PROGRAMA DE DOCTORADO EN BIOLOGÍA MOLECULAR, BIOMEDICINA E INVESTIGACIÓN CLÍNICA

UNIVERSIDAD DE SEVILLA

TESIS DOCTORAL

ACTIVACIÓN INMUNE, INFLAMACIÓN Y RECUPERACIÓN INMUNOLÓGICA EN DISTINTOS ESCENARIOS DE LA INFECCIÓN POR EL VIH.

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

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“Si supiéramos lo que estábamos haciendo,
no se llamaría investigación, ¿verdad?”

Albert Einstein

AGRADECIMIENTOS

Todo esfuerzo tiene su recompensa y todo comienzo tiene su fin. El recorrido hasta llegar al momento en el que estoy, escribiendo mi Tesis Doctoral, ha sido arduo y largo, pero no hubiera sido posible sin todas las personas que han formado parte de este camino, a las que quiero expresar mi más sincero agradecimiento.

En primer lugar, quiero agradecer a mis directores, Luis y Alicia. Luis, gracias por confiar en mí y darme la oportunidad de formar parte del grupo. Gracias por tu cercanía, esfuerzo, paciencia y conocimientos transmitidos. Ali, podría escribir hojas y hojas, y aun así no sería suficiente. Gracias por acompañarme en aquel entonces nuevo camino e ir aprendiendo a la par, juntas. Gracias por tu perseverancia, dedicación, guía, confianza, apoyo, empatía, cariño… Gracias por cuidar de mí. Sin ti, esta tesis no hubiera sido posible. Gracias jefa, compañera y amiga. Siempre te estaré agradecida.

Gracias a todos los compañeros del laboratorio 211, por hacer fácil y divertidas las largas jornadas de trabajo. A Esperanza por ser mi "cónyuge laboral", por aguantarme y escucharme, por nuestras importantísimas peleas sobre la elección de los colores de las gráficas, pero, sobre todo, por entendernos tan bien. A Ana por ser nuestra mami tanto científica como afectivamente, por cuidarnos. Por su ayuda sin necesidad de pedírsela, su alegría, sabiduría y consejos más allá del trabajo. A Alberto por su sinceridad y cariño. Por soportarme, aconsejarme y hacerme de rabiar. Por traer la diversión al laboratorio. A Carmen por su ternura, bondad y su sentido del humor. A Laura por su energía, apoyo y sabios consejos. A Abraham por su cariño y apoyo, y por qué no, por ser el pollito gruñón. A Reyes por ser mi primera compañera de fatigas y empezar juntas este largo viaje. A Ezequiel, Rafii, Joana, Sara, Fran, Isabel y las Martas, por contribuir y ayudarme en esta aventura.

A las niñas del CDT, Silvia, Montse e Inma. Gracias por vuestra paciencia infinita con los miles de consultas con las que os he acribillado sobre los participantes de los estudios y por estar siempre dispuestas a ayudar. Gracias a María del Mar por recibirme

siempre con una sonrisa. A Nuria, Cristina, Marta y César por vuestra colaboración en reclutar a los participantes y vuestro aprecio.

Gracias también a los que os marchasteis. A Tamara por ser los primeros brazos que me recibieron, por su ternura y amor. A Bea y Laura por acogerme como una más y enseñarme las primeras lecciones sobre citometría. A Sofía por su positivismo, vitalidad y afecto. A Yuya por su buen rollo, su ayuda estadística y su cariño.

Y por supuesto, gracias a los pacientes, porque son los auténticos protagonistas y sin ellos la investigación no sería posible.

A mis amigos, a los de siempre y a los que se han ido incorporando después, con los que comparto tantos momentos de alegría y felicidad. Gracias por vuestro tiempo y amistad.

