major components: i) interaction of Env spikes with CD4 receptor of the host cells (CD4+ T cells, monocytes, macrophages and dendritic cells) and ii) interaction with C-C chemokine receptor 5 (CCR5) (36) or C-X-C chemokine receptor 4 (CXCR4) (Figure 5A) (37). CCR5 and CXCR4 function as HIV-1 co-receptors and the viral tropism of the strain (R5-tropic, X4-tropic or dual-tropic) is determined by the ability of the virus to use these co-receptors (38).

2.3.1. Entry and fusion

Gp120 and gp41 interact to form the Env spikes, that permit the binding of gp120 to the cellular receptor CD4. This binding induces conformational changes in gp120 that expose its coreceptor-binding sites (39). Following gp120´s attachment to the coreceptor, either CCR5 or CXCR4, conformational changes in both gp120 and gp41 induce the fusion of viral and cellular membranes (Figure 5B), releasing the viral core into the cytoplasm of the target cell, initiating reverse transcription (40).

2.3.2. Nuclear entry, uncoating and reverse transcription

Viral capsid is transported along the microtubules of the host cell towards the nuclear pore complex (NPC) (Figure 5C). After the capsid docks to the NPC (Figure 5D), it enters the nucleoplasm and partially disassembles, releasing viral enzymes and nucleotides (Figure 5E) (41,42). Viral genome RNA (vRNA) retrotranscription into proviral double-stranded DNA (dsDNA) is completed within the host cell nucleus (41). Viral RT is a highly error-prone DNA

polymerase and lacks error-correcting mechanisms, resulting in extensive genetic diversity due to mutations that leads to an accumulation of a vast number of sequence variants within one single individual (43). Furthermore, a single cell can be infected by multiple viral particles, leading to possible recombination events that also enhance the variability of HIV (44).

2.3.3. Viral genome integration

The reverse transcribed viral DNA is incorporated into the host cell genome, mediated by the IN and RT (Figure 5F). Proviral DNA forms the pre-integration complex together with IN and Vpr. Then, two terminal nucleotides of the 3´-end of the provirus are removed and the host DNA is cleaved, which permits the integration of the provirus (45,46). Provirus can be integrated in any place within the host cell genome, but it targets preferentially active transcription units (47). Thus, HIV-1 establishes its latent reservoir in the form of a provirus that remains silent and resists elimination during ART, although it can be reactivated upon cell activation.

HIV-1 integration displays a non-random pattern, with preferential localization to gene-rich regions of the host genome, with active transcription and chromatin accessibility, facilitating more efficient expression of the viral genome (48,49). However, proviruses integrated in these sites tend to be recognized and eliminated by the immune response or the cell-intrinsic mechanisms (50). Integration in repressive chromatin locations may produce a protective effect for HIV, as these proviruses may not express viral proteins that could be detected by the immune system (51), leading to a long-term persistence of this integrated viral DNA. Centromeric DNA and zinc finger (ZNF) genes, in particular Krüppel-associated box domain-containing ZNF (KRAB-ZNF), exhibit heterochromatin signatures, marked by an enrichment of silencing histone modifications that maintain transcriptional repression (52). Proviral integration within these epigenetically silenced regions promotes a deeper state of latency, in which proviruses are more refractory to conventional reactivation signals (53–56).

2.3.4. Transcription and translation

The HIV-1 provirus utilizes host transcription factor such as nuclear factor-kappa B (NF-κB), that is induced when the immune response is activated, causing an increase in the replication of the virus when immune cells are in an active state (57). Transcription factors and RNA polymerase II (RNA Pol II) bind to the preinitiation complex at the 5´-LTR promoter, starting the transcription of the provirus and the synthesis of the first two viral proteins: Tat

and Rev (Figure 5G). Tat enables a positive feedback loop as a result of binding to TAR, activating positive transcription elongation factor b kinase (P-TEFb) (58–60). P-TEFb phosphorylates the C-terminal domain of RNA Pol II, increasing the affinity of RNA Pol II for proviral DNA, enabling elongation of viral transcripts (61,60). Rev modulated the transport of the viral transcripts to the cytoplasm and their binding to the ribosomes, facilitating the translation into viral proteins (Figure 5H) (62). Posttranscriptional modifications of viral proteins, such as phosphorylation, methylation and acetylation, play a crucial role in this process (63,64). Production of viral transcripts and proteins from defective proviruses do not lead to the formation of new virions, but they may contribute to the chronic inflammation characteristic of HIV-1 infection (65,66).

2.3.5. Assembly and budding

Gag and gag-pol are translated into polyproteins that migrate to the cell membrane. As the Env precursor protein, gp160, travels from the Golgi to the membrane, cellular proteases cleave it into its mature forms, gp120 and gp41. Both proteins are reorganized into Env trimers and attached to the surface of the new forming virion, together with host cell surface proteins. p7 and p6 will interact with vRNA to include it inside the new virion (Figure 5I and J) (67,68).

2.3.6. Release and virion maturation

Gag recruits the endosomal sorting complexes required for transport (ESCRT) (69) that form a complex that facilitates the release of the immature viral particle (Figure 5K). The viral proteins Nef and Vpu facilitate the internalization of CD4 receptors on the cell membrane, promoting their degradation, which avoids the anchoring of the new formed virions to the same cell. Nef also triggers the internalization of Major Histocompatibility Complex class I (MHC-I) to prevent antigen presentation to CD8+ lymphocytes that would activate the immune response against the infected cell (70). Therefore, the internalization of CD4 and MHC class I promotes the longevity of the latently infected cell and prevents new infections.

After release, the activation of PR results in the cleavage of Gag and Gag-Pol into the viral enzymes MA, CA, NC, RT and IN (71). Then, NC binds to the vRNA, stabilizing it (72,73) whereas CA proteins encapsulate the viral genome along with RT and IN (74). The virion is now prepared for a new replication cycle.

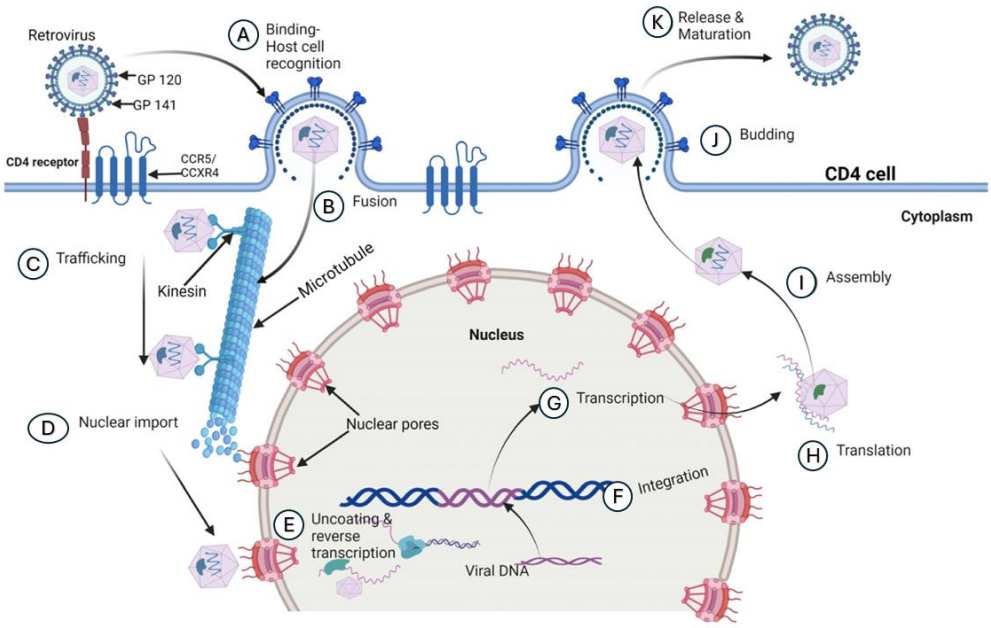


Figure 5. Schematic representation of the HIV life cycle. **(A)** The lifecycle begins when the envelope proteins gp120 and gp41 bind to the CD4 receptor and the CCR5/CXCR4 co-receptor, respectively. **(B)** This binding triggers fusion and release of viral core into the cytoplasm. **(C)** The core moves through the cytoplasm and is directed to the nuclear pores for import into nucleus while reverse transcription begins **(D)**. Uncoating of the viral capsid initiates and retrotranscription continues **I**. The obtained dsDNA is integrated into the cell genome **(F)**, which begins to transcript **(G)** and translate **(H)** into Gag polyproteins. Then, these proteins assemble **(I)** and are translocated and docked at the plasma membrane to form new virions by budding **(J)**. After release, the virion undergoes the final maturation **(K)** in which the viral protease cleaves the Gag polyprotein, transforming the virion into an infectious particle and completing the HIV life cycle. Adapted from Masenga et al. (75).

3. HIV-1 immunopathology

Currently, the most common route of HIV-1 infection is sexual transmission, followed by injection drug use, exposure to contaminated blood via transfusions and mother-fetus exposure (76). The HIV-1 disease course takes years for its progression and is mainly due to the destruction of CD4+ T cells and the chronic immune activation against infected cells. This course can be divided into three phases: i) acute, ii) chronic and iii) AIDS.

3.1. Natural course of HIV-1 infection

3.1.1. Acute HIV-1 infection

For HIV-1 infection establishment, the virus needs that individuals are exposed to fluids or tissues with high viral load and that the mucosal barrier is broken, which is usually produced during sexual intercourse (77). Thus, spread of the virus usually begins in the lamina propria of the vaginal or rectal mucosa, migrating to the proximal lymph nodes without generating clinical manifestations. A single virion called founder virus, is responsible for HIV-1 transmission in approximately 80% of heterosexual individuals, about 60% of MSM, and 40% of injection drug users (78).

Even in the absence of ART, the first three weeks of the acute phase are characterized by undetectable viral load and absence of immune response (Figure 6A). The establishment of latent viral reservoirs occurs early during this initial phase of infection. From lymph nodes, the virus migrates to gut-associated lymphoid tissue (GALT), the spleen and bone marrow, while also extending into blood stream. Thus, HIV-1 causes a massive depletion of CD4+ T cells and viremia reaches its peak (10^6-10^7 RNA copies/mL) (79). The CD4 count attains its lowest point, known as the nadir. The first antibodies against HIV-1 proteins can be detected 3 to 6 weeks after infection (80), so the control of viremia relies initially in Natural Killer (NK) cells and later, in CD8+ T cell cytotoxic response (81). Clinical symptoms during this first phase of the disease correspond to the acute retroviral syndrome (ARS), characterized by retro-orbital pain, fever, sore throat, muscle aches and non-pruritic macular erythematous rash (82).

3.1.2. Chronic HIV-1 infection

During this phase, the combined humoral and cellular immune responses are able to control the viremia that is reduced to the viral set point (83). However, the immune response is uncapable of eradicating the latent HIV-1 reservoir that has been promptly established in several tissues in the early phases of the acute infection. CD4 counts are partially recovered after the immune response and relative control of the viremia, but the continuous HIV-1 replication due to the persistence of the viral reservoir causes a progressive decay in CD4 counts during the chronic phase of the disease. When they reach a level below 200 cells/μL, the onset of AIDS begins (Figure 6A and B) (84,85). This phase may have an average lasting of 10 years, in which the continuous immune response develops a chronic inflammatory state that cannot resolve the infection and leads to a state of immune exhaustion in which

the elimination of CD4+ T cells by HIV-1 active replication and the senescence of CD4+ and CD8+ T cells result in alterations of immune activation and T cell maturation (86).

3.1.3. AIDS

AIDS is defined by a rapid increase in viremia, a decrease in CD4 count below 200 cels/μL and subsequent infections by opportunistic pathogens (Figure 6A,B) (87). The survival rate of people diagnosed with AIDS that do not receive ART is below 20% after 6 years (88).

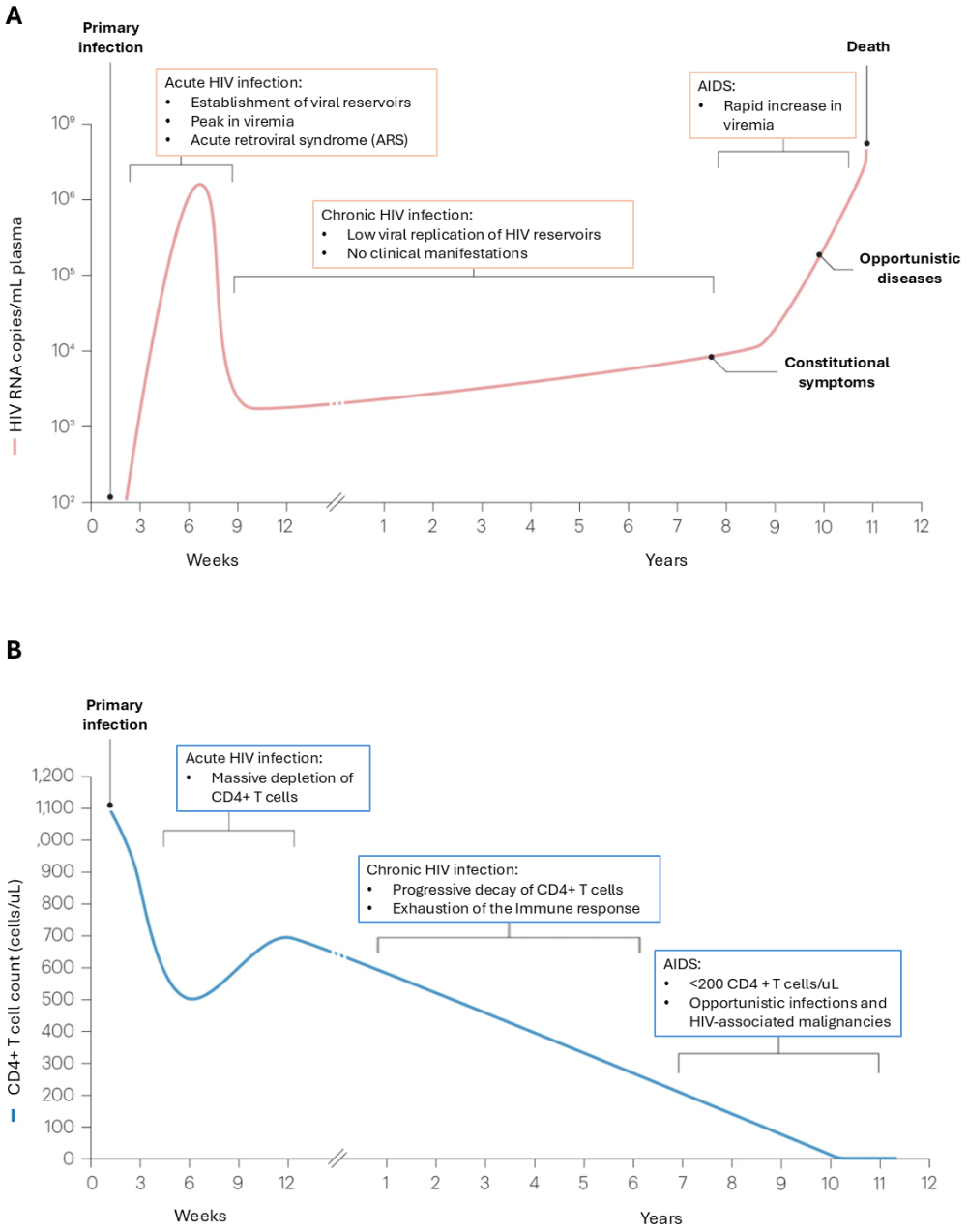


Figure 6. Natural course of HIV-1 infection. Changes in viral load (A) and CD4+ T cell counts (B) during progression of HIV-1 disease. Adapted from Bekker et al. (89).

3.2. Immune response against HIV-1

3.2.1. Innate immune response and antiviral factors

Innate immune cells utilize their pathogen-recognition receptors (PRRs) for the detection of pathogen-associated molecular patterns (PAMPs). In infected cells, HIV-1 activates PRRs such as Toll-like receptors (TLR 1-10) that can induce cell death on infected cells (90) or inflammatory activation (91) and can detect different components of HIV-1. TLR7 and TLR9 detect single-stranded RNA (sRNA) of HIV-1 (92), mucosa epithelial cells utilize TLR2 and TLR4 expression for the detection of gp120 (93), and TLR7 and TLR8 recognize vRNA (94).

Activation of PRRs usually triggers the production of interferon type I (IFN-α/β), emphasizing the importance of plasmacytoid dendritic cells (pDCs) in producing these antiviral cytokines. This, in turn, induces the expression of IFN-stimulated genes (ISGs) that activate the adaptive immune response (95,96). Tetherin is one ISG that is activated via IFN and may interfere with virion release (97), but also may induce the activity of NF-κB transcription factor and the release of proinflammatory cytokines (98). IFN also activates other essential antiviral factors such as i) the tripartite motif-containing protein 5 alpha (TRIM5α), that destabilizes virion capsid and provokes its degradation (99); ii) apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC3G), that generates hypermutation in viral DNA (100); and iii) S-adenosynelmethionine-dependent demethylase 1 (SAMHD1), that decreases the concentration of free triphosphate nucleotides (dNTPs) required for vRNA reverse transcription (101). In infected cells, detection of PAMPs of HIV-1 also stimulates the production of IFN that blocks the cellular cycle via STAT1/STAT2 and protein synthesis to stop HIV-1 lifecycle (102).

Therefore, the detection of HIV-1 via PRRs induces the activation of monocytes, macrophages and dendritic cells (DCs) and non-infected surrounding cells. Macrophages are a secondary cellular reservoir for HIV-1, following CD4+ T cells, and when infected, they initiate an inflammatory response by activating NF-κB transcription factor, that promotes the expression of proinflammatory cytokines (IL-1β, IL-6 and TNF-α), thereby contributing to vasodilation and the extravasation of immune cells (103). IFN-α activates NK cells that can detect infected cells through their loss of MHC-I expression (“missing self”) or via recognition by HIV-1-specific antibodies. These antibodies mediate antibody-dependent cell-mediated cytotoxicity (ADCC) through the interaction between the Fc domain of immunoglobulins and the CD16 receptor of NK cells (104). NK cells then release pro-inflammatory cytokines and cytolytic granules, inducing target cell death (105). NK cells are

the most dominant innate lymphoid cells (ILC) subset and represent 5-10% of peripheral blood mononuclear cells (PBMCs). Other cytokines released during innate and adaptive immune responses also stimulate the activity of NK cells, such as IL-12, IL-15, IL-2, and IL-18 (106).

3.2.2. Adaptive immune response

The adaptive immune response comprehends the humoral immunity, based on the production of HIV-1-specific antibodies by B cells, and cell-mediated response driven by CD4+ and CD8+ T cells. Both responses are developed in days to weeks after the peak of viremia, involving the expansion, maturation, and interaction of T and B cells in secondary lymphoid organs such as spleen or lymph nodes (107). The first antibodies produced during the acute infection are non-neutralizing and do not select for viral escape, but can induce ADCC responses (108). On the other hand, antibodies that neutralize the founder virus are not detected until 3 months or more after infection (109). Thus, by the time a potent and effective antibody response develops, HIV-1 has already established viral reservoirs. This underscores the importance of the innate immune response in preserving the integrity of the immune system until a more specialized adaptive response can take over.

CD4+ T cells are divided into T helper (Th), regulatory T cells (Treg), and follicular helper T cells (Tfh), with different functions in the immune response. Th cells are further subdivided in more specialized subsets, being the most relevant Th1, Th2, Th9, Th17, and Th22. Th1 cells play a crucial antiviral role by inducing the secretion of IL-2 and IFN-γ, promoting antigen presentation by increasing HLA class I and II gene expression, which is particularly important for antigen presenting cells (APCs) (110).

Th2 cells play an important role in protecting against helminths and also in repairing tissues, particularly by the production of IL-4, IL-13 and IL-10 (111,112). Th9 cells provide immunity against helminth parasites (113) and tumors by secreting IL-9, IL-3 and IL-21 (114). Th22 cells are involved in skin barrier function and the maintenance of the mucosal immunity through the production of IL-22, that drives the secretion of antimicrobial peptides and mucus (115). Th17 cells are also important in mucosal immune responses through the production of IL-17 that regulates both humoral and cellular responses in tissues (116). (117). Tfh cells are mainly found in the germinal centers of the secondary lymphoid organs, where they participate in antigen presentation by follicular dendritic cells (fDCs) and maturation of B cells (118). Conversely, Treg cells can inhibit T-cell activation and

proliferation through contact-dependent mechanisms and by suppressing IL-2 production (119).

The initial CD8+ T cell responses are directed against epitopes of Nef and Gag , but both proteins end up producing escape mutations that evade these responses (120). In contrast, later CD8+ T cell responses are characterized by cells that do not drive escape mutations, as they target viral proteins that are less prone to mutation but are still able to interfere with viral replication, thereby contributing to HIV-1 control (121). These CD8+ T cells produce cytotoxic molecules such as perforin and granzyme B that form pores and activate caspases in infected target cells (122).

Gamma delta T cells (γδT cells) are unconventional T cells that also contribute to the immune response against HIV-1, as they do not require antigen presentation via MHC to recognize and respond to infected cells. γδT cells primarily reside in tissues and can produce cytokines and chemokines that activate and recruit other immune cells, thereby regulating NK cell immunity, promoting B cell and DCs maturation and antigen presentation, and activating macrophages (123–126). However, HIV-1 interferes with the proliferation and distribution of certain γδT cell subtypes, impairing their ability to support DCs and B lymphocytes differentiation into APCs (127). Given that both the number and functions of γδT cells are significantly reduced during HIV-1 infection, several strategies are being explored to activate and expand these cells.

3.2.3. Chronic inflammation

Chronic inflammation is a hallmark of HIV infection, persisting even in individuals effectively treated with antiretroviral therapy, and contributes significantly to immune system dysfunction and disease progression. In fact, elevated inflammation markers in PWH are strongly associated with disease progression and predict earlier onset of non-AIDS events (128–130). This persistent inflammation relies on the production of viral transcripts and proteins from both defective and intact proviruses (131,132). The continuous production of viral particles contributes to IFN-α production (133) and immune activation through NF-κB pathway, which induces the overexpression of IL-6 and IL-8 (134,135), resulting in chronic immune activation. Immune activation leads to a decrease in the half-lives of CD4+ and CD8+ T cells, as well as T-cell exhaustion characterized by the expression of senescence markers, such as PD-1 (136,137). The activity of NK and B cells is also impaired during chronic HIV-1 infection, which contributes to immune system activation due to suboptimal viral control (138). Treg cells are also depleted during chronic HIV-1

infection as they are susceptible to viral infection, leading to a deficit of these cells that are necessary for inhibiting T-cell activation and proliferation(139–141). Moreover, the induction of pro-inflammatory cytokines promotes aberrant thymic Treg development, further compromising their immunoregulatory capacity (142).

4. Viral reservoir of HIV-1

Latent infection established by HIV-1 is characterized by the integration of viral DNA into the host genome without active viral transcription or translation; however, it retains the capacity to replicate upon cell stimulation. Viral reservoirs are defined as specific cell types or anatomical locations where replication-competent forms of the virus persist (143). Unlike other viruses capable of establishing latency, such as herpesviruses, which may produce viral proteins essential for initiating and sustaining the viral latency, HIV-1's persistence relies on the nature of the infected immune cells, which may be in a resting or activated state.

The proportion of resting CD4+ T cells containing integrated provirus is comparably low in both blood and lymph nodes (approximately, one reservoir cell per one million resting CD4+ T cells), and significantly lower than the frequency of cells carrying unintegrated viral genomes (144). These unintegrated genomes, though much shorter-lived (144), can complicate the assessment of the HIV-1 reservoir during untreated HIV-1 infection. Nonetheless, the reservoir is long-lived, with an estimated half-life of 44 months, and previous data has suggested that it may require more than 70 years of continuous ART to induce a significant decay of HIV-1 reservoir cells (145). Furthermore, immune pressure during two decades of ART drives the selection of intact proviruses that are integrated into repressive chromatin locations, such as KRAB-ZNF genes (51). This protective effect, along with the maintenance and proliferation of latently-infected cells, makes HIV-1 reservoir especially stable over time.

4.1. Methods for measuring HIV-1 reservoir size

Detecting changes in HIV-1 reservoir size during ART presents several major challenges: i) over 90% of proviruses in PWH on ART contain mutations or deletions, rendering them defective and replication-incompetent; ii) not all intact genomes (those without defects) can be induced to produce infectious virions; iii) as mentioned above, the frequency of latently infected cells is extremely low; iv) most of the HIV-1 reservoirs resides in inaccessible tissues, such as the central nervous system (146–151). Therefore, although

total reservoir size does not accurately reflect its functional capacity, measuring the reservoir adequately remains essential. This reservoir is quite stable over time (145) but, as infected cells tend to decay over time in PWH on long-term ART, the reservoir may become enriched with defective proviruses (51). It is also important to determine the anatomical localization of the reservoir, and which reservoirs are intact and capable of producing new virions, and which are defective but still produce viral particles, contributing to chronic immune activation and exhaustion (65,152). However, high inter-individual variability complicates the identification of specific tissue locations of the viral reservoir (153–155).

Measuring total and integrated HIV-1 DNA by quantitative PCR (qPCR) or droplet digital PCR (ddPCR) is a highly reproducible method that can be performed in both tissues and blood samples (156,157). This PCR method employs a two-step amplification strategy: the first PCR amplifies genomic segments between HIV-1 LTRs and proximal Alu elements, while the second round uses nested LTR-specific primers to enhance sensitivity by targeting the viral LTR region (156,157). However, this method tends to overestimate the replication-competent reservoir size, since most integrated proviruses are defective (158).

The quantitative viral outgrowth assay (QVOA) was one of the standard measurements of the replication-competent HIV-1 reservoir. In this technique, infected cells are stimulated and cultivated with other cells that are susceptible to infection. However, the need for a high number of cells and the fact that not all infected cells are reactivated (underestimating reservoir size), together with its high cost and labor requirements, makes this technology not ideal for measuring reservoir size (159,160). The viral protein spot (VIP-SPOT) is an *ex vivo* assay that allows the detection of p24 production at the single-cell level upon reactivation of latently infected cells, being especially sensitive for measuring the replication-competence of the reservoir in HIV-1 cure studies (161). The sensitivity and reproducibility of this assay also depends on the number of cells, and cells that are used for reactivation in this method cannot be analyzed phenotypically after p24 detection (161).

The intact proviral DNA assay (IPDA) is a ddPCR technique that solves the problems with the number of cells, cost and labor requirements that QVOA presented. This method differentiates between intact and defective provirus without the need for sequencing the DNA (162). However, it has been observed that less than 2% of the estimated intact proviruses are likely to be activated *ex vivo*, thus showing problems in determining the proportion of cells producing replication-competent virus (163). Another issue is sequence polymorphisms that result in the need for alternative primer/probe sets for this technique

(164). Additionally, this technique does not provide information about the integration sites of the provirus, which is important because intact provirus located in non-transcriptionally active locations in the chromatin will not induce effective replication cycles (51).

Proviral reactivation assays have been proposed as a solution, as they allow the detection of the provirus competence for its reactivation. The miniQVOA, described by Vigón et al (165), allows the reactivation of the proviruses in isolated CD4+ T cells by using CD3 plus anti-CD28 to induce T-cell activation in the presence of brefeldin A to inhibit protein transport from the endoplasmic reticulum to the Golgi apparatus (165), and then p24-gag production is measured by flow cytometry. Although this method cannot discriminate between reactivation from intact or defective proviruses, it permits the detection of provirus competence after measuring reservoir size by other techniques.

Several techniques also allow the detection of active forms of the reservoir. One example are those techniques that measure translationally-competent virus, such as the digital p24 molecule assay technology (SIMOA) immunoassay that detects the production of p24 molecules at single cell level, although it cannot differentiate p24 production between intact and defective provirus (166,167). Production of p24 can also be assessed using live cells in flow cytometry, as is the case with the single-cell FISH-flow cytometry technique (168,169). On the other hand, there are those techniques that measure levels of transcriptionally-competent virus, such as the Tat/Rev induced limiting dilution assay (TILDA) (170), or the HIV-DNAscope and RNAscope (171). RNAscope allows the detection of HIV-1 RNA , while the DNA scope detects viral DNA in cells and tissues (172). The sensitivity of both techniques closely matched qPCR measurements, but the preservation of tissues and cells allows the simultaneous assessment of viral reservoirs in their native tissue microenvironment, information that is destroyed by nucleic acid extraction methods (172). However, both techniques have a limited ability to quantify the viral reservoir in tissues due to the restricted tissue accessibility in clinical trial participants.

Real-time reverse transcription (RT-qPCR) has allowed the detection of total, integrated and episomal HIV-1 DNA, in cost- and labor-efficient manner. The fact that more than 90% of the integrated provirus is defective makes interpretation of the results obtained by this technique challenging (146,158,162). However, measuring total HIV-1 DNA is recommended when only a limited number of cells are available, and the results obtained in the detection of integrated HIV-1 DNA correlate with IPDA measurements (173).

4.2. Methods for measuring the integration sites

Analyzing HIV-1 proviral integration sites is essential because the genomic locations where proviruses insert directly influence clonal expansion and long-term persistence of infected cells, even under suppressive ART (174). Understanding these integration patterns can help identify mechanisms that sustain the reservoir and inform strategies aimed at its eradication (175).

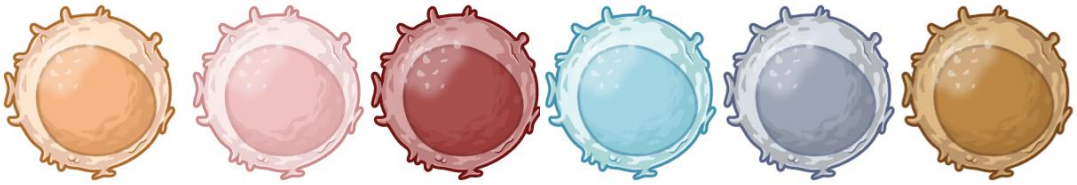
Techniques such as matched integration-site and proviral sequencing (MIP-seq) (176) or multiple-displacement-amplification single-genome sequencing (MDA-SGS) (177), permit the analysis of the distribution of HIV-1 DNA integration sites. Although these technologies require a vast number of isolated CD4+ T cells and a significant investment in labor and cost, they can provide information on reservoir maintenance. These techniques can help determine why certain HIV-1-infected cells exhibit a tendency to proliferate more actively *in vivo*, which may be useful for targeting these cells in HIV-1 cure strategies (167).

4.3. Locations of HIV-1 reservoirs

4.3.1. Cellular reservoirs

CD4+ T cells represent the major cellular reservoir of HIV-1, but some subtypes are more prone to being infected by HIV-1 and harboring more viral reservoirs. The reservoir in CD4+ T cells is recognized for its remarkable stability. CD4+T cells constitute the most significant obstacle to achieving a cure due to their high frequency and slow decay rate (145,178). HIV-1 infection is typically observed in resting memory CD4+ T cells and can be established either through infection of these resting memory cells or through infection of an activated CD4+ T cell that subsequently transitions into a memory cell (pre-activation latency) (179,180). However, HIV-1 preferentially infects activated CD4+ T cells, that later can shift to a resting state, establishing another pathway for latency (post-activation latency) (181). Latently infected cells produce molecules that promote their own survival, increasing their half-life, along with increased gene and marker expression for immune evasion, particularly the expression of immune checkpoints, such as PD-1 or TIGIT (182,183).

Resting memory cells can be divided into different subtypes: näive T cells (T_N), transitional memory T cells (T_{TM}), stem cell memory T cells (T_{SCM}), central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and terminally differentiated effector memory T cells (T_{EMRA}) (Figure 7) (184,185).



Näive (T_N)	Transitional memory (T_{TM})	Stem cell memory (T_{SCM})	Central memory (T_{CM})	Effector memory (T_{EM})	Terminally differentiated effector memory (T_{EMRA})
CD45RA+	CD45RA-	CD45RA+	CD45RA-	CD45RA-	CD45RA+
CD45RO-	CD45RO+	CD45RO-	CD45RO+	CD45RO+	CD45RO-
CCR7+	CCR7+	CCR7+	CCR7+	CCR7-	CCR7-
CD27+	CD27+	CD27+	CD27+	CD27+/-	CD27-

Figure 7. Main surface markers for identification of memory subpopulations in T cells. Surface markers are included for memory subpopulations that are usually found in blood. Created with Biorender and adapted from Corneau et al. (184).

It is well established that CD4+ T cell susceptibility to HIV-1 infection increases with T cell differentiation (186,187). Although viral DNA has been detected in all those subtypes, T_{CM}, T_{TM} and T_{EM} contain the highest proportion of proviruses (188,187). T_{SCM} cells exhibit an exceptionally long half-life, making them the most stable component of the latent reservoir (189). They also have the capacity to differentiate into T_{CM} and T_{EM} cells, while T_{TM} cells represent intermediate phenotypes between T_{CM} and T_{EM}, as T_{CM} can further differentiate into T_{EM} cells. T_{CM} cells exhibit a long half-life, possess self-renewal capabilities, and make the largest contribution to the overall population of provirus-containing cells (190). T_{EM} cells have a higher replication capacity and contain a greater proportion of intact proviruses (191–194).

Each of these cell types contributes to maintaining its own latent HIV-1 reservoir (195). T_N cells also serve as a significant source of the virus following treatment interruption or failure, suggesting that this subtype of CD4+ T cells is also important for latent HIV-1 infection (196). T_{EMRA} cells harbor higher proportions of unstable viral forms, contributing to T cell activation, which in turn promotes reservoir maintenance and viral rebound (197).

As was mentioned before, CD4+ T cells can be divided into subtypes according to their functionality. The Th1 subset preferentially harbors intact viral genomes maintained by clonal proliferation (191), Th17 cells also harbor viral reservoirs (198), and Tfh cells contain replication-competent proviruses in secondary lymphoid tissues (147,199).

Myeloid cells, specifically macrophages and DCs, have also been identified as cells that may harbor HIV-1 reservoir (Figure 8). Macrophages containing replication-competent viral DNA have been found in several tissues (200). fDCs are present in secondary lymphoid tissues and also contain replication-competent proviruses that, as has been observed in classic DCs, can be transferred to CD4+ T cells during antigen presentation (cell-to-cell transmission) (201). Viral DNA or RNA has also been found in astrocytes or microglia, which are long-lived cell types, but these reservoirs have not shown replication-competency (202).

4.3.2. Anatomic sites of the reservoir

CD4+ T cells of blood are fewer than 2% of the total CD4+ T cells found in the body, making tissues the main location for HIV-1 reservoirs. Latent infected cells have been found in lymph nodes (147), GALT (148,149,203), the central nervous system (204), lungs (205), bone marrow (206,207) and the genital tract (208,209) (Figure 8). Moreover, intact viral genomes have been found in at least 28 different tissues (154). Secondary lymphoid tissues, such as spleen, lymph nodes, and GALT, contain the major proportion of HIV-1 replication as a result of the large amounts of Tfh cells that are susceptible to HIV-1 infection (210) and the difficulty of trafficking cytotoxic T cells to these sites (211–213). The brain and testis are called “sanctuary sites” for the HIV-1 reservoir, as ART cannot penetrate them (214). Additionally, immune cells and responses cannot easily access the testis, and multiple mechanisms suppress the immune response in this tissue (215–217). Thus, finding a cure for HIV-1 requires controlling and eradicating HIV-1 reservoirs in all these tissues and organs. The Last Gift tissue donation research study is a program focused on end-of-life HIV-1 cure research, in which individuals choose to donate their bodies to science shortly before death from non-AIDS related causes (153). This clinical research maps the anatomical distribution of the HIV-1 reservoir in tissues rarely accessible in living patients and is producing important advances in understanding the reservoirs distribution (153–155).

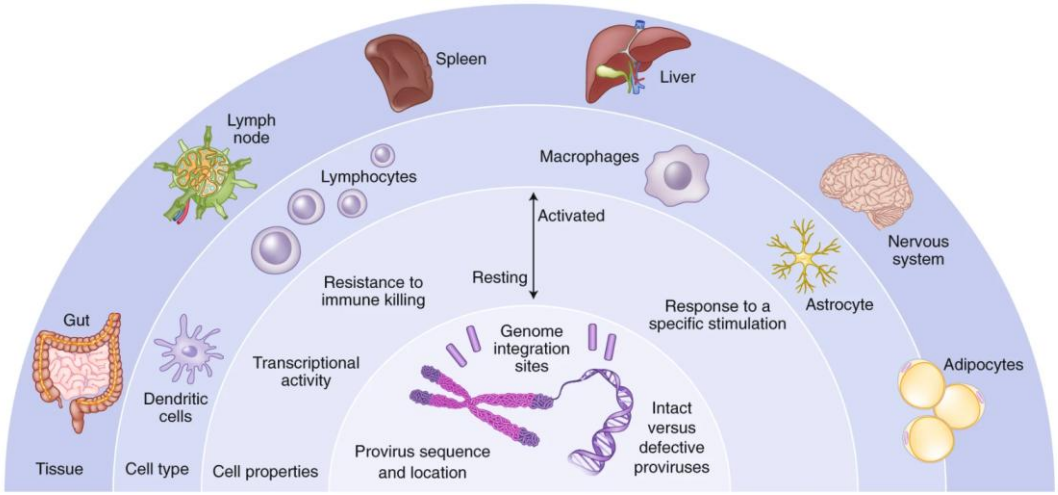


Figure 8. Localization of HIV-1 reservoirs. HIV-1 reservoir is characterized by its anatomical location, cell types that can harbor proviruses, properties that define those cell types as HIV-1 reservoirs, the integration sites within the host cell genome, and the characteristics of the provirus. Obtained from Deeks et al. (218).

4.3.3. HIV-1 reservoir maintenance and proliferation

4.3.3.1. Homeostatic proliferation

The site of HIV-1 integration within the host cell genome may impact the ability of the provirus to survive in latency. HIV-1 proviruses tend to integrate more often into introns of genes that are actively being transcribed (219,220), but those integrated in non-genic sites of the genome are likely to be latent for longer periods (176). HIV-1 reservoirs that are located in introns of active genes are also modulated by epigenetic silencing mechanisms, such as DNA methylation (221,222), and histone acetylation (223,224) and deacetylation (225,226).

The main mechanism for HIV-1 reservoir maintenance and proliferation is the clonal expansion of CD4+ T cells, resulting from activation of these cells via antigen presentation (TCR-mediated activation) or γc-cytokine stimulation (227). This process generates proviruses with identical integration sites in the genomes, even though these proviruses sometimes come from different cells (228). Moreover, in PWH on ART, the limited sequence evolution in the viral reservoir implies that ART prevents viral replication cycles, and only certain proviruses are maintained through T-cell proliferation (229).

Cytokine-stimulation of CD4+ T cells, mainly by IL-7 and IL-15, promotes their proliferation and survival, enabling the persistence of proviruses (Figure 9) (188). Both cytokines induce

T-cell expansion and maintenance by triggering the signal transducer and activator of transcription 5 (STAT5) via the JAK-STAT pathway (230).

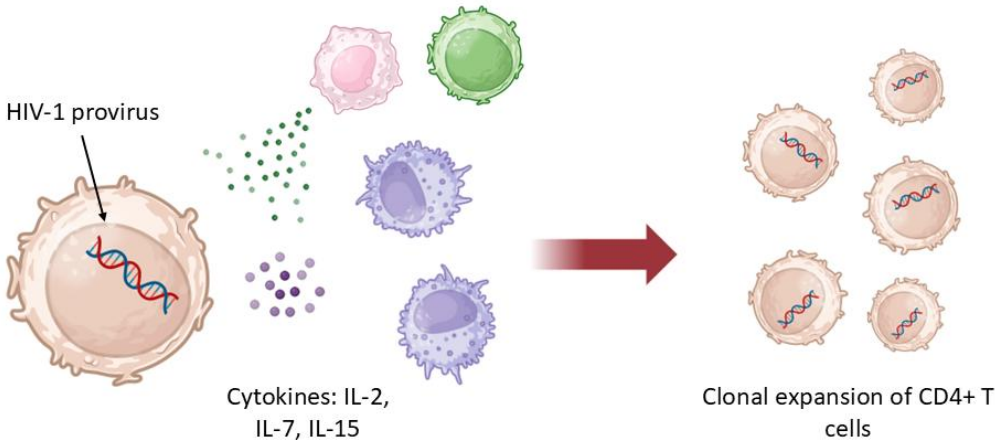


Figure 9. Clonal expansion of HIV-1-infected CD4+ T cells. Production of IL-2, IL7 or IL-15 by immune cells, such as macrophages, T cells and NK cells, drives the clonal expansion of latently infected CD4+ T cells, enabling the persistence of HIV-1 proviruses. Created with Biorender.

4.3.3.2. Antigen-driven reservoir replenishment

TCR-mediated activation of CD4+ T cells also mediates the proliferation and maintenance of the reservoir, as these cells are activated by antigen presentation, generating cellular clones in response to infection and tumor antigens (231,232). Therefore, TCR-mediated activation is responsible for CD4+ T cells differentiation into effector memory subsets that maintain virus clonotypes (Figure 10) (233).

Another mechanism is the active transcription and translation of proviruses, either defective or intact, which is detectable in PWH on ART (150,234). This mechanism results in the detection of infected cells by the immune response that leads to immune senescence. It is also an opportunity for TCR-mediated activation of other CD4+ T cells that leads to clonal expansion and immune senescence (235).

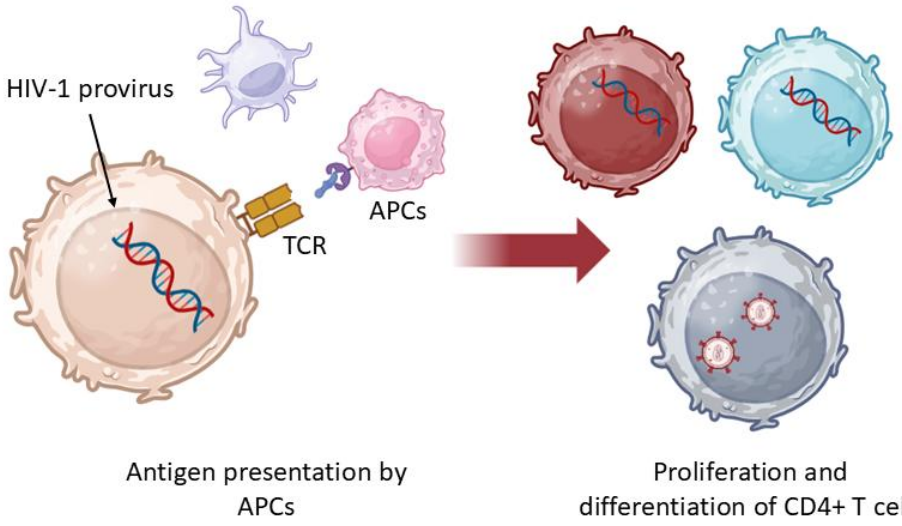


Figure 10. TCR-mediated activation of HIV-1-infected CD4+ T cells. Activation of CD4+ T cells is induced by the presentation of antigens by APCs, which promotes the differentiation of CD4+T cells into memory subsets and the reactivation of the provirus, producing new virion particles. Created with Biorender.

Thus, HIV-1 reservoir maintenance and proliferation are deeply linked to intrinsic T cell maintenance and immunity mechanisms, and reducing HIV-1 reservoir size involves modifications or alterations of these mechanisms. This maintenance of viral reservoirs despite long-term treatment with ART results in chronic immune activation that leads to non-infectious complications and co-morbidities such as cardiovascular diseases, liver and kidney diseases, neurocognitive detriment, and non-AIDS malignancies (236).