Agradecer a toda mi familia, en especial a los tres pilares fundamentales de mi vida. A mis padres, Pepi y Eduardo, por haberme educado en el esfuerzo y perseverancia. Gracias a vosotros soy quien soy. Gracias por vuestro incondicional apoyo, amor infinito y darme la vida. A mi hermana Laura, por estar siempre y ser mi confidente y mejor amiga. Eres espejo donde reflejarme. Y gracias por darme, junto con Manuel, el mayor regalo que existe y que, con tan solo unas semanas, llena nuestros días de felicidad. Carmen, no sabes lo que te quiere tu tita.

Por último, quiero dar las gracias a José Miguel, mi compañero de viaje, el motor de mi vida, mi apoyo más firme. Gracias por ayudarme a no perder la esperanza, aguantarme, escucharme y creer siempre en mí. Contigo todo es más fácil. Gracias por quererme y comprenderme, por compartir y disfrutar de la vida juntos.

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RESUMEN

1. Resumen

Esta tesis doctoral explora la activación inmunológica e inflamación crónica (AI/Ic) en distintos escenarios de la infección por el VIH: la coinfección por el VHC, la respuesta inmune discordante (RID) y la simplificación del tratamiento antirretroviral (TAR). Dicho estado se considera uno de los principales factores responsables de la aparición de los eventos no definitorios de SIDA (ENOS) y de la morbilidad y mortalidad de los pacientes con infección por el VIH.

En primer lugar, se realizó un estudio prospectivo para examinar el efecto de la erradicación del VHC sobre la activación y recuperación inmunológica en pacientes coinfectados por el VIH/VHC tras conseguir una respuesta virológica sostenida (RVS) tras completar tratamiento de 12 semanas con antivirales de acción directa (AAD). En segundo lugar, se analizó si la infusión intravenosa de cuatro dosis de células madre mesenquimales procedentes de tejido adiposo (CMTATAd) es segura y eficaz para mejorar la recuperación inmunológica y disminuir la inflamación y activación en pacientes con infección por el VIH y RID. Por último, en un ensayo clínico fase IV abierto, aleatorizado y unicéntrico, se ha evaluado el efecto en la recuperación inmunológica, inflamación y activación inmunológica del mantenimiento de una terapia antirretroviral triple (TT) basada en inhibidores de la integrasa (INI) versus la simplificación a doble terapia (DT) tras 48 y 96 semanas en pacientes con infección por el VIH y viremia indetectable de forma mantenida previa a la inclusión.

Los resultados del primer estudio han mostrado que la erradicación del VHC en pacientes coinfectados por el VIH/VHC ocasiona una disminución significativa en la activación inmunológica en linfocitos T $CD4^+$ y $CD8^+$, en monocitos, en el reservorio del VIH, en los marcadores de translocación microbiana y en los niveles plasmáticos de dímeros D. En cuanto al ensayo clínico con pacientes con infección por el VIH y RID,

los datos sugieren que las infusiones de CMTATAd no son eficaces para incrementar la recuperación inmunológica ni reducir la activación inmunológica e inflamación, al menos con la pauta posológica seleccionada. Por último, los resultados del ensayo clínico de simplificación del TAR han mostrado que la simplificación a DT [darunavir/cobicistat (DRVc) o dolutegravir (DTG) más lamivudina (3TC)] no tiene ninguna repercusión en la recuperación inmunológica, AI/Ic, reservorio o actividad transcripcional del VIH en comparación con el mantenimiento de una TT basado en INI en pacientes con infección por el VIH y supresión virológica.

En conclusión, estos estudios contribuyen a una mejor compresión y caracterización de la activación inmunológica e inflamación crónica en estos escenarios de la infección por el VIH.

INTRODUCCIÓN

2. Introducción

2.1 Evolución histórica de la infección por VIH y tratamiento antirretroviral

La infección por el virus de la inmunodeficiencia humana (VIH) continúa siendo, 40 años después, un problema de salud pública. Según el Programa Conjunto de las Naciones Unidas sobre el VIH/Sida (ONUSIDA), se estima que en 2020 había aproximadamente 37,7 millones de personas en el mundo con infección por el VIH, 1,5 millones de personas contrajeron el VIH y 680.000 murieron debido a enfermedades relacionadas con el VIH [1].

A pesar de seguir siendo una de las enfermedades infecciosas con mayor mortalidad a nivel mundial [2], la historia de la enfermedad ha cambiado por completo gracias a la aparición del tratamiento antirretroviral (TAR). En la era pre-TAR, el síndrome de inmunodeficiencia adquirida (SIDA) era la principal causa de muerte en pacientes con infección por el VIH. Sin embargo, gracias al TAR ha disminuido esta morbilidad y mortalidad asociada a SIDA, permitiendo que las personas con VIH que viven en países desarrollados con acceso a servicios de salud tengan una esperanza de vida mayor [3,4]. Así, la infección por el VIH ha pasado de ser una enfermedad devastadora y letal a una enfermedad crónica [5].

Los regímenes de TAR actuales reducen la replicación del VIH hasta niveles indetectables en plasma, logran un aumento del recuento de linfocitos T $CD4^+$ en la mayoría de los pacientes y una restauración parcial del sistema inmunológico [6], especialmente si se administra en fases tempranas de la infección, previniendo el agotamiento del sistema inmunológico asociado a SIDA y, finalmente, la muerte [7,8]. Sin embargo, el TAR no consigue la erradicación del virus y las personas con infección por el VIH continúan teniendo unas cifras de morbilidad y mortalidad más elevadas que

la población general [9,10], causadas, principalmente, por los eventos no definitorios de SIDA (ENOS) [11].

2.2 Patogénesis de los ENOS

Los ENOS son eventos clínicos que no cumplen con la definición de eventos definitorios de SIDA según los Centros para el Control y Prevención de Enfermedades (CDC) [12] e incluyen enfermedades cardiovasculares (ECV), neoplasias malignas no definitorias de SIDA, deterioro neurocognitivo sin criterios de demencia asociada a SIDA, enfermedad ósea, hepática y renal [13]. Representan las principales causas de muerte en los pacientes con infección por el VIH con TAR [5,14], siendo las más frecuentes las neoplasias malignas, los eventos cardiovasculares y las enfermedades relacionados con el hígado, aunque estas últimas han disminuido considerablemente desde la aparición de los antivirales de acción directa (AAD) frente al virus de la hepatitis C (VHC) [10,13]. Estas comorbilidades, asociadas con la edad avanzada, ocurren de manera más temprana en los pacientes con infección por el VIH que en la población general [15], existiendo motivos para afirmar que los pacientes con infección por el VIH sufren un envejecimiento prematuro o acelerado [16,17].

La activación inmunológica e inflamación crónica (AI/Ic) asociada a la persistencia del VIH es uno de los principales factores causantes de la aparición de los ENOS, además de las coinfecciones con otros patógenos y comorbilidades subyacentes, los estilos de vida insalubres como el tabaco, alcohol o consumo de drogas, y la toxicidad de los fármacos antirretrovirales (Figura 1) [13].