5. Cellular metabolism in HIV-1 infection

Metabolic pathways and immune activation are closely linked to the immune response against HIV-1 infection, as well as with the susceptibility of host cells to the virus. New therapeutic strategies aim to modulate cellular metabolism to increase CD4+ T cells resistance to HIV-1 infection and to strengthen the cellular immune response.

5.1. Metabolism and susceptibility to HIV-1 infection

Activated CD4+ T cells are more susceptible to HIV-1 infection, while resting CD4+ T cells exhibit strong resistance to infection (237,238). T_N cells, which show high resistance to HIV-1 infection, exist in a quiescent state in which cellular metabolism depends mainly on low levels of oxidative phosphorylation (OXPHOS) in the mitochondria (239). T-cell

differentiation into effector or memory T cells modifies cellular metabolism, increasing glutaminolysis and glycolysis (240). However, it has been observed that HIV-1 may not select CD4+ T cells based on cellular differentiation, but on higher mitochondrial biomass, elevated glucose uptake, or increased OXPHOS (241). Thus, HIV-1 preferentially selects CD4+ T cells with high metabolic activity. Moreover, mitochondrial respiration plays a critical role in regulating HIV-1 replication, as opposed to aerobic glycolysis (241), as CD4+ T cells with higher mitochondrial metabolism show increased susceptibility to HIV-1 (242). It has been proposed that inhibiting specific pathways in the cellular metabolism of CD4+ T cells may promote resistance to HIV-1 infection and interfere with HIV-1 provirus reactivation in latently infected cells.

5.2. Metabolism and the immune response against HIV-1

HIV-1 chronic infection is characterized by T-cell exhaustion of the immune response. In the first weeks of acute infection, oxidative stress and hyperactivation of mitochondrial metabolism produce CD8+ T cells characterized by mitochondrial impairment and reduced expression of genes involved in the tricarboxylic acid (TCA) cycle (243). These CD8+ T cells are incapable of developing memory subsets and have little potential for controlling HIV-1 infection in the absence of ART, relying on glucose metabolism (244). Similar alterations are observed in NK cells in PWH, where reduced OXPHOS and mitochondrial impairment reduce the production of IFNγ (245). It has been suggested that functional mitochondrial metabolism and the capacity to change metabolic activity are crucial for a CD8+ T-cell response capable of controlling HIV-1 infection (241). Therefore, restoring mitochondrial metabolism in CD8+ T and NK cells may be a good strategy to enhance a potent immune response that can control HIV-1 infection.

6. Antiretroviral therapy

ART is a combination of three to four antiviral compounds that control HIV-1 replication, but must be administered as a life-long treatment (246). ART reduces plasma viremia in almost all individuals to nearly undetectable levels, which significantly lowers the sexual transmission of HIV-1, thereby contributing to the prevention of new infections (247). Furthermore, initiating ART immediately or early after an HIV-1 diagnosis reduces the risk of acquiring HIV-1 within a serodiscordant relationship by 96% (248).

ART is divided into seven classes as they interfere with key steps of the HIV-1 replication cycle (249):

- Nucleoside reverse transcriptase inhibitors (NRTIs): interfere with the elongation of viral DNA. Some examples include abacir, lamivudine, tenofovir, and emtricitabine.
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs): block the DNA polymerase function of the viral RT. Some of them include doravirine, etravirine, and rilpivirine.
- Protease inhibitors (PIs): block the cleavage of Gag and Gag-pol. Some examples are darunavir, ritonavir, saquinavir, and tipranavir.
- Integrase inhibitors (INIs): target the IN and block its function, resulting in circularized vDNA that cannot be integrated into the host cell genome. These compounds are cabotegravir, dolutegravir and raltegravir.
- Entry inhibitors: this group includes three types of compounds: post-attachment inhibitors (fostemsavir and ibalizumab-uiyk), CCR5 receptor antagonists (maraviroc), and fusion inhibitors (enfuvirtide).

ART alone is incapable of eradicating HIV-1 infection, since viral reservoirs are maintained even in the presence of ART as a life-long treatment (250), and ART interruption leads to viral rebound (251). The long-acting ART approach is an injectable alternative to the treatment that may be used in PWH with poor adherence to daily oral ART or uncontrolled HIV-1 infection (252,253). The regimen of cabotegravir plus rilpivirine can be administered every 2 months, maintaining viral suppression. Lenacapavir treatment every 6 months was approved as an adjunctive treatment for multidrug-resistant HIV-1 (254).

However, ART has also facilitated the existence of two preventive methods. Pre-exposure prophylaxis (PrEP) is a successful preventive strategy for individuals at substantial risk of infection (255). Effectiveness varies depending on adherence to treatment levels. MSM have demonstrated higher efficacy rates compared to the results observed in studies involving only women (256). In addition to this preventive method, post-exposure prophylaxis (PEP), which contains three antiretrovirals (tenofovir, emtricitabine and raltegravir), should ideally be started as soon as possible, and no later than 72 hours, following exposure to the virus, reducing the transmission risk by 80% (257,258).

7. Strategies to tackle HIV-1 reservoirs

7.1. Natural control of the infection

Although natural control of the infection does not result from therapeutic interventions, certain individuals can control HIV-1 in the absence of ART. These individuals are known as elite controllers (ECs) and viremic controllers (VCs), and they represent 1-5% of all PWH. ECs are characterized by maintaining viremia below 50 copies/mL, while VCs suppress viral replication between 50 and 2,000 copies/mL (259). It is worth mentioning a third group, referred to as post-treatment controllers (PTCs) who can control viral replication between 50-400 copies/mL after ART interruption (260), although the mechanisms of viral control differ from those observed in ECs and VCs (261,262).

An enhanced immune response is responsible for viral control in ECs and VCs. NK cells in ECs usually present an increased expression of activation markers that trigger a heightened ADCC response (263), along with high numbers of cytotoxic NK populations (264). The primary factor in immune control among ECs and VCs is the differentiation of CD8+ T cells targeting conserved and vulnerable epitopes of HIV-1 (265). In ECs, it has been shown that CD8+ T cells also have the capacity to eliminate resting CD4+ T cells that are HIV-1-infected, which may be highly effective in reducing proviral reservoirs (266). Moreover, the presence of certain MHC-I alleles in ECs and VCs enhances antigen presentation of HIV-1, triggering a potent and durable CD8+ T cell response (267–269). However, not all ECs possess these alleles, and not all individuals who develop an efficient CD8+ T cell response (270).

It has been hypothesized that, in ECs, proviral integration into transcriptionally inactive genomic regions might be one of the factors enabling viral control (271). Initially, it was found that, in ECs, intact proviruses tend to be more integrated into KRAB-ZNF genes with repressive expression, which may contribute to controlling HIV-1 infection (271). However, this feature was also identified in clonally expanded CD4+ T cells of PWH on long-term ART (272), indicating that this feature is not unique to ECs. In contrast, defective proviruses and nonclonal intact proviruses lack preferential integration sites near KRAB-ZNF genes, and tend to integrate in proximity to cancer associated genes such as BACH2 or MKL2 (174,273,274). ZNF genes are actively transcribed in resting memory CD4+ T cells (176,271)), but their silencing during cellular activation might contribute to clonal expansion rather than viral expression (272). Thus, the role of proviral integration sites in the natural control of infection remains a matter of debate.

Many strategies aiming to cure HIV-1 seek to extrapolate the immunological features found in ECs and VCs using therapeutic interventions.

7.2. Allogeneic stem-cell transplantation via CCR5

Homozygosity for the deletion of 32 amino acids in *CCR5* allele (*CCR5*Δ32/Δ32) generates resistance against HIV-1 infection, blocking the entry of the virus into CD4+ T cells as they lack *CCR5* expression on the cell surface (275). Allogeneic stem-cell transplantation from *CCR5*Δ32/Δ32 donors is, to date, the only strategy that has led to a functional cure and a substantial reduction in the HIV-1 reservoir in some individuals (276–280). Several studies have shown that reservoir size reduction results from the effects of cancer treatments, graft-versus-host immune response and substitution of infected cells after transplantation (281,282), while the *CCR5*Δ32 mutation may not directly affect reservoir size (283). Recently, a case of stem-cell transplantation from a wild-type CCR5 donor was reported (284), which also demonstrated the role of graft-versus-host events in reducing the HIV-1 reservoir.

However, this cure approach presents several limitations, such as high mortality and morbidity associated with stem-cell transplantation, the need for HLA-compatible *CCR5*Δ32/Δ32 donors, the risk of viral reseeding from the small number of infected cells that may remain after transplantation, or the presence of CXCR4-tropic variants that can replenish the viral reservoir and are associated with increased pathogenicity (218). Therefore, stem-cell transplantation is not considered a viable curative strategy for all PWH.

7.3. Shock and kill

This pharmaceutical approach uses latency reversal agents (LRAs) for the reactivation of latently infected cells (the shock), thereby making them vulnerable to the cytotoxic immune response and ART (the kill) (Figure 11). Many substances can be used as LRAs; some types include: i) cytokines or receptors agonists (IL-7, IL-15 or TLR2, 3) such as flagellin or Pam3CSK4; ii) epigenetic modification enzyme inhibitors (histone deacetylase inhibitors such as trichostatin A or histone methyltransferase inhibitors such as chaetocin); or iii) cellular signaling modulators that promote activation of the protein kinase C (PKC) pathway (prostratin or bryostatin) (285,286). Although several LRAs have been tested in humans, they have failed to reduce reservoir size (287–292), and none of them is fully approved for their use in HIV-1 cure strategies. It should be noted that only a small proportion of the reservoir size consists of intact proviruses that are inducible by LRAs (293–295), and that latently infected cells are more resistant to killing by CD8+ T cells (296). Moreover, many

LRAs have some off-target effects that result in toxicity. Thus, new LRAs are necessary for reducing the off-target effects and improving the immune response of PWH (297).

Additionally, immunomodulatory strategies should also be implemented to enhance the capacity of cytotoxic cells for "the kill" part of this strategy. In this regard, several approaches are being evaluated such as 1) the IL-15 superagonist N-803 to boost NK and $CD8^+$ T cell activity (298); blockade of the PD-1/PD-L1 immune checkpoint pathway to reinvigorate exhausted $CD8^+$ T cells (299); adoptive transfer of HIV-specific CAR-T cells engineered to recognize viral envelope antigens (300); therapeutic dendritic cell vaccines loaded with HIV peptides to prime robust $CD8^+$ T cell responses (301); combinations of N-803 with broadly neutralizing antibodies (bNAbs) to maintain immune activation after ART interruption (302); or CTLA-4 blockade to enhance follicular helper T cell and $CD8^+$ T cell functions (303).

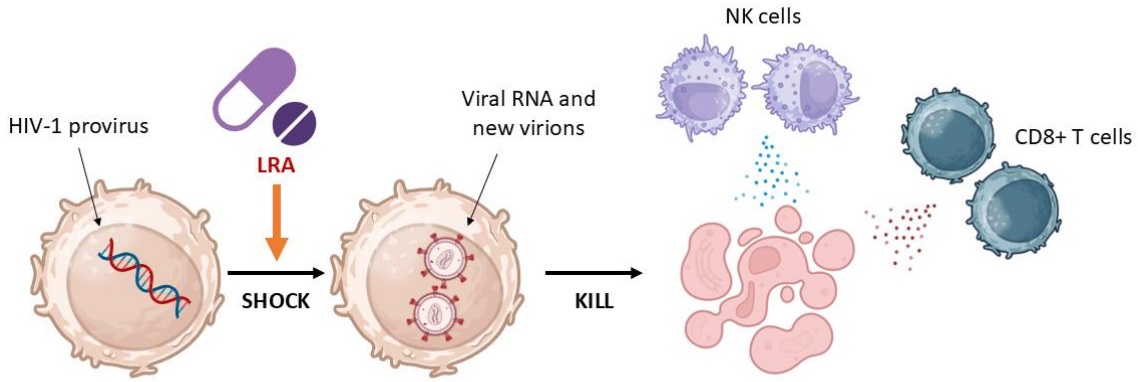


Figure 11. Graphic representation of the Shock & Kill strategy. LRAs induce the transcription of viral DNA and the generation of viral proteins (the shock). Thus, the activated cell becomes visible to the immune response, which eliminates it (the kill). Adapted from (297) and created with Biorender.

7.4. Block and lock

This strategy involves permanently pushing HIV-1 into a deeply latent epigenetic state that resists natural reactivation of the provirus, known as deep latency (304,305), using latency promoting agents (LPAs) (Figure 12). Some approaches within this strategy include the use of Tat-inhibitors such as dihydrocorstatin (306); short interfering RNA, which induce transcriptional gene silencing by targeting promoting regions (307); or inhibitors of mTOR, which block NF-κB pathway (308). Some LPAs are still in early phases of development, and

many lack the “lock” function, in which provirus reactivation is impeded even after LPA withdrawal (309). Although it is an interesting and promising strategy, clinical trials have not yet started or are still ongoing, and more data and compounds are necessary to advance this approach.

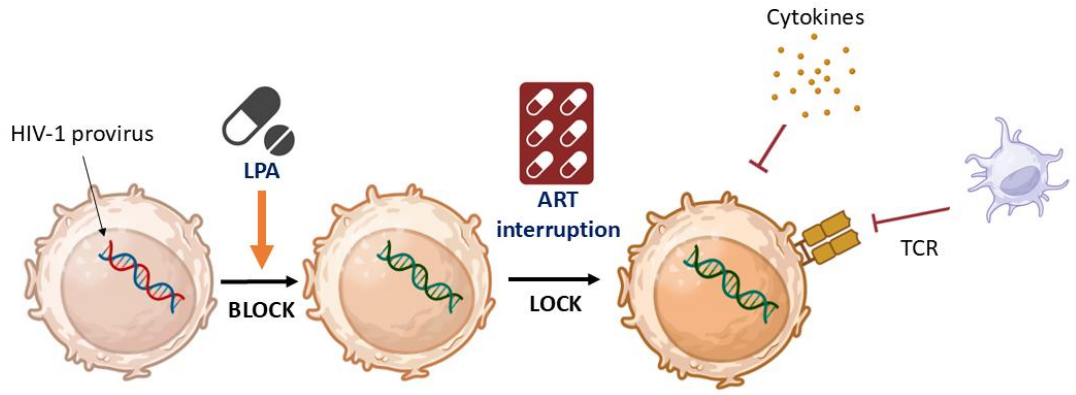


Figure 12. Graphic representation of Block & Lock strategy. LPAs induce the deep latency state in persistently infected cells, preventing natural reactivation of these cells via TCR-mediated activation or cytokine stimulation. In this strategy, the activity of LPAs is maintained even when ART is interrupted. Adapted from (304) and created with Biorender.

7.5. Immunotherapy with broadly neutralizing antibodies

Broadly neutralizing antibodies (bNAbs) have emerged as an opportunity to replace or combine with ART for the suppression or even eradication of the HIV-1 reservoir (310,311). bNAbs have been identified in individuals infected with highly mutable RNA viruses prone to escape through quasispecies formation such as HIV-1, Zika, influenza, Ebola and Marburg, SARS-CoV-2, and hepatitis C virus, because these antibodies can neutralize multiple co-circulating quasispecies of the same virus (312–314). Therefore, bNAbs have been isolated from PWH and can efficiently block a wide range of genetically diverse HIV-1 strains, and in PWH it has been observed that they can reduce viremia (315–318).

By targeting the HIV-1 envelope, bNAbs competitively inhibit viral gp120-CD4 interactions, thereby interfering with viral fusion and entry into susceptible cells (319). Additionally, these antibodies also facilitate recognition by cytotoxic cells, leading to elimination of infected cells and virions by cytotoxicity and phagocytosis (320). Nonetheless, the immune response can also produce anti-drug antibodies (ADAs) against the bNAbs that may reduce treatment efficacy by neutralizing the bNAbs or accelerating their clearance, which makes necessary the pre-screening for antibody-sensitivity (319).

The first generation of bNAbs, such as 2F5, 2G12, and 4E10, were tested in human clinical trials (321–324) and showed only a transient reduction in viral load. The rapid selection of viral escape mutations led to treatment failure and viral rebound (321,325). Second-generation bNAbs, such as VRC01, 3BN117, and 10-1074, have shown enhanced breadth, potency, safety, and tolerability (315,326–328). However, immunization with a single bNAbs has only produced a transient reduction in viral load, with a rapid viral rebound occurring in most individuals (319). In contrast, combination therapy with two or more bNAbs has achieved prolonged virologic control in most participants by effectively restricting viral escape mutations. Furthermore, combining bNAbs, such as 3BNC117 and 10-1074, targeting distinct epitopes, has enhanced neutralization breadth (319,329). Yet, immunotherapy with two or more bNAbs has still been insufficient in preventing viral escape. Obtaining bNAbs against every HIV-1 variant that can escape existing antibodies remains a major challenge, and the efficacy of bNAbs relies on ART regiment and antibody half-life (330).

Thus, bNAbs have also been used in combination with LRAs, inducing a reactivation effect in latently infected cells that exposes them to the bNAbs (331). Additionally, bNAbs have been used for the prevention of HIV-1 infection (332,333) with the objective of expanding the number of available preventive treatments. VRC01 was tested in humans, but it did not prevent HIV-1 infection (334).

Long-term delivery of anti-HIV-1 bNAbs via adeno-associated virus (AAV) vectors shows potential for HIV-1 prevention and treatment. The preliminary study described by Martinez-Navio et al. (331) demonstrated that one monkey infected with SHIV that received AAV-encoded bNAbs, maintained effective antibody levels for over two years, leading to sustained viral suppression. Although this provides proof-of-concept that this strategy could be feasible as a functional cure, it also highlights that the formation of anti-drug antibodies (ADAs) is a important challenge for the practical application of this approach in humans.

Nonetheless, bNAbs show promising characteristics and results that can help in controlling viremia and reducing reservoir size, but viral diversity, immune cell exhaustion and antibody half-life remain as challenges to be addressed in the future (335).

7.6. Vaccines

The high mutation and recombination rate of HIV-1 and its capacity to establish viral reservoirs have been great obstacles for the development of safe and effective HIV-1

vaccines (336,337). Several clinical trials have tried to implement vaccines to reduce the HIV-1 reservoir size, but no significant changes were observed (338–340). In 1986, the first phase I clinical trial for a prophylactic vaccine was performed in the Democratic Republic of Congo (341). In this study, a vaccinia vector expressing the unprocessed gp160 HIV-1 envelope protein was used. Two more trials focused on using the Env protein, VAX003 (342) and VAX004 (343), but the high variability of Env caused that infection rates were similar between vaccine and placebo groups. Thus, vaccine trials were later focused on inducing T-cell responses. The Step Study and the Phambili study used an adenoviral vector that encoded for Gag, Nef, and Pol, but both failed to reduce the risk of HIV-1 infection (344–346).

The next clinical trials were aimed at inducing both cellular and humoral immunity. The HTVN 505 trial used three DNA primes that induced antibodies and CD4+/CD8+ T cell responses, but again failed to prevent HIV-1 infection (347). However, the RV144 efficacy trial later showed positive results for preventing HIV-1 acquisition (348). The HVTN 702 clinical trial in South Africa derived from the initial RV144 clinical trial that was developed in Thailand with a vaccine containing gp120-SU subunit (349). In HVTN 702, clinical phase III was achieved, but the vaccine failed to prevent HIV-1 infection (350). In another clinical trial, called Imbokodo/HTVN705, in which a mosaic vaccine was used (composed of different epitopes from HIV-1 variants), the low efficacy of the vaccine in preventing new cases resulted in the early termination of the study (351).

One novel approach for preventive vaccines is the use of immunogens that induce the maturation of B lymphocytes, triggering the production of potent bNAbs (352,353). One of these immunogens is eOD-GT8, which focuses on inducing VRC01-like bNAbs (354).

Nevertheless, new approaches in this field are emerging as future candidates for a vaccine that achieves a significant and durable HIV-1 suppression. One of these approaches is the induction of HLA-E specific CD8+ T cells using cytomegalovirus (CMV)-vectored vaccine in non-human primates, with potential for reducing HIV-1 replication (355). The HIVACAT T-cell is a novel HIV-1 vaccine immunogen focused on inducing cellular immune responses. In this approach, the AELIX-002 trial has showed that a combination of three of these immunogens elicited robust T-cell responses with no serious adverse effects (356). Another approach in rhesus macaques used an Ankara vaccine with TLR7 agonists plus anti-PD-1 blockade (one of the immune checkpoints expressed by exhausted T cells), which induced a potent CD8+ T cell response with capacity to migrate to lymphoid nodes and reduce

reservoir size (357). In the same way, using TLR7 agonists but in a modified adenovirus vaccine, another study showed an induction of the innate immune response that controlled viral rebound after ART interruption, and also decreased viral DNA (358). These new approaches show promising results, but the resistance of latently infected cells to killing by the cytotoxic immune response still represents a major issue in vaccine development.

Currently, the development of vaccines with novel technologies, such as mRNA vaccines, represents new candidates that may result in efficient prophylactic HIV-1 vaccines.

7.7. Cancer immunotherapy: tyrosine kinase inhibitors

Cancer immunotherapy has emerged as a strategy that may enhance the immune response and interfere with reservoir maintenance. In this regard, tyrosine kinase inhibitors (TKIs), which are used for the treatment of chronic myeloid leukemia (CML), have shown promising results in inducing HIV-1 suppression effects and interfering with HIV-1 integration.

CML is a myeloproliferative neoplasm that originates from the translocation between the Abelson murine leukemia gene (ABL1) on chromosome 9 and the breakpoint cluster region (BCR) gene on chromosome 22, resulting in the formation of the Philadelphia (Ph) chromosome abnormality, which produces the oncoprotein BCR::ABL1 (359). The permanently active tyrosine kinase activity of BCR::ABL1 causes unlimited replication of myeloid cells (360–364). TKIs block the interaction between BCR::ABL1 and ATP, which impedes the uncontrolled cellular proliferation (365,366).

Currently, six TKIs have been approved for CML treatment: imatinib (1st generation); nilotinib, dasatinib and bosutinib (2nd generation); ponatinib and asciminib (3rd generation). The efficacy of TKIs is determined by their ability to induce a deep molecular response (DMR), in which BCR::ABL1 cancer transcript expression is less than 0.01%, corresponding to a 4-log reduction. The synthesis of the first TKI, imatinib, substantially increased the life expectancy of PWH due to its high efficacy in inhibiting BCR::ABL1, but second-generation TKIs (dasatinib, nilotinib and bosutinib) were later generated for the treatment of CML patients with resistance mutations against imatinib (367). These second-generation TKIs showed enhanced effects against leukemia, but also some adverse effects, although they all were approved as first-line treatment. Nilotinib, a derivative of imatinib, has shown similar or slightly increased antileukemic effects compared to imatinib, but also an augmented risk of vascular side effects (368). Bosutinib has demonstrated higher inhibitory effects against BCR::ABL1 than imatinib, establishing enhanced antileukemic responses,

but also a higher incidence of cardiovascular side effects (369) or gastrointestinal toxicity (370).

Dasatinib is 350 times more potent than imatinib in inhibiting BCR::ABL1, inducing accelerated and enhanced antileukemic responses, with a recommended dose of 100 mg daily. Dasatinib also shows an increased probability of pleural effusions (up to 30% of CML patients), but this can be addressed with a lower dose (50mg per day), or corticosteroids (368). Thus, second generation TKIs induce an enhanced response against leukemia compared to imatinib, but the survival rates of people treated with imatinib or second generation TKIs are similar. Although there is an increased probability of severe toxicities with all second generation TKIs, all of them can be managed with dose readjustments and therapeutic interventions.

Ponatinib is a third-generation TKI that exhibits a 500 times greater inhibitory capacity than imatinib against BCR::ABL1 kinase activity (371). It is used in people with CML who has developed resistance mutations to dasatinib or nilotinib, including the mutation T315I in BCR::ABL1 (368). The stronger antileukemic effects of ponatinib are penalized by adverse effects, such as thrombotic events, vascular occlusions, heart failure, and hepatotoxicity (372). Ponatinib is not used as a first line treatment due to the elevated incidence of arterial ischemic events (373). To avoid these adverse effects, it is recommended to use low-dose ponatinib of 30 mg daily or even 15 mg (the approved dose is 45 mg daily) (368)

Asciminib is the last TKI that has been approved and it is recommended for CML patients who have been treated previously with 2 or more TKIs, and like ponatinib, in cases with the T315I mutation (374). In a study in which it was compared to imatinib and all second generation TKIs, asciminib showed better antileukemic response and fewer adverse effects than the rest of the TKIs (375).

All TKIs, except asciminib, are ATP-competitive inhibitors that compete with ATP for binding to ABL1 (376). Asciminib is an allosteric inhibitor that induces a conformational change locking the kinase in an inactive state (376). TKIs are also differentiated by their off-target effects. Dasatinib exhibits a significantly broader kinase inhibition profile compared to imatinib or nilotinib, targeting not only BCR::ABL1 but also KIT, PDGFR, SRC family kinases, EPHA, EGFR and MAP kinases (377). Bosutinib does not inhibit KIT or PDGFRA, although it does interfere with cell cycle regulation (378,379). Ponatinib shows the broadest kinase inhibition profile, targeting ABL1, KIT, PDGFR, SRC family kinases, VEGFR, EGFR, HER2, FLT3, FGFR and JAK2 (Figure 13). Asciminib´s unique binding to the ABL1 myristoyl pocket,

distinct from conventional ATP-competitive inhibitors, confers unparalleled selectivity, causing no off-target effects (380).

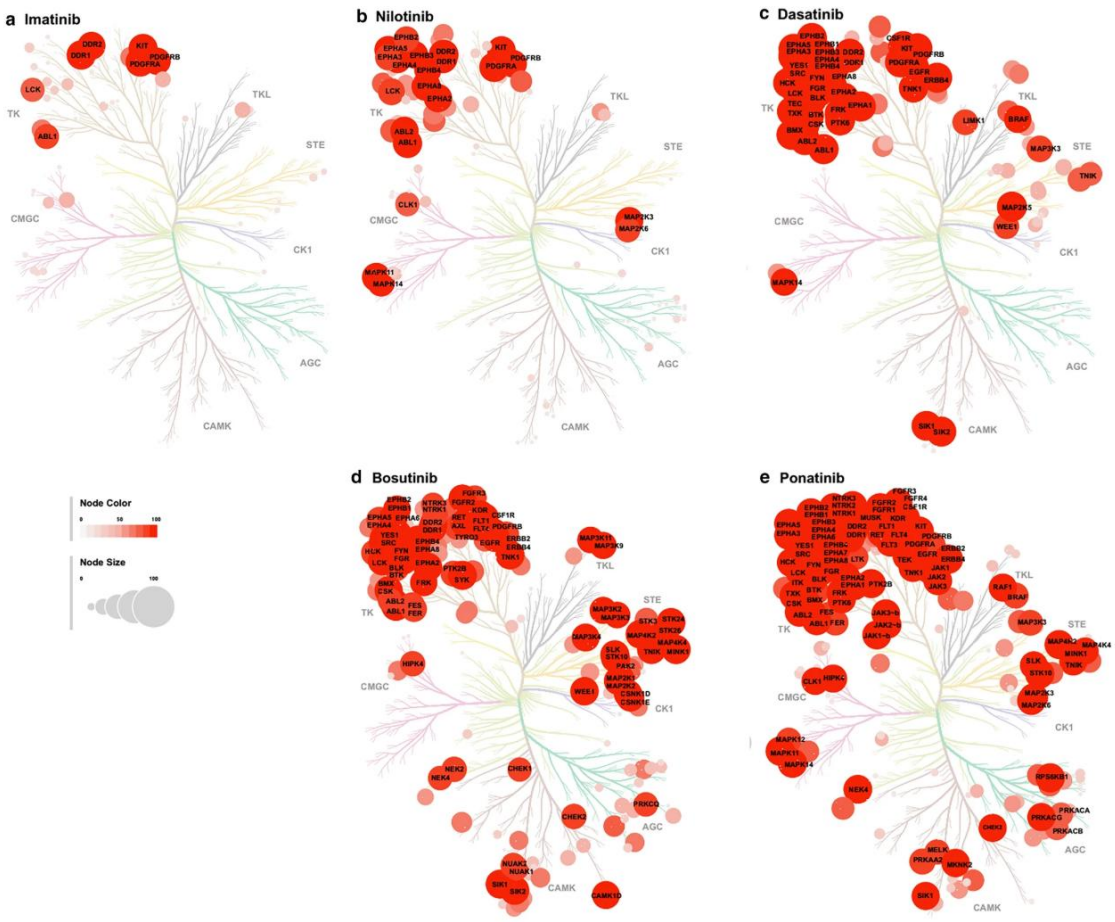


Figure 13. Comparison of the off-target spectrum of ATP-competitive tyrosine kinase inhibitors: a) imatinib, b) nilotinib, c) dasatinib, d) bosutinib, and e) ponatinib. Highlighted targets are labeled when their activity is inhibited more than 80%. Size and color intensity of each node increases with the degree of inhibition. Obtained from Lee et al., 2021 (376).

Despite the TKIs potent cytostatic and inhibitory effects in T cells, the reports of opportunistic infections are sporadic (381).

7.7.1. Use of TKIs against HIV-1 infection

Some off-target effects of TKIs can interfere with T cell activation and proliferation, HIV-1 replication, and can also enhance the cytotoxic immune response against infected cells. The resistance of latently infected cells and the nature of HIV-1 reservoir maintenance highlight the need for cure strategies that disrupt the maintenance and proliferation of these cellular reservoirs.

7.7.1.1. TKIs as senolytics in chronic HIV-1 infection

Some TKIs reduce senescent cell burden and decrease the production of proinflammatory cytokines (382), which may critically impact the effects of chronic inflammation in HIV-1 infection. Dasatinib was selected as a senolytic drug due to its potential to inhibit the pathways that protect senescent cells from proapoptotic signals (383). It has also been observed that this TKI reduces the production of proinflammatory cytokines that promote chronic inflammation, such as IL-6, IL8, monocyte chemotactic protein 1 (MCP-1), and granulocyte macrophage colony-stimulating factor (GM-CSF) (382). Reducing both proinflammatory cytokines and the proportion of senescent cells resulted in an increase in health and lifespan (382). These effects are not observed in other TKIs, such as imatinib or nilotinib, which have less potential for inhibiting different kinases (384,385). Nonetheless, imatinib and dasatinib have shown potential in reducing PD-1 expression in CD8+ T cells, which may improve the cytotoxic response by CD8+ T cells in HIV-1 infection (386). The reduction in PD-1-positive CD8+ T cells by dasatinib may be due to its induction of lymphocytosis (386,387). Furthermore, dasatinib reduces the number of Treg cells (386), which may reduce the induction of aberrant thymic Treg cells in chronic HIV-1 infection (142).

Thus, dasatinib could also be used as a senolytic drug in chronic HIV-1 infection, reducing chronic inflammation and the number of senescent immune cells with impaired cytotoxic and immune functions that fail to eliminate latently infected cells.

7.7.1.2. Interference with TCR-mediated activation of T cells

Dasatinib and bosutinib inhibit the Src family of tyrosine kinases (SFKs), which are involved in T cell proliferation and maturation (388,389). One of these SFKs is Lck, mainly expressed in T cells and one of the major contributors to TCR-mediated activation of these cells, as it interacts with CD4 and CD8 receptors (390) (Figure 12). Lck is also implicated in the phosphorylation of the SAMHD1 antiviral immune factor. Phosphorylation at threonine 592 (T592) by cyclin A2/Cdk1 induces the change to phosphorylated SAMHD1 (pSAMHD1), the inactive form of the protein that stops degradation of dNTPs, which facilitates HIV-1 transcription and increases cell susceptibility to infection (101).

Another target of Lck that is the protein kinase C theta (PKCθ), which is triggered after TCR and CD28 activation, resulting in activation of NF-κB, which ultimately is involved in T cell activation (391–393). Lck phosphorylates PKCθ at threonine 538 (T538) and at tyrosine 90 (Y90), triggering PKCθ activation that leads to T-cell activation and HIV-1 replication (394–

396). Thus, inhibition of Lck interferes with HIV-1 transcription and T cell activation, which disrupts provirus reactivation and the establishment of new HIV-1 infections. The reduction of T-cell activation may also contribute to reducing persistent inflammation and secretion of proinflammatory cytokines in HIV-1 infection.

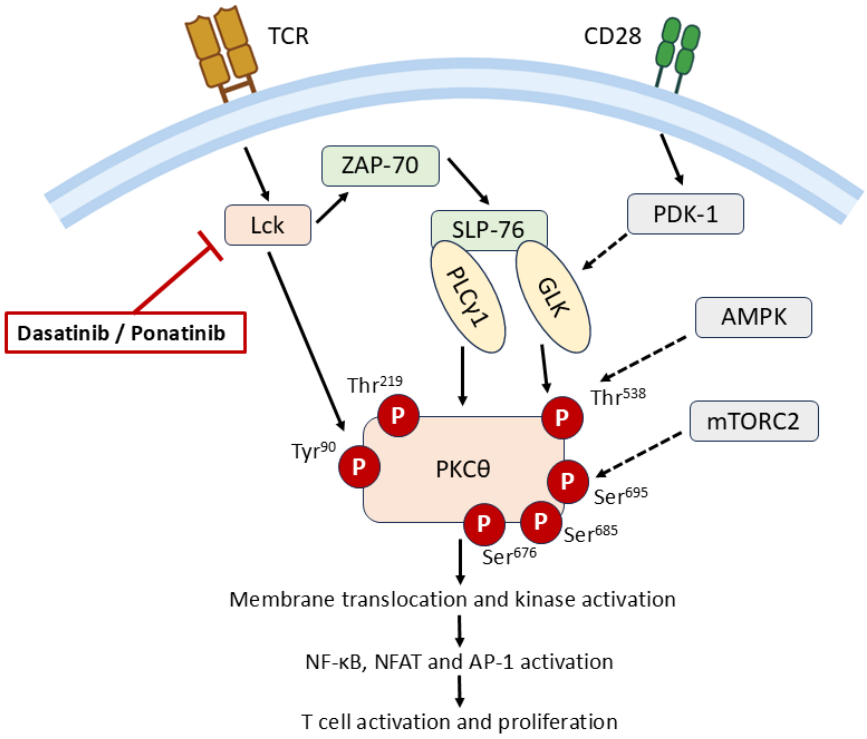


Figure 14. Phosphorylation of PKCθ during TCR-mediated activation and inhibition by dasatinib and ponatinib. TCR engagement initiates Lck-dependent phosphorylation of ZAP-70, which activates the adaptor protein SLP-76 to recruit and stimulate PLCγ1 and GLK. PLCγ1 activation results in conformational changes in PKCθ. PKCθ activation is regulated by sequential phosphorylation events: GLK (potentiated by CD28-PDK1 signaling) phosphorylates T538, Lck directly phosphorylates Y90, and phosphorylation at T219 facilitates PKCθ membrane translocation. Additional TCR-induced phosphorylation events further modulate PKCθ activity. AMPK also contributes to T538 phosphorylation. Once activated and membrane-localized, PKCθ drives downstream transcription factors (NF-κB, NFAT, and AP-1) to trigger T-cell activation, proliferation, and effector functions. Dasatinib and ponatinib directly inhibit Lck activity, impeding PKCθ activation, which interferes with T cell activation. Adapted from Wang et al. (397).

7.7.1.3. Interference with homeostatic proliferation of T cells

Stimulation by γc-cytokines, such as IL-7 and IL-15, results in the clonal proliferation of T cells (227), which also leads to the clonal expansion of latently infected T cells, enabling the persistence of HIV-1 proviruses (188). The proliferation of T cells by both cytokines is mediated by the activation of STAT5 through the JAK-STAT pathway (230). Furthermore, IL-2 and IL-7 induce the phosphorylation of SAMHD1 in CD4+ T cells through mechanisms different from TCR-mediated activation (398). Both dasatinib and ponatinib strongly inhibit IL-7 induced proliferation of T cells (399) and block the phosphorylation of SAMHD1 induced by IL-7 (400). However, they do not interfere with IL-15 induced phosphorylation of SAMHD1, indicating that both cytokines use distinct pathways for inducing T-cell proliferation and SAMHD1 phosphorylation (399). Although the exact mechanisms by which dasatinib blocks IL-7-induced proliferation of T cells are still not elucidated, it has been shown that this TKI interferes with STAT5 phosphorylation in chronic myelogenous leukemia cells (K562 human CML cells) (401). It was later confirmed that dasatinib inhibits STAT5 phosphorylation induced by IL-7 in CD4+ T cells (399). These effects were not observed with other TKIs, or were not strong enough to produce a significant reduction in clonal expansion of CD4+ T cells and inhibition of SAMHD1, which would result in interference with HIV-1 reservoir replenishment (399).

Furthermore, the inhibition of the JAK-STAT pathway has been considered for the reduction of the pool of senescent cells and the secretion of proinflammatory cytokines (402). The JAK-STAT pathway controls cytokine production (403,404), and its inhibition may reduce the secretion of IL-6 and TNF-α, which contribute to chronic inflammation in PWH (134,135). This mechanism would also be important for the potential use of TKIs as senolytic drugs in chronic HIV-1 infection.

7.7.1.4. TKIs enhance the cytotoxic immune response

Another important characteristic of dasatinib is its ability to induce the proliferation and maturation of cytotoxic immune cell populations, called large granular lymphocytes (LGLs), which have immunomodulatory effects in CML and HIV-1 infection. LGLs are cytotoxic cells (CD8+ T, γδT, and NK cells) that are able to induce a better antileukemic response (405–407). In CML individuals, dasatinib treatment induces the proliferation of NK cells (CD3-CD56+) and matured NK cells (CD3-CD56+CD57+) (408) with less production of inhibitory markers such as KIR2DL5 or NKG2A (409,410). In fact, it has been hypothesized that the

expansion of these NK cell subsets is driven by the combination of CMV reactivation, CML, and dasatinib treatment (411,412).

Dasatinib treatment also induces the proliferation of effector memory CD8+ T cells and γδT cells in almost half of CML patients (413,414). Dasatinib treatment interferes with and reduces Treg cell activity, which may allow the maturation and enhancement of the cytotoxic cell response in the context of exhausted immune responses (415). The proliferation of LGLs and enhancement of the cytotoxic response may play a critical role in controlling HIV-1 infection in PWH who interrupt ART treatment, as these cellular populations are important for infection control in ECs. In a previously published work from our group, we observed that mature NK and γδT cells of individuals with CML have the capacity to develop an antiviral immune response against HIV-1-infected-CD4+ T cells (416). In fact, mature NK cells are associated with the control of HIV-1 infection (417,418).

7.7.1.5. Dasatinib and ponatinib are the preferred choices against HIV-1

As was mentioned above, not all TKIs have the same spectrum of off-target effects, resulting in different interference with HIV-1 reservoir maintenance and provirus reactivation. Second generation TKIs are less specific for the BCR::ABL1 kinase and have a wider spectrum of tyrosine kinase inhibition. Imatinib has shown little capacity to inhibit SAMHD1 phosphorylation and NF-κB activity and it does not interfere with HIV-1 integration. Nilotinib inhibits SAMHD1 phosphorylation but still does not interfere with the reverse transcription of HIV-1 (419). Despite its potent cytostatic effect, bosutinib would need twice the recommended dose *in vivo* for PWH in order to suppress HIV-1 replication, which may increase the probability of adverse side effects (419).

On the other hand, dasatinib and ponatinib efficiently block SAMHD1 phosphorylation and interfere with HIV-1 replication and integration (419). Interestingly, these effects are achieved with dasatinib even when it is used at one-fifth of the recommended dose in CML patients, which makes it a safer option than ponatinib for its use in PWH (419). Ponatinib is less efficient than dasatinib in blocking other tyrosine kinases such as Lck, although its potent cytostatic effect is caused by inhibition of tyrosine kinases whose role in HIV-1 reservoir maintenance is not yet fully understood (420). Nonetheless, dasatinib and ponatinib meet four inclusion criteria (Figure 13) that make them viable candidates for adjuvant treatment in PWH aimed at reducing the HIV-1 reservoir size or even eradicating it.

In previous work, our group established that dasatinib use is recommended in PWH with ART that does not include ritonavir or cobicistat to avoid interactions, as both compounds

inhibit cytochrome P450 3A4 (CYP3A4) that is needed for processing dasatinib (419). The anti-proliferative effects of dasatinib and ponatinib in CD4+ T cells were further described (398,399,419), showing that both TKIs have the strongest inhibition of TCR-mediated activation. Additionally, CD4+ T cells and monocyte-derived macrophages treated with dasatinib *in vitro* are resistant against HIV-1 infection (419,421), even after they recover their capacity to become activated and despite SAMHD1 being phosphorylated (416). In a study published by our group, *in vivo* pre-treatment with dasatinib of humanized mice rendered CD4+ T cells resistant to HIV-1 infection and impaired their maturation into effector memory subsets, which are crucial for HIV-1 reservoir maintenance (422). In a small cohort of PWH diagnosed with CML who initiated TKI therapy while on ART, we observed a markedly reduced reservoir size, with no intact proviruses detected in two participants (165). This reduction was associated with the potent cytostatic effect of dasatinib, which maintains SAMHD1 in its active form and disrupts the maintenance of latently infected CD4+ T cells.

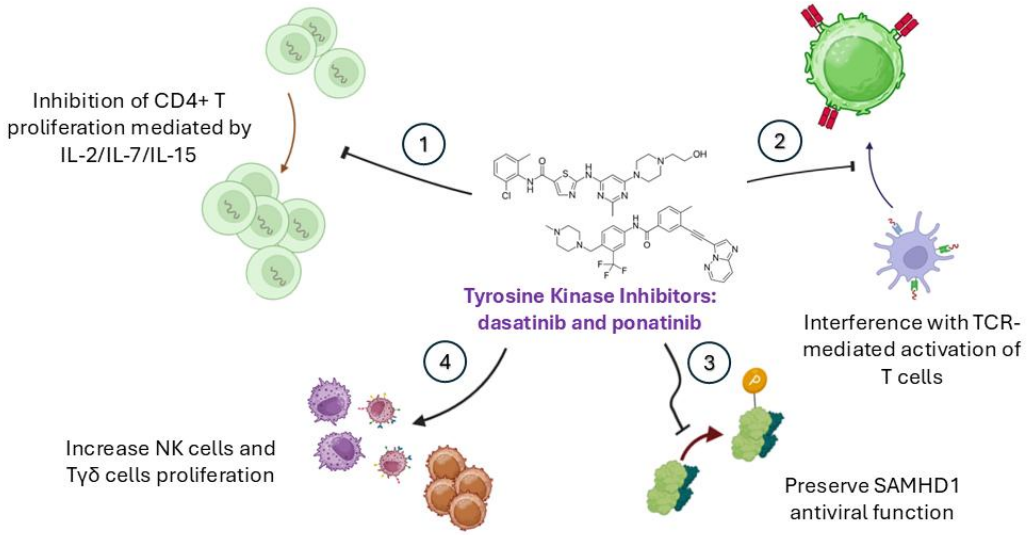


Figure 15. Dasatinib and ponatinib meet four characteristics that make them the preferred choices for adjuvant treatment in PWH on ART: 1) they inhibit CD4+ T cell proliferation induced by homeostatic cytokines, such as IL-2, IL-15 and IL-7, which are essential for reservoir maintenance and replenishment; 2) they interfere with TCR-mediated activation of T cells, which is implicated in the clonal proliferation of the reservoir and provirus reactivation; 3) they block SAMHD1 phosphorylation at T592, which preserves SAMHD1 antiviral function by degrading dNTPs necessary for viral transcription; 4) they increase the proliferation of NK and γδT cells with cytotoxic antiviral potential. Created with Biorender.