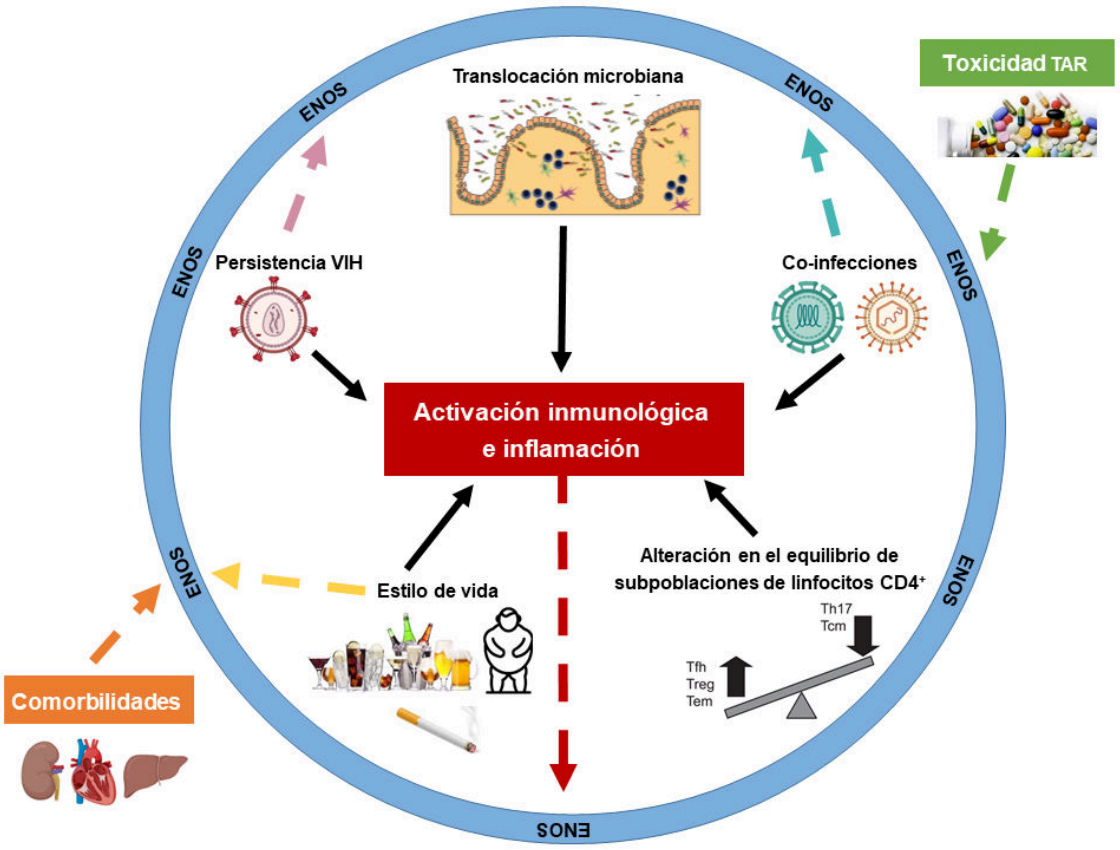

Figura 1. Factores implicados en la patogénesis de los ENOS y en la activación inmunológica e inflamación.

2.3 Activación inmunológica e inflamación

a. Definición y marcadores

La inflamación es una respuesta del sistema inmune ante una amenaza, lesión o invasión de patógenos que se caracteriza por la activación/estimulación de las células del sistema inmune innato y adaptativo, incluidos los monocitos/macrófagos, las células dendríticas y los linfocitos NK, B y T. Estas células secretan mediadores solubles inflamatorios que activan a su vez a otras células inflamatorias con el fin de eliminar el patógeno o reparar el daño y restablecer el estado saludable del organismo [18]. Esta actividad inflamatoria, en condiciones normales, se resuelve una vez que la amenaza desaparece. Sin embargo, la inflamación puede volverse crónica si el desencadenante persiste o si los mecanismos de control supresores no funcionan correctamente.

En la fase crónica de la infección por el VIH, todas las células del sistema inmune pueden adquirir un estado de activación y secretar diferentes citoquinas inflamatorias (Figura 2).