7.7.1.6. Interruption of TKI treatment

When a stable DMR is achieved, TKI treatment can be interrupted, leading to treatment free remission (TFR), in which CML is controlled and DMR maintained without TKIs. Until now, only 50% of CML individuals who have interrupted treatment with second-generation TKIs maintain TFR and do not relapse into CML (423). The probability of interrupting treatment while not relapsing into CML may be determined by the potency of the TKI in inhibiting BCR::ABL1 (the greater the potency, the higher the probability of maintaining DMR) and by the proliferation of LGLs (424,425). In fact, expansion of NK cells and mature NK cells is associated with better control of CML after dasatinib treatment is interrupted (426). In CML participants maintaining TFR, TKI treatment resulted in the maintenance and proliferation of CD3-CD56+CD16+ NK cells (implicated in the ADCC response) and mature NK cells, with enhanced cytotoxic activity and the capacity to interfere with HIV-1 integration (416). Thus, these observations support the crucial role of these cytotoxic cell populations in maintaining the antileukemic response during TFR and suggests that they also have antiviral potential against HIV-1 infection.

As part of Study 1 of this thesis, we investigated if one year of treatment with ponatinib in CML participants induces resistance to HIV-1 in CD4+ T cells, assessing the maintenance of resistance effects during TFR. In Study 2, we analyzed the reservoir size, and characterized it by IPDA, in two PWH with CML who were on long-term treatment with dasatinib. In Study 3, we measured the effects of both dasatinib and ponatinib on the cellular metabolism of CD4+ and CD8+ T cells and NK cells, which is crucial in both HIV-1 infection and the development of the antiviral immune response.

HYPOTHESIS AND OBJECTIVES

Hypothesis

Although many strategies have tried to reduce or eradicate the reservoir size of HIV-1, none has been shown to be sufficiently effective to achieve a functional cure or the eradication of the infection. Furthermore, ART alone fails to completely eliminate the virus from the body and, when treatment is discontinued, HIV-1 viremia rebounds. Chronic HIV-1 infection leads to non-infectious complications and comorbidities, which makes it necessary to search for new strategies that significantly reduce HIV-1 reservoirs.

We hypothesize that TKIs such as dasatinib and ponatinib have important off-target effects that may impact the maintenance of viral reservoirs and interfere with HIV-1 infection. Consequently, transient treatment with TKIs in PWH may enhance the resistance of CD4+ T cells against HIV-1 infection and induce the proliferation of cytotoxic populations that may control the maintenance of HIV-1-infected cells.

Objectives

Our main objective was to investigate if transient treatment with TKIs dasatinib and ponatinib can reduce the HIV-1 reservoir and enhance antiviral immune responses in PWH, by promoting resistance of CD4+ T cells to HIV-1 infection and inducing the proliferation of cytotoxic immune cell populations.

Our specific objectives were:

1. To evaluate if one year of treatment with ponatinib may induce sustained resistance against HIV-1 infection and to assess its role in enhancing cytotoxic immunity against viral infections and cancer.
2. To examine the effects of long-term treatment with dasatinib on HIV-1 infection in PWH with CML. This objective is further divided into:
    a. To measure the HIV-1 reservoir size and its composition.
    b. To assess the competence of the HIV-1 provirus for reactivation.
3. To determine the impact of TKI treatment on mitochondrial and glycolytic metabolism of CD4+ and CD8+ T cells, and NK cells.

MATERIAL AND METHODS

1. Study cohorts

1.1. Study I

This was a substudy of the multicenter, open-label, single-arm, Phase II exploratory trial NCT04043676, which enrolled individuals with CML that were Philadelphia chromosome-positive (n=11). The objective of the clinical trial was to assess the ability of ponatinib to enhance the antileukemic response that could maintain CML remission without relapsing following the discontinuation of TKI treatment, in a cohort of individuals that were on imatinib treatment. All participants gave informed written consent in compliance with the principles of the Helsinki Declaration. The study protocol received review and approval from the Ethical Committee for Clinical Research with Medicines at Hospital Universitario La Princesa (Madrid, Spain) (protocol number 17/18), as well as from the ethical committees of the other participant hospitals.

The primary endpoint of the substudy was to evaluate the potential of ponatinib to induce the generation of cytotoxic cell populations that are implicated in maintaining the antileukemic response after TKI treatment interruption and that could have both anticancer and antiviral activity. Inclusion criteria were as follows: to be over 18 years old, not pregnant, to have received treatment with 400mg of imatinib for at least 4 years, to demonstrate DMR4 (>4 log reduction in BCR::ABL1; corresponding to a 99,99% reduction compared to baseline levels established at diagnosis) for at least the last 4 months, and to provide written informed consent. Recruitment of the selected participants was conducted at the Hospital Universitario Ramón y Cajal (Madrid, Spain), Hospital Regional de Málaga (Málaga, Spain) and Hospital Universitario Virgen de la Salud (Toledo, Spain). Upon enrollment, participants discontinued imatinib and initiated the intensification treatment with 15mg of ponatinib daily for 12 months, after which ponatinib treatment was stopped. Follow-up continued for an additional of 12 months, during which participants received no TKI treatment, as CML relapse typically occurs within the first 6 to 8 months after treatment cessation (426–429).

PBMCs were obtained from blood samples using Ficoll-Hypaque gradient centrifugation (Sigma Aldrich, St. Louis, MO). Plasma was also isolated and stored at -80ºC. The isolated PBMCs were then cryopreserved in liquid nitrogen. The initial sample was collected prior to initiating ponatinib treatment and before imatinib treatment discontinuation. The second sample was taken after 12 months of ponatinib treatment, coinciding with the discontinuation of ponatinib. Subsequent samples were collected at approximately 3, 6 and

12 months following the cessation of ponatinib. The sample collection is represented in Figure 16.

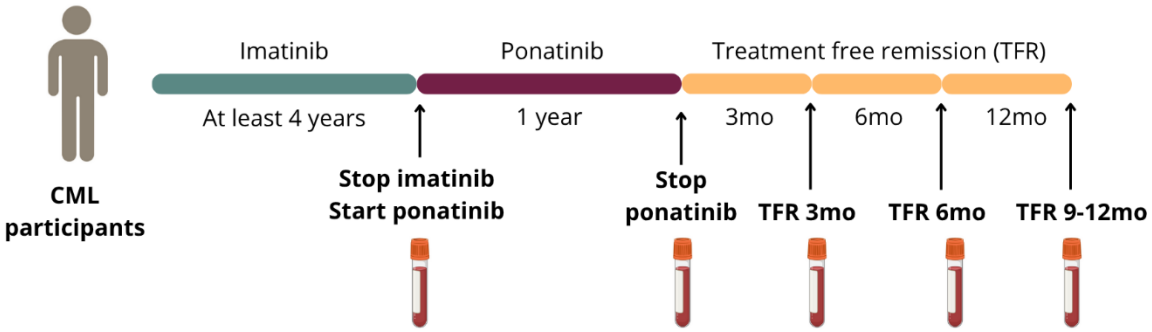


Figure 16. Representation of sample collection for CML participants included in Study I.

1.2. Study II

This study involved blood sample collection from two individuals with chronic HIV-1 infection who were subsequently diagnosed with CML, starting dasatinib treatment. First participant (participant #004) was recruited at ICH Study Center (Hamburg, Germany) whereas second participant (participant #006) was enrolled at University Hospital of Cologne (Cologne, Germany). Blood samples were obtained at different time points for both participants.

A control group of 20 PWH was also recruited at Hospital Clinico San Carlos (Madrid, Spain). These controls were divided into two subgroups: 10 matched to participant #004 and 10 matched to participant #006 based on age at sampling, age at HIV-1 diagnosis, duration of HIV-1 infection, and time on ART.

All subjects gave written informant consent in accordance with the declaration of Helsinki. The study was approved by the Ethic Committee of Instituto de Salud Carlos III (IRB IORG0006384) as wells as by all Ethics Committees of all participating hospitals.

PBMCs were isolated using Ficoll-Hypaque gradient centrifugation (Sigma Aldrich, St. Louis, MO) and cryopreserved for subsequent analysis. Plasma was also isolated and stored at -80ºC.

1.3. Study III

Blood samples from 8 healthy donors were obtained from the French blood bank (Etablissement Français du Sang) and processed at Institut Pasteur (Paris, France). PBMCs were isolated using Ficoll-Hypaque gradient centrifugation (Sigma Aldrich, St. Louis, MO) and cryopreserved for further analysis.

1.4. Flow cytometry antibodies

The antibodies used for all flow cytometry experiments are included in table 1.

Marker	Fluorochrome	Reference	Vendor
CD3	Bv510	564713	Becton Dickinson
CD8	APC-H7	560273	Becton Dickinson
CD45RA	PE-Cy7	561216	Becton Dickinson
CCR7	Bv421	562555	Becton Dickinson
CD56	Bv605	562780	Becton Dickinson
TCRγδ	PE	331209	BioLegend
CD107a	PE-Cy7	561348	Becton Dickinson
*p24-gag	FITC	6604665	Beckman Coulter
*pSAMHD1	PE	875935	Cell Signaling

*p24-gag core antigen was stained using the KC57 clone.
*pSAMHD1 is determined by phosphorylation of SAMHD1 at T592.

Table 1. Flow cytometry antibodies used in Studies I and II.

1.5. Flow cytometry staining

Approximately, $5x10^5$-10^6 PBMCs were collected in 5mL polystyrene round-bottom tubes and washed with 2mL of phosphate-buffered saline (PBS). The antibody cocktail for surface marker staining was prepared in PBS1x supplemented with 2% fetal bovine serum (FBS) for Fc blocking and added to all tubes. Cells were incubated at 4ºC in the dark for 20-30 minutes and then washed with 1mL PBS1x. Subsequently, cells were fixed with 300mL paraformaldehyde (PFA) 1% for 20mins at 4ºC in the dark.

For intracellular staining, IntraPrep permeabilization reagent (Beckman Coulter, Marseille, France) was used instead of PFA, following the protocol according to the manufacturer: cells were fixed for 20 minutes in the dark at 4ºC using fixation buffer, and then washed with PBS1x. Cells were subsequently permeabilized with permeabilization buffer, and the intracellular antibody cocktail was added. The mixture incubated for 15-20 minutes at room

temperature in the dark. Cells were washed with PBS1x, fixed with PFA 1% for 20 minutes at 4ºC in the dark, and acquired using a flow cytometer.

1.6. HIV-1 infection of PBMCs

PBMCs were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 µg/mL streptomycin and 100IU/mL penicillin (Biowhittaker, Walkersville, MD). Cells were activated with 10 µg/mL of phytohemagglutinin (PHA) (Sigma-Aldrich) and 300 IU/mL of IL-2 (Chiron, Emeryville, CA) for 48 hours. Following activation, cells were subjected to *in vitro* infection with the HIV-1 strain NL4-3_wt (430) using spinoculation: cells were centrifuged at 600g for 30 minutes at 25ºC in the presence of the virus, and then washed twice with PBS1x. Infected cells were then cultured for 72 hours in round-bottom 96-well plates containing RPMI supplemented with 300 IU/mL of IL-2. Cells were collected and processed for intracellular flow cytometry staining using the following antibodies: CD3-Bv510, CD8-APCH7, CD45RA-PE-Cy7, CCR7-Bv421, pSAMHD1-PE and p24-gag-PE. CD4+ T cells were defined as CD3+CD8-, as HIV-1 infections induces the downregulation of the CD4 receptor (431). CD4+ T subpopulations were designated as follows: CD45RA+CCR7+ (T_N), CD45RA-CCR7+ (T_{CM}); CD45RA-CCR7- (T_{EM}); CD45RA+CCR7- (T_{EMRA}). Gating strategy is shown in supplementary Figure S1. Samples were acquired using a LSR Fortessa flow cytometer and data analyzed with FlowJo v.10.7.0 software.

1.7. Evaluation of the cytotoxic response against HIV-1 infected cells

TZM-bl luciferase-reporter target cells (obtained from human cervix; NIH AIDS Research and Reference Reagent Program, no. 8129) were infected with the HIV-1 strain NL4_3_wt for 48 hours and cultured in DMEM medium supplemented with 10% FCS, 2mM L-glutamine, 100ug/mL streptomycin and 100IU/mL penicillin (Biowhittaker, Walkersville, MD). After 48 hours, PBMCs were added to the TZM-bl monolayer at a 1:1 ratio and co-cultured for 1 hour. After co-culturing, TZM-bl cells were detached using trypsin-EDTA solution (Sigma Aldrich-Merck, Darmstadt, Germany). Caspase-3 activity, used as a marker of cellular apoptosis, was measured in TZM-bl cells using the Caspase-Glo 3/7 analysis system (Promega), as previously described (432). Direct cellular cytotoxicity (DCC) fold-change was determined using the following formula:

$$\frac{\text{Average caspase} - 3 \text{ activity (RLUs) in HIV} - \text{infected TZM cocultured with PBMCs obtained after ponatinib treatment and during TFR}}{\text{Average caspase} - 3 \text{ activity (RLUs) in HIV} - \text{infected TZM cells cocultured with PBMCs obtained before ponatinib treatment}}$$

PBMCs from study participants were also collected after the 1-hour co-culturing with infected TZM-bl cells. For determination of cytotoxic immune cells (CD8+ T cells, NK cells and γδ T cells) and their degranulation activity, we used the following antibodies for flow cytometry staining of surface markers: CD3-Bv510, CD8-APCH7, CD56-Bv605, TCRγδ-PE, CD107a-PE-Cy7. Gating strategy is shown in supplementary Figure S2. Data was acquired using the Cytek Aurora spectral flow cytometer and SpectroFlo software. Data and gating were analyzed using FlowJo v10.7.1.

1.8. Determination of cytotoxic immune response against autologous HIV-1 infected CD4+ T cells

CD4+ T cells were isolated from PBMCs of participants using the CD4+ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), following manufacturer´s instructions: PBMCs were washed with PBS1x and resuspended in isolation buffer (50mL PBS1x + 200mL EDTA + 250mL FBS) and then incubated with anti-CD4 magnetic beads for 20 minutes at 4ºC. After washing with PBS1x, PBMCs were subjected to a magnetic field to deplete non CD4-expressing cells. Cells that remained bound to the magnetic beads were collected by elution once the magnetic field was removed. CD4 negative cells were were also collected separately.

Isolated CD4+ T cells were activated with 10 ug/mL of PHA (Sigma-Aldrich) and 300 units/mL of IL-2 (Chiron, Emeryville, CA) for 48 hours and then infected *in vitro* with NL4-3_wt for 72 hours in the presence or absence of autologous cytotoxic cells (CD8+ T, NK and γδ T cells) at 1:1 ratio, and cultured in RPMI medium with 300 units/mL of IL-2. Cytotoxic cells were not purified in previous steps to reduce cells loss. Cells then were collected, and intracytoplasmic production of p24-gag in CD4+ T cells was assessed using flow cytometry with the next antibodies for intracellular staining: CD3-Bv510, CD8-APCH7, CD45RA-PE-Cy7, CCR7-Bv421, pSAMHD1-PE and p24-gag-FITC. CD4+ T subpopulations and gating strategy were designated as in *1.6. HIV-1 infection of PBMCs*. Samples were acquired on an LSR Fortessa flow cytometer and data analyzed using FlowJo v.10.7.0 software.

1.9. *Ex vivo* reactivation of HIV-1 provirus from CD4+ T cells of PWH

CD4+ T cells were isolated from PBMCs and cultured in RPMI 1640 medium enriched with 10% FCS, 2 mM L-glutamine, 100 µg/mL streptomycin and 100IU/mL penicillin (Biowhittaker, Walkersville, MD). Cells were cultured with Dynabeads Human T Activator CD3/CD8 (ThermoFisher Scientific) and 300 IU/mL of IL-2 (Chiron, Emeryville, USA) for 5 days for TCR-mediated activation of the CD4+ T cells. Provirus reactivation was then

induced using 25 ng/mL of phorbol 12-myristate 13-acetate (PMA) and 1.5 µg/mL inomycin for 16-18 hours, and brefeldin A (BD GolgiPlug, BD Biosciences) was also included in the culture medium to prevent the release of the viral particles from the CD4+ T cells. PMA and ionomycin robustly activate CD4+ T cells by stimulating several transcription factors, such as PKCθ or NF-κB, that lead to the transcription of the HIV-1 provirus. Cells were then collected for intracellular staining for flow cytometry, following the same procedure as in *1.6. HIV-1 infection of PBMCs*. Gating strategy is shown in supplementary Figure S3.

1.10. Quantification of integrated and total HIV-1 DNA

PBMCs from PWH were used for DNA extraction with the QIAamp DNA Blood Mini Kit (Qiagen Iberia, Madrid, Spain). DNA was quantified with Nanodrop 2000C (Thermo Fisher Scientific, Waltham, MA). Integrated provirus DNA was quantified using TaqMan probes conjugated with the FAM fluorochrome through a nested Alu-LTR PCR, as previously described (156,165,433) on a ddPCR system (BioRad laboratories). The list of primers and probes is included in table 2. First, oligonucleotides for the Alu conserved sequence and the HIV-1 LTR were used for a conventional PCR under the following conditions: 95ºC for 8 minutes, 12 cycles of 95ºC for 1 minute, 60ºC for 1 minute, 72ºC for 10 minutes and 72ºC for 15 minutes. This was followed by a quantitative PCR (qPCR) using TaqMan probes with FAM/ZEN/Iowa Black quenchers (Integrated DNA Technologies, IDT) and TaqMan Master Mix (Life Technologies). In this qPCR, the HIV-1 LTR and Lambda T primers were used for nested amplification of products of the first PCR. qPCR cycling was as follows: 95ºC 8 minutes, 50 cycles of 95ºC 10 seconds, 60ºC for 10 seconds and 72ºC for 9 seconds).

Primers for CCR5 housekeeping gene, conjugated with the VIC fluorochrome were used as control for quantification of total DNA in the qPCR. In the same qPCR, total HIV-1 DNA was determined using previously described primers (434): U5 region of the HIV-1 LTR (probe MH 531) and 5´end of the gag gene (probe MH 532). The Two-LTR circles (2-LTR) were amplified with primers targeting both LTRs (HIV F and HIV R1). For determination of copy numbers of both integrated and total HIV-1 DNA, the DNA of the 8E5 cell line was used as reference. Integrated HIV-1 DNA is expressed as copy numbers per 10^6 PBMCs.

Primer/Probe	Sequence	Target
L-M667 (HIV-1-LTR primer)	ATGCCACGTAAGCGAAACTCTGGCTAACTAGGGAACCCACTG	Integrated HIV-1 DNA (first PCR)
Alu 1	TCCCAGCTACTGGGGAGGCTGAGG	Alu sequence
Alu 2	GCCTCCCAAAGTGCTGGGATTACAG	Alu sequence
Lambda T	ATGCCACGTAAGCGAAACT	Integrated HIV-1 DNA (qPCR)
AA55M(LTR primer)	GCTAGAGATTTTCCACACTGACTAA	Integrated HIV-1 DNA (qPCR)
MH-603	ACACTACTTGAAGCACTCAAGGCAAGCTTT	LTR
CCR5	CAAAAAGAAGGTCTTCATTACACC	CCR5 human gene
MH 531	TGTGTGCCCGTCTGTTGTGT	U5 region of LTR
MH 532	GAGTCCTGCGTCGAGAGAGC	5´end of gag

Table 2. List of primers used for quantification of integrated and total HIV-1 DNA.

1.11. Intact proviral DNA measurements

The IPDA assay was performed following the protocol described in (162,435). A summary of the primers and probes used in the protocol is included in Table 3. The procedure involved two ddPCR steps: first, proviral DNA was quantified and classified as intact and defective by targeting the ψ (psi) region (frequent site of small deletions) and the env region of the HIV-1 genome. For the ψ region, FAM-labelled probes were used, whereas VIC probes were used for the env region. Droplets positive for FAM were scored as 3´-defective, droplets positive for VIC were classified as 5´-defective, and double-positive droplets were scored as intact proviruses.

In the second step, the input cell number was determined by quantifying the cellular *RPP30* gene (located at chromosome 10:90,880,081 on GRCh38), which was also used to calculate the DNA shearing index (DSI), which allows the determination of intra-amplicon

shearing (that usually occurs during the DNA isolation procedure). DSI is used for shearing correction, as DNA shearing typically reduces intact provirus counts. For the quantification of proviral DNA, up to 500ng of genomic DNA was analyzed per reaction, with up to four replicates performed. For input cell quantification, up to 50 ng of genomic DNA was used. DNA from cryopreserved PBMCs of HIV-1 negative donors was used as a template control, while water was used as no-template control. DNA of the 8E5 cell line was used as an HIV-1 positive control. ddPCR was performed on the BioRad QX200 AutoDG Digital Droplet PCR system using the ddPCR Supermix for Probes (no dUTPs) (BioRad laboratories, Madrid, Spain). PCR conditions were as follows: 95ºC 10 minutes, 45 cycles of 94ºC for 30 seconds and 59ºC for 1 minute, 98ºC for10 minutes. Droplets were assessed using the QX200 droplet reader (Bio-Rad). Data was analyzed using QX Manager Software 2.0 (Bio-Rad). Samples that showed a DSI<0.50 were included for analysis. IPDA results of intact and defective proviruses are reported as proviral counts/10^6 PBMCs. Representations of graphical analysis are shown in supplementary Figures S4 and S5.

Primer/Probe	Sequence	Target
Ψ Forward	CAGGACTCGGCTTGCTGAAG	HIV-1 Ψ region
Ψ Reverse	GCACCCATCTCTCTCCTTCTAGC	HIV-1 Ψ region
Ψ Probe	TTTTGGCGTACTCACCAGT	HIV-1 Ψ region
env forward	AGTGGTGCAGAGAGAAAAAAGAGC	HIV-1 env region
env reverse	GTCTGGCCTGTACCGTCAGC	HIV-1 env region
env intact probe	CCTTGGGTTCTTGGGA	HIV-1 env region
env hypermut	CCTTAGGTTCTTAGGAGC	HIV-1 env region (APOBEC-3G gene)
RPP30 probe	CTGACCTGAAGGCTCT	Cellular RPP30 gene
RPP30 Forward	GATTTGGACCTGCGAGCG	Cellular RPP30 gene
RPP30 Reverse	GCGGCTGTCTCCACAAGT	Cellular RPP30 gene

Table 3. List of primers and probes used in the ddPCR for the IPDA.

1.12. CMV serology

The levels of CMV-specific IgG antibodies were measured in the plasma samples of participants in collaboration with the Serology Service of the Instituto de Salud Carlos III. The commercial kit Liaison CMV IgG (Diasorin, Italy) was used following the manufacturer´s instructions. Values above 230 IU/mL were considered positive. Results are expressed as positive (Pos) or negative (Neg).

1.13. Cellular metabolism assay

PBMCs from healthy donors were used to isolate CD4+ and CD8+ T cells with EasySep enrichment kits (StemCell Technologies), and NK cells with NK cell isolation kit (Miltenyi Biotec). CD4+ and CD8+ T cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 µg/mL streptomycin and 100 IU/mL penicillin (Biowhittaker, Walkersville, MD), in the presence of 200 IU/mL IL-2 (Miltenyi). Depending on the condition, cells were cultured for 72 hours in the presence/absence of 75 nM dasatinib, 150 nM ponatinib and/or PHA 2ug/mL, generating six conditions for CD4+ and CD8+ T cells:

- Basal (RPMI + IL-2)
- Dasatinib: dasatinib 75 nM
- Ponatinib: ponatinib 150 nM
- PHA-activated: PHA 2 µg/mL
- Dasatinib + PHA: dasatinib 75 nM + PHA 2µg/mL
- Ponatinib + PHA: ponatinib 150 nM + PHA 2µg/mL

Purified NK cells were cultured for 16-18 hours in RPMI 1640 medium in the presence or absence of dasatinib and/or 100 ng/mL IL-15, 50 ng/mL IL-12 and 50 ng/mL IL-18 (Miltenyi Biotec).

After culture, CD4+T, CD8+ T and NK cells were washed and resuspended in Seahorse XF DMEM medium (pH 7.4, Agilent Technologies) supplemented with 10 mM glucose, 2 mM glutamine and 1 mM pyruvate. XF96 V3 PS plates (Seahorse Biosciences) were coated with 0.5 mg/mL Cell-Tak (Corning). CD4+ and CD8+ T cells were plated in 5 to 6 replicates at a concentration of 250,000 cells/well, while NK cells were plated at 200,000 cells/well, then incubated for a minimum of 45 minutes in a CO_2-free incubator at 37ºC. Oxygen consumption rate (OCR), extracellular acidification rate (ECAR) and proton efflux rate (PER) were measured using a Seahorse XFe96 Extracellular Flux Analyzer (Agilent Technologies).

The Seahorse XF Cell Mito Stress Test (Agilent Technologies) was performed on all cell types following manufacturer´s instructions for drug injections: i) Seahorse XF DMEM medium; ii) 2.5 µM oligomycin; iii) 0,9 µM carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) and iv) 1 µM rotenone/antimycin A. The Seahorse XF Glycolytic Rate Assay Kit was used for CD4+ and CD8+ T cells following manufacturer´s indications: i) Seahorse XF DMEM medium; ii) 2.65 µM oligomycin; iii) 100 mM 2-Deoxyglucose (2-DG). Functions of reagents from the Mito Stress test Kit and the Glycolysis Stress Test kit are detailed in Supplementary Table 1. Data was analyzed using Agilent Seahorse Analytics.

1.14. Statistical analysis

Statistical analysis was performed using GraphPad Prism v10.2.1 (GraphPad Software Inc., San Diego, CA) and Stata 17 (StataCorp, College Station, TX). Data normality was assessed using the Shapiro-Wilk normality test. Comparisons between group medians were calculated using the Paired t-test when data followed a normal distribution, while the Wilcoxon matched pairs signed rank test was used when data normality was not assumed. One-way ANOVA was used to calculate statistical significance among groups when data followed a normal distribution. P values (p) < 0.05 were considered statistically significant. For correlations between intracellular levels of p24-gag and pSAMHD1, Pearson´s or nonparametric Spearman´s rank correlation coefficients (r) were determined.

RESULTS

Study I

Sustained antiviral response against *in vitro* HIV-1 infection in peripheral blood mononuclear cells from people with chronic myeolid leukemia treated with ponatinib

1.1. Study participants

Eleven individuals with CML from the NCT04043676 phase II clinical trial were recruited for this substudy with the objective of determining if one-year treatment with ponatinib is able to induce the development of cytotoxic cell populations with anticancer and antiviral activity. Additionally, we studied if these cytotoxic populations could maintain their cytotoxic activity after treatment interruption. Main sociodemographic and clinical data are detailed in Table 4. Most participants were male (82%) with a median age at sampling of 50 years old (Interquartile range (IQR) 40-57) and median age at CML diagnosis of 39 years old (IQR 22-43). The median time since CML diagnosis was 11 years (IQR 7-19), while the median duration of imatinib treatment was 10.2 years (IQR 4.7-16.3). 64% of participants showed a low Sokal risk score, which is a parameter used in clinical practice for the prediction of patient outcomes and treatment strategies (436).

Ponatinib treatment did not produce an immunosuppressive effect as most participants did not present opportunistic infections or CMV reactivation during ponatinib treatment and TFR, despite most of them being seropositive for CMV (91%). None of the participants experienced a relapsed of CML, and all maintained DMR after a medium follow-up period of 2.9 years (IQR 2.24-3.51).

ID of participants	Gender (M/F)	Age	Age at CML diagnosis	Sokal risk score before ponatinib	Time from CML diagnosis	Time on treatment with imatinib	IgG against CMV
02_02	M	62	40	UD	21	18.0	Pos
02_03	M	57	51	UD	7	4.2	Pos
04_01	M	41	22	Low	20	16.3	Pos
04_02	F	53	43	Intermediate	9	4.7	Pos
04_03	M	48	30	Low	19	15.2	Pos
04_04	M	39	21	Low	18	14.5	Pos
04_06	M	29	19	Low	10	6.5	Neg
04_07	F	40	32	Low	7	6.5	Pos
04_08	M	56	51	Low	5	4.7	Pos
04_09	M	50	39	Low	11	10.2	Pos
10_01	M	59	43	UD	16	16.4	Pos

CML, Chronic Myeloid Leukemia; F, Female; M, male; Neg, negative; Pos, Positive; UD, Undetermined.

Table 4. Sociodemographic and clinical data of the recruited participants.

1.2. Treatment with ponatinib induced resistance to HV-1 infection *in vitro*

As treatment with ponatinib may induce lymphopenia due to its potent cytostatic effect on T cells, we first assessed if levels of CD4+ T cells in blood were modified by this treatment. The median percentage of CD4+ T cells was 33.3% (IQR 18.7-44.1) during imatinib treatment. After the 1-year period of treatment with ponatinib, the median percentage of

CD4+ T cells was 32.0% (IQR 15.6-45.1). During the 1-year follow-up after ponatinib treatment was discontinued, the median percentage of CD4+ T cells was 32.8% (IQR 16.9-46.7). Thus, ponatinib treatment did not significantly reduce the levels of CD4+ T cells.

To estimate the capacity of ponatinib to induce resistance against HIV-1 infection in CD4+ T cells, PBMCs obtained from different time points were infected *in vitro* with the NL4-3_wt strain for 72 hours after cellular activation, and intracellular p24-gag levels were measured in CD4+ T cells by flow cytometry. The gating strategy is included in supplementary Figure S1. The percentage of p24-gag+ CD4+ T cells was 3.7-fold (p=0.0180) lower after 12 months of treatment with ponatinib, compared to previous treatment with imatinib (Figure 17A). p24-gag CD4+ T cells levels remained significantly reduced at 3 and 12 months after discontinuing ponatinib (3.7-fold, p=0.0092 and 4.7-fold, p=0.0006, respectively). Analysis of CD4+ memory subpopulations revealed similar results in T_N and T_{CM}, as percentage of p24-gag+ cells was significantly lower after 1-year treatment with ponatinib (-3.2-fold, p=0.0098 in T_N and -4.5-fold, p=0.0098 in T_{CM}) (Figure 17B). The percentage of p24-gag CD4+ T cells was significantly lower 12 months after treatment interruption in all CD4+ T subpopulations: T_N (-2.8-fold, p=0.0420), T_{CM} (-4.5-fold, p=0.0186), T_{EM} (-2.7-fold, p=0.0186) and T_{EMRA} (-3.0-fold, p=0.0313). Interestingly, these findings suggest that ponatinib induced a resistant effect in all CD4+ T subpopulations, and this effect was maintained after treatment discontinuation.

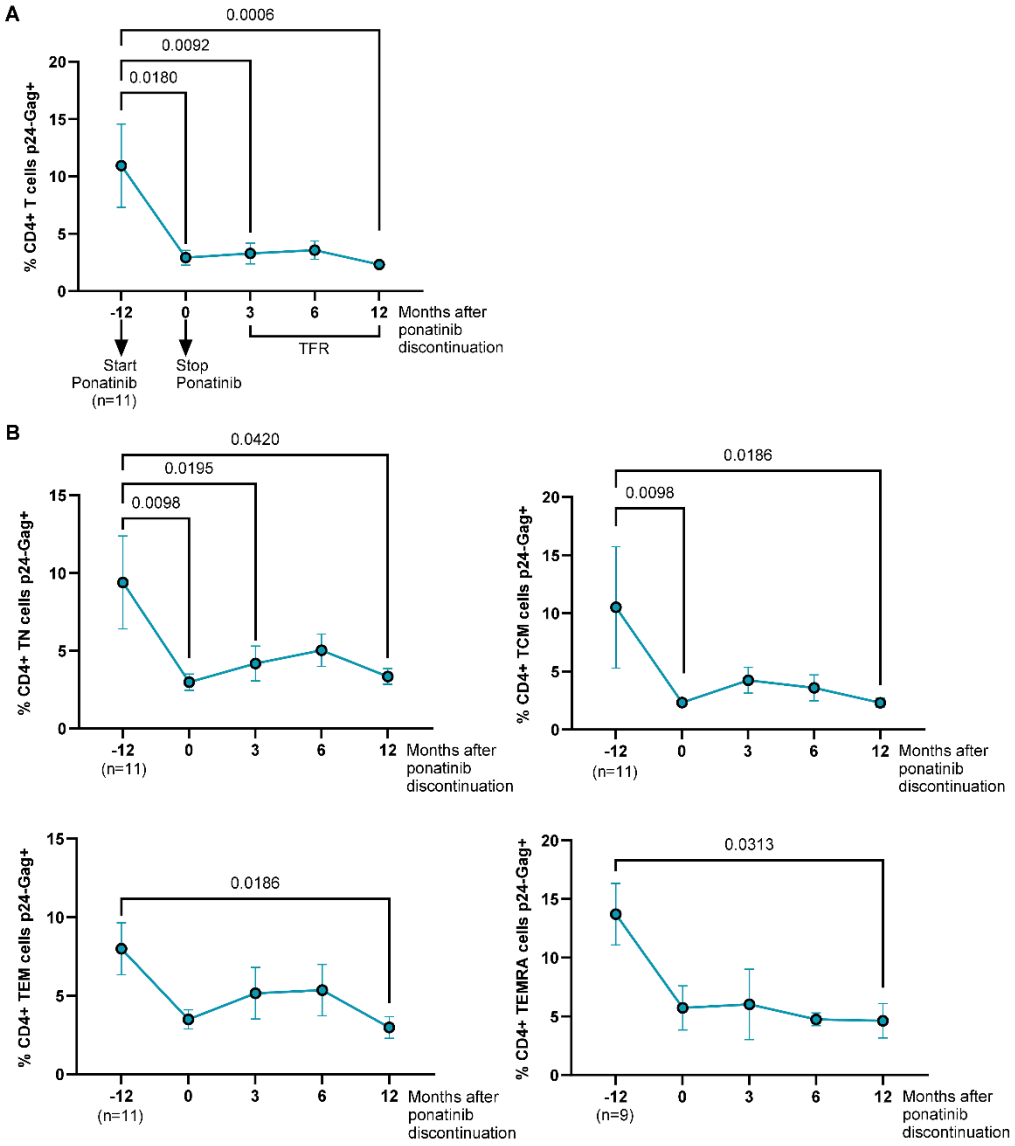


Figure 17. Levels of p24-gag+ CD4+ T cells during imatinib treatment, after 1-year ponatinib and treatment free remission (TFR). After 72 hours of infection *in vitro* of PBMCs, intracellular levels of p24-gag in total CD4+ T cells (A) and in CD4+ memory subpopulations (B) were determined. Each dot represents the median of all samples and vertical lines are the standard error of the mean (SEM). Wilcoxon matched-pairs signed rank test and paired t-test were used to assess the statistical significance.

1.3. Ponatinib reduced the phosphorylation of SAMHD1 in CD4+ T cells.

To further explore the observed resistance effect generated by ponatinib, we determined if the antiviral immune factor SAMHD1 remained in its active form. The phosphorylation at T592 of SAMHD1 in CD4+ T cells from all time points of the study after 72 hours of infection

with NL4-3_wt strain was analyzed. The percentage of pSAMHD1+ CD4+ T cells was 5.7-fold lower (p=0.0050) after 12 months of treatment with ponatinib compared to imatinib (Figure 18A). This analysis was also extended to CD4+ subpopulations, in which a significant reduction in pSAMHD1+ CD4+ T cells was observed in T_{CM} and T_{EMRA} cells (2.8-fold, p=0.0397 and 3.3-fold, p=0.0372) after ponatinib treatment compared to previous imatinib treatment (Figure 18B). No significant reductions were found during TFR. These results indicate that the levels of the inactive form, pSAMHD1, are impacted after one-year of ponatinib treatment in total CD4+ T cells. However, this effect was not observed in all CD4+ T subsets and was not maintained after ponatinib treatment discontinuation.

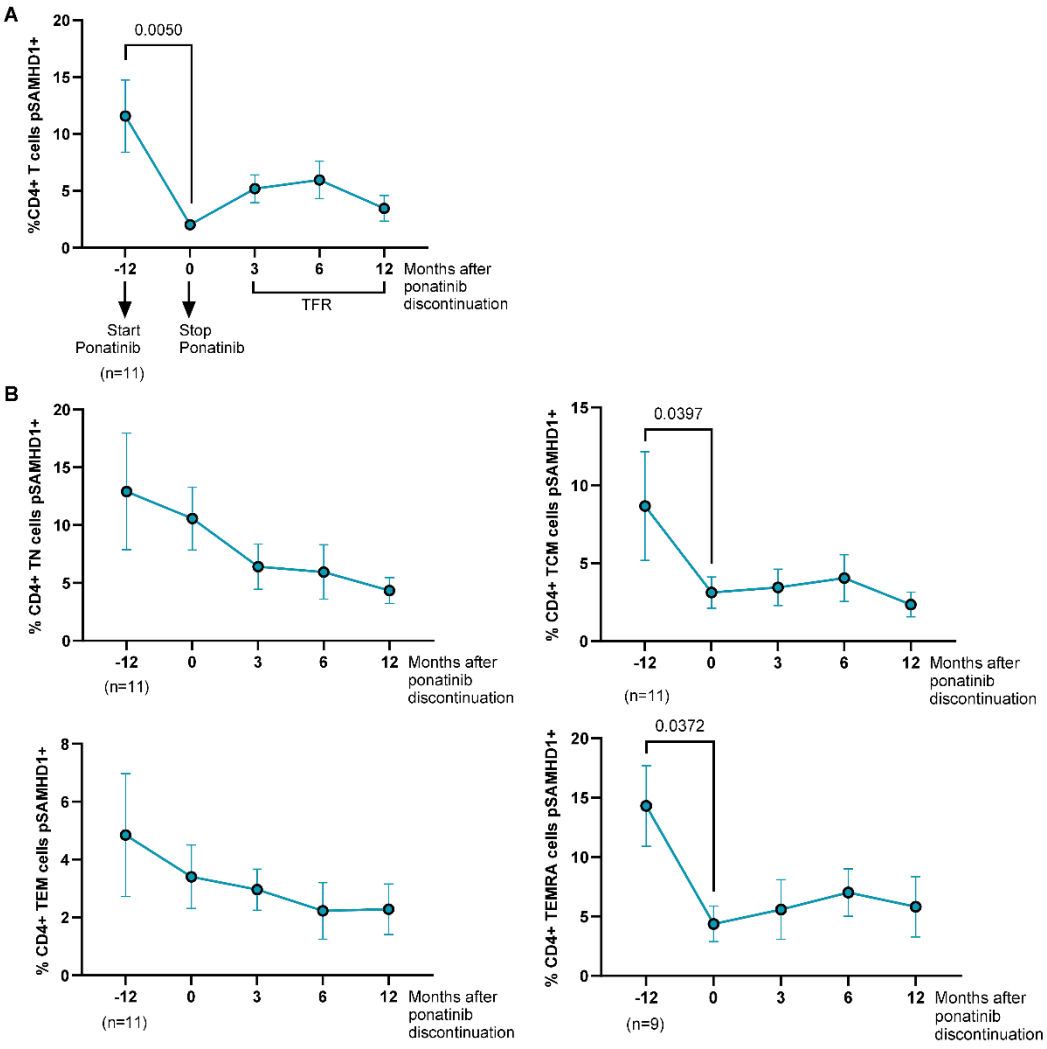


Figure 18. Phosphorylation of SAMHD1 at T592 before and after one-year treatment with ponatinib and during TFR. After 48 hours of activation with PHA and 72 hours of infection with NL4-3_wt, the intracellular levels of pSAMHD1 in total CD4+ T cells (A) and in CD4+ memory T cell subpopulations (B) were analyzed. Each dot corresponds to the mean

value of all samples and vertical lines represent the SEM. Wilcoxon matched-pairs signed rank test and paired t-test were used for assessment of statistical significance.

1.4. Cytotoxic activity is enhanced against HIV-1 infected cells after ponatinib treatment

Since TKI treatment may increase the development of cytotoxic cell populations, we explored the potential of ponatinib to induce such an effect after 12 months of treatment. PBMCs obtained from the 11 participants at different time points were cocultured with HIV-1-infected TZM-bl cells for 1 hour to determine their DCC. Higher DCC was observed in PBMCs after 12 months of ponatinib treatment (2.5-fold, p=0.0049) compared to previous treatment with imatinib (Figure 19). This enhanced DCC was also detected in PBMCs obtained after 12 months of TFR (2.6-fold, p=0.0098).

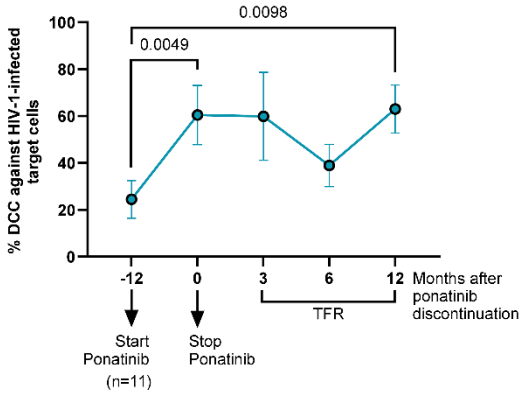


Figure 19. Analysis of DCC in PBMCs after coculture with HIV-1 infected cells. Caspase-3 activity in HIV-1-infected TZM-bl cells was analyzed as an apoptosis indicator after coculture with PBMCs. Each dot corresponds to the mean value of all samples and vertical lines represent the SEM. Wilcoxon matched-pairs signed rank test and paired t-test were used for assessment of statistical significance.

1.5. Correlation between p-24-gag, pSAMHD1 and DCC

Given the increased DCC in PBMCs, we next asked whether the antiviral factor SAMHD1 or the increased DCC were the factors implicated in the resistance of CD4+ against HIV-1 *in vitro* infection. Only a positive correlation was found between the intracellular levels of pSAMHD1 and p24-gag (r = 0.7448, p=0.0135) in CD4+ T cells obtained after 3 months of ponatinib treatment interruption (Figure 20A). Between DCC and p24-gag levels, we found a significative negative correlation after 12 months of ponatinib treatment (r = -0.6858,

p=0.0286), and after 3 (r = -0.7043, p=0.0266) and 6 months (r = -0.7800, p=0.0046) of ponatinib treatment discontinuation (Figure 20B). These results suggest a significant role of DCC in interfering with HIV-1 infection *in vitro* in CD4+ T cells obtained from these participants.

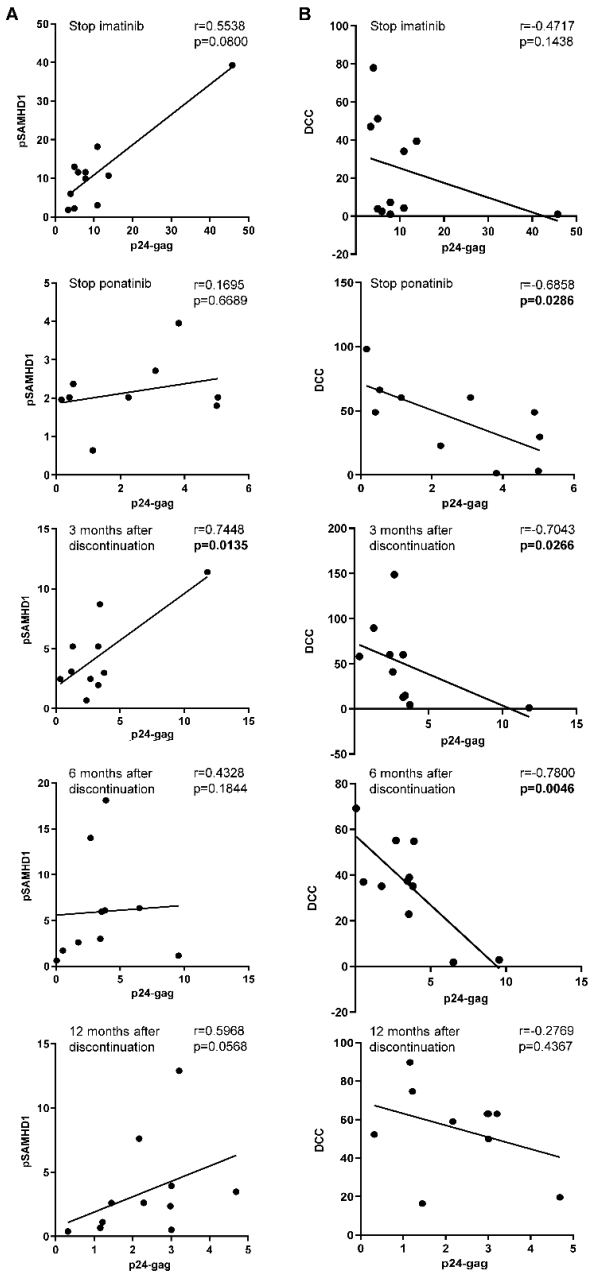


Figure 20. Analysis of correlation of p24-gag levels with pSAMHD1 or DCC. The association between the levels of p24-gag and pSAMHD1 (A) and DCC (B) was studied in total CD4+ T cells in all timepoints of the study, using Pearson´s or nonparametric Spearman´s rank correlation coefficients. Each dot corresponds to one sample. The straight line in the graph represents a simple linear regression.

1.6. Treatment with ponatinib modifies the levels of cytotoxic cells

Cytotoxic cell populations were studied in the PBMCs that were cocultured with HIV-1-infected TZM-bl cells to determine their role in inducing DCC. The gating strategy is shown in supplementary Figure S2. After 12 months on TFR, we observed 1.3-fold (p=0.0322) higher levels of NK cells (CD3-CD56+) compared to treatment with imatinib (Figure 21A, left graph). However, during ponatinib treatment and throughout TFR, the degranulation capacity, as assessed by CD107a expression, was not significantly altered (Figure 21A, right graph).

CD8-Tγδ+ cells (CD3+CD8-TCRγδ+) were increased after 12 months of treatment with ponatinib (2.0-fold, p=0.0020), and at 3 (2.1-fold, p=0.0195), 6 (2.3-fold, p=0.0273) and 12 (2.4-fold, p=0.0020) months after treatment interruption (Figure 21B, left graph). The expression of CD107a in these cells remained unchanged during ponatinib or during TFR (Figure 21B, right graph). Similarly, the levels of CD8+Tγδ+ cells (CD3+CD8+ TCRγδ+) and their CD107a expression remained unaltered during and after ponatinib treatment (Figure 21C).

Across all time points of the study, no significant differences were detected in the levels of CD8+ T cells (Figure 21D, left graph), although the expression of CD107a marker was higher in CD8+ T cells 12 months after ponatinib discontinuation compared to treatment with imatinib (1.7-fold, p=0.0469) (Figure 21C, right graph).

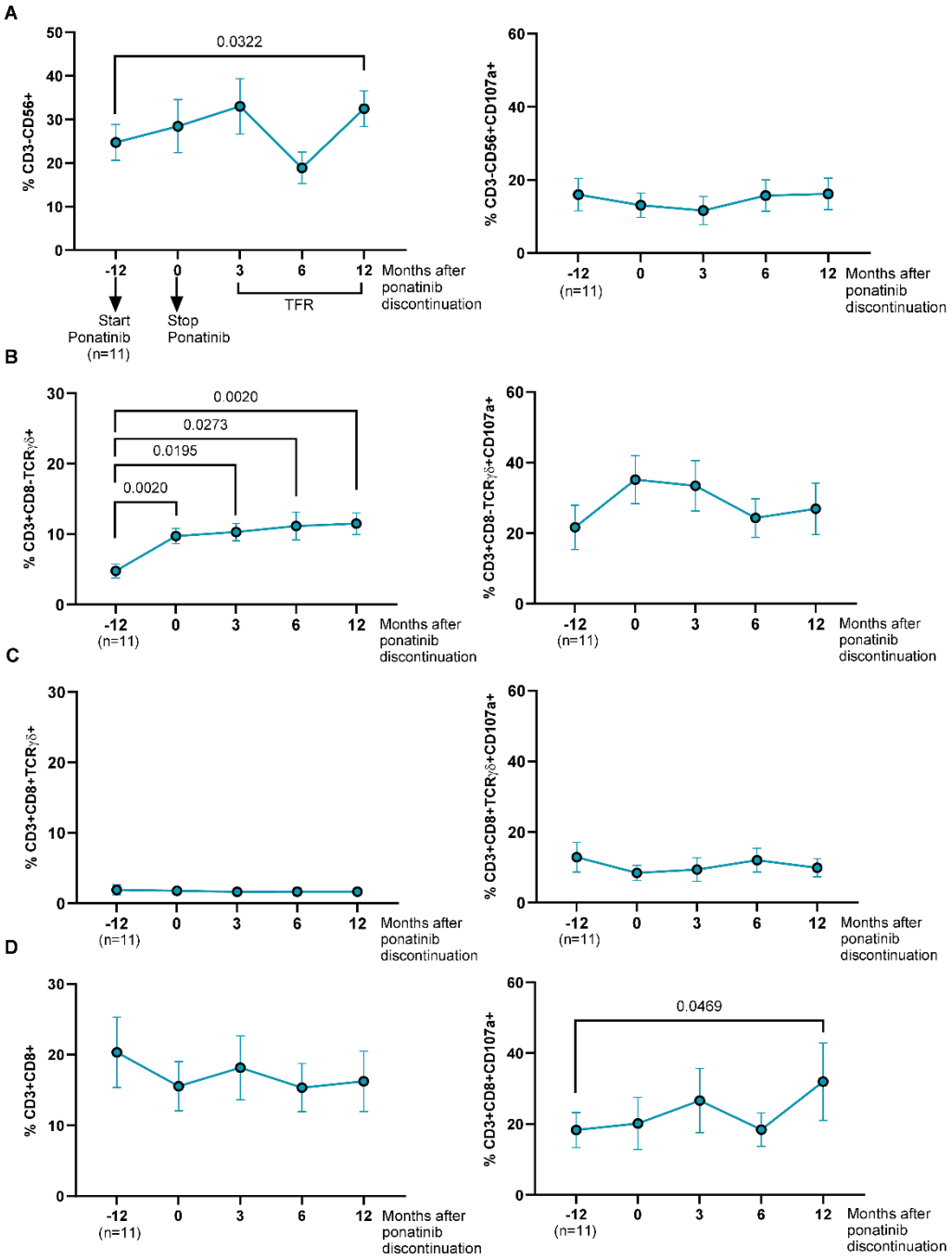


Figure 21. Levels and degranulation capacity of cytotoxic cells cocultured with HIV-1-infected TZM-bl cells. Using flow cytometry, we analyzed the levels of NK cells (CD3-CD56+) (A), CD8-Tγδ+ cells (CD3+ CD8-TCRγδ+) (B), CD8+Tγδ+ (CD3+CD8+TCRγδ+) (C) and CD8+ T cells (CD3+CD8+) (D), and their degranulation capacity assessed by CD107a expression (represented in the right graphs). Each dot represents the mean value of all samples, with vertical lines indicating the SEM. Statistical analysis was conducted using the Wilcoxon matched-pairs signed rank test and paired t-test.

1.7. DCC against autologous HIV-1-infected CD4+ T cells

To validate the impact of DCC in the resistance of CD4+ T cells against HIV-1 infection, we isolated CD4+ T cells obtained after 12 months of ponatinib discontinuation, activated them and then infected with the NL4-3_wt strain in the presence or absence of autologous cytotoxic cells (mainly, NK, Tγδ and CD8+ T cells) for 72 hours. In total CD4+ T cells, the expression of p24-gag was 1.6-fold lower (p=0.0469) when they were cocultured with the cytotoxic cells in comparison to CD4+ T cells infected alone (Figure 22A). Similar results were observed in all CD4+ T subpopulations that we analyzed: T_N (2.4-fold, p=0.0391); T_{CM} (1.7-fold, p=0.0352); T_{EM} (1.4-fold, p=0.0313) and T_{EMRA} (2.7-fold, p=0.0313) (Figure 22B). Thus, we confirmed the role of cytotoxic cells in interfering with HIV-1 infection *in vitro* after 12-month of ponatinib treatment. Furthermore, this effect is maintained after 12 months of treatment discontinuation.

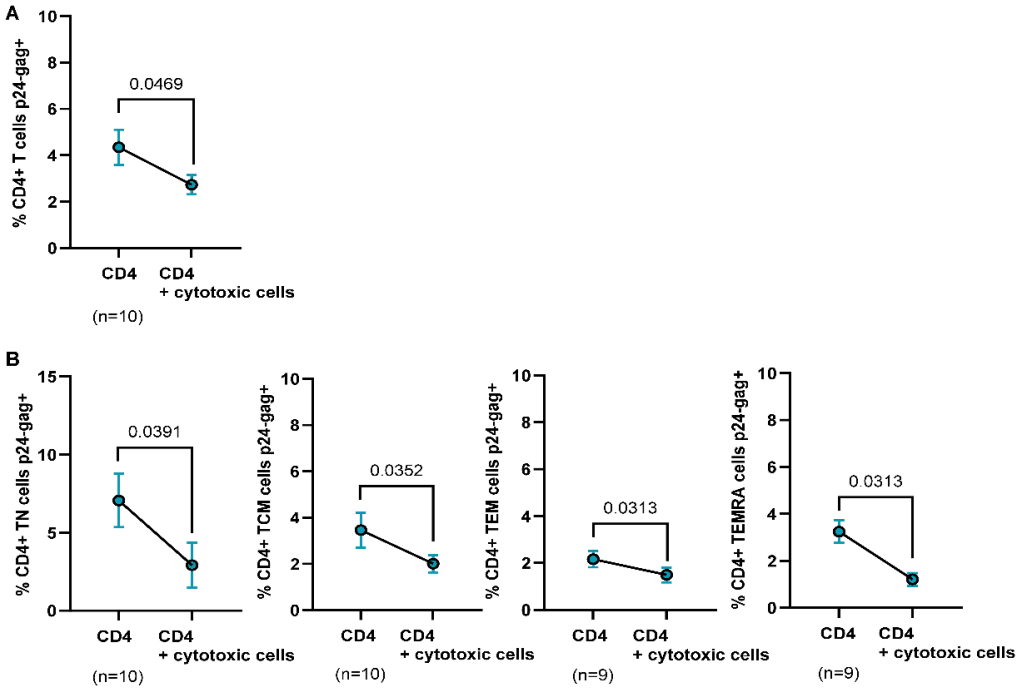


Figure 22. Analysis of p24-gag expression in CD4+ T cells obtained 12 months after ponatinib discontinuation and after coculture with autologous cytotoxic cells. Using the NL4-3_wt strain and in the presence or absence of autologous cytotoxic cells, we infected CD4+ T cells for 72 hours and measured p24-gag expression by flow cytometry in total CD4+ T cells (A) and CD4+ memory subpopulations (B). Each dot corresponds to the mean of all samples and vertical lines represent the SEM. Comparison between both conditions was performed using the paired t-test.

Study II

Analysis of reservoir size and its competency in people with HIV and chronic myeloid leukemia under long-term treatment with dasatinib

1.1. Study participants

Clinical and sociodemographic data of all participants in this study are shown in Table 5 and Supplementary table 2. A total of 22 participants were recruited: 2 PWH and CML that were on ART and TKI treatment, and 20 PWH that were only on ART and that were recruited as the control group.

The first participant with HIV and CML (participant #004) is a 51-year old male who was diagnosed with HIV-1 infection 17 years ago and with CML 13 years ago. He was recruited at ICH Study Center (Hamburg, Germany). He started ART regime of raltegravir, abacavir and lamivudine (RAL/ABC/3TC), and after 9 years he transitioned to bictegravir, emtricitabine and tenofovir (BIC/FTC/TAF). After CML diagnosis, he started and maintained TKI treatment with imatinib for 9 years, but a change to dasatinib was necessary due to fatigue and polyneuropathy. He continued the treatment with dasatinib for 3 years and 4 months, in which he maintained the DMR. Given the strong and sustained antileukemic response, he interrupted dasatinib in order to observe if this response could be preserved after treatment discontinuation. The DMR was maintained for 4.3 months, but he relapsed in CML and dasatinib was reintroduced, controlling CML. Due to fatigue and eczema, after 3.7 months he switched to asciminib and has remained on it since then. Median CD4 and CD8 counts were 1014 cells/μL (IQR 733-1236) and 639 cells/μL (IQR 442-824) respectively, with a median ratio of CD4/CD8 of 1.6 (IQR 1.1-1.7). A scheme of the particpant´s follow-up is included in Figure 23.

The second participant (participant #006) is a 63-year old male who was diagnosed with HIV-1 infection 23 years ago and with CML 5 years ago. He was enrolled at the University Hospital of Cologne (Cologne, Germany). Before CML diagnosis, he was on treatment with zidovudine, rilpivirine and tenofovir (AZT/RPV/TDF). As dasatinib was chosen as TKI treatment once CML was diagnosed, he changed his ART regimen to bictegravir, emtricitabine and tenofovir (BIC/FTC/TAF) to avoid interactions with dasatinib. During all dasatinib treatment, he maintained a molecular response of 3.0 (which corresponds to a 3-log reduction in BCR::ABL1 transcript levels). HIV-1 viral load was undetectable. Median CD4 and CD8 counts were 812 cells/μL (IQR 787-1309) and 3251 cells/μL (IQR 2231-3909), respectively. Median CD4/CD8 ratio was 0.3 (IQR 0.2-0.4).

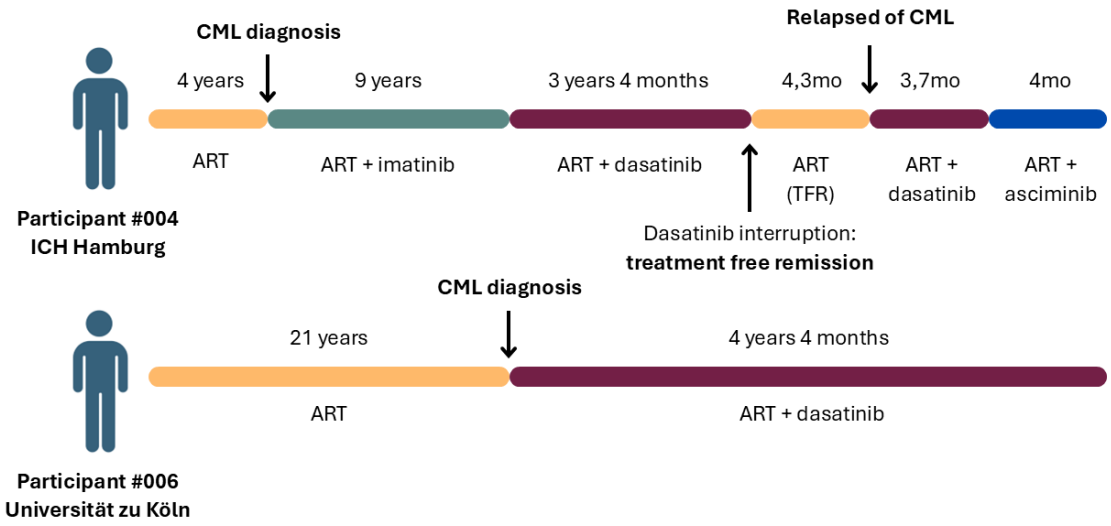


Figure 23. Scheme of follow-up for participants #004 and #006**.**

Ten PWH on ART with undetectable viral load were recruited as controls for participant #004. They were selected based on matching criteria of age, time on HIV-1 infection and time on ART. Eight out of ten participants were males. The median age was 47.5 years (IQR 41-52.5). Median infection time with HIV-1 was 15 years (IQR 12.75-19), while median time on ART was 14.5 years (IQR 12.0-17.5). Median CD4 count was 870 cells/μL (IQR 570.5-1034.5), median CD8 count was 818 cells/μL (IQR 456.5-1046.5), and median CD4/CD8 ratio was 1.08 (IQR 0.8-1.43).

For participant #006, 10 PWH on ART were also selected based on matched age, time with HIV-1 infection and time on ART. 70% of the participants were males, with a median age of 58.0 years (IQR 54-64). Median infection time was 29.5 years (IQR 23.8-32.3), whereas median time on ART was 28 years (IQR 22-31.5). Median CD4 count was 934.5 (IQR 800.5-1047.75) and median CD8 count was 1206.5 (IQR 760.5-1591.5). Median CD4/CD8 ratio was 0.77 (IQR 0.54-0.95).

	Participant #004	Controls for participant #004	Participant #006	Controls for participant #006
Gender, n (%)				
Male	1 (100%)	8 (80%)	1 (100%)	7 (70%)
Female	-	2 (20%)	-	3 (30%)
Age	50	47.5 (41-52.5)	62	58 (54-64)
Age at HIV diagnosis	29	32 (24.75-40.5)	33	30.5 (24.5-37.8)
Infection time (years)	17	15(12.75-19)	25	29.5 (23.8-32.3)
Time on ART (years)	Tim	14.5(12-17.5)	25	28 (22-31.5)
Current ART regime				
BIC/FTC/TAF	1 (100%)	2 (20%)	1 (100%)	2 (20%)
DTG/3TC	-	5 (50%)	-	4 (40%)
DTG/RPV	-	1 (10%)	-	-
DRV/COBI/FTC/TAF	-	1 (10%)	-	2 (20%)
DOR/TDF/FTC	-	1 (10%)	-	-
RPV/FTC/TAF	-	-	-	2 (20%)
Time on current ART regime (years)	2.1	3.24 (1.91-4.43)	5.5	3.79 (1.17-5.37)
Nadir CD4+ per mL	563	181(111.5-312)	-	181 (175-283)
CD4+ T lymphocytes per mL	944	870 (570.5-1034.5)	812.5	934.5(800.5-1047.5)
CD8+ T lymphocytes per mL	639	818 (456.5-1046.5)	3251.8	1206.5(760.5-1591.5)
CD4/CD8 ratio	1.6	1.08 (0.8-1.43)	0.36	0.77 (0.54-0.95)
Sokal risk score	Undetermined	NA	High	NA
Time from CML diagnosis (years)	13	NA	5	NA
Time on dasatinib (years)	4.1	NA	4.3	NA
Previous treatment with TKI (Yes/No,which)	Yes, imatinib	NA	No	NA
Time on previous TKI (years)	9	NA	-	NA

3TC, lamivudine; ART, antiretroviral treatment; BIC, bictegravir; COBI, cobicistat; CML, chronic myeloid leukemia; DRV, darunavir; DOR, doravirine; DTG, dolutegravir; FTC, emtricitabine; NA, not applicable; RPV, rilpivirine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TKI, tyrosine kinase inhibitor.

Table 5. Sociodemographic and clinical data of all participants recruited for this study. Continuous variables are represented as median (Interquartile range), whereas qualitative variables are represented as number and percentage of participants.

1.2. Effect of dasatinib treatment in CD4+ T cell proliferation

Dasatinib treatment may induce cytopenia as it inhibits the homeostatic maintenance of T cells by IL-2 and IL-7 and their activation induced via TCR stimulation (399,419). We determined the number of CD4+ T cells and the frequency of memory subpopulations in order to evaluate if changes in HIV-1 infection or reservoir size could be caused by a strong reduction in CD4 counts or a modification of the distribution of memory subpopulations. In participant #004, CD4+ T cells were maintained at a median of 925.16 cells/µL (IQR 742.25-1180.5) during all dasatinib treatment, while in the control group CD4+ count was 808.2

cells/µL (IQR 570.5-1034.5) (Figure 24A). In participant #006, CD4+ levels decreased after starting dasatinib treatment, but they were maintained at 839.5 cells/µL (IQR 787.0-919.0) during all dasatinib treatment, whereas in the control group, CD4 count was 930.0 cells/µL (IQR 800.5-1047.75) (Figure 24A). Thus, in both participants the number of CD4+ T cells was always above 500 cells/µL and at similar levels to the control groups, indicating that dasatinib treatment did not cause a strong reduction of CD4+ counts.

We also studied the frequencies of CD4+ T subpopulations in several blood samples of both participants and control groups using flow cytometry. We determined CCR7 and CD45RA expression on total CD4+ T cells that were defined as T_N (CD45RA+CCR7+), T_{CM} (CD45RA-CCR7+), T_{EM} (CD45RA-CCR7-) and T_{EMRA} (CD45RA+CCR7-). In both participants, T_{EM} and T_{EMRA} were the most frequent subpopulations during all dasatinib treatment. In participant #004, T_{EM} and T_{EMRA} represented a median of 48.65% (IQR 33.78-65.25) and 35.07% (IQR 22.25-45.6), respectively, of all CD4+ subpopulations during all dasatinib treatment. In the control group, T_{EM} and T_{EMRA} cells also represented 31.6% (IQR 28.4-34.93) and 34.63% (IQR 29.98-42.23), respectively. In participant #006, TEM cells alone represented 74.14% (IQR 70.85-76.9) of all CD4+ during dasatinib, while in the control group TEM cells represented 53.33% (IQR 48.3-57.5) (Figure 24B).

In the control group for participant #004, we found 2.92-fold ($p<0.0001$) higher levels of T_{EM} compared to T_{CM} cells. T_{EMRA} cells were found at 1.51-fold ($p=0.0386$) and 3.20-fold ($p=0.005$) higher levels than T_N and T_{CM}. In the control group for participant #006, we found similar results with increased levels of effector memory subpopulations. T_{EM} cells were the most frequent CD4+ subpopulation, with 5.36-fold ($p<0.0001$), 2.35-fold ($p<0.0001$) and 3.79-fold ($p<0.0001$) higher levels of T_{EM} compared to T_N, T_{CM} and T_{EMRA}, respectively. Together, these data show that dasatinib is not modifying the distribution of CD4+ subpopulations in both participants on treatment with ART and dasatinib, compared to PWH only on ART, suggesting that reservoir size should not be modified by a reduction in CD4+ counts or proliferation of näive subpopulations that are more resistant to HIV-1 infection.

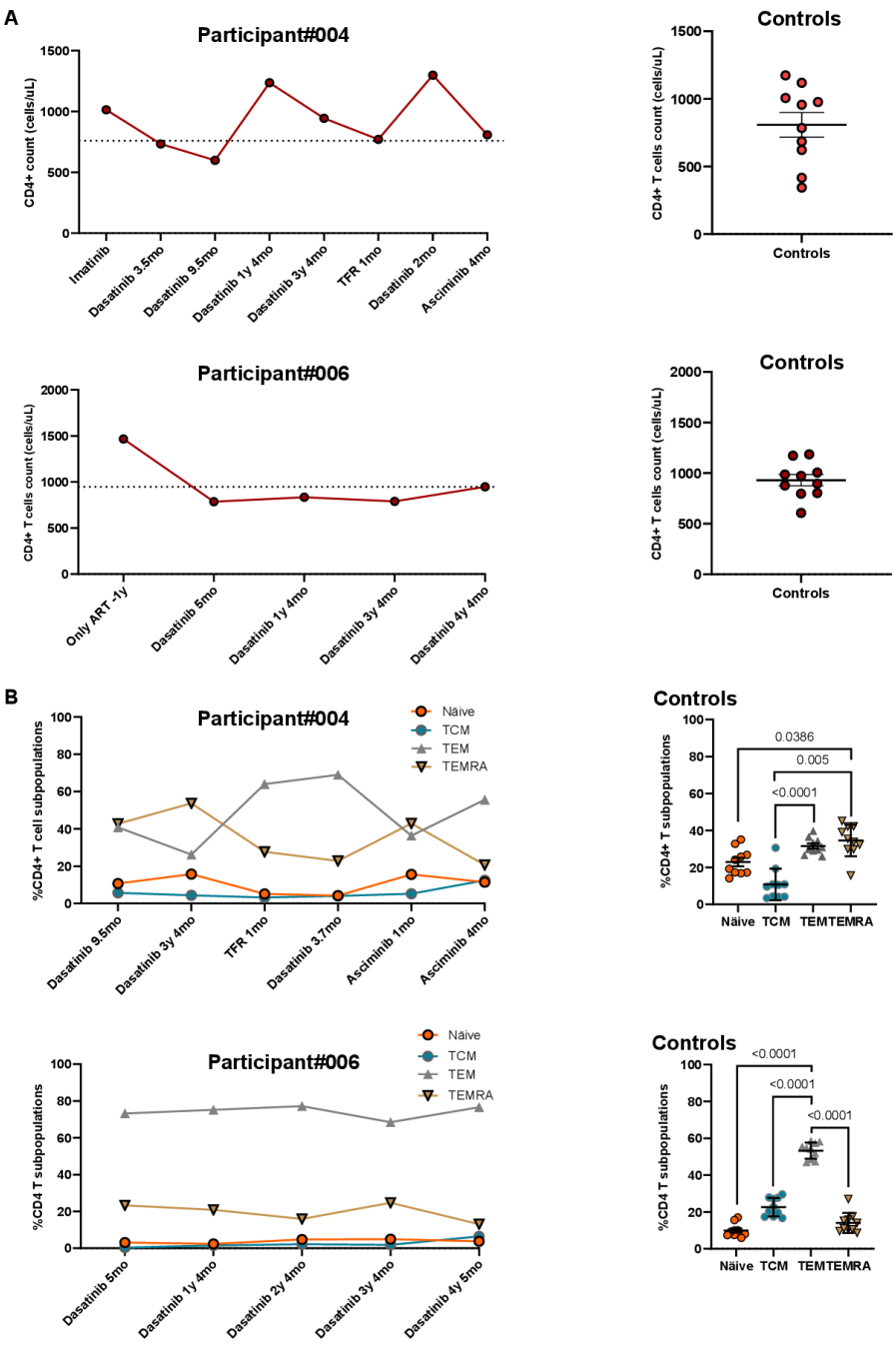


Figure 24. Analysis of CD4+ T cell count and distribution of subpopulations during dasatinib treatment and in control groups. (A) CD4+ T cell count expressed as cells/μL in participant #004 and #006 (left) and in control groups of PWH only on ART (right). (B) Distribution of CD4+ T cell subpopulations T Näive (T_N), T central memory (T_{CM}), T effector memory (T_{EM}) and terminally differentiated effector memory (T_{EMRA}), expressed as percentages in participants #004 and #006 (left) and in control groups (right). Each dot corresponds to one sample. Vertical lines in control groups represent the median and ± SEM. Statistical significance among control groups was calculated using one-way ANOVA as the data were normally distributed.

1.3. Decay of HIV-1 integrated DNA after dasatinib treatment introduction

To determine whether long-term treatment with dasatinib was interfering with HIV-1 reservoir maintenance and proliferation, the number of integrated HIV-1 proviral copies was calculated by Alu-LTR PCR, using DNA isolated from PBMCs from all participants included in the study.

In participant #004, after 3.5 months of dasatinib treatment, we observed a 7.94-fold decrease of HIV-1 integrated DNA compared to imatinib treatment, which represented a reduction from 1737.80 copies/10^6 cells to 218.78 copies/10^6 cells. This decrease was more pronounced after 9.5 months of dasatinib, when integrated proviruses diminished to 1.50 copies/10^6 cells, and was maintained at a median of 0.88 copies/10^6 cells (IQR 0.40-1.00) during all dasatinib treatment, TFR, dasatinib reintroduction and asciminib treatment. Thus, HIV-1 integrated proviruses were maintained at lower levels after 9.5-months of dasatinib compared to the control group, which had a median of 30.98 copies/10^6 cells (IQR: 2.25-316.23), (Figure 25A).

In participant #006, the number of copies of integrated provirus was at a median of 160.09 copies/10^6 cells (IQR 10.88-335.11). Dasatinib treatment for 5 months reduced HIV-1 integrated DNA by 1.60-fold compared to treatment only on ART. This decline was more marked after 1 year and 4 months of dasatinib, with a 29.17-fold decrease. Integrated HIV-1 DNA was then maintained at a median of 6.85 copies/10^6 cells (IQR 3.63-11.75) during all dasatinib treatment. The copies of integrated provirus in the control group were determined at a 14.50-fold higher levels compared to median levels during dasatinib treatment of participant #006 (Figure 25B).

Together, dasatinib treatment induced a strong reduction in the reservoir size of both participants. Remarkably, in participant #004, this reduction was maintained when dasatinib treatment was interrupted, indicating that its effects on interfering with reservoir maintenance persist.

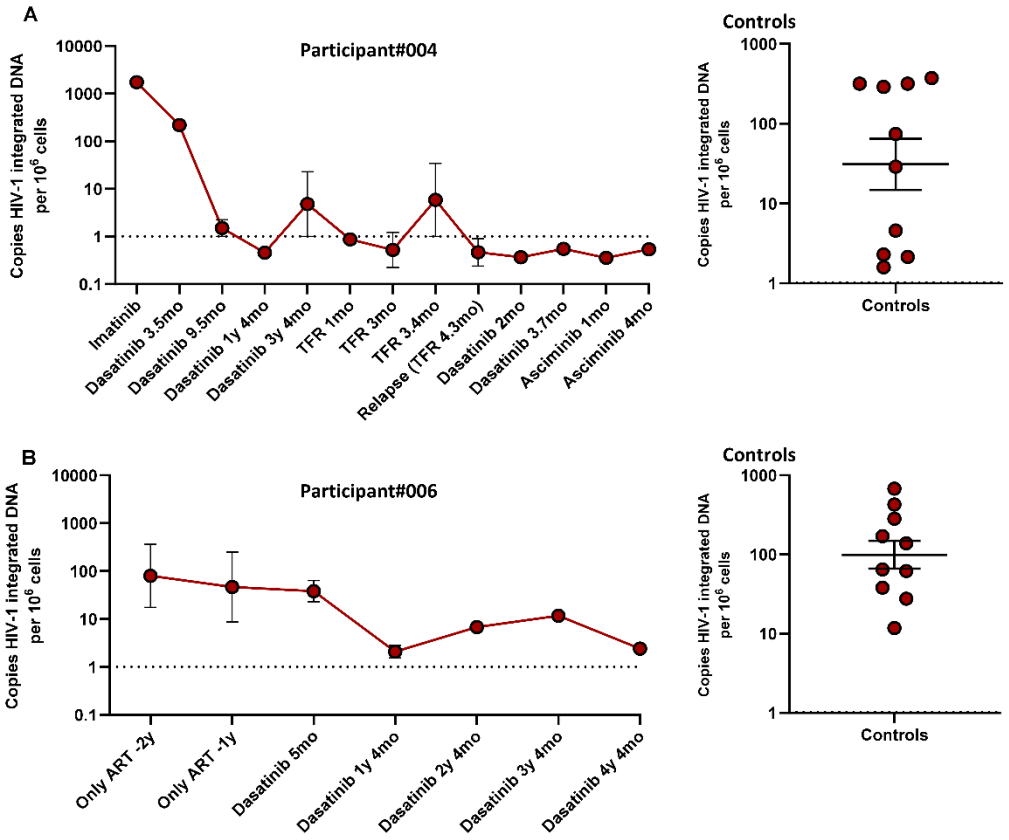


Figure 25. Dasatinib treatment substantially decreases the reservoir size in both participants with HIV and CML. (A) Quantification by Alu-LTR PCR of proviral DNA per 10^6 cells in participant #004 (left) and in control group of PWH only on ART (right). (B) Proviral DNA quantification in participant #006 (left) and the control group of PWH only on ART (right). Each dot corresponds to one sample. Vertical lines in control groups represent median ± SEM.

1.4. Dasatinib treatment reduces both intact and defective proviruses

In a previous work (165), we described in two PWH with CML that were one year on treatment with dasatinib, finding that number of copies of intact and defective proviruses were not modified after dasatinib treatment introduction. However, we still have not determined whether long-term treatment might interfere with the maintenance of intact and defective proviruses. For this purpose, we analyzed integrated proviruses with IPDA in DNA extracted from PBMCs of all the participants included in the study. Due to scarcity of the samples, IPDA could not be done in samples of participant #004 obtained before dasatinib treatment.

In participant #004, copies of intact proviral DNA were 158.0 copies/10^6 PBMCs after 3.5 months on dasatinib treatment, and after 9.5 months intact HIV-1 DNA was undetectable.

It was only detectable again after 3 years and 4 months of dasatinib treatment (19.7 copies/10^6 PBMCs) and 1 month after dasatinib interruption (10.33 copies/10^6 PBMCs). Thus, after 9.5 months of dasatinib, levels of intact proviruses were lower compared to the control group, in which the number of intact copies was 158.6 copies/10^6 PBMCs (IQR 1.0-256.0) (Figure 26A). When analyzing defective proviruses, 3´-defective were at a median of 62.02 copies/10^6 PBMCs (IQR 27.06-98.68) after 9.5 months on dasatinib, and decreased to a median of 19.05 copies/10^6 PBMCs (IQR 3.80-31.47) from 1 year and 4 months of dasatinib onwards. Levels of 3´-defective proviruses were 9.41-fold higher in the control group, in which the median was 179.29 copies/10^6 PBMCs (IQR 60.54-289.96) (Figure 26A). 5´-defective proviruses appeared to remain stable in participant #004 throughout dasatinib treatment, TFR, dasatinib reintroduction and asciminib treatment. After 9.5 months of dasatinib, 5´-defective proviruses were at a median of 16.82 copies/10^6 PBMCs (IQR 7.34-26.3), and were maintained at a median of 14.87 copies/10^6 PBMCs (IQR 2.76-27.28) from 1 year and 4 months on dasatinib onwards. The number of 5´-defective proviruses was 10.66-fold higher in the control group (Figure 26A).

In participant #006, the median intact proviruses was 39.83 copies/10^6 PBMCs (IQR 1.0-65.5) during treatment with ART only. Then, intact proviruses remained undetectable during all dasatinib treatment. In the control group, the median of intact proviruses was 27.39 copies/10^6 PBMCs (IQR 0.0-54.5) (Figure 26B). We also found a substantial reduction in defective proviruses once dasatinib treatment started. 3´-defective proviruses decreased 5.21-fold after dasatinib treatment started compared to treatment only on ART, and the median of 3´-defective proviruses was maintained at 24.74 copies/10^6 PBMCs (IQR 11.36-48.65). Levels of 3´-defective proviruses in control group were 3.57-fold higher compared to treatment with ART and dasatinib of participant #006 (Figure 26B). The number of 5´-defective proviruses was 7.55-fold lower after dasatinib treatment started compared to treatment only on ART. 5´-defective proviruses were maintained at a median of 16.33 copies/10^6 PBMCs (IQR 7.69-16.80) during all dasatinib treatment. The number of copies of 5´-defective HIV-1 DNA was 6.76-fold higher in the control group compared to treatment with ART and dasatinib in participant #006 (Figure 26B).

Our findings indicate that dasatinib was able to reduce both intact and defective proviruses, and this effect was maintained even when dasatinib treatment was interrupted in participant #004. Surprisingly, intact proviruses were undetectable in both participants during most of the dasatinib treatment.

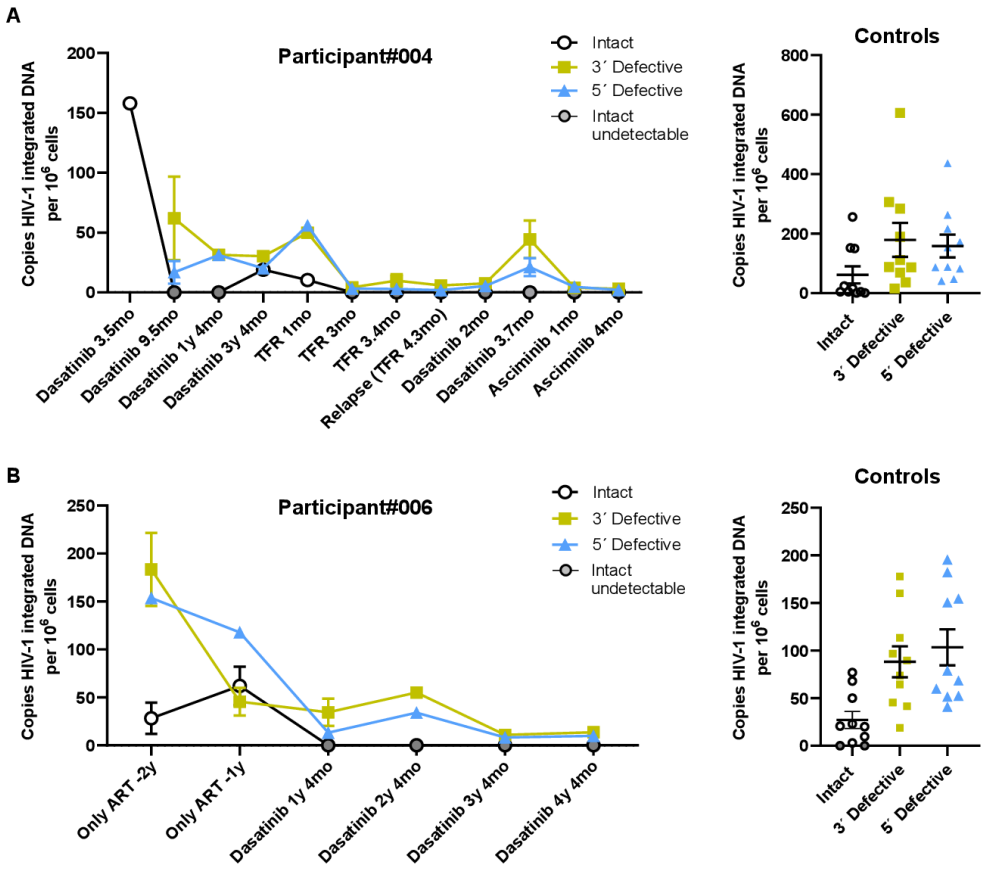


Figure 26. Strong reduction of intact and defective proviruses after initiation of dasatinib treatment. Intact and defective proviruses were quantified using the IPDA in a ddPCR. (A) Quantification of intact, 3´-defective and 5´-defective proviruses in participant #004 (left) and the control group of PWH only on ART (right). (B) Quantification of intact, 3´-defective and 5´-defective proviruses in participant #006 (left) and in the control group of PWH only on ART (right). Each dot corresponds to one sample. Vertical lines in control groups represent median ± SEM.

1.5. Dasatinib treatment interfered with proviral reactivation

After analyzing the reservoir size in both participants, we characterized the reservoir functionality by inducing proviral reactivation in isolated CD4+ T cells. These cells were first activated with CD3/CD28 and IL-2 for 5 days, and then provirus was reactivated using PMA and ionomycin for 18 hours, combined with brefeldin A to inhibit the release of viral particles into the culture medium. p24-Gag production in total CD4+ T cells and CD4+ subpopulations was assessed by flow cytometry. Median p24-Gag+ in total CD4+ T cells was 2.49% (IQR 0.91-4.39) in participant #004, including both dasatinib and asciminib treatment. Of note, the lowest proviral reactivation was observed during asciminib

treatment. In the control group, p24-Gag+ CD4+ T cells levels were 3.31-fold higher, with a median of 8.22% (IQR 1.50-13.45). Thus, proviral reactivation in participant #004 was always lower than in the control group (Figure 27A). In participant #006, we found a median of p24-Gag+ CD4+ T cells that was 4.03% (IQR 1.97-5.73) during all dasatinib treatment, whereas median proviral reactivation in control group was 5.62% (IQR 2.79-7.53). Thus, proviral reactivation in participant #006 was similar to participant #004, but it was higher than in the control group only after 2 years and 4 months (Figure 27A).

In participant #004, median proviral reactivation in CD4+ T subpopulations was 3.23% (IQR 1.69-4.72) in T_N, 3.01% (IQR 0.85-5.29) in T_{CM}, 1.37% (IQR 0.83-1.79) in T_{EM} and 2.0% (IQR 0.84-3.07) in T_{EMRA}. Thus, proviral reactivation in the subpopulations was lower than in total CD4+ T cells during all dasatinib treatment, TFR and asciminib. Furthermore, it was not above the median proviral reactivation of T_{CM} cells of the control group, which was found to be the main source of viral reactivation. In the control group, reactivation of provirus was 1.43-fold (p=0.0488) higher in T_N than in T_{EM}, and 8.32-fold (p=0.0406) higher in T_{CM} cells than in T_{EM} (Figure 27B). Almost identical results were obtained in participant #006, in which median p24-Gag+ CD4+ T cells was 2.83% (IQR 0.99-5.15) in T_N, 2.80% (IQR 0.46-4.56) in T_{CM}, 2.01% (IQR 1.25-2.90) in T_{EM} and 3.18% (IQR 1.39-5.22) in T_{EMRA}, resulting in a proviral reactivation that was not above the detected reactivation in T_{CM} of the control group. Again, the proviral reactivation in the control group was higher in T_N and T_{CM} cells. p24-Gag+ detection in T_N was 3.45-fold (p=0.0216) and 3.07-fold (p=0.0089) higher than in T_{EM} and T_{EMRA}, respectively. In T_{CM}, proviral reactivation was 4.66-fold (p=0.00251) and 4.15-fold (p=0.0208) higher than in T_{EM} and T_{EMRA}, respectively (Figure 27B).

Altogether, our results link the decrease in the reservoir size with a reduction in the proviral reactivation in both participants, in comparison with PWH only on ART. Moreover, this reduced proviral reactivation is detected in all CD4+ subpopulations that were analyzed, suggesting that the effect of dasatinib against HIV-1 infection is extended to all CD4+ subsets.

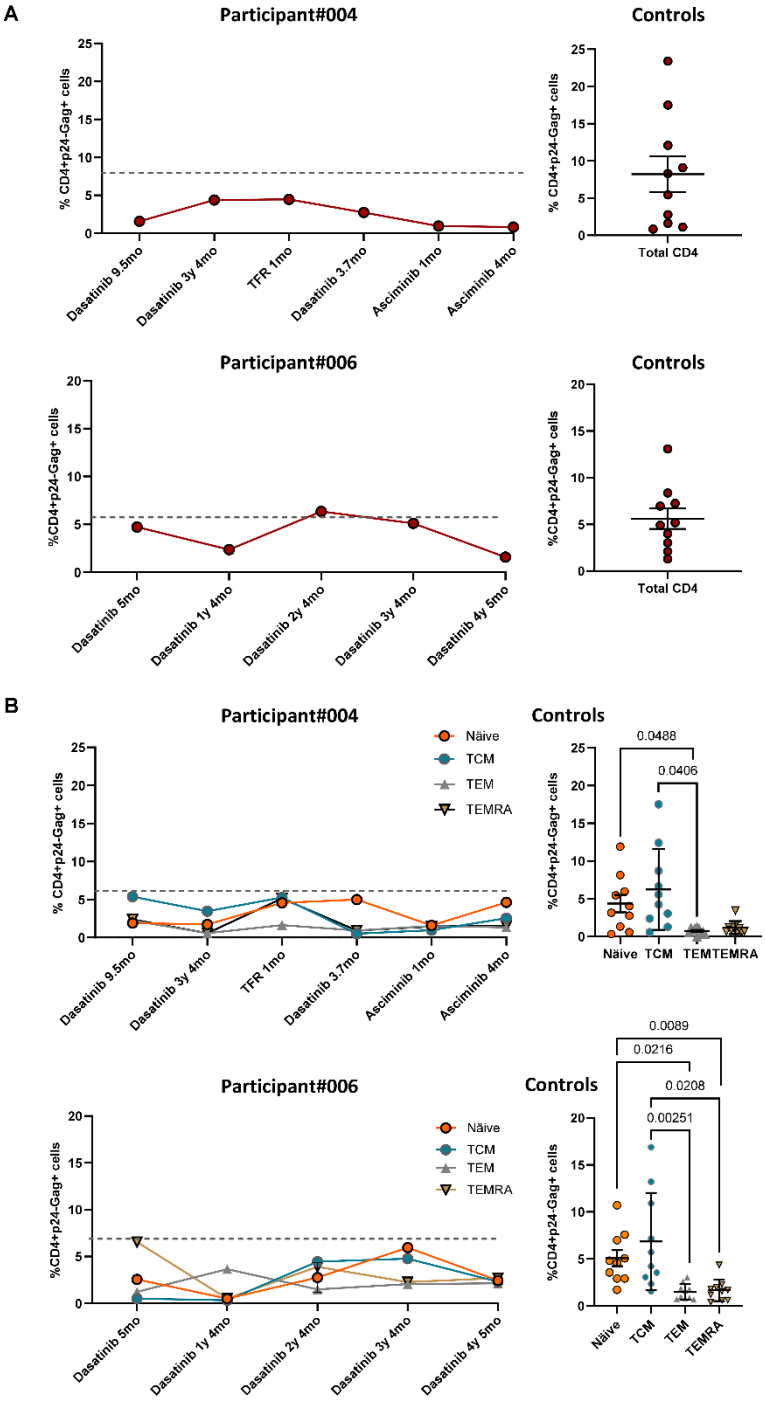


Figure 27. Dasatinib treatment interferes with proviral reactivation in total CD4+ T cells and memory subpopulations. Reactivation of HIV-1 provirus was induced by activation of CD4+ T cells with CD3/CD28 and subsequent stimulation with PMA and Ionomycin. p24-Gag production was assessed by flow cytometry. (A) Percentage of p24-Gag+ total CD4+ T cells in participants #004 and #006 (left) and in control groups (right). (B) Percentage of p24-Gag production in T_N, T_{CM}, T_{EM} and T_{EMRA} cell subpopulations in both participants (left) and in control groups of PWH only on ART (right). Each dot corresponds to one sample. Vertical

lines in the control groups represent the median ± SEM. Statistical significance in control groups was evaluated using one-way ANOVA.

1.6. SAMHD1 antiviral activity was maintained during all dasatinib treatment

Given the strong reduction in both reservoir size and the reactivation of the provirus, we next tested if long-term treatment with dasatinib was able to inhibit the phosphorylation at T592 of the antiviral immune factor SAMHD1, which would maintain its antiviral activity. After CD4+ T cells isolation and activation with CD3/CD28 for 5 days, we analyzed the frequency of pSAMHD1 in total CD4+ T cells and memory subpopulations.

In total CD4+ T cells of participant #004, SAMHD1 phosphorylation did not increase during all dasatinib treatment and remained below control levels, with a median of 1.56% (IQR 0.37-2.82) of pSAMHD1. Median pSAMHD1+ in total CD4+ T cells of the control group was 12.52% (5.08-20.4). Similar results were found in total CD4+ T cells of participant #006, in which median percentage of pSAMHD1 was 4.03% (IQR 1.97-5.73), whereas in the control group median was 11.33% (IQR 6.35-15.33) (Figure 28A). Therefore, the phosphorylation of SAMHD1 in total CD4+ T cells was always lower in both participants than in control groups. These results can also be extended to the different CD4+ T subpopulations that were analyzed, in which pSAMHD1 in both participants was never above the detected levels in both control groups, with no differences between subpopulations. However, we did find statistical differences in the subpopulations of control groups. In the control group for participant #004, the percentage of pSAMHD1 was 37.12-fold (p=0.0069) and 11.63-fold (0.0127) higher in T_N than in T_{EM} and T_{EMRA}, respectively. We also found 22.18-fold (p=0.0370) higher percentage of pSAMHD1 in T_{CM} than in T_{EM}, that was also observed in the control group for participant #006, in which pSAMHD1 was 2.60-fold (p=0.0127) higher (Figure 28B). We found no correlation between p24-Gag production and phosphorylation of SAMHD1 in CD4+ T cell during all dasatinib treatment.

These results provide evidence of the inhibition of the phosphorylation of SAMHD1 by dasatinib treatment in CD4+ T cells, which would maintain the antiviral effects of SAMHD1, resulting in an interference with HIV-1 infection and proviral reactivation. Furthermore, the effects of dasatinib are extended to all CD4+ subpopulations, enabling possible the maintenance of SAMDH1 function in all CD4+ T cells, regardless of their differentiation state.

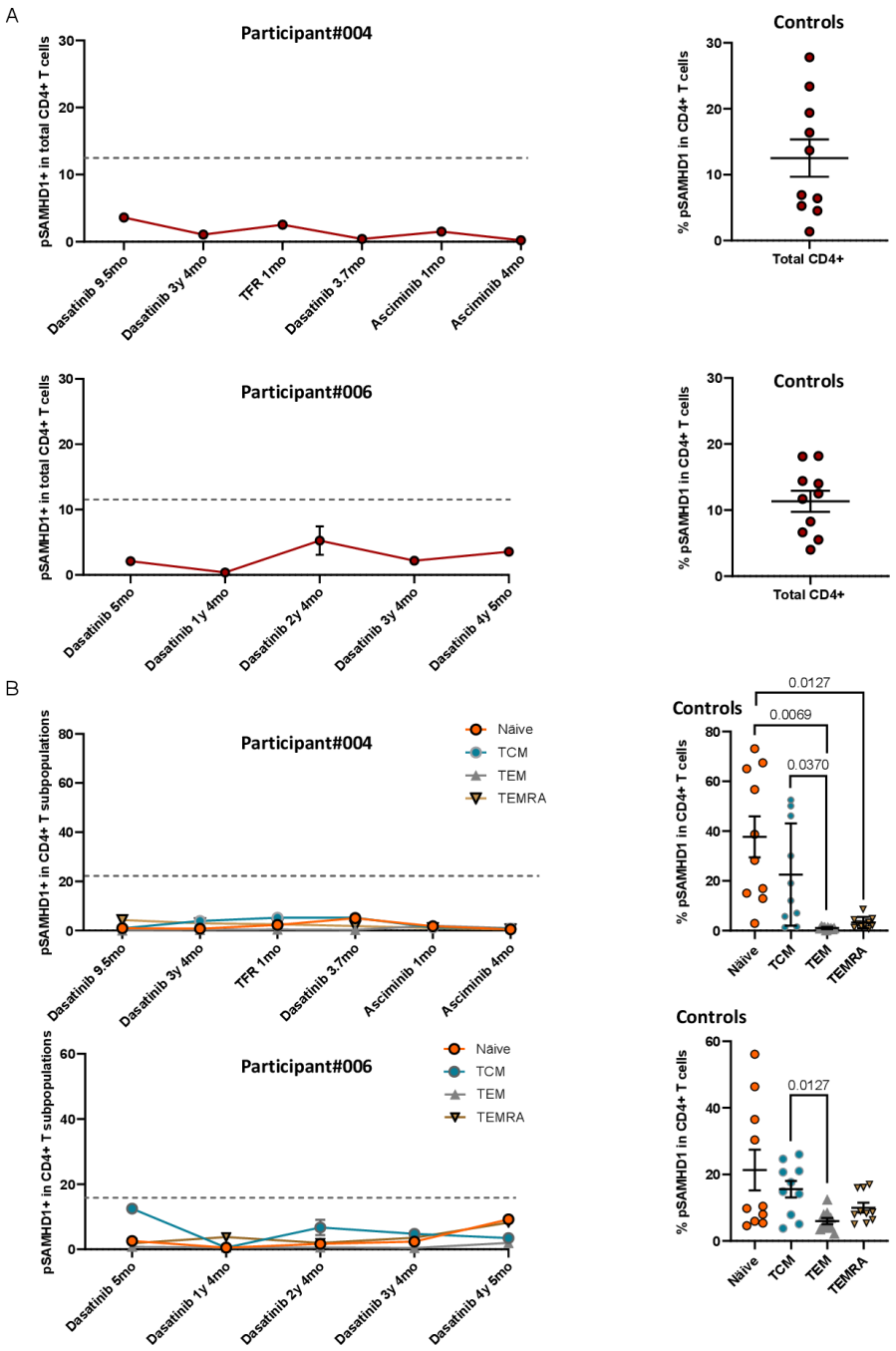


Figure 28. SAMHD1 phosphorylation at T592 was blocked during dasatinib treatment. The percentage of pSAMHD1 was measured in isolated CD4+ T cells by flow cytometry after activation with CD3/CD28 for 5 days and subsequent stimulation with PMA/Ionomycin for 18h. (A) Percentage of phosphorylation at T592 of SAMHD1 in total CD4+ T cells in participants #004 and #006 (left) and in control groups of PWH receiving only ART (right). (B) Percentage of pSAMHD1 in CD4+ T subpopulations of both participants (left) and control groups (right). Each dot corresponds to one sample. Vertical lines in control groups represent the median ± SEM. Statistical significance in control groups was evaluated using one-way ANOVA.

Study III

Analysis of the effects of tyrosine kinase inhibitors on immunometabolism of CD4+ T, CD8+ T and NK cells

1. Dasatinib and ponatinib treatment *in vitro* impaired mitochondrial and glycolytic activity in CD4+ T cells.

In order to evaluate the impact of dasatinib and ponatinib treatment on cell metabolism, we determined the metabolic activity of CD4+ T cells that were isolated from PBMCs of 5 different healthy donors. Cells were cultured for 72 hours in the presence or absence of dasatinib, ponatinib, and also in the presence of PHA to induce cellular activation.

The metabolic activity of unstimulated CD4+ T cells was at low levels in both OCR and ECAR measurements, in accordance with their quiescent state (Figure 29A, left graphs). Treatment with dasatinib or ponatinib of non-stimulated CD4+ T cells did not induce changes in mitochondrial or glycolytic metabolism, although cells remained in a susceptible state in which they could respond to inhibitory or activating compounds. Thus, in these treated cells, oligomycin was able to reduce mitochondrial respiration, while FCCP could induce maximum cell respiration. This was also observed in the glycolytic metabolism, where the use of rotenone plus antimycin A induced an increase in ECAR, indicating an increase in the glycolytic activity of these cells (Figure 29A, lower left graph).

Treatment with dasatinib or ponatinib together with PHA produced a strong inhibitory effect on cell metabolism, showing low OCR and ECAR values that indicated a suppression of mitochondrial and glycolytic metabolism (Figure 29A, right graphs). Again, these cells remained susceptible to inhibitory and activating stimuli despite their low metabolic activity, although their capacity to respond to the different drugs was substantially reduced in comparison with CD4+ T cells that were activated with PHA. As a result, dasatinib and ponatinib treatment of PHA-stimulated cells induced a 2.99-fold ($p<0.0001$) and 3.01-fold ($p<0.0001$) reduction of OCR compared to PHA-only treated cells, respectively.

This reduction was also observed in ECAR measurements, where dasatinib and ponatinib provoked a 4.11-fold ($p<0.0001$) and 4.7-fold ($p=0.0002$) reduction compared to PHA-only treated CD4+ T cells (Figure 29B). Therefore, our results suggest that dasatinib and ponatinib induce a potent cytostatic effect that interferes with the activation of CD4+ T cells via mitochondrial and glycolytic metabolism, which is crucial for the establishment and maintenance of HIV-1 infection.

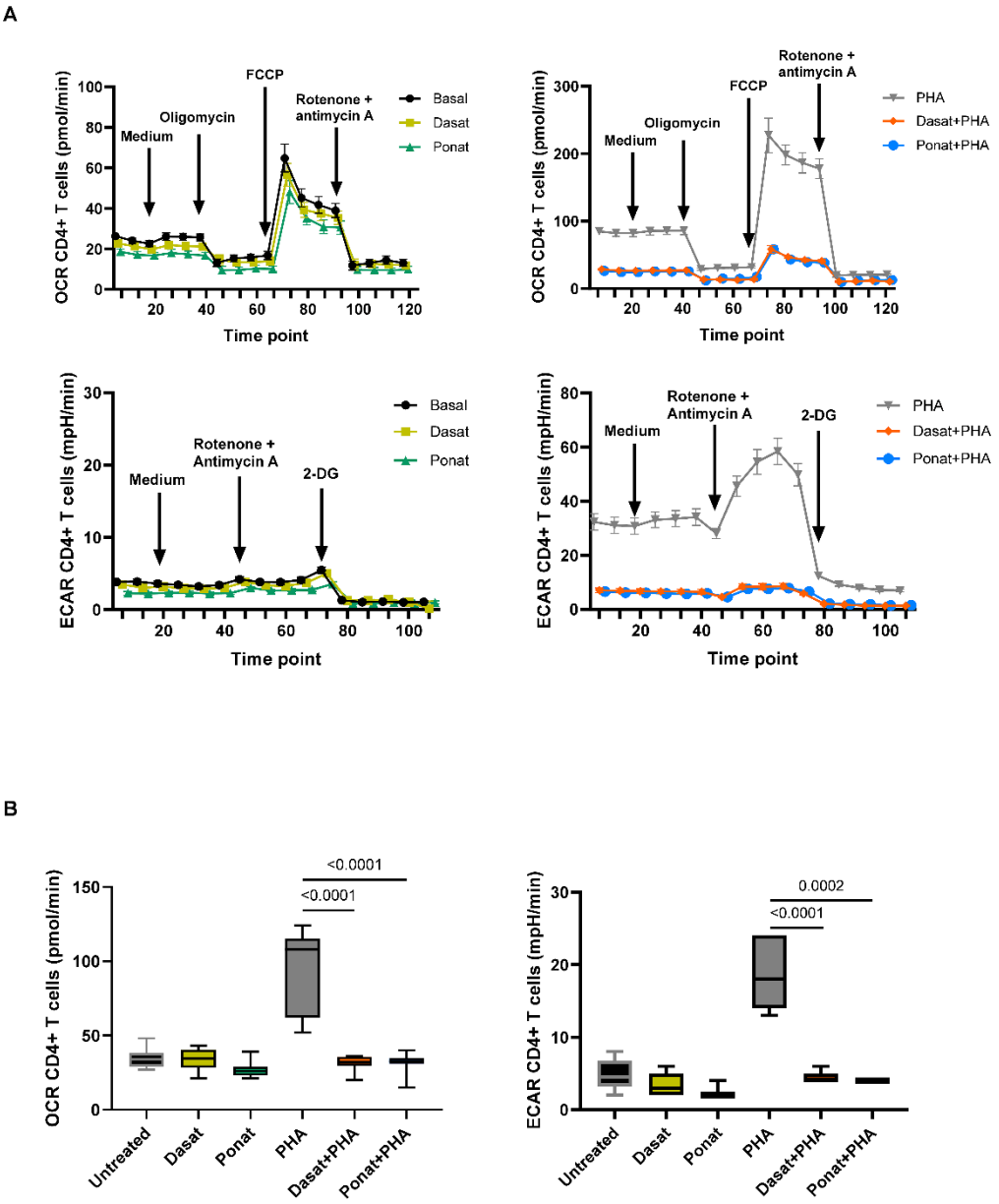


Figure 29. ***In vitro*** **treatment with dasatinib or ponatinib strongly reduces cell metabolism.** Isolated CD4+ T cells from PBMCs of healthy donors were treated in the presence or absence of dasatinib, ponatinib and PHA for 72 hours. The graphs show the median results from different experiments with 5 donors. (A) Representative experiment of Seahorse XF Analyzer with MitoStress Test kit for OCR and Glycolytic Stress kit for ECAR measurements in non-activated CD4+ T cells (left) and PHA-activated cells (right). (B) Representative analysis of OCR and ECAR (based on the same measurements as in panel A). Bar graphs represent the median ± SEM. Paired t-tests were used for differences between conditions in CD4+ T cells.

2. Metabolic suppression of CD8+T cells by treatment *in vitro* with dasatinib and ponatinib

Following the same procedure as with CD4+ T cells, we next tested if the cytostatic effect induced by dasatinib and ponatinib extended to CD8+ T cells, as their activity is necessary for control and elimination of HIV-1-infected cells. Again, OCR was measured as an indicator of OXHPOS, while ECAR was measured as an indicator of glycolytic metabolism.

The quiescent state of non-stimulated CD8+ T cells resulted in low levels of OCR and ECAR in basal conditions that were not modified by dasatinib or ponatinib treatment (Figure 30A, left graphs). As with CD4+ T cells, CD8+ T cells responded to activating and inhibitory drugs for both mitochondrial and glycolytic metabolism, allowing maximum respiratory capacity of OCR (after FCCP injection) or an increase in ECAR (after rotenone plus antimycin A) (Figure 30A, left).

Treatment with PHA and dasatinib or ponatinib interfered with the activation of CD8+ T cells, resulting in a decrease in both OCR and ECAR in comparison with PHA-stimulated cells in which we observed an increase in their mitochondrial and glycolytic metabolism (Figure 30A, right graphs). The effects of dasatinib of ponatinib still permitted CD8+ T cells to respond to the injection drugs, but not as strongly as activated CD8+ T cells. Consequently, we observed a 2.48-fold ($p<0.0001$) and 2.78-fold ($p<0.0001$) reduction of the OCR of dasatinib + PHA and ponatinib + PHA treated CD8+ T cells in comparison with PHA-only treated cells, respectively. A similar decrease was observed in ECAR of dasatinib + PHA and ponatinib + PHA CD8+ T cells, in which a 3.05-fold ($p<0.0001$) and 3.20-fold ($p<0.0001$) reduction was observed in comparison to PHA-only treated cells (Figure 30B).

Altogether, these results confirm the strong cytostatic effects of dasatinib and ponatinib on T cells, which remained in a quiescent state when they were treated with either of both TKIs. Although these results may imply that TKIs may interfere with CD8+ T cell activation, the cytotoxic capacity of these cells remains to be determined.

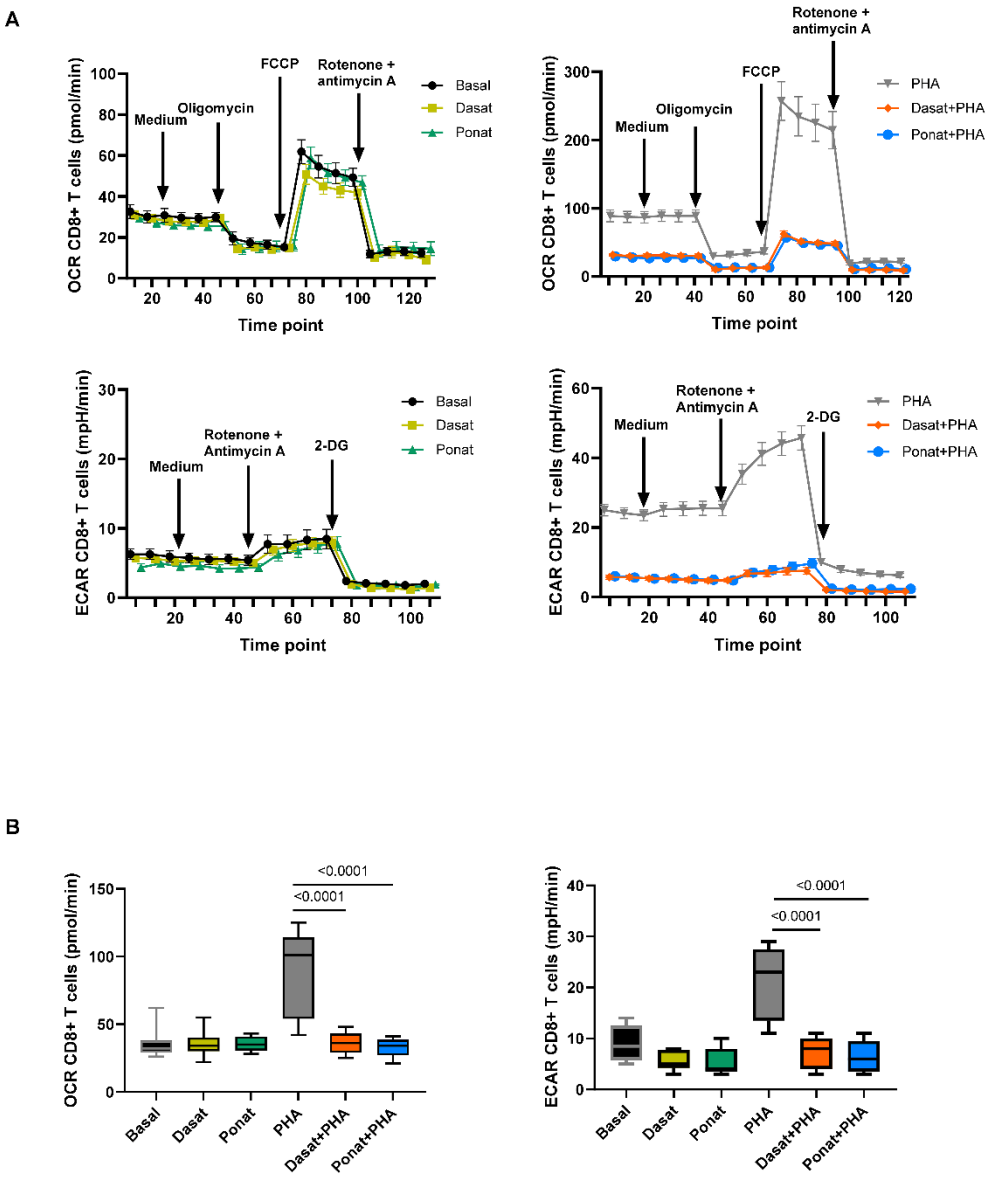


Figure 30. Dasatinib and ponatinib *in vitro* treatment blocked the activation of cell metabolism. Isolated CD8+ T cells from healthy donors were treated in the presence or absence of dasatinib, ponatinib and PHA for 72 hours. Data from 5 independent experiments are shown. (A) Median values of the experiment in the Seahorse XF Analyzer using Mito Stress test kit for OCR and Glycolytic Stress kit for ECAR measurements in non-stimulated (left) and PHA-activated (right) CD8+ T cells. (B) Analysis of global results of OCR and ECAR that were represented in (A). Bar graphs represent the mean ± SEM. Paired t-tests were used for analysis of differences between conditions.

3. Dasatinib reduces cytokine-induced metabolic activation in NK cells while preserving metabolic plasticity

To determine whether the metabolism and activation of NK cells are modified by TKI treatment, we isolated NK cells from healthy donors and treated them in the presence or absence of dasatinib. IL-15, IL-12, and IL-18 were also used to stimulate their metabolic activity. Given the previous results in which ponatinib induced the same effects as dasatinib, we hypothesized that any effects observed with dasatinib could be observed with ponatinib treatment of NK cells. We measured OCR as indicator of OXPHOS, ECAR as indicator of glycolysis, and PER as an indicator of shifts between OXPHOS and glycolysis. PER is linked to lactate production (via glycolysis), indicating that an increase in PER is usually caused by high glycolytic activity, while low PER values correspond to reliance on OXPHOS or a quiescent state of the cell.

Our results showed that dasatinib treatment reduced the cell metabolism of un-stimulated NK cells, as demonstrated by OCR, ECAR and PER measurements (Figure 31A). The maximal respiratory capacity obtained after FCCP drug injection of dasatinib-treated cells was lower than in basal NK cells. However, OCR, ECAR, and PER were increased when NK cells were activated with IL-15/12/18, and the addition of dasatinib to the culture of these stimulated cells only reduced ECAR and PER, although not to basal levels. Furthermore, we found that dasatinib reduced OCR by 1.18-fold ($p=0.0221$) in NK cells not stimulated with cytokines. The use of IL15/12/18 induced a 1.72-fold ($p<0.0066$) and 2.13-fold ($p<0.0001$) increase of the mitochondrial metabolism in comparison to un-activated and dasatinib treated NK cells, respectively. When dasatinib was added with IL15/12/18, we found no differences in the OCR of these cells in comparison with un-stimulated NK cells, but their OCR increased 1.83-fold ($p=0.0022$) when compared to dasatinib-only treated NK cells (Figure 31B, left graph).

Notably, the analysis of ECAR revealed a similar trend, in which treatment *in vitro* with dasatinib reduced 1.92-fold ($p=0.0012$) the glycolytic metabolism of un-stimulated cells. The use of dasatinib with IL15/12/18 reduced ECAR by 1.60-fold ($p=0.0006$) compared to stimulated NK cells (Figure 31B, right graph). However, we also observed that culture of NK cells with dasatinib and IL15/12/18 produced a 1.65-fold ($p=0.0005$) increase in ECAR compared to NK cells cultured only with dasatinib.

Overall, our results suggest that dasatinib may interfere with cytokine-driven activation of the mitochondrial and glycolytic metabolism of NK cells, but still permits some degree of

metabolic plasticity, allowing NK cells to shift to glycolytic metabolism and avoid remaining in a quiescent state.

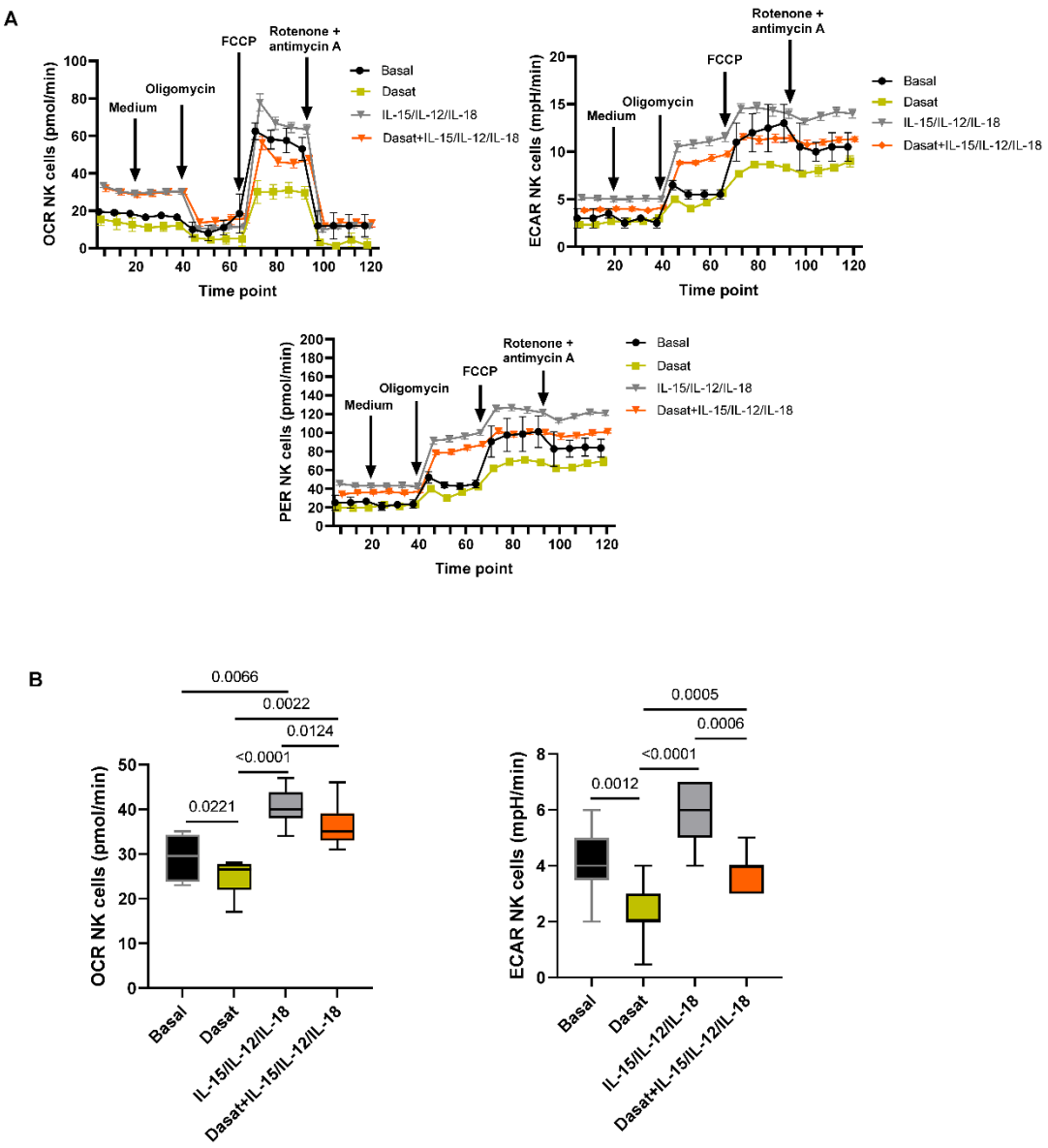


Figure 31. Dasatinib does not interfere with mitochondrial metabolism and permits low-level activation of glycolytic metabolism in NK cells. After isolating NK cells from PBMCs of healthy donors, cells were cultured in the presence or absence of dasatinib, IL-15, IL-12 and IL-18. Data from 3 independent experiments are shown. (A) OCR, ECAR and PER measurements in NK cells under different conditions. (B) Statistical analysis of OCR and ECAR. Bar graphs represent the mean ± SEM. Paired t-tests were used for analysis of differences between conditions.

DISCUSSION

Since the first described cases in the early 1980s, which were initially associated with rare types of immunosuppression, more than 88.4 million people have been infected with HIV, and 42.3 million people have died of AIDS-related causes (4). The fact that 1.3 million people are newly diagnosed with HIV each year shows that, despite the development of new antiviral drugs and vaccines, HIV remains one of the biggest threats to global health. The existence and unique characteristics of the HIV viral reservoir constitute a major challenge for the achievement of a cure for HIV infection. The early initiation of ART in SIV infection in simians (437,438) or HIV infection in humans (160) has demonstrated that ART alone cannot prevent the formation of the viral reservoirs. The detection of HIV-1 infected cells in different tissues and organs (147–149,154,203–209), which even ART or cytotoxic T cells cannot reach (214) complicates the elimination of latently infected cells. The HIV-1 reservoir has an estimated half-life of 44 months, and ART alone would need more than 70 years to eliminate or significantly reduce the reservoir (439).

In PWH on ART, latently infected cells are primarily composed of clonally expanded CD4+ T cells (191,220,227,440,441). The maintenance and proliferation of these cells via cytokine stimulation (188) and TCR-mediated activation (233) enable the maintenance of the HIV-1 viral reservoir through intrinsic T-cell maintenance and mechanisms linked to the development of the immune response. Thus, the persistence of latently infected CD4+ T cells results in chronic immune activation, which leads to immune exhaustion, non-infectious complications and associated co-morbidities (236).

Multiple intervention strategies have been implemented to achieve a meaningful reduction in the HIV-1 latent reservoir. The Shock & Kill (287–292) and the Block & Lock (304,305) strategies are being studied in laboratories all over the world, but these approaches still need to be tested in controlled clinical trials (309). Several LRAs have been analyzed in clinical trials, such as bryostatin-1 (288) or vesatolimod (292), but they failed in significantly reducing reservoir size. In the case of LPAs, only ruxolitinib has progressed to clinical trials, although its potential as a Block & Lock compound was not evaluated (442). Therefore, the only approach that has been effective so far in producing a real cure for HIV-1 infection is the transplantation of stem-cell from bone marrow or umbilical cord blood obtained from CCR5Δ32/Δ32 donors (276–280) and, apparently, also from a wild-type CCR5 donor (284), although this remains to be confirmed in other cases. Unfortunately, the difficulty of finding HLA-compatible donors and the elevated risk of the stem-cell transplantation procedure make this cure approach difficult to implement for all PWH. Consequently, alternatives that

are more accessible for everyone and easier to implement are necessary to achieve a real reduction and control of the HIV-1 reservoir that may lead to a functional cure.

It is well known that the maintenance of normal T cell counts by homeostatic proliferation (443) allows the persistence of latently infected cells in PWH (444–446). Moreover, the differentiation of latently infected CD4+ T cells by antigen-driven proliferation leads to the reactivation of the provirus and triggers the expansion of T-cell clonotypes that contain proviruses (447–450). Thus, blocking CD4+ T cell proliferation and maintenance appears to be essential for interfering with the maintenance of the HIV-1 reservoir and progressing toward an HIV cure (188,451,452).

Our laboratory has been studying for several years the potential use of TKIs, a cancer immunotherapy option, to tackle the HIV-1 reservoir. The cytostatic capacity of TKIs to interfere with CD4+ T cell homeostatic proliferation and TCR-mediated activation, to inhibit the phosphorylation of SAMHD1, and even their capacity to enhance the cytotoxic immune response, make these drugs potential candidates to disrupt HIV-1 infection in PWH (419). Dasatinib and ponatinib have been shown to be the most potent TKIs against HIV-1; therefore, this thesis has been centered on evaluating the efficacy of these drugs in containing the infection, and also in inducing a potent antiviral, cytotoxic response in CD8+ T cells and NK cells.

Dasatinib and ponatinib are both able to interfere with the formation and maintenance of the HIV-1 reservoir (399,419,422), but they may differ in efficacy. Ponatinib has been described as the most potent TKI, so we hypothesized that a one-year treatment with ponatinib may modify the susceptibility of CD4+ T cells to HIV-1 infection and even stimulate cytotoxic cell responses against the infected cells. In people with CML on long-term treatment with imatinib, CD4+ T cells showed persistent susceptibility to HIV-1 infection, which correlated with increased SAMHD1 phosphorylation that reduces its antiviral activity (419). Switching to ponatinib for one year rendered CD4+ T cells resistant to HIV-1 infection, and this resistance was maintained during one year of treatment interruption. Ponatinib affected all CD4+ T subpopulations, independently of their differentiation status, which means that it could interfere with the establishment, maintenance and proliferation of the HIV-1 reservoir, and also with the expression of defective integrated proviruses that trigger chronic immune activation (453).

Ponatinib efficiently interfered with proviral integration (419), but also with the homeostasis and activation of CD4+ T cells driven by cytokines and TCR-mediated activation (399).

Despite the potent cytostatic effect of ponatinib, the participants did not show a significant reduction in the levels of CD4+T cells. However, although these mechanisms could achieve the reduction of the reservoir size, as has been previously determined with dasatinib (165), they do not explain the resistance of CD4+ T cells to HIV-1 infection. The lack of a significant correlation between HIV-1 infection levels in CD4+ T cells and the preservation of SAMHD1 antiviral activity suggested the existence of additional antiviral mechanisms that were triggered by ponatinib treatment and that could remain active even in the TFR phase.

Similar results were found in the 2 PWH and CML that were on long-term treatment with dasatinib, in which the CD4+ T cell count remained at similar levels to those of control groups of PWH for 5 years. Thus, the strong reduction in reservoir size that was observed in both individuals may not be caused by a significant decrease in CD4+ T cell counts. In fact, it has been observed that dasatinib preserves thymic function for regenerating lymphocytes, resulting in a low incidence of opportunistic infections (454). In both individuals, the distribution of CD4+ T subpopulations was similar to control groups, with more than 50% of the CD4+ T cells characterized as T_{EM} or T_{EMRA} subsets. In contrast, opposite results were found in mice engrafted with CD34+ hematopoietic stem cells that were treated with dasatinib before infecting them with HIV-1 (422). We hypothesize that the more frequent effector memory subpopulations found in our study were already differentiated in the context of chronic HIV-1 infection and cancer, and dasatinib treatment did not modify their distribution. HIV-1 reservoir frequency is highest in memory CD4+ T cell subpopulations (188,191,455–457), but it is still unknown whether the differentiation of CD4+ T cells towards memory phenotypes affects the reservoir size and its competence in PWH on ART, although CD4+ T susceptibility to HIV-1 infection increases with T cell differentiation (186,187).

Nonetheless, long-term treatment with dasatinib resulted in a significant reduction in reservoir size that was maintained even after treatment interruption and the switch to asciminib. The decrease in reservoir size is observed in intact and defective proviruses, with intact proviruses being undetectable in PBMCs of both participants during most of the dasatinib treatment. Of note, intact reservoirs were undetectable in PBMCs of blood samples in both participants, but HIV-1 preferentially persists within tissue-resident memory T cells (458). Although we were not able to measure HIV-1 reservoir size in tissues, the cytostatic effect of dasatinib may extend to tissue-resident memory CD4+ T cells, controlling HIV-1 reservoir maintenance. The decrease in defective proviruses in both participants, which are implicated in the chronic immune activation through the production

of viral transcripts and proteins (66,152,459), may involve reductions in both the chronic inflammation and associated comorbidities of HIV-1 infection.

The observed decrease in both intact and defective proviruses could be explained by the inhibition of homeostatic proliferation and TCR activation of CD4+ T cells, which may block clonal proliferation of T cells, as defective and intact genomes are found in clonal populations (231,447,460,461). One additional mechanism that may be involved in reducing reservoir size and the acquired resistance of CD4+ T cells to HIV-1 infection after treatment for one year with ponatinib is the reduction of metabolic activity in CD4+ T cells. In the absence of activation, CD4+ T cells are in a quiescent state with minimal metabolic activity (462), during which they are highly resistant to HIV-1 infection (187,242). HIV-1 selectively infects CD4+T cells that show a phenotype with high OXPHOS and glycolytic metabolism (462). In this activated state, cells upregulate dNTP production and SAMHD1 phosphorylation, favoring HIV-1 replication (462). The quiescent state induced by dasatinib and ponatinib treatment interfered with the metabolic activation of CD4+ T cells, which may drastically reduce the susceptibility of CD4+ T cells to HIV-1 infection. Our results indicate that dasatinib and ponatinib blocked HIV-1 infection not only by inhibiting the phosphorylation of SAMHD1, but also both mitochondrial and glycolytic metabolism through the induction of a quiescent state in CD4+ T cells.

These cytostatic effects are also extended to interfere with proviral reactivation in PWH and CML, which was maintained throughout all dasatinib treatment in the 2 PWH and CML. (165). Of note, we found residual proviral reactivation in total CD4+ T cells and also in the CD4+ subpopulations, independently of their differentiation status. This residual reactivation may proceed from defective proviruses, as we did not detect intact proviruses in PBMCs during all dasatinib treatment in the IPDA analysis. As we observed in total CD4+ T cells, the preservation of the antiviral activity of SAMHD1 and the inhibition of TCR-mediated activation in CD4+ T subpopulations may block HIV-1 replication, while the inhibition of mitochondrial and glycolytic metabolism induces a quiescent state of the cell that interferes with HIV-1 infection. Thus, the cytostatic effect of dasatinib is extended to all CD4+ subpopulations, which may interfere with the replication of intact (more frequent in T_{EM} cells (191–194)) and defective proviruses with unstable expression (more frequent in T_{EMRA} cells (197). We may also suggest that the strong cytostatic effect of dasatinib may counteract the increased susceptibility of CD4+ T cells as they differentiate into memory subpopulations (186,187), protecting them from HIV-1 infection. Furthermore, these effects were maintained after dasatinib treatment interruption or switch to asciminib.

However, there are other mechanisms involved in interfering with HIV-1 infection. In fact, it has been previously observed that TKIs may induce the proliferation and maturation of cytotoxic cell populations (405,406,416,463,464). In the cohort of individuals with CML from study I, treatment with ponatinib for one year resulted in increased cytotoxic activity against HIV-1-infected cells that was maintained after treatment interruption, which we correlated with the increased proliferation of NK and γδT CD8- cells. This enhanced cytotoxic activity was responsible for the resistance of CD4+T cells against HIV-1 one year after treatment interruption. Remarkably, CML individuals who discontinued dasatinib treatment have also shown increased cytotoxic activity that interfered with HIV-1 infection in autologous CD4+ T cells (416). In fact, dasatinib treatment induces the proliferation of NK and γδT cells with activated profiles (409,410,413,414) that may emerge after CMV reactivation (411,412) and that may be associated with the control of HIV-1 infection (417,418). In study I, most individuals were seropositive to CMV, which may have contributed to the proliferation of NK and γδT CD8- cells, as it has been previously observed that CMV reactivation during TKI treatment in CML individuals drives NK cell maturation into memory-like phenotypes that control the leukemia during treatment interruption (411,412). Thus, a low level of persistent CML reactivation may have induced the maturation of NK cells during ponatinib treatment, but we cannot discard that other mechanisms, such as the modulation of NK and γδT cell activity via the IL-15 signaling pathway (465,466), may be involved. The enhanced cytotoxic response after ponatinib treatment might also contribute to the antileukemic response, as has been described before (416,424,425), which could explain the absence of CML relapse after ponatinib interruption in all participants. However, more studies are necessary that include participants that relapse of CML during treatment interruption in order to establish the main factors that impact on the antileukemic response during TFR.

It has been described before that the expansion and maturation of NK cells is a key factor in the control of CML after TKI treatment discontinuation (467–469) and, in the present study, we demonstrated their antiviral potential to interfere with HIV-1 infection. Interestingly, we have also observed that dasatinib treatment suppresses the metabolic activity of untreated NK cells, but does not induce a cytostatic effect that could block activation by IL-15/IL-12/IL-18. Upon inhibition of mitochondrial metabolism, NK cells that were treated with IL15/12/18 and in the presence or absence of dasatinib could shift to glycolytic metabolism while showing increased OXPHOS and glycolysis compared to NK cells treated only with dasatinib. Prior studies have demonstrated that activation of NK cells triggers increased

glycolytic activity (470–475), and that this activation is maintained after the stimulation (476). The shift and increased glycolysis allow for rapid production of ATP, cellular proliferation, and synthesis of immune effector molecules (477). Nonetheless, NK cells still depend on both OXPHOS and glycolysis for the development of their immune response, as inhibition of either of both metabolic pathways resulted in decreased IFN-γ production (475,478). Our data suggest that dasatinib treatment may permit the activation of both mitochondrial and glycolytic metabolism upon cellular stimulation only in NK cells, which could lead to an increase in the cytotoxic response. Thus, due to the similarities to dasatinib in its mechanisms of action and off-target effects, we hypothesize that ponatinib may induce similar effects in the metabolism of NK cells, allowing cellular activation and the proliferation of these cells that are involved in interfering with HIV-1 infection and the elimination of latently infected cells.

Interestingly, the potent cytostatic effect of ponatinib on CD4+ T cells did not produce a significant decrease in the levels of CD8+ T cells. Notably, it has been reported that dasatinib treatment triggers the expansion of memory CD8+ T cells (479), and that memory CD8+ T cells are implicated in the deep antileukemic control after dasatinib treatment interruption (480). As in CD4+ T cells, we observed that treatment with either dasatinib or ponatinib produces a strong inhibitory effect on mitochondrial and glycolytic metabolism of CD8+ T cells. However, treatment with dasatinib can induce the proliferation of CD8+ T cells (405,407,408), which suggests that TKIs may inhibit the metabolic activation of these cells but not their proliferation. Remarkably, it has been reported that dasatinib treatment triggers the expansion of memory CD8+ T cells (479), which are also implicated in the deep antileukemic control after dasatinib treatment interruption (480). Effector memory and terminal memory subpopulations are characterized by their higher cytotoxic capacity (481). Thus, we hypothesize that ponatinib may induce similar effects to dasatinib in CD8+ T cells, and instead of causing lymphopenia, may be prompting the proliferation of memory subpopulations.

Treatment with imatinib in people with CML or in participant #004 with HIV and CML did not result in a significant interference with HIV-1 infection or the proliferation of cytotoxic cell populations. These observations suggest that imatinib may not induce the expansion of NK cells with antiviral potential, as ponatinib and dasatinib do. This could be related to the more specific activity of imatinib against BCR::ABL1 and the reduced off-target effects of this TKI compared to dasatinib and ponatinib (377,381,419,420). This expansion and maturation of NK cells as a consequence of TKI reprogramming seems to be a key factor in

the control of CML after treatment discontinuation (467–469), and also to stimulate their antiviral potential against HIV-1 infection, as described in Study I. Thus, the lack of NK cell expansion during imatinib treatment could at least partially explain the higher susceptibility of CD4+ T cells to HIV-1 infection in Study I. Furthermore, our results from Study II showed that imatinib treatment did not interfere with the maintenance and proliferation of HIV-1 reservoir. In fact, imatinib was determined to not block proviral integration in PBMCs of CML individuals infected *ex vivo* with HIV-1 (419), and also does not interfere with SAMHD1 phosphorylation (419). Moreover, imatinib showed low efficiency in interfering with homeostatic proliferation and TCR-mediated activation of T cells (399), which is necessary to inhibit HIV-1 reservoir replenishment.

An important limitation of Study II is the low incidence of CML in PWH (approximately, 1 person with CML for each 65,000 PWH) who are receiving dasatinib treatment. Therefore, more studies are necessary to directly confirm the impact of dasatinib treatment on the HIV-1 reservoir size and its competence in PWH. Notably, the recommended dose of dasatinib is 100mg per day, but our prior research identified 16nM (8.26ng/mL) as the threshold for achieving the antiviral efficacy of dasatinib (419). Consequently, the use of dasatinib in reduced concentrations in PWH would be a safer option while reducing the probability of side effects when used as adjuvant of ART in PWH. Indeed, the drastic reduction in the reservoir size of the two participants included in this study was achieved after one year of dasatinib treatment, and the antiviral effects of dasatinib were maintained after discontinuation in participant #004. These findings suggest that the administration of dasatinib in short periods of time may be a valid strategy for the induction of a potent antiviral response (422,482). Future studies are necessary to assess these hypotheses and also to analyze the effects of dasatinib in other cells that contain proviruses, such as macrophages or dendritic cells.

Overall, the intensified treatment with ponatinib in CML individuals resulted in a protective effect on CD4+ T cells against HIV-1 infection *in vitro*. This effect mostly relied on factors such as the inhibition of mitochondrial and glycolytic metabolism of CD4+ T cells, the preservation of the antiviral activity of SAMHD1, and the maintenance during treatment interruption of the expansion of cytotoxic cell populations, primarily NK and γδT cells, with antiviral potential. Moreover, the potent cytostatic effect of dasatinib and ponatinib was not extended to NK cells, which can be activated and proliferate even after treatment with both TKIs. These factors may also be involved in the low frequency of intact and defective proviruses in both participants with HIV and CML who were on long-term treatment with

dasatinib. However, due to the wide range of off-target effects of dasatinib, other still-undetermined mechanisms may also contribute to interfering with HIV-1 reservoir maintenance and proliferation, such as the inhibition of homeostatic proliferation of T cells induced by IL-7 or IL15, and the blockade of TCR-mediated activation through Lck inhibition.

CONCLUSIONS

1. Intensification treatment for one year with ponatinib in CML individuals was effective to protect CD4+ T cells against HIV-1 infection *in vitro* mostly due to its cytostatic effect that preserved SAMHD1 function.
2. The antiviral effects of ponatinib were extended to all CD4+ T cell subpopulations, independently of their differentiation status, which is crucial to interfere with HIV-1 reservoir maintenance.
3. The proliferation of cytotoxic populations, primarily NK and γδT cells, supported the persistence of antiviral protection after ponatinib treatment discontinuation.
4. Long-term treatment with dasatinib in two PWH and CML efficiently reduced HIV-1 reservoir size by interfering with homeostatic proliferation of CD4+ T cells and preserving SAMHD1 antiviral activity.
5. Intact proviruses were undetectable in PBMCs from PWH and CML after one year of treatment with dasatinib.
6. The inhibition of SAMHD1 phosphorylation by dasatinib treatment caused the reduction of proviral reactivation in PWH and CML, which was maintained during dasatinib discontinuation or after modification of the TKI regimen.
7. Dasatinib and ponatinib induced a strong cytostatic effect that inhibited mitochondrial and glycolytic metabolism in CD4+ T cells.
8. The cytostatic effect also blocked the metabolic activity of CD8+ T cells, although apparently dasatinib did not affect the proliferative capacity of these cells.
9. NK cells treated with dasatinib and IL-15/IL-12/IL-18 presented increased OXPHOS and glycolytic activity, confirming the enhanced proliferative effect of dasatinib on these cells.
10. Reprogramming the immune system via transient treatment with dasatinib or ponatinib as adjuvants with ART may produce a persistent antiviral state in PWH. This approach may significantly reduce the reservoir size and suppress proviral reactivation, efficiently controlling HIV-1 infection. This strategy holds promise for achieving a functional cure in individuals with chronic HIV-1 infection.

CONCLUSIONES

1. El tratamiento de intensificación con ponatinib durante un año en personas con LMC fue efectivo en proteger a los linfocitos T CD4+ contra la infección por VIH-1 *in vitro* gracias al efecto citostático que preservó la función de SAMHD1.
2. Los efectos antivirales de ponatinib se extendieron a todas las subpoblaciones de linfocitos T CD4+, independientemente de su estado de diferenciación, siendo crucial para interferir con el mantenimiento del reservorio de VIH-1.
3. La proliferación de poblaciones celulares citotóxicas, principalmente células NK y γδT, apoyó la persistencia de la protección antiviral tras la discontinuación del tratamiento con ponatinib.
4. El tratamiento a largo plazo con dasatinib en dos PCV y LMC redujo eficientemente el tamaño del reservorio de VIH-1 gracias a la interferencia de este ITK con la proliferación homeostática de los linfocitos T CD4+ y la preservación de la actividad antiviral de SAMHD1.
5. Los provirus intactos fueron indetectables en ambas PCV y LMC tras un año de tratamiento con dasatinib.
6. La inhibición de la fosforilación de SAMHD1 por el tratamiento con dasatinib causó la reducción de la reactivación proviral en PCV y LMC, lo cual se mantuvo durante la discontinuación del tratamiento con dasatinib o la modificación del ITK utilizado en el tratamiento.
7. Dasatinib y ponatinib indujeron un potente efecto citostático que inhibió el metabolismo mitocondrial y glicolítico de los linfocitos T CD4+.
8. El efecto citostático también bloqueó la actividad metabólica de los linfocitos T CD8+, aunque aparentemente dasatinib no afectaba a la capacidad proliferativa de estas células.
9. Las células NK tratadas con dasatinib junto con IL-15/IL-12/IL-18 presentaron incrementos en la actividad de OXPHOS y la glicólisis, confirmando el efecto proliferativo de dasatinib en estas células.
10. La reprogramación del sistema inmunitario mediante el tratamiento transitorio con dasatinib o ponatinib como adyuvantes de TAR podría generar un estado antiviral persistente en PCV. Este enfoque podría reducir significativamente el tamaño del reservorio viral y suprimir la reactivación proviral, controlando eficazmente la infección por VIH-1. Esta estrategia representa un avance prometedor hacia la consecución de una cura funcional en individuos con infección crónica por VIH-1.

REFERENCES

1. Centers for Disease Control (CDC). Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. MMWR Morb Mortal Wkly Rep. 1981;30(25):305–8.

2. What to call the AIDS virus? Nature. 1986;321(6065):10–10.

3. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). Science. 1983;220(4599):868–71.

4. 2024 global AIDS report — The Urgency of Now: AIDS at a Crossroads | UNAIDS

5. Report UNAIDS 2023.

6. Soriano B, Krupovic M, Llorens C. ICTV Virus Taxonomy Profile: Belpaoviridae 2021. J Gen Virol. 2021;102(11):001688.

7. Hahn BH, Shaw GM, De KM, Cock, Sharp PM. AIDS as a Zoonosis: Scientific and Public Health Implications. Science. 2000;287(5453):607–14.

8. Simon F, Mauclère P, Roques P, Loussert-Ajaka I, Müller-Trutwin MC, Saragosti S, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med. 1998;4(9):1032–7.

9. Roques P, Robertson DL, Souquière S, Apetrei C, Nerrienet E, Barré-Sinoussi F, et al. Phylogenetic characteristics of three new HIV-1 N strains and implications for the origin of group N. AIDS. 2004;18(10):1371.

10. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemée V, et al. A new human immunodeficiency virus derived from gorillas. Nat Med. 2009;15(8):871–2.

11. Ayouba A, Mauclère P, Martin PMV, Cunin P, Mfoupouendoun J, Njinku B, et al. HIV-1 Group O Infection in Cameroon, 1986–1998. Infect Genet Evol. 2015; 36: 210-216.

12. Vallari A, Holzmayer V, Harris B, Yamaguchi J, Ngansop C, Makamche F, et al. Confirmation of Putative HIV-1 Group P in Cameroon. J Virol. 2011;85(3):1403–7.

13. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature. 1999;397(6718):436–41.

14. Keele BF, Van Heuverswyn F, Li Y, Bailes E, Takehisa J, Santiago ML, et al. Chimpanzee Reservoirs of Pandemic and Nonpandemic HIV-1. Science. 2006;313(5786):523–6.

15. Eberle J, Gürtler L. HIV Types, Groups, Subtypes and Recombinant Forms: Errors in Replication, Selection Pressure and Quasispecies. Intervirology. 2012;55(2):79–83.

16. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, et al. Timing the Ancestor of the HIV-1 Pandemic Strains. Science. 2000;288(5472):1789–96.

17. Barin F, Denis F, Allan JS, M'Boup S, Kanki P, Lee TH, et al. Serological evidence for virus related to simian T-lymphotropic retrovirus III in residents of West Africa. The Lancet. 1985;326(8469):1387–9.

18. Gottlieb GS, Sow PS, Hawes SE, Ndoye I, Coll-Seck AM, Curlin ME, et al. Molecular Epidemiology of Dual HIV-1/HIV-2 Seropositive Adults from Senegal, West Africa. AIDS Res Human Retroviruses. 2003; 19(7):575-84.

19. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. Curr Opin HIV AIDS. 2019; (3):153.

20. de Mendoza C, Cabezas T, Caballero E, Requena S, Amengual MJ, Peñaranda M, et al. HIV type 2 epidemic in Spain: challenges and missing opportunities. AIDS. 2017 ;31(10):1353.

21. HIV-2 Infection Surveillance --- United States, 1987--2009

22. Werner A, Staszewski S, Helm EB, Stille W, Weber K, Kurth R. HIV-2 (WEST GERMANY, 1984). The Lancet. 1987;329(8537):868–9.

23. Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, et al. Reduced Rate of Disease Development After HIV-2 Infection as Compared to HIV-1. Science. 1994;265(5178):1587–90.

24. Jaffar S, Wilkins A, Ngom PT, Sabally S, Corrah T, Bangali JE, et al. Rate of Decline of Percentage CD4+ Cells Is Faster in HIV-1 Than in HIV-2 Infection. JAIDS J Acquir Immune Defic Syndr. 1997;16(5):327.

25. MacNeil A, Sarr AD, Sankalé JL, Meloni ST, Mboup S, Kanki P. Direct Evidence of Lower Viral Replication Rates In Vivo in Human Immunodeficiency Virus Type 2 (HIV-2) Infection than in HIV-1 Infection. J Virol. 2007;81(10):5325–30.

26. Leligdowicz A, Feldmann J, Jaye A, Cotten M, Dong T, McMichael A, et al. Direct Relationship between Virus Load and Systemic Immune Activation in HIV-2 Infection. J Infect Dis. 2010;201(1):114–22.

27. Ceccarelli G, Giovanetti M, Sagnelli C, Ciccozzi A, d'Ettorre G, Angeletti S, et al. Human Immunodeficiency Virus Type 2: The Neglected Threat. Pathogens. 2021;10(11):1377.

28. Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich B, Josephs SF, et al. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature. 1985;313(6000):277–84.

29. Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. Annu Rev Biochem. 1998;67:1–25.

30. Rabson AB, Martin MA. Molecular organization of the AIDS retrovirus. Cell. 1985 ;40(3):477–80.

31. Greene WC, Peterlin BM. Charting HIV's remarkable voyage through the cell: Basic science as a passport to future therapy. Nat Med. 2002;8(7):673–80.

32. Li G, Clercq ED. HIV Genome-Wide Protein Associations: a Review of 30 Years of Research. Microbiol Mol Biol Rev. 2016.

33. van Heuvel Y, Schatz S, Rosengarten JF, Stitz J. Infectious RNA: Human Immunodeficiency Virus (HIV) Biology, Therapeutic Intervention, and the Quest for a Vaccine. Toxins. 2022;14(2):138.

34. Freed EO. HIV-1 assembly, release and maturation. Nat Rev Microbiol. 2015 ;13(8):484–96.

35. Chojnacki J, Eggeling C. Super-resolution fluorescence microscopy studies of human immunodeficiency virus. Retrovirology. 2018;15(1):41.

36. Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, et al. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature. 1996;384(6605):179–83.

37. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, et al. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature. 1996;382(6594):833–5.

38. Fauci AS. Host factors and the pathogenesis of HIV-induced disease. Nature. 1996 ;384(6609):529–34.

39. Moscoso CG, Sun Y, Poon S, Xing L, Kan E, Martin L, et al. Quaternary structures of HIV Env immunogen exhibit conformational vicissitudes and interface diminution elicited by ligand binding. Proc Natl Acad Sci U S A. 2011;108(15):6091–6.

40. El-Zohairy MA, Zlotos DP, Berger MR, Adwan HH, Mandour YM. Discovery of Novel CCR5 Ligands as Anticolorectal Cancer Agents by Sequential Virtual Screening. ACS Omega. 2021;6(16):10921–35.

41. Dharan A, Bachmann N, Talley S, Zwikelmaier V, Campbell EM. Nuclear pore blockade reveals that HIV-1 completes reverse transcription and uncoating in the nucleus. Nat Microbiol. 2020;5(9):1088–95.

42. Zila V, Margiotta E, Turoňová B, Müller TG, Zimmerli CE, Mattei S, et al. Cone-shaped HIV-1 capsids are transported through intact nuclear pores. Cell. 2021 Feb 18;184(4):1032-1046.e18.

43. Domingo E, Perales C. Quasispecies and virus. Eur Biophys J EBJ. 2018;47(4):443–57.

44. Hu WS, Hughes SH. HIV-1 reverse transcription. Cold Spring Harb Perspect Med. 2012;2(10):a006882.

45. Chiu TK, Davies DR. Structure and function of HIV-1 integrase. Curr Top Med Chem. 2004;4(9):965–77.

46. Hazuda DJ. HIV integrase as a target for antiretroviral therapy. Curr Opin HIV AIDS. 2012;7(5):383–9.

47. Craigie R, Bushman FD. HIV DNA integration. Cold Spring Harb Perspect Med. 2012;2(7):a006890.

48. Schröder ARW, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. Cell. 2002;110(4):521–9.

49. Debyser Z, Christ F, De Rijck J, Gijsbers R. Host factors for retroviral integration site selection. Trends Biochem Sci. 2015;40(2):108–16.

50. Li P, Kaiser P, Lampiris HW, Kim P, Yukl SA, Havlir DV, et al. Stimulating the RIG-I pathway to kill cells in the latent HIV reservoir following viral reactivation. Nat Med. 2016;22(7):807–11.

51. Lian X, Seiger KW, Parsons EM, Gao C, Sun W, Gladkov GT, et al. Progressive transformation of the HIV-1 reservoir cell profile over two decades of antiviral therapy. Cell Host Microbe. 2023;31(1):83-96.e5.

52. Roadmap Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518(7539):317–30.

53. Jordan A, Defechereux P, Verdin E. The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transactivation. EMBO J. 2001;20(7):1726–38.

54. Battivelli E, Dahabieh MS, Abdel-Mohsen M, Svensson JP, Tojal Da Silva I, Cohn LB, et al. Distinct chromatin functional states correlate with HIV latency reactivation in infected primary CD4+ T cells. eLife. 2018;7:e34655.

55. Einkauf KB, Osborn MR, Gao C, Sun W, Sun X, Lian X, et al. Parallel analysis of transcription, integration, and sequence of single HIV-1 proviruses. Cell. 2022;185(2):266-282.e15.

56. Dragoni F, Kwaa AK, Traut CC, Veenhuis RT, Woldemeskel BA, Camilo-Contreras A, et al. Proviral location affects cognate peptide-induced virus production and immune recognition of HIV-1-infected T cell clones. J Clin Invest. 2023;133(21):e171097.

57. Chen BK, Feinberg MB, Baltimore D. The kappaB sites in the human immunodeficiency virus type 1 long terminal repeat enhance virus replication yet are not absolutely required for viral growth. J Virol. 1997;71(7):5495–504.

58. Kao SY, Calman AF, Luciw PA, Peterlin BM. Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product. Nature. 1987;330(6147):489–93.

59. Barboric M, Peterlin BM. A new paradigm in eukaryotic biology: HIV Tat and the control of transcriptional elongation. PLoS Biol. 2005;3(2):e76.

60. McNamara RP, McCann JL, Gudipaty SA, D'Orso I. Transcription factors mediate the enzymatic disassembly of promoter-bound 7SK snRNP to locally recruit P-TEFb for transcription elongation. Cell Rep. 2013;5(5):1256–68.

61. Peterlin BM, Price DH. Controlling the elongation phase of transcription with P-TEFb. Mol Cell. 2006;23(3):297–305.

62. Malim MH, Hauber J, Le SY, Maizel JV, Cullen BR. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature. 1989;338(6212):254–7.

63. Kiernan RE, Vanhulle C, Schiltz L, Adam E, Xiao H, Maudoux F, et al. HIV-1 tat transcriptional activity is regulated by acetylation. EMBO J. 1999;18(21):6106–18.

64. Dahiya S, Nonnemacher MR, Wigdahl B. Deployment of the human immunodeficiency virus type 1 protein arsenal: combating the host to enhance viral transcription and providing targets for therapeutic development. J Gen Virol. 2012;93(Pt 6):1151–72.

65. Imamichi H, Dewar RL, Adelsberger JW, Rehm CA, O'Doherty U, Paxinos EE, et al. Defective HIV-1 proviruses produce novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy. Proc Natl Acad Sci U S A. 2016;113(31):8783–8.

66. Imamichi H, Smith M, Adelsberger JW, Izumi T, Scrimieri F, Sherman BT, et al. Defective HIV-1 proviruses produce viral proteins. Proc Natl Acad Sci U S A. 2020;117(7):3704–10.

67. Klein JS, Bjorkman PJ. Few and far between: how HIV may be evading antibody avidity. PLoS Pathog. 2010;6(5):e1000908.

68. Izquierdo-Useros N, Puertas MC, Borràs FE, Blanco J, Martinez-Picado J. Exosomes and retroviruses: the chicken or the egg? Cell Microbiol. 2011;13(1):10–7.

69. Hurley JH, Cada AK. Inside job: how the ESCRTs release HIV-1 from infected cells. Biochem Soc Trans. 2018;46(5):1029–36.

70. Schaefer MR, Wonderlich ER, Roeth JF, Leonard JA, Collins KL. HIV-1 Nef targets MHC-I and CD4 for degradation via a final common beta-COP-dependent pathway in T cells. PLoS Pathog. 2008;4(8):e1000131.

71. Könnyű B, Sadiq SK, Turányi T, Hírmondó R, Müller B, Kräusslich HG, et al. Gag-Pol processing during HIV-1 virion maturation: a systems biology approach. PLoS Comput Biol. 2013;9(6):e1003103.

72. Mouhand A, Pasi M, Catala M, Zargarian L, Belfetmi A, Barraud P, et al. Overview of the Nucleic-Acid Binding Properties of the HIV-1 Nucleocapsid Protein in Its Different Maturation States. Viruses. 2020;12(10):1109.

73. Blakemore RJ, Burnett C, Swanson C, Kharytonchyk S, Telesnitsky A, Munro JB. Stability and conformation of the dimeric HIV-1 genomic RNA 5'UTR. Biophys J. 2021;120(21):4874–90.

74. Kleinpeter AB, Freed EO. HIV-1 Maturation: Lessons Learned from Inhibitors. Viruses. 2020;12(9):940.

75. Masenga SK, Mweene BC, Luwaya E, Muchaili L, Chona M, Kirabo A. HIV–Host Cell Interactions. Cells. 2023;12(10).

76. Shaw GM, Hunter E. HIV transmission. Cold Spring Harb Perspect Med. 2012 Nov 1;2(11):a006965.

77. Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. Nat Rev Microbiol. 2003;1(1):25–34.

78. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. N Engl J Med. 2011;364(20):1943–54.

79. Simon V, Ho DD, Karim QA. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. Lancet. 2006;368(9534):489–504.

80. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci U S A. 2003;100(7):4144–9.

81. Alcamí J, Coiras M. Immunopathogenesis of HIV infection. Enferm Infecc Microbiol Clin. 2011;29(3):216–26.

82. Macneal RJ, Dinulos JGH. Acute retroviral syndrome. Dermatol Clin. 2006;24(4):431–8, v.

83. Huang X, Chen H, Li W, Li H, Jin X, Perelson AS, et al. Precise determination of time to reach viral load set point after acute HIV-1 infection. J Acquir Immune Defic Syndr 1999. 2012;61(4):448–54.

84. McCune JM. The dynamics of CD4+ T-cell depletion in HIV disease. Nature. 2001;410(6831):974–9.

85. Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. Annu Rev Immunol. 2003;21:265–304.

86. Desai S, Landay A. Early immune senescence in HIV disease. Curr HIV/AIDS Rep. 2010;7(1):4–10.

87. Melhuish A, Lewthwaite P. Natural history of HIV and AIDS. Medicine. 2022;50(5):298–303.

88. Poorolajal J, Hooshmand E, Mahjub H, Esmailnasab N, Jenabi E. Survival rate of AIDS disease and mortality in HIV-infected patients: a meta-analysis. Public Health. 2016;139:3–12.

89. Bekker LG, Beyrer C, Mgodi N, Lewin SR, Delany-Moretlwe S, Taiwo B, et al. HIV infection. Nat Rev Dis Primer. 2023;9(1):1–21.

90. Chang JJ, Lacas A, Lindsay RJ, Doyle EH, Axten KL, Pereyra F, et al. Differential regulation of toll-like receptor pathways in acute and chronic HIV-1 infection. AIDS Lond Engl. 2012;26(5):533–41.

91. Shuai K, Liu B. Regulation of JAK-STAT signalling in the immune system. Nat Rev Immunol. 2003;3(11):900–11.

92. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124(4):783–801.

93. Nazli A, Kafka JK, Ferreira VH, Anipindi V, Mueller K, Osborne BJ, et al. HIV-1 gp120 induces TLR2- and TLR4-mediated innate immune activation in human female genital epithelium. J Immunol Baltim Md 1950. 2013;191(8):4246–58.

94. Berg RK, Melchjorsen J, Rintahaka J, Diget E, Søby S, Horan KA, et al. Genomic HIV RNA induces innate immune responses through RIG-I-dependent sensing of secondary-structured RNA. PloS One. 2012;7(1):e29291.

95. Stetson DB, Medzhitov R. Type I interferons in host defense. Immunity. 2006 ;25(3):373–81.

96. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature. 2011;472(7344):481–5.

97. Neil SJD, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature. 2008;451(7177):425–30.

98. Hotter D, Sauter D, Kirchhoff F. Emerging role of the host restriction factor tetherin in viral immune sensing. J Mol Biol. 2013;425(24):4956–64.

99. Pertel T, Hausmann S, Morger D, Züger S, Guerra J, Lascano J, et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. Nature. 2011;472(7343):361–5.

100. Stopak K, de Noronha C, Yonemoto W, Greene WC. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol Cell. 2003;12(3):591–601.

101. Cribier A, Descours B, Valadão ALC, Laguette N, Benkirane M. Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. Cell Rep. 2013;3(4):1036–43.

102. Doyle T, Goujon C, Malim MH. HIV-1 and interferons: who's interfering with whom? Nat Rev Microbiol. 2015;13(7):403–13.

103. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023-.

104. Fauci AS, Mavilio D, Kottilil S. NK cells in HIV infection: paradigm for protection or targets for ambush. Nat Rev Immunol. 2005;5(11):835–43.

105. Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with $CD8^+$ T cells. Nat Rev Immunol. 2011;11(10):645–57.

106. Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, et al. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. J Virol. 2009;83(8):3719–33.

107. Kazer SW, Walker BD, Shalek AK. Evolution and Diversity of Immune Responses during Acute HIV Infection. Immunity. 2020;53(5):908–24.

108. McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. The immune response during acute HIV-1 infection: clues for vaccine development. Nat Rev Immunol. 2010;10(1):11–23.

109. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. Nature. 2003;422(6929):307–12.

110. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol. 2015;135(3):626–35.

111. Punnonen J, Yssel H, de Vries JE. The relative contribution of IL-4 and IL-13 to human IgE synthesis induced by activated CD4+ or CD8+ T cells. J Allergy Clin Immunol. 1997;100(6 Pt 1):792–801.

112. Sallusto F. Heterogeneity of Human CD4(+) T Cells Against Microbes. Annu Rev Immunol. 2016;34:317–34.

113. Neurath MF, Kaplan MH. Th9 cells in immunity and immunopathological diseases. Semin Immunopathol. 2017;39(1):1–4.

114. Végran F, Berger H, Boidot R, Mignot G, Bruchard M, Dosset M, et al. The transcription factor IRF1 dictates the IL-21-dependent anticancer functions of TH9 cells. Nat Immunol. 2014;15(8):758–66.

115. Jia L, Wu C. The biology and functions of Th22 cells. Adv Exp Med Biol. 2014;841:209–30.

116. Archer NK, Adappa ND, Palmer JN, Cohen NA, Harro JM, Lee SK, et al. Interleukin-17A (IL-17A) and IL-17F Are Critical for Antimicrobial Peptide Production and Clearance of Staphylococcus aureus Nasal Colonization. Infect Immun. 2016;84(12):3575–83.

117. Valverde-Villegas JM, Matte MCC, de Medeiros RM, Chies JAB. New Insights about Treg and Th17 Cells in HIV Infection and Disease Progression. J Immunol Res. 2015;2015:647916.

118. Jeger-Madiot R, Heredia M, Graff-Dubois S. Germinal centers B-cell reaction and T follicular helper cells in response to HIV-1 infection. Curr Opin HIV AIDS. 2019;14(4):246–52.

119. Terzieva V, Popova D, Kicheva M, Todorova Y, Markova R, Martinova F, et al. Correlation between the degree of immune activation, production of IL-2 and FOXP3 expression in CD4+CD25+ T regulatory cells in HIV-1 infected persons under HAART. Int Immunopharmacol. 2009;9(7–8):831–6.

120. Goonetilleke N, Liu MKP, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, et al. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J Exp Med. 2009;206(6):1253–72.

121. Leslie AJ, Pfafferott KJ, Chetty P, Draenert R, Addo MM, Feeney M, et al. HIV evolution: CTL escape mutation and reversion after transmission. Nat Med. 2004;10(3):282–9.

122. Demers KR, Makedonas G, Buggert M, Eller MA, Ratcliffe SJ, Goonetilleke N, et al. Temporal Dynamics of CD8+ T Cell Effector Responses during Primary HIV Infection. PLoS Pathog. 2016;12(8):e1005805.

123. He Y, Wu K, Hu Y, Sheng L, Tie R, Wang B, et al. γδ T cell and other immune cells crosstalk in cellular immunity. J Immunol Res. 2014;2014:960252.

124. Vermijlen D, Ellis P, Langford C, Klein A, Engel R, Willimann K, et al. Distinct cytokine-driven responses of activated blood gammadelta T cells: insights into unconventional T cell pleiotropy. J Immunol Baltim Md 1950. 2007;178(7):4304–14.

125. Miyagawa F, Tanaka Y, Yamashita S, Minato N. Essential requirement of antigen presentation by monocyte lineage cells for the activation of primary human gamma delta T cells by aminobisphosphonate antigen. J Immunol Baltim Md 1950. 2001;166(9):5508–14.

126. Ismaili J, Olislagers V, Poupot R, Fournié JJ, Goldman M. Human gamma delta T cells induce dendritic cell maturation. Clin Immunol Orlando Fla. 2002;103(3 Pt 1):296–302.

127. Sacchi A, Rinaldi A, Tumino N, Casetti R, Agrati C, Turchi F, et al. HIV infection of monocytes-derived dendritic cells inhibits Vγ9Vδ2 T cells functions. PloS One. 2014;9(10):e111095.

128. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, et al. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis. 2008;197(1):126–33.

129. Rodger AJ, Fox Z, Lundgren JD, Kuller LH, Boesecke C, Gey D, et al. Activation and coagulation biomarkers are independent predictors of the development of opportunistic disease in patients with HIV infection. J Infect Dis. 2009;200(6):973–83.

130. Ganesan A, Chattopadhyay PK, Brodie TM, Qin J, Gu W, Mascola JR, et al. Immunologic and virologic events in early HIV infection predict subsequent rate of progression. J Infect Dis. 2010;201(2):272–84.

131. Lv T, Cao W, Li T. HIV-Related Immune Activation and Inflammation: Current Understanding and Strategies. J Immunol Res. 2021;2021(1):7316456.

132. Zicari S, Sessa L, Cotugno N, Ruggiero A, Morrocchi E, Concato C, et al. Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. Viruses. 2019;11(3):200.

133. Katusiime MG, Van Zyl GU, Cotton MF, Kearney MF. HIV-1 Persistence in Children during Suppressive ART. Viruses. 2021;13(6):1134.

134. Shah A, Verma AS, Patel KH, Noel R, Rivera-Amill V, Silverstein PS, et al. HIV-1 gp120 induces expression of IL-6 through a nuclear factor-kappa B-dependent mechanism: suppression by gp120 specific small interfering RNA. PloS One. 2011;6(6):e21261.

135. Henrick BM, Yao XD, Rosenthal KL, INFANT study team. HIV-1 Structural Proteins Serve as PAMPs for TLR2 Heterodimers Significantly Increasing Infection and Innate Immune Activation. Front Immunol. 2015;6:426.

136. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12(12):1365–71.

137. Mogensen TH, Melchjorsen J, Larsen CS, Paludan SR. Innate immune recognition and activation during HIV infection. Retrovirology. 2010;7:54.

138. Müller-Trutwin M, Hosmalin A. Role for plasmacytoid dendritic cells in anti-HIV innate immunity. Immunol Cell Biol. 2005;83(5):578–83.

139. Herbein G, Khan KA. Is HIV infection a TNF receptor signalling-driven disease? Trends Immunol. 2008;29(2):61–7.

140. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin Dev Immunol. 2012;2012:670957.

141. Sokoya T, Steel HC, Nieuwoudt M, Rossouw TM. HIV as a Cause of Immune Activation and Immunosenescence. Mediators Inflamm. 2017;2017(1):6825493.

142. Bandera A, Ferrario G, Saresella M, Marventano I, Soria A, Zanini F, et al. CD4+ T cell depletion, immune activation and increased production of regulatory T cells in the thymus of HIV-infected individuals. PloS One. 2010;5(5):e10788.

143. Blankson JN, Persaud D, Siliciano RF. The Challenge of Viral Reservoirs in HIV-1 Infection. Annu Rev Med. 2002;53(Volume 53, 2002):557–93.

144. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature. 1997;387(6629):183–8.

145. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med. 2003 Jun;9(6):727–8.

146. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, et al. Defective proviruses rapidly accumulate during acute HIV-1 infection. Nat Med. 2016;22(9):1043–9.

147. Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, Ohmiti K, et al. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nat Med. 2016;22(7):754–61.

148. Chun TW, Nickle DC, Justement JS, Meyers JH, Roby G, Hallahan CW, et al. Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. J Infect Dis. 2008;197(5):714–20.

149. Yukl SA, Gianella S, Sinclair E, Epling L, Li Q, Duan L, et al. Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. J Infect Dis. 2010;202(10):1553–61.

150. Estes JD, Kityo C, Ssali F, Swainson L, Makamdop KN, Del Prete GQ, et al. Defining total-body AIDS-virus burden with implications for curative strategies. Nat Med. 2017;23(11):1271–6.

151. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature. 1993;362(6418):355–8.

152. Pollack RA, Jones RB, Pertea M, Bruner KM, Martin AR, Thomas AS, et al. Defective HIV-1 Proviruses Are Expressed and Can Be Recognized by Cytotoxic T Lymphocytes, which Shape the Proviral Landscape. Cell Host Microbe. 2017;21(4):494-506.e4.

153. The Last Gift Study. Available from: https://lastgift.ucsd.edu/

154. Chaillon A, Gianella S, Dellicour S, Rawlings SA, Schlub TE, De Oliveira MF, et al. HIV persists throughout deep tissues with repopulation from multiple anatomical sources. J Clin Invest. 2020;130(4):1699–712.

155. Wang C, Schlub TE, Yu WH, Tan CS, Stefic K, Gianella S, et al. Landscape of Human Immunodeficiency Virus Neutralization Susceptibilities Across Tissue Reservoirs. Clin Infect Dis Off Publ Infect Dis Soc Am. 2022;75(8):1342–50.

156. Brussel A, Sonigo P. Analysis of early human immunodeficiency virus type 1 DNA synthesis by use of a new sensitive assay for quantifying integrated provirus. J Virol. 2003;77(18):10119–24.

157. Brussel A, Delelis O, Sonigo P. Alu-LTR real-time nested PCR assay for quantifying integrated HIV-1 DNA. Methods Mol Biol Clifton NJ. 2005;304:139–54.

158. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DIS, et al. Replication-competent non-induced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell. 2013;155(3):540.

159. Brodin J, Zanini F, Thebo L, Lanz C, Bratt G, Neher RA, et al. Establishment and stability of the latent HIV-1 DNA reservoir. eLife. 2016;5:e18889.

160. Henrich TJ, Hatano H, Bacon O, Hogan LE, Rutishauser R, Hill A, et al. HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study. PLoS Med. 2017;14(11):e1002417.

161. Puertas MC, Bayón-Gil Á, Garcia-Guerrero MC, Salgado M, Urrea V, Morón-López S, et al. VIP-SPOT: an Innovative Assay To Quantify the Productive HIV-1 Reservoir in the Monitoring of Cure Strategies. mBio. 12(3):e00560-21.

162. Bruner KM, Wang Z, Simonetti FR, Bender AM, Kwon KJ, Sengupta S, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. Nature. 2019;566(7742):120–5.

163. Kwon KJ, Timmons AE, Sengupta S, Simonetti FR, Zhang H, Hoh R, et al. Different human resting memory CD4+ T cell subsets show similar low inducibility of latent HIV-1 proviruses. Sci Transl Med. 2020;12(528):eaax6795.

164. Kinloch NN, Ren Y, Conce Alberto WD, Dong W, Khadka P, Huang SH, et al. HIV-1 diversity considerations in the application of the Intact Proviral DNA Assay (IPDA). Nat Commun. 2021;12(1):165.

165. Vigón L, Martínez-Román P, Rodríguez-Mora S, Torres M, Puertas MC, Mateos E, et al. Provirus reactivation is impaired in HIV-1 infected individuals on treatment with dasatinib and antiretroviral therapy. Biochem Pharmacol. 2021;192.

166. Passaes CPB, Bruel T, Decalf J, David A, Angin M, Monceaux V, et al. Ultrasensitive HIV-1 p24 Assay Detects Single Infected Cells and Differences in Reservoir Induction by Latency Reversal Agents. J Virol. 2017;91(6):e02296-16.

167. Abdel-Mohsen M, Richman D, Siliciano RF, Nussenzweig MC, Howell B, Martinez-Picado J, et al. Recommendations for measuring HIV reservoir size in cure-directed clinical trials. Nat Med. 2020;26(9):1339–50.

168. Grau-Expósito J, Serra-Peinado C, Miguel L, Navarro J, Curran A, Burgos J, et al. A Novel Single-Cell FISH-Flow Assay Identifies Effector Memory CD4+ T cells as a Major Niche for HIV-1 Transcription in HIV-Infected Patients. mBio. 2017;8(4):e00876-17.

169. Baxter AE, Niessl J, Fromentin R, Richard J, Porichis F, Massanella M, et al. Multiparametric characterization of rare HIV-infected cells using an RNA-flow FISH technique. Nat Protoc. 2017;12(10):2029–49.

170. Procopio FA, Fromentin R, Kulpa DA, Brehm JH, Bebin AG, Strain MC, et al. A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals. EBioMedicine. 2015;2(8):874–83.

171. Deleage C, Chan CN, Busman-Sahay K, Estes JD. Next-generation in situ hybridization approaches to define and quantify HIV and SIV reservoirs in tissue microenvironments. Retrovirology. 2018;15(1):4.

172. Deleage C, Wietgrefe SW, Del Prete G, Morcock DR, Hao XP, Piatak M, et al. Defining HIV and SIV Reservoirs in Lymphoid Tissues. Pathog Immun. 2016;1(1):68–106.

173. Papasavvas E, Azzoni L, Ross BN, Fair M, Yuan Z, Gyampoh K, et al. Intact Human Immunodeficiency Virus (HIV) Reservoir Estimated by the Intact Proviral DNA Assay Correlates With Levels of Total and Integrated DNA in the Blood During Suppressive Antiretroviral Therapy. Clin Infect Dis Off Publ Infect Dis Soc Am. 2021;72(3):495–8.

174. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, et al. HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014;345(6193):179–83.

175. Anderson EM, Maldarelli F. The role of integration and clonal expansion in HIV infection: live long and prosper. Retrovirology. 2018;15(1):71.

176. Einkauf KB, Lee GQ, Gao C, Sharaf R, Sun X, Hua S, et al. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. J Clin Invest. 2019;129(3):988–98.

177. Patro SC, Brandt LD, Bale MJ, Halvas EK, Joseph KW, Shao W, et al. Combined HIV-1 sequence and integration site analysis informs viral dynamics and allows reconstruction of replicating viral ancestors. Proc Natl Acad Sci U S A. 2019;116(51):25891–9.

178. Crooks AM, Bateson R, Cope AB, Dahl NP, Griggs MK, Kuruc JD, et al. Precise Quantitation of the Latent HIV-1 Reservoir: Implications for Eradication Strategies. J Infect Dis. 2015;212(9):1361–5.

179. Shan L, Deng K, Gao H, Xing S, Capoferri AA, Durand CM, et al. Transcriptional Reprogramming during Effector-to-Memory Transition Renders CD4+ T Cells Permissive for Latent HIV-1 Infection. Immunity. 2017;47(4):766-775.e3.

180. Saleh S, Solomon A, Wightman F, Xhilaga M, Cameron PU, Lewin SR. CCR7 ligands CCL19 and CCL21 increase permissiveness of resting memory CD4+ T cells to HIV-1 infection: a novel model of HIV-1 latency. Blood. 2007;110(13):4161–4.

181. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat Med. 1995;1(12):1284–90.

182. Clark IC, Mudvari P, Thaploo S, Smith S, Abu-Laban M, Hamouda M, et al. HIV silencing and cell survival signatures in infected T cell reservoirs. Nature. 2023;614(7947):318–25.

183. Sun W, Gao C, Hartana CA, Osborn MR, Einkauf KB, Lian X, et al. Phenotypic signatures of immune selection in HIV-1 reservoir cells. Nature. 2023;614(7947):309–17.

184. Corneau A, Cosma A, Even S, Katlama C, Le Grand R, Frachet V, et al. Comprehensive Mass Cytometry Analysis of Cell Cycle, Activation, and Coinhibitory Receptors Expression in CD4 T Cells from Healthy and HIV-Infected Individuals. Cytometry B Clin Cytom. 2017;92(1):21–32.

185. Gálvez C, Grau-Expósito J, Urrea V, Clotet B, Falcó V, Buzón MJ, et al. Atlas of the HIV-1 Reservoir in Peripheral CD4 T Cells of Individuals on Successful Antiretroviral Therapy. mBio. 2021;12(6):e0307821.

186. Schnittman SM, Lane HC, Greenhouse J, Justement JS, Baseler M, Fauci AS. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc Natl Acad Sci U S A. 1990;87(16):6058–62.

187. Buzon MJ, Sun H, Li C, Shaw A, Seiss K, Ouyang Z, et al. HIV-1 persistence in CD4+ T cells with stem cell-like properties. Nat Med. 2014;20(2):139–42.

188. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med. 2009;15(8):893–900.

189. Cartwright EK, Palesch D, Mavigner M, Paiardini M, Chahroudi A, Silvestri G. Initiation of Antiretroviral Therapy Restores CD4+ T Memory Stem Cell Homeostasis in Simian Immunodeficiency Virus-Infected Macaques. J Virol. 2016;90(15):6699–708.

190. De Boer RJ, Perelson AS. Quantifying T lymphocyte turnover. J Theor Biol. 2013;327:45–87.

191. Hiener B, Horsburgh BA, Eden JS, Barton K, Schlub TE, Lee E, et al. Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants. Cell Rep. 2017;21(3):813–22.

192. De Scheerder MA, Vrancken B, Dellicour S, Schlub T, Lee E, Shao W, et al. HIV Rebound Is Predominantly Fueled by Genetically Identical Viral Expansions from Diverse Reservoirs. Cell Host Microbe. 2019;26(3):347-358.e7.

193. Wonderlich ER, Subramanian K, Cox B, Wiegand A, Lackman-Smith C, Bale MJ, et al. Effector memory differentiation increases detection of replication-competent HIV-l in resting CD4+ T cells from virally suppressed individuals. PLoS Pathog. 2019;15(10):e1008074.

194. Musick A, Spindler J, Boritz E, Pérez L, Crespo-Vélez D, Patro SC, et al. HIV Infected T Cells Can Proliferate in vivo Without Inducing Expression of the Integrated Provirus. Front Microbiol. 2019;10.

195. Kulpa DA, Talla A, Brehm JH, Ribeiro SP, Yuan S, Bebin-Blackwell AG, et al. Differentiation into an Effector Memory Phenotype Potentiates HIV-1 Latency Reversal in CD4+ T Cells. J Virol. 2019;93(24):e00969-19.

196. Zerbato JM, McMahon DK, Sobolewski MD, Mellors JW, Sluis-Cremer N. Naive CD4+ T Cells Harbor a Large Inducible Reservoir of Latent, Replication-competent Human Immunodeficiency Virus Type 1. Clin Infect Dis Off Publ Infect Dis Soc Am. 2019;69(11):1919–25.

197. Trémeaux P, Lenfant T, Boufassa F, Essat A, Mélard A, Gousset M, et al. Increasing contribution of integrated forms to total HIV DNA in blood during HIV disease progression from primary infection. EBioMedicine. 2019;41:455–64.

198. Gosselin A, Wiche Salinas TR, Planas D, Wacleche VS, Zhang Y, Fromentin R, et al. HIV persists in CCR6+CD4+ T cells from colon and blood during antiretroviral therapy. AIDS Lond Engl. 2017;31(1):35–48.

199. Perreau M, Savoye AL, De Crignis E, Corpataux JM, Cubas R, Haddad EK, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med. 2013;210(1):143–56.

200. Wong ME, Jaworowski A, Hearps AC. The HIV Reservoir in Monocytes and Macrophages. Front Immunol. 2019;10:1435.

201. Keele BF, Tazi L, Gartner S, Liu Y, Burgon TB, Estes JD, et al. Characterization of the follicular dendritic cell reservoir of human immunodeficiency virus type 1. J Virol. 2008;82(11):5548–61.

202. Marban C, Forouzanfar F, Ait-Ammar A, Fahmi F, El Mekdad H, Daouad F, et al. Targeting the Brain Reservoirs: Toward an HIV Cure. Front Immunol. 2016;7:397.

203. Belmonte L, Olmos M, Fanin A, Parodi C, Baré P, Concetti H, et al. The intestinal mucosa as a reservoir of HIV-1 infection after successful HAART. AIDS Lond Engl. 2007;21(15):2106–8.

204. Churchill MJ, Gorry PR, Cowley D, Lal L, Sonza S, Purcell DFJ, et al. Use of laser capture microdissection to detect integrated HIV-1 DNA in macrophages and astrocytes from autopsy brain tissues. J Neurovirol. 2006 Apr;12(2):146–52.

205. Costiniuk CT, Salahuddin S, Farnos O, Olivenstein R, Pagliuzza A, Orlova M, et al. HIV persistence in mucosal CD4+ T cells within the lungs of adults receiving long-term suppressive antiretroviral therapy. AIDS Lond Engl. 2018;32(16):2279–89.

206. Carter CC, Onafuwa-Nuga A, McNamara LA, Riddell J, Bixby D, Savona MR, et al. HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. Nat Med. 2010;16(4):446–51.

207. Durand CM, Ghiaur G, Siliciano JD, Rabi SA, Eisele EE, Salgado M, et al. HIV-1 DNA is detected in bone marrow populations containing CD4+ T cells but is not found in purified CD34+ hematopoietic progenitor cells in most patients on antiretroviral therapy. J Infect Dis. 2012;205(6):1014–8.

208. Deleage C, Moreau M, Rioux-Leclercq N, Ruffault A, Jégou B, Dejucq-Rainsford N. Human Immunodeficiency Virus Infects Human Seminal Vesicles in Vitro and in Vivo. Am J Pathol. 2011;179(5):2397–408.

209. Ganor Y, Real F, Sennepin A, Dutertre CA, Prevedel L, Xu L, et al. HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. Nat Microbiol. 2019;4(4):633–44.

210. Miller SM, Miles B, Guo K, Folkvord J, Meditz AL, McCarter MD, et al. Follicular Regulatory T Cells Are Highly Permissive to R5-Tropic HIV-1. J Virol. 2017;91(17):e00430-17.

211. Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, et al. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. Nat Med. 2015;21(2):132–9.

212. Connick E, Folkvord JM, Lind KT, Rakasz EG, Miles B, Wilson NA, et al. Compartmentalization of simian immunodeficiency virus replication within secondary lymphoid tissues of rhesus macaques is linked to disease stage and inversely related to localization of virus-specific CTL. J Immunol Baltim Md 1950. 2014;193(11):5613–25.

213. Connick E, Mattila T, Folkvord JM, Schlichtemeier R, Meditz AL, Ray MG, et al. CTL fail to accumulate at sites of HIV-1 replication in lymphoid tissue. J Immunol Baltim Md 1950. 2007;178(11):6975–83.

214. Fletcher CV, Staskus K, Wietgrefe SW, Rothenberger M, Reilly C, Chipman JG, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A. 2014;111(6):2307–12.

215. Le Tortorec A, Le Grand R, Denis H, Satie AP, Mannioui K, Roques P, et al. Infection of semen-producing organs by SIV during the acute and chronic stages of the disease. PloS One. 2008;3(3):e1792.

216. Mital P, Hinton BT, Dufour JM. The blood-testis and blood-epididymis barriers are more than just their tight junctions. Biol Reprod. 2011;84(5):851–8.

217. Jenabian MA, Costiniuk CT, Mehraj V, Ghazawi FM, Fromentin R, Brousseau J, et al. Immune tolerance properties of the testicular tissue as a viral sanctuary site in ART-treated HIV-infected adults. AIDS Lond Engl. 2016;30(18):2777–86.

218. Deeks SG, Archin N, Cannon P, Collins S, Jones RB, de Jong MAWP, et al. Research priorities for an HIV cure: International AIDS Society Global Scientific Strategy 2021. Nat Med. 2021;27(12):2085–98.

219. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, et al. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014;345(6193):179–83.

220. Wagner TA, McLaughlin S, Garg K, Cheung CYK, Larsen BB, Styrchak S, et al. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014;345(6196):570–3.

221. Kauder SE, Bosque A, Lindqvist A, Planelles V, Verdin E. Epigenetic regulation of HIV-1 latency by cytosine methylation. PLoS Pathog. 2009;5(6):e1000495.

222. Blazkova J, Trejbalova K, Gondois-Rey F, Halfon P, Philibert P, Guiguen A, et al. CpG methylation controls reactivation of HIV from latency. PLoS Pathog. 2009;5(8):e1000554.

223. Sheridan PL, Mayall TP, Verdin E, Jones KA. Histone acetyltransferases regulate HIV-1 enhancer activity in vitro. Genes Dev. 1997;11(24):3327–40.

224. Lusic M, Marcello A, Cereseto A, Giacca M. Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter. EMBO J. 2003;22(24):6550–61.

225. Coull JJ, Romerio F, Sun JM, Volker JL, Galvin KM, Davie JR, et al. The Human Factors YY1 and LSF Repress the Human Immunodeficiency Virus Type 1 Long Terminal Repeat via Recruitment of Histone Deacetylase 1. J Virol. 2000;74(15):6790–9.

226. Archin NM, Keedy KS, Espeseth A, Dang H, Hazuda DJ, Margolis DM. Expression of latent human immunodeficiency type 1 is induced by novel and selective histone deacetylase inhibitors. AIDS. 2009;23(14):1799.

227. Seddon B, Tomlinson P, Zamoyska R. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. Nat Immunol. 2003;4(7):680–6.

228. Kim M, Siliciano RF. Reservoir expansion by T-cell proliferation may be another barrier to curing HIV infection. Proc Natl Acad Sci. 2016;113(7):1692–4.

229. Murray AJ, Kwon KJ, Farber DL, Siliciano RF. The Latent Reservoir for HIV-1: How Immunologic Memory and Clonal Expansion Contribute to HIV-1 Persistence. J Immunol. 2016;197(2):407–17.

230. Chetoui N, Boisvert M, Gendron S, Aoudjit F. Interleukin-7 promotes the survival of human CD4+ effector/memory T cells by up-regulating Bcl-2 proteins and activating the JAK/STAT signalling pathway. Immunology. 2010;130(3):418–26.

231. Simonetti FR, Sobolewski MD, Fyne E, Shao W, Spindler J, Hattori J, et al. Clonally expanded CD4+ T cells can produce infectious HIV-1 in vivo. Proc Natl Acad Sci U S A. 2016;113(7):1883–8.

232. Henrich TJ, Hobbs KS, Hanhauser E, Scully E, Hogan LE, Robles YP, et al. Human Immunodeficiency Virus Type 1 Persistence Following Systemic Chemotherapy for Malignancy. J Infect Dis. 2017;216(2):254–62.

233. Simonetti FR, Zhang H, Soroosh GP, Duan J, Rhodehouse K, Hill AL, et al. Antigen-driven clonal selection shapes the persistence of HIV-1-infected CD4+ T cells in vivo. J Clin Invest. 2021;131(3):e145254, 145254.

234. Buzón MJ, Massanella M, Llibre JM, Esteve A, Dahl V, Puertas MC, et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nat Med. 2010;16(4):460–5.

235. Kreider EF, Bar KJ. HIV-1 Reservoir Persistence and Decay: Implications for Cure Strategies. Curr HIV/AIDS Rep. 2022;19(3):194–206.

236. Hsu DC, Sereti I. Serious Non-AIDS Events: Therapeutic Targets of Immune Activation and Chronic Inflammation in HIV Infection. Drugs. 2016;76(5):533–49.

237. Stevenson M, Stanwick TL, Dempsey MP, Lamonica CA. HIV-1 replication is controlled at the level of T cell activation and proviral integration. EMBO J. 1990;9(5):1551–60.

238. Pan X, Baldauf HM, Keppler OT, Fackler OT. Restrictions to HIV-1 replication in resting CD4+ T lymphocytes. Cell Res. 2013;23(7):876–85.

239. Chapman NM, Boothby MR, Chi H. Metabolic coordination of T cell quiescence and activation. Nat Rev Immunol. 2020;20(1):55–70.

240. Patsoukis N, Bardhan K, Weaver J, Herbel C, Seth P, Li L, et al. The role of metabolic reprogramming in T cell fate and function. Curr Trends Immunol. 2016;17:1–12.

241. Sáez-Cirión A, Sereti I. Immunometabolism and HIV-1 pathogenesis: food for thought. Nat Rev Immunol. 2021;21(1):5–19.

242. Clerc I, Abba Moussa D, Vahlas Z, Tardito S, Oburoglu L, Hope TJ, et al. Entry of glucose- and glutamine-derived carbons into the citric acid cycle supports early steps of HIV-1 infection in CD4 T cells. Nat Metab. 2019;1(7):717–30.

243. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol. 2019;37:457–95.

244. Angin M, Volant S, Passaes C, Lecuroux C, Monceaux V, Dillies MA, et al. Metabolic plasticity of HIV-specific CD8+ T cells is associated with enhanced antiviral potential and natural control of HIV-1 infection. Nat Metab. 2019;1(7):704–16.

245. Moreno-Cubero E, Alrubayyi A, Balint S, Ogbe A, Gill US, Matthews R, et al. IL-15 reprogramming compensates for NK cell mitochondrial dysfunction in HIV-1 infection. JCI Insight. 2024;9(4):e173099.

246. Warnke D, Barreto J, Temesgen Z. Antiretroviral Drugs. J Clin Pharmacol. 2007;47(12):1570–9.

247. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Antiretroviral Therapy for the Prevention of HIV-1 Transmission. N Engl J Med. 2016;375(9):830–9.

248. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 Infection with Early Antiretroviral Therapy. N Engl J Med. 2011;365(6):493–505.

249. van Heuvel Y, Schatz S, Rosengarten JF, Stitz J. Infectious RNA: Human Immunodeficiency Virus (HIV) Biology, Therapeutic Intervention, and the Quest for a Vaccine. Toxins. 2022;14(2):138.

250. Chun TW, Davey RT, Engel D, Lane HC, Fauci AS. Re-emergence of HIV after stopping therapy. Nature. 1999;401(6756):874–5.

251. Davey RT, Bhat N, Yoder C, Chun TW, Metcalf JA, Dewar R, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci. 1999;96(26):15109–14.

252. Orkin C, Oka S, Philibert P, Brinson C, Bassa A, Gusev D, et al. Long-acting cabotegravir plus rilpivirine for treatment in adults with HIV-1 infection: 96-week results of the randomised, open-label, phase 3 FLAIR study. Lancet HIV. 2021;8(4):e185–96.

253. Overton ET, Richmond G, Rizzardini G, Jaeger H, Orrell C, Nagimova F, et al. Long-acting cabotegravir and rilpivirine dosed every 2 months in adults with HIV-1 infection (ATLAS-2M), 48-week results: a randomised, multicentre, open-label, phase 3b, non-inferiority study. Lancet Lond Engl. 2021 Dec 19;396(10267):1994–2005.

254. Segal-Maurer S, DeJesus E, Stellbrink HJ, Castagna A, Richmond GJ, Sinclair GI, et al. Capsid Inhibition with Lenacapavir in Multidrug-Resistant HIV-1 Infection. N Engl J Med. 2022;386(19):1793–803.

255. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach, 2nd ed. Available from: https://www.who.int/publications/i/item/9789241549684

256. Fonner VA, Dalglish SL, Kennedy CE, Baggaley R, O'Reilly KR, Koechlin FM, et al. Effectiveness and safety of oral HIV preexposure prophylaxis for all populations. AIDS. 2016;30(12):1973.

257. Rey D. Post-exposure prophylaxis for HIV infection. Expert Rev Anti Infect Ther. 2011;9(4):431–42.

258. Thomas R, Galanakis C, Vézina S, Longpré D, Boissonnault M, Huchet E, et al. Adherence to Post-Exposure Prophylaxis (PEP) and Incidence of HIV Seroconversion in a Major North American Cohort. PloS One. 2015;10(11):e0142534.

259. Yang OO, Cumberland WG, Escobar R, Liao D, Chew KW. Demographics and natural history of HIV-1-infected spontaneous controllers of viremia. AIDS Lond Engl. 2017;31(8):1091–8.

260. Mastrangelo A, Banga R, Perreau M. Elite and posttreatment controllers, two facets of HIV control. Curr Opin HIV AIDS. 2022;17(5):325–32.

261. Sharaf R, Lee GQ, Sun X, Etemad B, Aboukhater LM, Hu Z, et al. HIV-1 proviral landscapes distinguish posttreatment controllers from noncontrollers. J Clin Invest. 2018;128(9):4074–85.

262. Etemad B, Esmaeilzadeh E, Li JZ. Learning From the Exceptions: HIV Remission in Post-treatment Controllers. Front Immunol. 2019;10:1749.

263. Zhang Z, Zhou Y, Lu J, Chen YF, Hu HY, Xu XQ, et al. Changes in NK Cell Subsets and Receptor Expressions in HIV-1 Infected Chronic Patients and HIV Controllers. Front Immunol. 2021;12.

264. Dominguez-Molina B, Ferrando-Martinez S, Tarancon-Diez L, Hernandez-Quero J, Genebat M, Vidal F, et al. Immune Correlates of Natural HIV Elite Control and Simultaneous HCV Clearance-Supercontrollers. Front Immunol. 2018;9:2897.

265. Sáez-Cirión A, Bacchus C, Hocqueloux L, Avettand-Fenoel V, Girault I, Lecuroux C, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog. 2013;9(3):e1003211.

266. Monel B, McKeon A, Lamothe-Molina P, Jani P, Boucau J, Pacheco Y, et al. HIV Controllers Exhibit Effective CD8+ T Cell Recognition of HIV-1-Infected Non-activated CD4+ T Cells. Cell Rep. 2019;27(1):142-153.e4.

267. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci U S A. 2000;97(6):2709–14.

268. Kiepiela P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S, et al. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature. 2004;432(7018):769–75.

269. Bailey JR, Williams TM, Siliciano RF, Blankson JN. Maintenance of viral suppression in HIV-1-infected HLA-B*57+ elite suppressors despite CTL escape mutations. J Exp Med. 2006;203(5):1357–69.

270. Koofhethile CK, Ndhlovu ZM, Thobakgale-Tshabalala C, Prado JG, Ismail N, Mncube Z, et al. CD8+ T Cell Breadth and Ex Vivo Virus Inhibition Capacity Distinguish between

Viremic Controllers with and without Protective HLA Class I Alleles. J Virol. 2016;90(15):6818–31.

271. Jiang C, Lian X, Gao C, Sun X, Einkauf KB, Chevalier JM, et al. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. Nature. 2020;585(7824):261–7.

272. Huang AS, Ramos V, Oliveira TY, Gaebler C, Jankovic M, Nussenzweig MC, et al. Integration features of intact latent HIV-1 in CD4+ T cell clones contribute to viral persistence. J Exp Med. 2021;218(12):e20211427.

273. Liu R, Yeh YHJ, Varabyou A, Collora JA, Sherrill-Mix S, Talbot CC, et al. Single-cell transcriptional landscapes reveal HIV-1-driven aberrant host gene transcription as a potential therapeutic target. Sci Transl Med. 2020;12(543):eaaz0802.

274. Wagner TA, McLaughlin S, Garg K, Cheung CYK, Larsen BB, Styrchak S, et al. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014;345(6196):570–3.

275. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996;86(3):367–77.

276. Hütter G, Nowak D, Mossner M, Ganepola S, Müssig A, Allers K, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med. 2009;360(7):692–8.

277. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature. 2019;568(7751):244–8.

278. Hsu J, Van Besien K, Glesby MJ, Pahwa S, Coletti A, Warshaw MG, et al. HIV-1 remission and possible cure in a woman after haplo-cord blood transplant. Cell. 2023;186(6):1115–26.

279. Jensen BEO, Knops E, Cords L, Lübke N, Salgado M, Busman-Sahay K, et al. In-depth virological and immunological characterization of HIV-1 cure after CCR5Δ32/Δ32 allogeneic hematopoietic stem cell transplantation. Nat Med. 2023; 29(3):583-587.

280. Dickter JK, Aribi A, Cardoso AA, Gianella S, Gendzekhadze K, Li S, et al. HIV-1 Remission after Allogeneic Hematopoietic-Cell Transplantation. N Engl J Med. 2024;390(7):669–71.

281. Salgado M, Kwon M, Gálvez C, Badiola J, Nijhuis M, Bandera A, et al. Mechanisms That Contribute to a Profound Reduction of the HIV-1 Reservoir After Allogeneic Stem Cell Transplant. Ann Intern Med. 2018;169(10):674–83.

282. Wu HL, Busman-Sahay K, Weber WC, Waytashek CM, Boyle CD, Bateman KB, et al. Allogeneic immunity clears latent virus following allogeneic stem cell transplantation in SIV-infected ART-suppressed macaques. Immunity. 2023;56(7):1649-1663.e5.

283. Salgado M, Gálvez C, Nijhuis M, Kwon M, Cardozo-Ojeda EF, Badiola J, et al. Dynamics of virological and immunological markers of HIV persistence after allogeneic

haematopoietic stem-cell transplantation in the IciStem cohort: a prospective observational cohort study. Lancet HIV. 2024;11(6):e389–405.

284. Sáez-Cirión A, Mamez AC, Avettand-Fenoel V, Nabergoj M, Passaes C, Thoueille P, et al. Sustained HIV remission after allogeneic hematopoietic stem cell transplantation with wild-type CCR5 donor cells. Nat Med. 2024;30(12):3544–54.

285. Chen J, Zhou T, Zhang Y, Luo S, Chen H, Chen D, et al. The reservoir of latent HIV. Front Cell Infect Microbiol. 2022; 12.

286. Rodari A, Darcis G, Lint CMV. The Current Status of Latency Reversing Agents for HIV-1 Remission. Annu Rev Virol. 2021;8:491–514.

287. Elliott JH, McMahon JH, Chang CC, Lee SA, Hartogensis W, Bumpus N, et al. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. Lancet HIV. 2015;2(12):e520-529.

288. Gutiérrez C, Serrano-Villar S, Madrid-Elena N, Pérez-Elías MJ, Martí ME, Barbas C, et al. Bryostatin-1 for latent virus reactivation in HIV-infected patients on antiretroviral therapy. AIDS Lond Engl. 2016; 30(9):1385-92.

289. Vibholm L, Schleimann MH, Højen JF, Benfield T, Offersen R, et al. Short-Course Toll-Like Receptor 9 Agonist Treatment Impacts Innate Immunity and Plasma Viremia in Individuals With Human Immunodeficiency Virus Infection. Clin Infect Dis Off Publ Infect Dis Soc Am. 2017;64(12): 1686-1695.

290. Gay CL, Kuruc JD, Falcinelli SD, Warren JA, Reifeis SA, Kirchherr JL, et al. Assessing the impact of AGS-004, a dendritic cell-based immunotherapy, and vorinostat on persistent HIV-1 Infection. Sci Rep. 2020 Mar 20;10(1):5134.

291. Fidler S, Stöhr W, Pace M, Dorrell L, Lever A, Pett S, et al. Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): a phase 2, randomised trial. Lancet Lond Engl. 2020;395(10227):888–98.

292. Riddler SA, Para M, Benson CA, Mills A, Ramgopal M, DeJesus E, et al. Vesatolimod, a Toll-like Receptor 7 Agonist, Induces Immune Activation in Virally Suppressed Adults Living With Human Immunodeficiency Virus-1. Clin Infect Dis Off Publ Infect Dis Soc Am. 2021;72(11):e815–24.

293. Cillo AR, Sobolewski MD, Bosch RJ, Fyne E, Piatak M, Coffin JM, et al. Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy. Proc Natl Acad Sci U S A. 2014;111(19):7078–83.

294. Laird GM, Bullen CK, Rosenbloom DIS, Martin AR, Hill AL, Durand CM, et al. Ex vivo analysis identifies effective HIV-1 latency-reversing drug combinations. J Clin Invest. 2015;125(5):1901–12.

295. Zerbato JM, Khoury G, Zhao W, Gartner MJ, Pascoe RD, Rhodes A, et al. Multiply spliced HIV RNA is a predictive measure of virus production ex vivo and in vivo following reversal of HIV latency. EBioMedicine. 2021;65:103241.

296. Huang SH, Ren Y, Thomas AS, Chan D, Mueller S, Ward AR, et al. Latent HIV reservoirs exhibit inherent resistance to elimination by CD8+ T cells. J Clin Invest. 2018;128(2):876–89.

297. Kim Y, Anderson JL, Lewin SR. Getting the "kill" into "shock and kill": strategies to eliminate latent HIV. Cell Host Microbe. 2018;23(1):14–26.

298. Howard JN, Bosque A. IL-15 and N-803 for HIV Cure Approaches. Viruses. 2023;15(9):1912.

299. Li J, Huang HH, Tu B, Zhou MJ, Hu W, Fu YL, et al. Reversal of the CD8+ T-Cell Exhaustion Induced by Chronic HIV-1 Infection Through Combined Blockade of the Adenosine and PD-1 Pathways. Front Immunol. 2021;12:687296.

300. Peterson CW. HIV-specific CAR T cells return to the clinic. J Clin Invest. 2021 Oct 1;131(19).

301. Lévy Y, Thiébaut R, Montes M, Lacabaratz C, Sloan L, King B, et al. Dendritic cell-based therapeutic vaccine elicits polyfunctional HIV-specific T-cell immunity associated with control of viral load. Eur J Immunol. 2014;44(9):2802–10.

302. Lim SY, Lee J, Osuna CE, Vikhe P, Schalk DR, Chen E, et al. Induction of durable remission by dual immunotherapy in SHIV-infected ART-suppressed macaques. Science. 2024;383(6687):1104-1111

303. Harper J, Gordon S, Chan CN, Wang H, Lindemuth E, Galardi C, et al. CTLA-4 and PD-1 dual blockade induces SIV reactivation without control of rebound after antiretroviral therapy interruption. Nat Med. 2020;26(4):519–28.

304. Moranguinho I, Valente ST. Block-And-Lock: New Horizons for a Cure for HIV-1. Viruses. 2020;12(12):1443.

305. Vansant G, Bruggemans A, Janssens J, Debyser Z. Block-And-Lock Strategies to Cure HIV Infection. Viruses. 2020;12(1):84.

306. Kessing CF, Nixon CC, Li C, Tsai P, Takata H, Mousseau G, et al. In Vivo Suppression of HIV Rebound by Didehydro-Cortistatin A, a "Block-and-Lock" Strategy for HIV-1 Treatment. Cell Rep. 2017;21(3):600–11.

307. Ahlenstiel C, Mendez C, Lim STH, Marks K, Turville S, Cooper DA, et al. Novel RNA Duplex Locks HIV-1 in a Latent State via Chromatin-mediated Transcriptional Silencing. Mol Ther Nucleic Acids. 2015;4(10):e261.

308. Besnard E, Hakre S, Kampmann M, Lim HW, Hosmane NN, Martin A, et al. The mTOR Complex Controls HIV Latency. Cell Host Microbe. 2016;20(6):785–97.

309. Pellaers E, Debyser Z. EDITORIAL: Putting HIV in the big sleep with the block-and-lock strategy. Curr Opin HIV AIDS. 2024;19(3):93–4.

310. Sneller MC, Blazkova J, Justement JS, Shi V, Kennedy BD, Gittens K, et al. Combination anti-HIV antibodies provide sustained virological suppression. Nature. 2022;606(7913):375–81.

311. Gaebler C, Nogueira L, Stoffel E, Oliveira TY, Breton G, Millard KG, et al. Prolonged viral suppression with anti-HIV-1 antibody therapy. Nature. 2022;606(7913):368–74.

312. Law M, Maruyama T, Lewis J, Giang E, Tarr AW, Stamataki Z, et al. Broadly neutralizing antibodies protect against hepatitis C virus quasispecies challenge. Nat Med. 2008;14(1):25–7.

313. Bozhanova NG, Sangha AK, Sevy AM, Gilchuk P, Huang K, Nargi RS, et al. Discovery of Marburg virus neutralizing antibodies from virus-naïve human antibody repertoires using large-scale structural predictions. Proc Natl Acad Sci U S A. 2020;117(49):31142–8.

314. Saied AA, Nascimento MSL, do Nascimento Rangel AH, Skowron K, Grudlewska-Buda K, Dhama K, et al. Transchromosomic bovines-derived broadly neutralizing antibodies as potent biotherapeutics to counter important emerging viral pathogens with a special focus on SARS-CoV-2, MERS-CoV, Ebola, Zika, HIV-1, and influenza A virus. J Med Virol. 2022;94(10):4599–610.

315. Caskey M, Klein F, Lorenzi JCC, Seaman MS, West AP, Buckley N, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature. 2015;522(7557):487–91.

316. Schoofs T, Klein F, Braunschweig M, Kreider EF, Feldmann A, Nogueira L, et al. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. Science. 2016;352(6288):997–1001.

317. Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, Millard K, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. Nature. 2018;561(7724):479–84.

318. Caskey M, Klein F, Nussenzweig MC. Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic. Nat Med. 2019;25(4):547–53.

319. Thavarajah JJ, Hønge BL, Wejse CM. The Use of Broadly Neutralizing Antibodies (bNAbs) in HIV-1 Treatment and Prevention. Viruses. 2024;16(6):911.

320. Irani V, Guy AJ, Andrew D, Beeson JG, Ramsland PA, Richards JS. Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases. Mol Immunol. 2015;67(2 Pt A):171–82.

321. Stiegler G, Armbruster C, Vcelar B, Stoiber H, Kunert R, Michael NL, et al. Antiviral activity of the neutralizing antibodies 2F5 and 2G12 in asymptomatic HIV-1-infected humans: a phase I evaluation. AIDS Lond Engl. 2002;16(15):2019–25.

322. Armbruster C, Stiegler GM, Vcelar BA, Jäger W, Michael NL, Vetter N, et al. A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1. AIDS Lond Engl. 2002;16(2):227–33.

323. Armbruster C, Stiegler GM, Vcelar BA, Jäger W, Köller U, Jilch R, et al. Passive immunization with the anti-HIV-1 human monoclonal antibody (hMAb) 4E10 and the hMAb combination 4E10/2F5/2G12. J Antimicrob Chemother. 2004;54(5):915–20.

324. Joos B, Trkola A, Kuster H, Aceto L, Fischer M, Stiegler G, et al. Long-term multiple-dose pharmacokinetics of human monoclonal antibodies (MAbs) against human immunodeficiency virus type 1 envelope gp120 (MAb 2G12) and gp41 (MAbs 4E10 and 2F5). Antimicrob Agents Chemother. 2006;50(5):1773–9.

325. Trkola A, Kuster H, Rusert P, Joos B, Fischer M, Leemann C, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med. 2005;11(6):615–22.

326. Frattari GS, Caskey M, Søgaard OS. Broadly neutralizing antibodies for HIV treatment and cure approaches. Curr Opin HIV AIDS. 2023;18(4):157–63.

327. Gaudinski MR, Coates EE, Houser KV, Chen GL, Yamshchikov G, Saunders JG, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. PLoS Med. 2018;15(1):e1002493.

328. Caskey M, Schoofs T, Gruell H, Settler A, Karagounis T, Kreider EF, et al. Antibody 10-1074 suppresses viremia in HIV-1-infected individuals. Nat Med. 2017;23(2):185–91.

329. Bar-On Y, Gruell H, Schoofs T, Pai JA, Nogueira L, Butler AL, et al. Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. Nat Med. 2018;24(11):1701–7.

330. Gruell H, Schommers P. Broadly neutralizing antibodies against HIV-1 and concepts for application. Curr Opin Virol. 2022;54:101211.

331. Martinez-Navio JM, Fuchs SP, Pantry SN, Lauer WA, Duggan NN, Keele BF, et al. Adeno-Associated Virus Delivery of Anti-HIV Monoclonal Antibodies Can Drive Long-Term Virologic Suppression. Immunity. 2019;50(3):567-575.e5.

332. Hessell AJ, Rakasz EG, Tehrani DM, Huber M, Weisgrau KL, Landucci G, et al. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. J Virol. 2010;84(3):1302–13.

333. Gautam R, Nishimura Y, Pegu A, Nason MC, Klein F, Gazumyan A, et al. A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. Nature. 2016;533(7601):105–9.

334. Stephenson KE, Wagh K, Korber B, Barouch DH. Vaccines and Broadly Neutralizing Antibodies for HIV-1 Prevention. Annu Rev Immunol. 2020;38:673–703.

335. Schriek AI, Aldon YLT, van Gils MJ, de Taeye SW. Next-generation bNAbs for HIV-1 cure strategies. Antiviral Res. 2024;222:105788.

336. Larijani MS, Sadat SM, Ramezani* A. HIV-1 Immune evasion: The main obstacle toward a successful vaccine. Arch Asthma Allergy Immunol. 2018;2(1):013–5.

337. Cohn LB, Chomont N, Deeks SG. The Biology of the HIV-1 Latent Reservoir and Implications for Cure Strategies. Cell Host Microbe. 2020;27(4):519–30.

338. Mothe B, Manzardo C, Sanchez-Bernabeu A, Coll P, Morón-López S, Puertas MC, et al. Therapeutic Vaccination Refocuses T-cell Responses Towards Conserved Regions of HIV-1 in Early Treated Individuals (BCN 01 study). EClinicalMedicine. 2019;11:65–80.

339. Colby DJ, Sarnecki M, Barouch DH, Tipsuk S, Stieh DJ, Kroon E, et al. Safety and immunogenicity of Ad26 and MVA vaccines in acutely treated HIV and effect on viral rebound after antiretroviral therapy interruption. Nat Med. 2020;26(4):498–501.

340. Bailón L, Llano A, Cedeño S, Escribà T, Rosás-Umbert M, Parera M, et al. Safety, immunogenicity and effect on viral rebound of HTI vaccines in early treated HIV-1 infection: a randomized, placebo-controlled phase 1 trial. Nat Med. 2022;28(12):2611–21.

341. Zagury D, Léonard R, Fouchard M, Réveil B, Bernard J, Ittelé D, et al. Immunization against AIDS in humans. Nature. 1987;326(6110):249–50.

342. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis. 2006;194(12):1661–71.

343. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis. 2005;191(5):654–65.

344. Priddy FH, Brown D, Kublin J, Monahan K, Wright DP, Lalezari J, et al. Safety and immunogenicity of a replication-incompetent adenovirus type 5 HIV-1 clade B gag/pol/nef vaccine in healthy adults. Clin Infect Dis Off Publ Infect Dis Soc Am. 2008;46(11):1769–81.

345. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet Lond Engl. 2008;372(9653):1881–93.

346. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis. 2011;11(7):507–15.

347. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med. 2013;369(22):2083–92.

348. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009;361(23):2209–20.

349. Pitisuttithum P, Rerks-Ngarm S, Bussaratid V, Dhitavat J, Maekanantawat W, Pungpak S, et al. Safety and reactogenicity of canarypox ALVAC-HIV (vCP1521) and HIV-1 gp120 AIDSVAX B/E vaccination in an efficacy trial in Thailand. PloS One. 2011;6(12):e27837.

350. Gray GE, Bekker LG, Laher F, Malahleha M, Allen M, Moodie Z, et al. Vaccine Efficacy of ALVAC-HIV and Bivalent Subtype C gp120-MF59 in Adults. N Engl J Med. 2021;384(12):1089–100.

351. Gray GE, Mngadi K, Lavreys L, Nijs S, Gilbert PB, Hural J, et al. Mosaic HIV-1 vaccine regimen in southern African women (Imbokodo/HVTN 705/HPX2008): a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Infect Dis. 2024;24(11):1201–12.

352. Jardine JG, Kulp DW, Havenar-Daughton C, Sarkar A, Briney B, Sok D, et al. HIV-1 broadly neutralizing antibody precursor B cells revealed by germline-targeting immunogen. Science. 2016;351(6280):1458–63.

353. Haynes BF, Wiehe K, Borrow P, Saunders KO, Korber B, Wagh K, et al. Strategies for HIV-1 vaccines that induce broadly neutralizing antibodies. Nat Rev Immunol. 2023;23(3):142–58.

354. Leggat DJ, Cohen KW, Willis JR, Fulp WJ, deCamp AC, Kalyuzhniy O, et al. Vaccination induces HIV broadly neutralizing antibody precursors in humans. Science. 2022;378(6623):eadd6502.

355. Yang H, Rei M, Brackenridge S, Brenna E, Sun H, Abdulhaqq S, et al. HLA-E-restricted, Gag-specific CD8+ T cells can suppress HIV-1 infection, offering vaccine opportunities. Sci Immunol. 2021;6(57):eabg1703.

356. Bailón L, Llano A, Cedeño S, Escribà T, Rosás-Umbert M, Parera M, et al. Safety, immunogenicity and effect on viral rebound of HTI vaccines in early treated HIV-1 infection: a randomized, placebo-controlled phase 1 trial. Nat Med. 2022;28(12):2611–21.

357. Rahman SA, Yagnik B, Bally AP, Morrow KN, Wang S, Vanderford TH, et al. PD-1 blockade and vaccination provide therapeutic benefit against SIV by inducing broad and functional CD8+ T cells in lymphoid tissue. Sci Immunol. 2021;6(63):eabh3034.

358. Borducchi EN, Cabral C, Stephenson KE, Liu J, Abbink P, Ng'ang'a D, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. Nature. 2016;540(7632):284–7.

359. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243(5405):290–3.

360. Ilaria RL, Van Etten RA. P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. J Biol Chem. 1996;271(49):31704–10.

361. Mandanas RA, Leibowitz DS, Gharehbaghi K, Tauchi T, Burgess GS, Miyazawa K, et al. Role of p21 RAS in p210 bcr-abl transformation of murine myeloid cells. Blood. 1993;82(6):1838–47.

362. Raitano AB, Halpern JR, Hambuch TM, Sawyers CL. The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc Natl Acad Sci U S A. 1995;92(25):11746–50.

363. Bovolenta C, Camorali L, Lorini AL, Ghezzi S, Vicenzi E, Lazzarin A, et al. Constitutive Activation of STATs Upon In Vivo Human Immunodeficiency Virus Infection. Blood. 1999;94(12):4202–9.

364. Carlesso N, Frank DA, Griffin JD. Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl. J Exp Med. 1996;183(3):811–20.

365. Huang X, Cortes J, Kantarjian H. Estimations of the increasing prevalence and plateau prevalence of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. Cancer. 2012;118(12):3123–7.

366. Andreieva SV, Korets KV, Kyselova OA, Ruzhinska OE, Serbin IM. Chronic myeloid leukemia in patient with the Klinefelter syndrome. Exp Oncol. 2016;38(3):195–7.

367. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293(5531):876–80.

368. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2025 update on diagnosis, therapy, and monitoring. Am J Hematol. 2024;99(11):2191–212.

369. Brümmendorf TH, Cortes JE, Milojkovic D, Gambacorti-Passerini C, Clark RE, le Coutre P, et al. Bosutinib versus imatinib for newly diagnosed chronic phase chronic myeloid leukemia: final results from the BFORE trial. Leukemia. 2022;36(7):1825–33.

370. Isfort S, Manz K, Teichmann LL, Crysandt M, Burchert A, Hochhaus A, et al. Step-in dosing of bosutinib in pts with chronic phase chronic myeloid leukemia (CML) after second-generation tyrosine kinase inhibitor (TKI) therapy: results of the Bosutinib Dose Optimization (BODO) Study. Ann Hematol. 2023 Oct 1;102(10):2741–52.

371. Zhou T, Commodore L, Huang WS, Wang Y, Thomas M, Keats J, et al. Structural mechanism of the Pan-BCR-ABL inhibitor ponatinib (AP24534): lessons for overcoming kinase inhibitor resistance. Chem Biol Drug Des. 2011;77(1):1–11.

372. Müller MC, Cortes JE, Kim DW, Druker BJ, Erben P, Pasquini R, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: analysis of responses according to preexisting BCR-ABL mutations. Blood. 2009;114(24):4944–53.

373. Kantarjian HM, Cortes J, Guilhot F, Hochhaus A, Baccarani M, Lokey L. Diagnosis and management of chronic myeloid leukemia: a survey of American and European practice patterns. Cancer. 2007;109(7):1365–75.

374. Hochhaus A, Réa D, Boquimpani C, Minami Y, Cortes JE, Hughes TP, et al. Asciminib vs bosutinib in chronic-phase chronic myeloid leukemia previously treated with at least two tyrosine kinase inhibitors: longer-term follow-up of ASCEMBL. Leukemia. 2023;37(3):617–26.

375. Hochhaus A, Wang J, Kim DW, Kim DDH, Mayer J, Goh YT, et al. Asciminib in Newly Diagnosed Chronic Myeloid Leukemia. N Engl J Med. 2024;391(10):885–98.

376. Lee H, Basso IN, Kim DDH. Target spectrum of the BCR-ABL tyrosine kinase inhibitors in chronic myeloid leukemia. Int J Hematol. 2021;113(5):632–41.

377. Hantschel O, Rix U, Superti-Furga G. Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. Leuk Lymphoma. 2008;49(4):615–9.

378. Remsing Rix LL, Rix U, Colinge J, Hantschel O, Bennett KL, Stranzl T, et al. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia. 2009;23(3):477–85.

379. Mancini M, Brusa G, Zuffa E, Corrado P, Martinelli G, Grafone T, et al. Persistent Cdk2 inactivation drives growth arrest of BCR-ABL-expressing cells in response to dual inhibitor of SRC and ABL kinases SKI606. Leuk Res. 2007;31(7):979–87.

380. Wylie AA, Schoepfer J, Jahnke W, Cowan-Jacob SW, Loo A, Furet P, et al. The allosteric inhibitor ABL001 enables dual targeting of BCR-ABL1. Nature. 2017;543(7647):733–7.

381. Rodriguez GH, Ahmed SI, Al-akhrass F, Rallapalli V, Safdar A. Characteristics of, and risk factors for, infections in patients with cancer treated with dasatinib and a brief review of other complications. Leuk Lymphoma. 2012;53(8):1530–5.

382. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. Nat Med. 2018;24(8):1246–56.

383. Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell. 2015;14(4):644–58.

384. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol. 2007;25(9):1035–44.

385. Rix U, Hantschel O, Dürnberger G, Remsing Rix LL, Planyavsky M, Fernbach NV, et al. Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. Blood. 2007;110(12):4055–63.

386. Lee MY, Park CJ, Cho YU, You E, Jang S, Seol CA, et al. Differences in PD-1 expression on CD8+ T-cells in chronic myeloid leukemia patients according to disease phase and TKI medication. Cancer Immunol Immunother CII. 2020;69(11):2223–32.

387. Yong ASM, Keyvanfar K, Eniafe R, Savani BN, Rezvani K, Sloand EM, et al. Hematopoietic stem cells and progenitors of chronic myeloid leukemia express leukemia-associated antigens: implications for the graft-versus-leukemia effect and peptide vaccine-based immunotherapy. Leukemia. 2008;22(9):1721–7.

388. Steegmann JL, Cervantes F, le Coutre P, Porkka K, Saglio G. Off-target effects of BCR-ABL1 inhibitors and their potential long-term implications in patients with chronic myeloid leukemia. Leuk Lymphoma. 2012;53(12):2351–61.

389. Yaqub S, Abrahamsen H, Zimmerman B, Kholod N, Torgersen KM, Mustelin T, et al. Activation of C-terminal Src kinase (Csk) by phosphorylation at serine-364 depends on the Csk-Src homology 3 domain. Biochem J. 2003;372(Pt 1):271–8.

390. Palacios EH, Weiss A. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene. 2004;23(48):7990–8000.

391. Baier-Bitterlich G, Überall F, Bauer B, Fresser F, Wachter H, Grunicke H, et al. Protein Kinase C-θ Isoenzyme Selective Stimulation of the Transcription Factor Complex AP-1 in T Lymphocytes. Mol Cell Biol. 1996;16(4):1842–50.

392. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, et al. PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes. Nature. 2000;404(6776):402–7.

393. Coudronniere N, Villalba M, Englund N, Altman A. NF-κB activation induced by T cell receptor/CD28 costimulation is mediated by protein kinase C-θ. Proc Natl Acad Sci. 2000;97(7):3394–9.

394. Coiras M, López-Huertas MR, Pérez-Olmeda M, Alcamí J. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. Nat Rev Microbiol. 2009;7(11):798–812.

395. Liu Y, Witte S, Liu YC, Doyle M, Elly C, Altman A. Regulation of protein kinase Ctheta function during T cell activation by Lck-mediated tyrosine phosphorylation. J Biol Chem. 2000;275(5):3603–9.

396. Gruber T, Pfeifhofer-Obermair C, Baier G. PKCtheta is necessary for efficient activation of NFkappaB, NFAT, and AP-1 during positive selection of thymocytes. Immunol Lett. 2010;132(1–2):6–11.

397. Wang X, Chuang HC, Li JP, Tan TH. Regulation of PKC-θ function by phosphorylation in T cell receptor signaling. Front Immunol. 2012;3:197.

398. Coiras M, Bermejo M, Descours B, Mateos E, García-Pérez J, López-Huertas MR, et al. IL-7 induces SAMHD1 phosphorylation in CD4+ T lymphocytes, improving early steps of HIV-1 life cycle. Cell Rep. 2016;14(9):2100–7.

399. Innis EA, Levinger C, Szaniawski MA, Williams ESCP, Alcamí J, Bosque A, et al. Pharmacologic control of homeostatic and antigen-driven proliferation to target HIV-1 persistence. Biochem Pharmacol. 2021;194:114816.

400. Manganaro L, Hong P, Hernandez MM, Argyle D, Mulder LCF, Potla U, et al. IL-15 regulates susceptibility of CD4+ T cells to HIV infection. Proc Natl Acad Sci U S A. 2018;115(41):E9659–67.

401. Nam S, Williams A, Vultur A, List A, Bhalla K, Smith D, et al. Dasatinib (BMS-354825) inhibits Stat5 signaling associated with apoptosis in chronic myelogenous leukemia cells. Mol Cancer Ther. 2007;6(4):1400–5.

402. Xu M, Tchkonia T, Kirkland JL. Perspective: Targeting the JAK/STAT pathway to fight age-related dysfunction. Pharmacol Res. 2016;111:152–4.

403. Meyer SC, Levine RL. Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. Clin Cancer Res Off J Am Assoc Cancer Res. 2014;20(8):2051–9.

404. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009;9(11):798–809.

405. Kreutzman A, Juvonen V, Kairisto V, Ekblom M, Stenke L, Seggewiss R, et al. Mono/oligoclonal T and NK cells are common in chronic myeloid leukemia patients at diagnosis and expand during dasatinib therapy. Blood. 2010;116(5):772–82.

406. Mustjoki S, Auvinen K, Kreutzman A, Rousselot P, Hernesniemi S, Melo T, et al. Rapid mobilization of cytotoxic lymphocytes induced by dasatinib therapy. Leukemia. 2013 27(4):914–24.

407. Schiffer CA, Cortes JE, Hochhaus A, Saglio G, le Coutre P, Porkka K, et al. Lymphocytosis after treatment with dasatinib in chronic myeloid leukemia: Effects on response and toxicity. Cancer. 2016;122(9):1398–407.

408. El Missiry M, Adnan Awad S, Rajala HL, Al-Samadi A, Ekblom M, Markevän B, et al. Assessment of bone marrow lymphocytic status during tyrosine kinase inhibitor therapy and its relation to therapy response in chronic myeloid leukaemia. J Cancer Res Clin Oncol. 2016;142(5):1041–50.

409. Chang MC, Cheng HI, Hsu K, Hsu YN, Kao CW, Chang YF, et al. NKG2A down-regulation by dasatinib enhances natural killer cytotoxicity and accelerates effective treatment responses in patients with chronic myeloid leukemia. Front Immunol. 2018;9:3152.

410. Kreutzman A, Jaatinen T, Greco D, Vakkila E, Richter J, Ekblom M, et al. Killer-cell immunoglobulin-like receptor gene profile predicts good molecular response to dasatinib therapy in chronic myeloid leukemia. Exp Hematol. 2012;40(11):906-913.e1.

411. Kadowaki N, Ishiyama K, Kitawaki T. Cytomegalovirus pulls strings behind NK cells. Oncotarget. 2017;8(55):93297–8.

412. Ishiyama K, Kitawaki T, Sugimoto N, Sozu T, Anzai N, Okada M, et al. Principal component analysis uncovers cytomegalovirus-associated NK cell activation in Ph+ leukemia patients treated with dasatinib. Leukemia. 2017;31(1):203–12.

413. Kim DH, Kamel-Reid S, Chang H, Sutherland R, Jung CW, Kim HJ, et al. Natural killer or natural killer/T cell lineage large granular lymphocytosis associated with dasatinib therapy for Philadelphia chromosome positive leukemia. Haematologica. 2009;94(1):135–9.

414. Mustjoki S, Ekblom M, Arstila TP, Dybedal I, Epling-Burnette PK, Guilhot F, et al. Clonal expansion of T/NK-cells during tyrosine kinase inhibitor dasatinib therapy. Leukemia. 2009;23(8):1398–405.

415. Najima Y, Yoshida C, Iriyama N, Fujisawa S, Wakita H, Chiba S, et al. Regulatory T cell inhibition by dasatinib is associated with natural killer cell differentiation and a favorable molecular response-The final results of the D-first study. Leuk Res. 2018;66:66–72.

416. Vigón L, Rodríguez-Mora S, Luna A, Sandonís V, Mateos E, Bautista G, et al. Cytotoxic cell populations developed during treatment with tyrosine kinase inhibitors protect autologous CD4+ T cells from HIV-1 infection. Biochem Pharmacol. 2020; 182:114203.

417. Gondois-Rey F, Chéret A, Granjeaud S, Mallet F, Bidaut G, Lécuroux C, et al. NKG2C+ memory-like NK cells contribute to the control of HIV viremia during primary infection: Optiprim-ANRS 147. Clin Transl Immunol. 2017;6(7):e150.

418. Flórez-Álvarez L, Hernandez JC, Zapata W. NK Cells in HIV-1 Infection: From Basic Science to Vaccine Strategies. Front Immunol. 2018;9:2290.

419. Bermejo M, Ambrosioni J, Bautista G, Climent N, Mateos E, Rovira C, et al. Evaluation of resistance to HIV-1 infection ex vivo of PBMCs isolated from patients with chronic myeloid leukemia treated with different tyrosine kinase inhibitors. Biochem Pharmacol. 2018;156:248–64.

420. Miller GD, Bruno BJ, Lim CS. Resistant mutations in CML and Ph(+)ALL - role of ponatinib. Biol Targets Ther. 2014;8:243–54.

421. Szaniawski MA, Spivak AM, Cox JE, Catrow JL, Hanley T, Williams ESCP, et al. SAMHD1 Phosphorylation Coordinates the Anti-HIV-1 Response by Diverse Interferons and Tyrosine Kinase Inhibition. mBio. 2018;9(3):e00819-18.

422. Salgado M, Martinez-picado J, Gálvez C, Rodríguez-mora S. Dasatinib protects humanized mice from acute HIV-1 infection. Biochem Pharmacol. 2019;174:113625.

423. Mahon FX. Is going for cure in chronic myeloid leukemia possible and justifiable? Hematol Am Soc Hematol Educ Program. 2012;2012:122–8.

424. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boqué C, et al. Final 5-year study results of DASISION: the dasatinib versus imatinib study in treatment-naïve chronic myeloid leukemia patients trial. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(20):2333–40.

425. Kimura S. Current status of ABL tyrosine kinase inhibitors stop studies for chronic myeloid leukemia. Stem Cell Investig. 2016;3:36.

426. Okada M, Imagawa J, Tanaka H, Nakamae H, Hino M, Murai K, et al. Final 3-year results of the dasatinib discontinuation trial in patients with chronic myeloid leukemia who received dasatinib as a second-line treatment. Clin Lymphoma Myeloma Leuk. 2018;18(5):353-360.e1.

427. Rousselot P, Charbonnier A, Cony-Makhoul P, Agape P, Nicolini FE, Varet B, et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. J Clin Oncol. 2014;32(5):424–30.

428. Etienne G, Guilhot J, Rea D, Rigal-Huguet F, Nicolini F, Charbonnier A, et al. Long-term follow-up of the french stop imatinib (STIM1) study in patients with chronic myeloid leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2017;35(3):298–305.

429. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747–57.

430. Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, Rabson A, et al. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J Virol. 1986;59(2):284–91.

431. Morón-López S, Puertas MC, Gálvez C, Navarro J, Carrasco A, Esteve M, et al. Sensitive quantification of the HIV-1 reservoir in gut-associated lymphoid tissue. PloS One. 2017;12(4):e0175899.

432. Jerome KR, Sloan DD, Aubert M. Measurement of CTL-induced cytotoxicity: the caspase 3 assay. Apoptosis Int J Program Cell Death. 2003;8(6):563–71.

433. Dismuke DJ, Aiken C. Evidence for a functional link between uncoating of the human immunodeficiency virus type 1 core and nuclear import of the viral preintegration complex. J Virol. 2006;80(8):3712–20.

434. Butler SL, Hansen MS, Bushman FD. A quantitative assay for HIV DNA integration in vivo. Nat Med. 2001;7(5):631–4.

435. Buchholtz NVEJ, Nühn MM, de Jong TCM, Stienstra T a. T, Reddy K, Ndung'u T, et al. Development of a highly sensitive and specific intact proviral DNA assay for HIV-1 subtype B and C. Virol J. 2024;21(1):36.

436. Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, et al. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood. 1984;63(4):789–99.

437. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature. 2014;512(7512):74–7.

438. Cadena AM, Ventura JD, Abbink P, Borducchi EN, Tuyishime H, Mercado NB, et al. Persistence of viral RNA in lymph nodes in ART-suppressed SIV/SHIV-infected Rhesus Macaques. Nat Commun. 2021;12(1):1474.

439. Hill AL. Mathematical models of HIV latency HHS Public Access. Curr Top Microbiol Immunol. 2018;417:131–56.

440. Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, Learn GH, et al. HIV-1 integration landscape during latent and active infection. Cell. 2015;160(3):420–32.

441. Wagner TA, McKernan JL, Tobin NH, Tapia KA, Mullins JI, Frenkel LM. An increasing proportion of monotypic HIV-1 DNA sequences during antiretroviral treatment suggests proliferation of HIV-infected cells. J Virol. 2013;87(3):1770–8.

442. Marconi VC, Moser C, Gavegnano C, Deeks SG, Lederman MM, Overton ET, et al. Randomized Trial of Ruxolitinib in Antiretroviral-Treated Adults With Human Immunodeficiency Virus. Clin Infect Dis Off Publ Infect Dis Soc Am. 2022;74(1):95.

443. Boyman O, Purton JF, Surh CD, Sprent J. Cytokines and T-cell homeostasis. Curr Opin Immunol. 2007;19(3):320–6.

444. Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, et al. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. Blood. 2009;113(25):6304–14.

445. Bosque A, Famiglietti M, Weyrich AS, Goulston C, Planelles V. Homeostatic proliferation fails to efficiently reactivate HIV-1 latently infected central memory CD4+ T cells. PLoS Pathog. 2011;7(10):e1002288.

446. Logerot S, Rancez M, Charmeteau-De Muylder B, Figueiredo-Morgado S, Rozlan S, Tambussi G, et al. HIV reservoir dynamics in HAART-treated poor immunological responder patients under IL-7 therapy. AIDS. 2018;32(6):715–20.

447. Hosmane NN, Kwon KJ, Bruner KM, Capoferri AA, Beg S, Rosenbloom DIS, et al. Proliferation of latently infected CD4+ T cells carrying replication-competent HIV-1: Potential role in latent reservoir dynamics. J Exp Med. 2017;214(4):959–72.

448. Spina CA, Anderson J, Archin NM, Bosque A, Chan J, Famiglietti M, et al. An in-depth comparison of latent HIV-1 reactivation in multiple cell model systems and resting CD4+ T cells from aviremic patients. PLoS Pathog. 2013;9(12):e1003834.

449. Laird GM, Rosenbloom DIS, Lai J, Siliciano RF, Siliciano JD. Measuring the Frequency of Latent HIV-1 in Resting $CD4^+$ T Cells Using a Limiting Dilution Coculture Assay. Methods Mol Biol Clifton NJ. 2016;1354:239–53.

450. Gantner P, Pagliuzza A, Pardons M, Ramgopal M, Routy JP, Fromentin R, et al. Single-cell TCR sequencing reveals phenotypically diverse clonally expanded cells harboring inducible HIV proviruses during ART. Nat Commun. 2020;11(1):4089.

451. Reeves DB, Duke ER, Hughes SM, Prlic M, Hladik F, Schiffer JT. Anti-proliferative therapy for HIV cure: a compound interest approach. Sci Rep. 2017;7(1):4011.

452. Reeves DB, Duke ER, Wagner TA, Palmer SE, Spivak AM, Schiffer JT. A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation. Nat Commun. 2018;9(1).

453. Imamichia H, Dewar RL, Adelsberger JW, Rehm CA, O'doherty U, Paxinos EE, et al. Defective HIV-1 proviruses produce novel proteincoding RNA species in HIV-infected patients on combination antiretroviral therapy. Proc Natl Acad Sci U S A. 2016;113(31):8783–8.

454. Marinelli Busilacchi E, Costantini A, Viola N, Costantini B, Olivieri J, Butini L, et al. Immunomodulatory Effects of Tyrosine Kinase Inhibitor In Vitro and In Vivo Study. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2018;24(2):267–75.

455. Bacchus-Souffan C, Fitch M, Symons J, Abdel-Mohsen M, Reeves DB, Hoh R, et al. Relationship between CD4 T cell turnover, cellular differentiation and HIV persistence during ART. PLoS Pathog. 2021;17(1):e1009214.

456. Morcilla V, Bacchus-Souffan C, Fisher K, Horsburgh BA, Hiener B, Wang XQ, et al. HIV-1 Genomes Are Enriched in Memory CD4+ T-Cells with Short Half-Lives. mBio. 2021;12(5):e0244721.

457. Duette G, Hiener B, Morgan H, Mazur FG, Mathivanan V, Horsburgh BA, et al. The HIV-1 proviral landscape reveals that Nef contributes to HIV-1 persistence in effector memory CD4+ T cells. J Clin Invest. 2022;132(7):e154422.

458. Cantero-Pérez J, Grau-Expósito J, Serra-Peinado C, Rosero DA, Luque-Ballesteros L, Astorga-Gamaza A, et al. Resident memory T cells are a cellular reservoir for HIV in the cervical mucosa. Nat Commun. 2019;10(1):4739.

459. Fisher K, Wang XQ, Lee A, Morcilla V, de Vries A, Lee E, et al. Plasma-Derived HIV-1 Virions Contain Considerable Levels of Defective Genomes. J Virol. 2022;96(6):e0201121.

460. Lorenzi JCC, Cohen YZ, Cohn LB, Kreider EF, Barton JP, Learn GH, et al. Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. Proc Natl Acad Sci U S A. 2016;113(49):E7908–16.

461. Bui JK, Sobolewski MD, Keele BF, Spindler J, Musick A, Wiegand A, et al. Proviruses with identical sequences comprise a large fraction of the replication-competent HIV reservoir. PLoS Pathog. 2017;13(3):e1006283.

462. Valle-Casuso JC, Angin M, Volant S, Passaes C, Monceaux V, Mikhailova A, 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. 2019;29(3):611-626.e5.

463. Hayashi Y, Nakamae H, Katayama T, Nakane T, Koh H, Nakamae M, et al. Different immunoprofiles in patients with chronic myeloid leukemia treated with imatinib, nilotinib or dasatinib. Leuk Lymphoma. 2012;53(6):1084–9.

464. Lachota M, Siernicka M, Pilch Z, Graczyk-Jarzynka A, Winiarska M. Dasatinib effect on NK Cells and anti-tumor immunity. Blood. 2018;132:3004–3004.

465. Harrington P, Radia D, Lim M, Dillon R, Kordasti S, Rezvani K, et al. Immunological effects of ponatinib therapy in patients with chronic myeloid leukaemia. 2020;189:72–3.

466. Van Acker HH, Anguille S, Willemen Y, Van den Bergh JM, Berneman ZN, Lion E, et al. Interleukin-15 enhances the proliferation, stimulatory phenotype, and antitumor effector functions of human gamma delta T cells. J Hematol OncolJ Hematol Oncol. 2016;9(1):101.

467. Mizoguchi I, Yoshimoto T, Katagiri S, Mizuguchi J, Tauchi T, Kimura Y, et al. Sustained upregulation of effector natural killer cells in chronic myeloid leukemia after discontinuation of imatinib. Cancer Sci. 2013;104(9):1146–53.

468. Imagawa J, Tanaka H, Okada M, Nakamae H, Hino M, Murai K, et al. Discontinuation of dasatinib in patients with chronic myeloid leukaemia who have maintained deep molecular response for longer than 1 year (DADI trial): a multicentre phase 2 trial. Lancet Haematol. 2015;2(12):e528-535.

469. Rea D, Henry G, Khaznadar Z, Etienne G, Guilhot F, Nicolini F, et al. Natural killer-cell counts are associated with molecular relapse-free survival after imatinib

discontinuation in chronic myeloid leukemia: the IMMUNOSTIM study. Haematologica. 2017;102(8):1368–77.

470. Keating SE, Zaiatz-Bittencourt V, Loftus RM, Keane C, Brennan K, Finlay DK, et al. Metabolic Reprogramming Supports IFN-γ Production by CD56bright NK Cells. J Immunol Baltim Md 1950. 2016 Mar 15;196(6):2552–60.

471. Mah AY, Rashidi A, Keppel MP, Saucier N, Moore EK, Alinger JB, et al. Glycolytic requirement for NK cell cytotoxicity and cytomegalovirus control. JCI Insight. 2017;2(23):e95128, 95128.

472. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, et al. mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. J Immunol Baltim Md 1950. 2014;193(9):4477–84.

473. Velásquez SY, Himmelhan BS, Kassner N, Coulibaly A, Schulte J, Brohm K, et al. Innate Cytokine Induced Early Release of IFNγ and CC Chemokines from Hypoxic Human NK Cells Is Independent of Glucose. Cells. 2020;9(3):734.

474. Almutairi SM, Ali AK, He W, Yang DS, Ghorbani P, Wang L, et al. Interleukin-18 up-regulates amino acid transporters and facilitates amino acid-induced mTORC1 activation in natural killer cells. J Biol Chem. 2019;294(12):4644–55.

475. Wang Z, Guan D, Wang S, Chai LYA, Xu S, Lam KP. Glycolysis and Oxidative Phosphorylation Play Critical Roles in Natural Killer Cell Receptor-Mediated Natural Killer Cell Functions. Front Immunol. 2020;11:202.

476. Terrén I, Orrantia A, Mosteiro A, Vitallé J, Zenarruzabeitia O, Borrego F. Metabolic changes of Interleukin-12/15/18-stimulated human NK cells. Sci Rep. 2021;11(1):6472.

477. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. Science. 2009;324(5930):1029–33.

478. Keppel MP, Saucier N, Mah AY, Vogel TP, Cooper MA. Activation-specific metabolic requirements for NK Cell IFN-γ production. J Immunol Baltim Md 1950. 2015;194(4):1954–62.

479. Lissina A, McLaren JE, Ilander M, Andersson EI, Lewis CS, Clement M, et al. Divergent roles for antigenic drive in the aetiology of primary versus dasatinib-associated CD8+ TCR-Vβ+ expansions. Sci Rep. 2018;8(1):2534.

480. Jo T, Noguchi K, Hayashi S, Irie S, Hayase R, Shioya H, et al. Long-lasting memory of cellular immunity in a chronic myeloid leukemia patient maintains molecular response 5 after cessation of dasatinib. Oncol Lett. 2018;15(3):2935–8.

481. Romero P, Zippelius A, Kurth I, Pittet MJ, Touvrey C, Iancu EM, et al. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. J Immunol Baltim Md 1950. 2007;178(7):4112–9.

482. Ambrosioni J, Coiras M, Alcamí J, Miró JM. Potential role of tyrosine kinase inhibitors during primary HIV-1 infection. Expert Rev Anti Infect Ther. 2017;15(5):421–3.

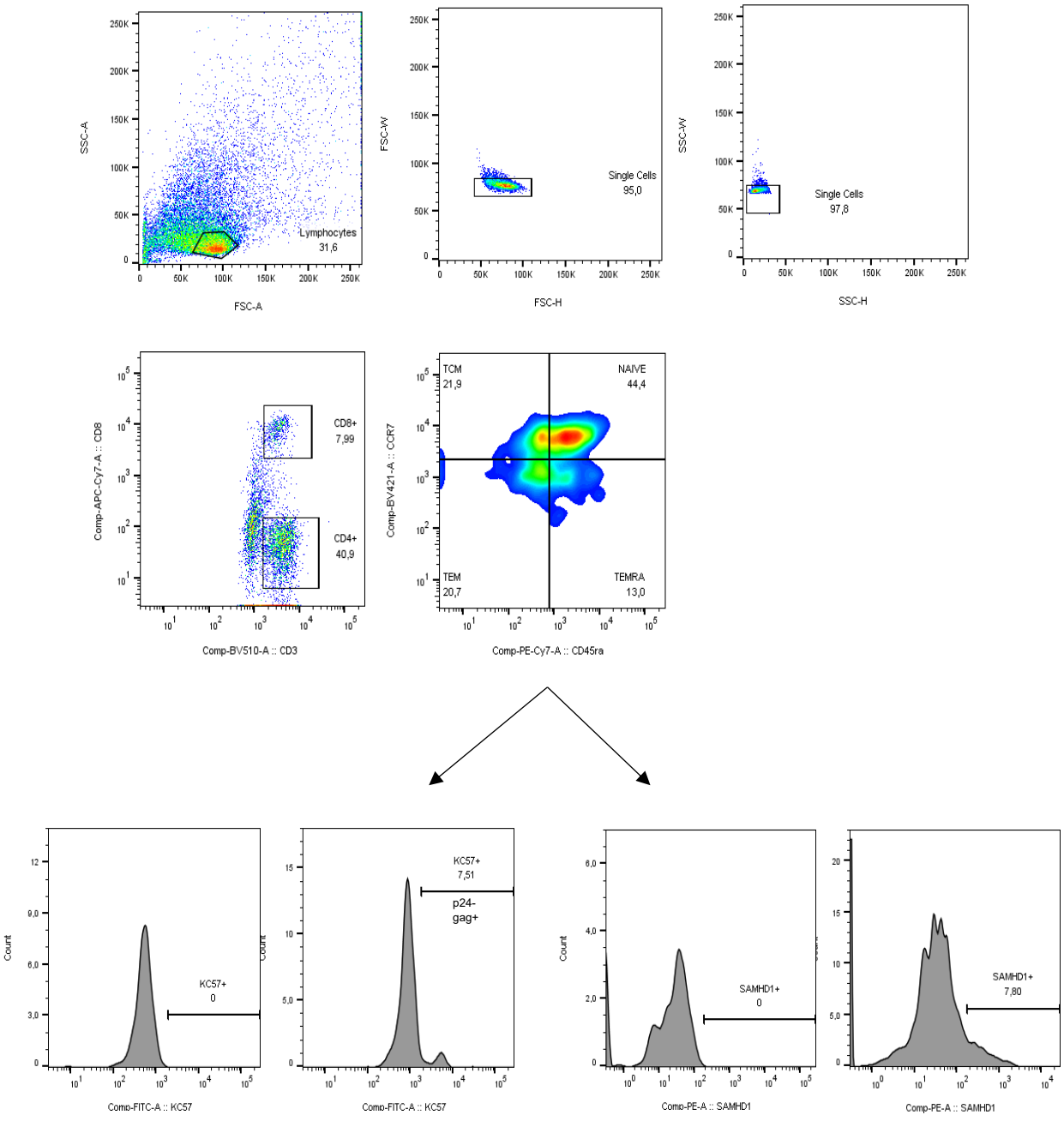
Reactive	Function
Oligomycin	Inhibits ATP synthase (Complex V), decreasing electron flow through the electron transport chain, which reduces mitochondrial respiration (decreasing OCR).
Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP)	Collapses the proton gradient and impairs the mitochondrial membrane potential. Consequently, electron flow through the electron transport chain proceeds uninhibited, driving oxygen consumption by ATP synthase (Complex IV) to its maximum rate. This reflects the maximal respiratory capacity of the cell.
Rotenone/antimycin A	This combination is used to quantify non-mitochondrial respiration by completely suppressing mitochondrial electron transport. By blocking mitochondrial respiration, it allows the measurement of OCR attributable to non-mitochondrial cellular processes.
2-Deoxyglucose (2-DG)	This inhibitor targets glycolysis, leading to a measurable reduction of ECAR and PER. It acts as a glucose analog that blocks glycolysis through competitive binding of glucose hexokinase (first enzyme in the glycolytic pathway).

Supplementary Table 1. List of compounds used in the Mito Stress Test Kit and the Glycolytic Rate Assay Kit for the induction of changes in the mitochondrial respiration or the glycolytic metabolism.

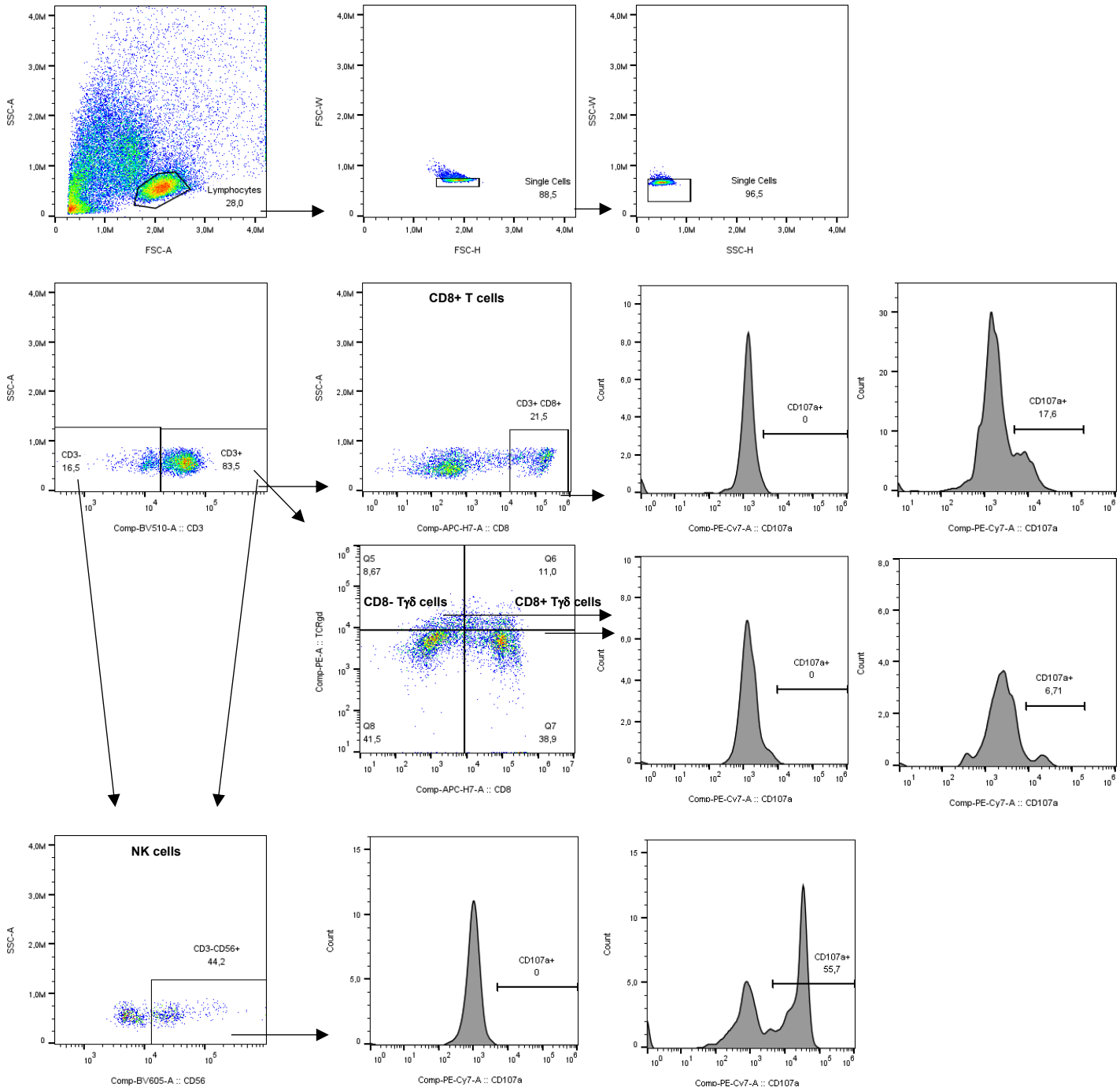
Participant code	Gender (M/F)	Age	Age at HIV diagnosis	Time on ART	Time on current ART regime	CD4 Nadir	CD4 per mililiter	CD8 per mililiter	CD4/CD8 ratio	ART
Participant#004	M	50	33	17	2.1	563	944	639	1.6	BIC/FTC/TAF
Participant#006	M	62	37	25	5.5	-	812.5	3251.8	0.36	BIC/FTC/TAF
Control 4.1	M	38	25	13	4,20	414	977	659	1.48	DTG/RPV
Control 4.2	M	54	42	13	4,17	-	956	462	2.06	BIC/FTC/TAF
Control 4.3	M	42	24	18	1,73	70	684	853	0.80	DTG/3TC
Control 4.4	F	36	21	16	4,89	181	344	1371	0.25	DRV/COBI/FTC/TAF
Control 4.5	M	72	58	16	0,01	153	416	439	0.95	DOR/TFV/FTC
Control 4.6	M	45	31	15	1,42	210	622	440	1.41	DTG/3TC
Control 4.7	M	49	36	13	3,62	-	1119	964	1.16	BIC/FTC/TAF
Control 4.8	M	51	40	12	2,99	-	784	783	1	DTG/3TC
Control 4.9	F	46	27	20	3,40	-	1174	855	1.37	DTG/3TC
Control 4.10	M	52	33	20	3,42	-	1006	1293	0.78	DTG/3TC
Control 6.1	F	53	53	37	3,89	175	1185	1345	0.88	DTG/3TC
Control 6.2	F	54	54	24	0,00	181	606	645	0.94	FTC/RPV/TAF
Control 6.3	M	81	50	32	5,08	-	986	2077	0.47	BIC/FTC/TAF
Control 6.4	M	58	30	29	5,07	36	877	43	20.39	DRV/COBI/FTC/TAF
Control 6.5	M	61	23	34	4,95	273	802	1115	0.72	DRV/COBI/FTC/TAF
Control 6.6	M	63	43	21	4,52	228	1173	1430	0.82	BIC/FTC/TAF
Control 6.7	M	58	35	23	5,19	310	896	925	0.97	FTC/RPV/TAF
Control 6.8	M	54	25	23	0,52	180	796	1298	0.61	DTG/3TC
Control 6.9	F	67	36	31	1,04	-	973	2395	0.41	DTG/3TC
Control 6.10	M	54	26	29	5,21	-	1006	799	1.26	DTG/3TC

ART, antiretroviral treatment; CML, chronic myeloid leukemia; F, female; M, male; Neg, negative; Pos, positive; UD, undetermined; BIC, bictegravir; FTC, emtricitabine, TAF, tenofovir alafenamide; DTG, dolutegravir; RPV, rilpivirine; 3TC, lamiduvine; COBI, cobicistat; DOR, doravidine; DRV, darunavir.

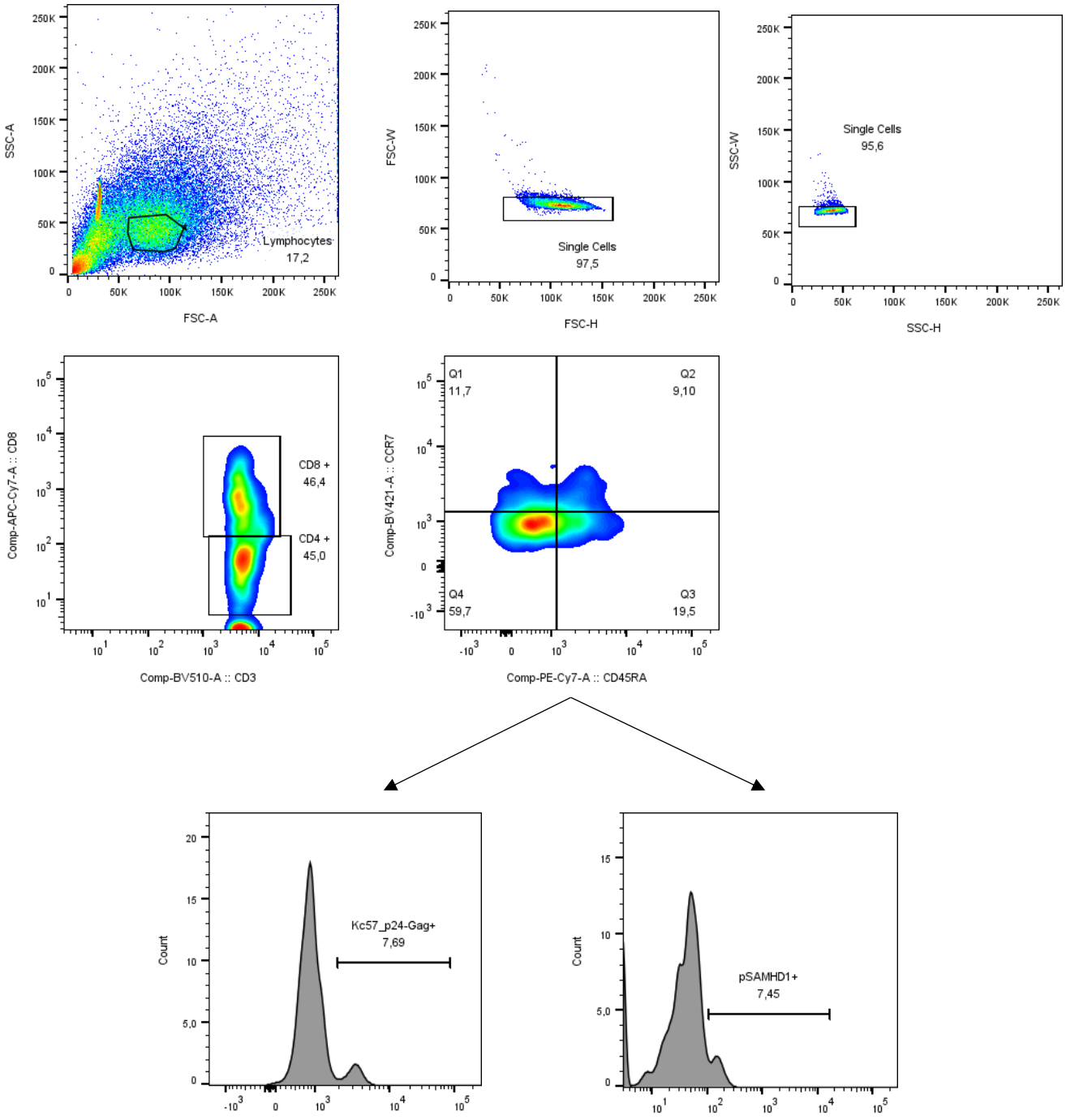
Supplementary table 2. Sociodemographic and clinical data of the participants recruited for the study of Study II



Supplementary Figure S1. Gating strategy to analyze the intracellular expression of p24-gag and pSAMHD1 in CD4+ T cells by flow cytometry. After gating lymphocytes in SSC/FSC dot plot, CD4+ T cells (CD3+CD8-) were gated into 4 memory subpopulations: T_N (CD45RA+CCR7+), T_{CM} (CD45RA-CCR7+), T_{EM} (CD45RA-CCR7-), and T_{EMRA} (CD45RA+CCR7-). In each subpopulation, percentages of p24-gag and SAMHD1 phosphorylation were obtained. Data of negative controls is shown.

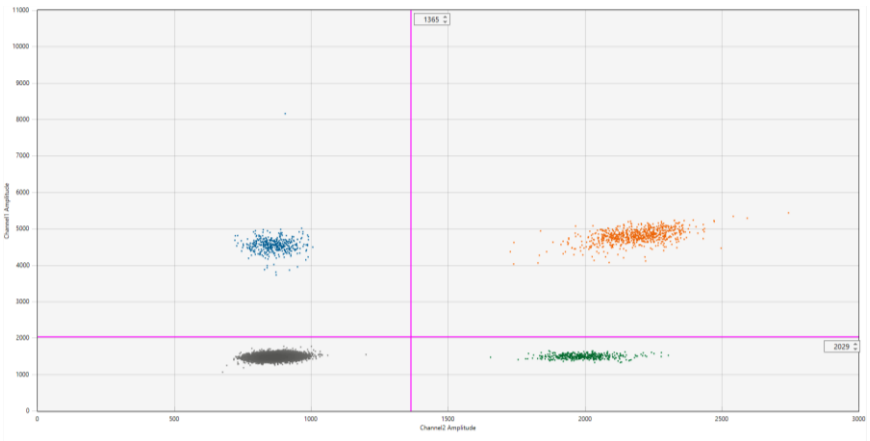


Supplementary Figure S2. Gating strategy to analyze cytotoxic cell populations (CD8+ T cells, Tγδ+ cells, and NK cells), and their degranulation capacity (CD107a+). After gating lymphocytes in SSC/FSC dot plot, CD3- cells were gated into CD56+ to obtain NK cells (CD3-CD56+) and their percentage of CD107a. From CD3+ cells we obtained CD8+ T, CD8+TCRγδ+ and CD8-TCRγδ+ cells, as well as the percentage of CD107a+ in each population.

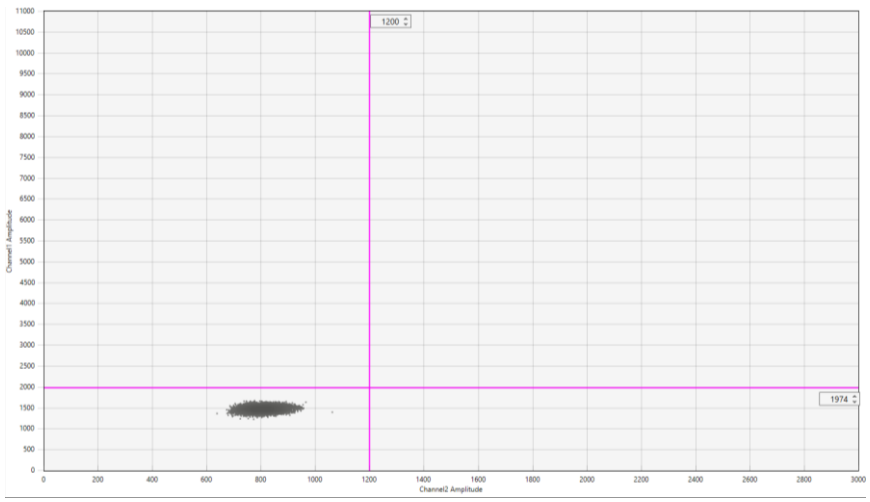


Supplemental Figure S3. Gating strategy for the analysis of the intracellular expression of p24-gag and pSAMHD1 in CD4+ T cells after HIV-1 provirus reactivation. After gating lymphocytes in SSC/FSC dot plot, CD4+ T cells (CD3+CD8-) were gated into 4 memory subpopulations: T_N (CD45RA+CCR7+), T_{CM} (CD45RA-CCR7+), T_{EM} (CD45RA-CCR7-) and T_{EMRA} (CD45RA+CCR7-). In each subpopulation, percentages of p24-gag and pSAMHD1 were obtained.

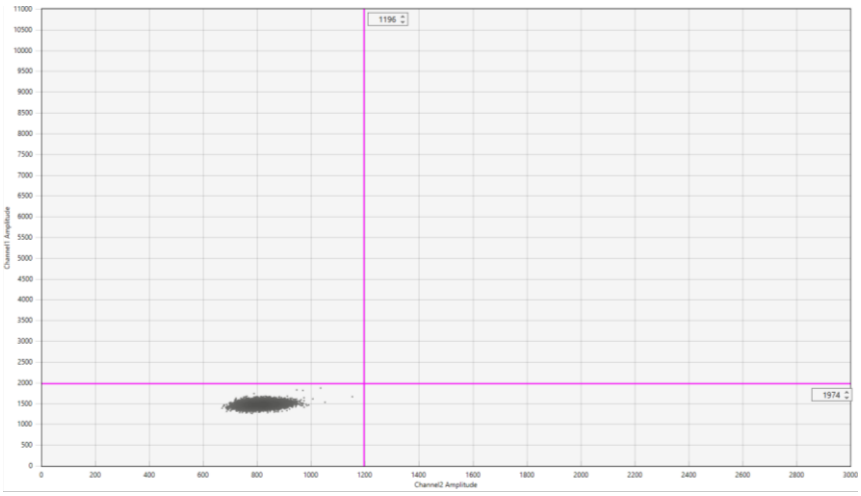
A



B

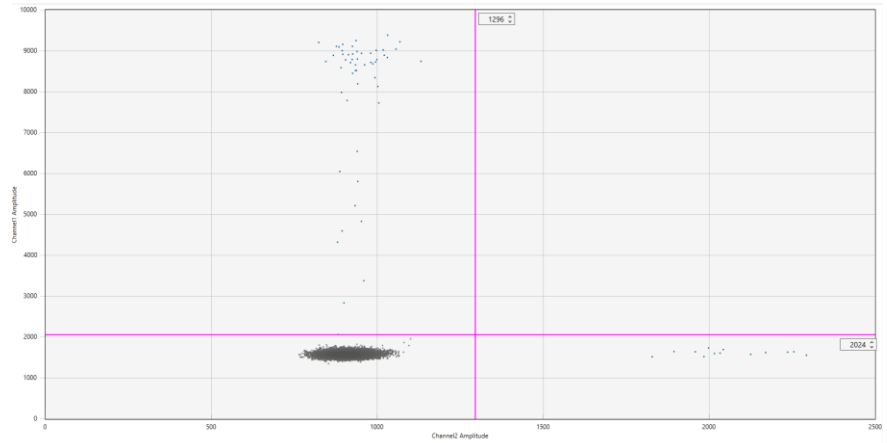


C

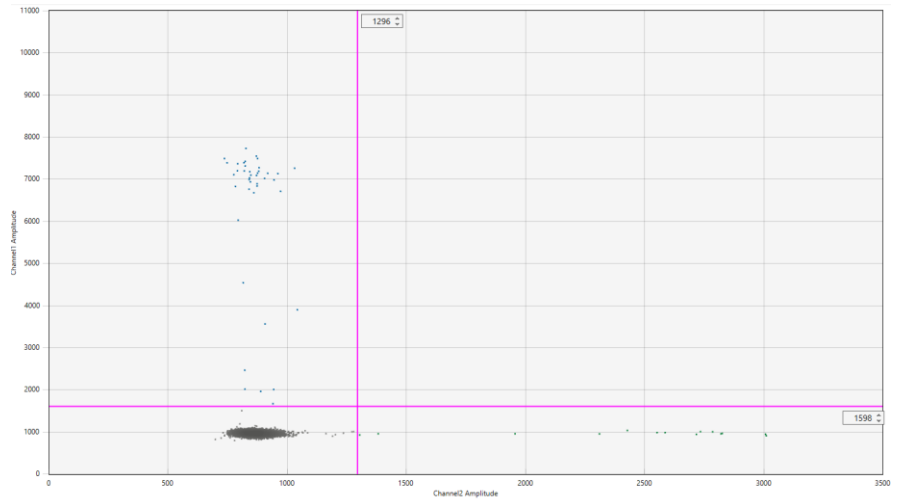


Supplemental Figure S4. Representative results for IPDA controls. Three control materials are analyzed in each IPDA experiment: A) 8E5 cell (positive control), B) purified water and C) PBMCs from healthy donors. Graphs represent the IPDA results for the HIV-1 IPDA.

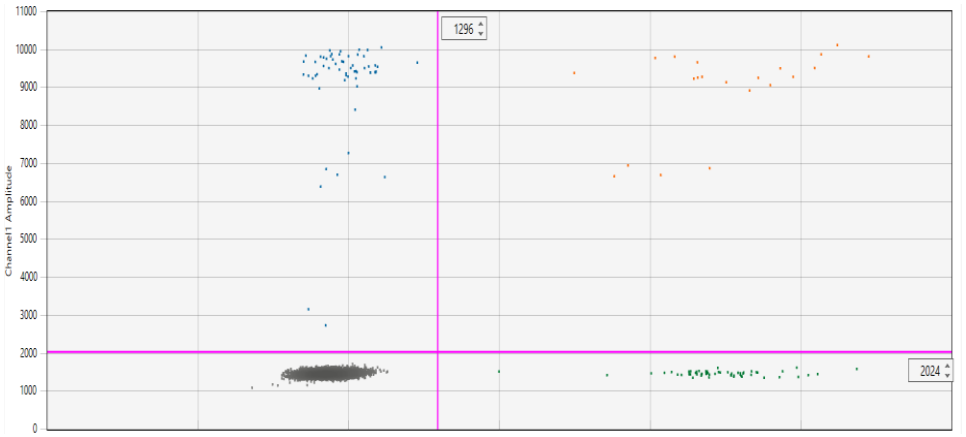
A



B



C



Supplemental Figure S5. HIV-1 IPDA results of participants included in Study II. Graphical representation includes: A) participant #004 after 9,5 months on dasatinib, B) participant #006 after 1 year and 4 months on dasatinib, C) Individual with HIV-1 on ART for 14 years. Identical gates were applied for each subject.

Publications

Publications related to this thesis

1. Manzanares M, Ramos-Martín F, Rodríguez-Mora S, Casado-Fernández G, Sánchez-Menéndez C, Simón-Rueda A, et al. Sustained antiviral response against in vitro HIV-1 infection in peripheral blood mononuclear cells from people with chronic myeloid leukemia treated with ponatinib. Front Pharmacol. 2024 [In press].

2. Pérez-Lamas L*, Manzanares M*, Hernández-Boluda JC, Casado-Montero LF, Gómez-Casares MT, Ayala R, et al. Efficacy of one-year consolidation therapy with ponatinib 15 mg in treatment-free remission rate in chronic myeloid leukemia patients previously achieving a deep molecular response with imatinib: findings from the phase II PONAZERO trial [In preparation].

3. Manzanares M, Casado-Fernández G, Simón-Rueda A, Mateos E, Torres M, Wyen C, et al. Long-term dasatinib treatment reduces reservoir size and competency in people with HIV and chronic myeloid leukemia [In preparation].

4. Manzanares M, Casado-Fernández G, Romero-Martín L, Gourves M, Monceaux V, Sáez-Cirión A, Coiras M. Dasatinib and ponatinib inhibit CD4+ T cell metabolism while allowing the activation of NK cells [In preparation].

Other publications

1. Rodríguez-Mora S, Corona M, Torres M, Casado-Fernández G, García-Pérez J, Ramos-Martín F, Manzanares M, et al. Early cellular and humoral responses developed in oncohematological patients after vaccination with one dose against COVID-19. J Clin Med. 2022. 16;11(10) [In press].

2. Casado-Fernández G, Corona M, Torres M, Saez AJ, Ramos-Martín F, Manzanares M, et al. Sustained cytotoxic response of peripheral blood mononuclear cells from unvaccinated individuals admitted to the ICU due to critical COVID-19 is essential to avoid a fatal outcome. Int J Environ Res Public Health. 2023. 20(3):1947 [In press].

3. Casado-Fernández G, Cantón J, Nasarre L, Ramos-Martín F, Manzanares M, Sánchez-Menéndez C, et al. Pre-existing cell populations with cytotoxic activity against SARS-CoV-2 in people with HIV and normal CD4/CD8 ratio previously unexposed to the virus. Front Immunol. 2024. 15 [In press].