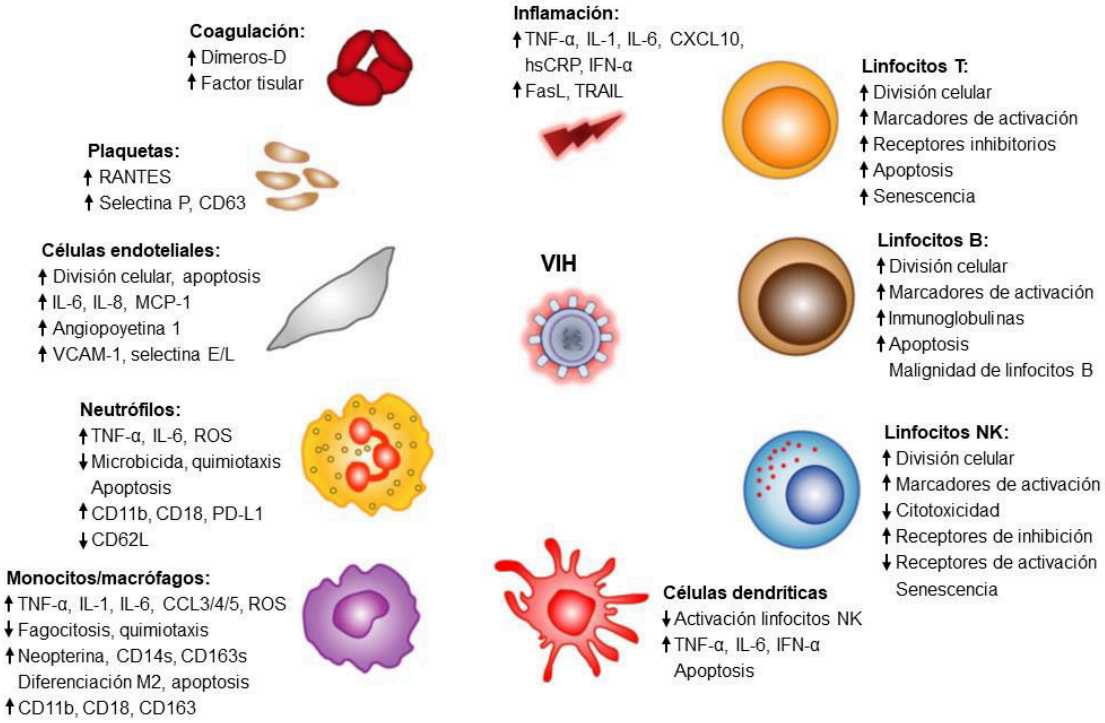

Figura 2. Signos y marcadores de activación en la infección por el VIH.

Modificada de Younas M, Psomas C, Reynes J, Corbeau P. Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. HIV Medicine. 2016 Feb;17(2):89–105.

Dentro de las distintas poblaciones celulares del sistema inmune innato, los monocitos juegan un papel muy importante en el proceso inflamatorio. En los pacientes con infección crónica por el VIH, se observa una expansión de la subpoblación inflamatoria que expresa el clúster de diferenciación (CD)-16, presentando una mayor producción de citoquinas proinflamatorias, como el factor de necrosis tumoral alfa (TNF-α), la interleuquina (IL)-1, IL-6, y de los ligandos de quimioquinas con motivo C-C (CCL)-3, CCL-4 y CCL-5. Además, se ha observado un aumento de la expresión de las moléculas de adhesión CD11b y CD18 y de la concentración plasmática de los marcadores solubles CD14 (CD14s) y CD163 (CD163s) [19,20]. Respecto a las células

dendríticas, existe un aumento de la producción de TNF-α, IL-6 e interferón (IFN) de tipo I, una mayor expresión de la molécula CD40 en la superficie celular, lo que nos indica su grado de activación, y una disminución en el número de células dendríticas circulantes debido a un alto nivel de apoptosis o a la acumulación en órganos linfoides secundarios [19–21]. La activación de los neutrófilos se caracteriza, entre otros, por un incremento en la producción de especies reactivas de oxígeno, una elevada expresión en la superficie celular del ligando 1 de muerte programada (PD-L1) y de las moléculas de adhesión CD11b y CD18, y una disminución en la expresión de CD62L y de la capacidad quimiotáctica [19,22]. En relación a la activación de los linfocitos NK, éstos muestran niveles bajos de mediadores citotóxicos intracitoplasmáticos y receptores activadores, pero sobreexpresan marcadores de activación, senescencia y receptores inhibidores [19,23].

En el sistema inmune adaptativo, la homeostasis de los linfocitos T se altera tras su estimulación. Las células naives se dividen, se activan y se diferencian en células memoria, las cuales se expanden y expresan en su superficie moléculas de activación, como CD38 y antígeno leucocitario humano (HLA)-DR. La estimulación persistente conlleva a un aumento de receptores inhibidores en la superficie de los linfocitos T, como la proteína de muerte celular programada 1 (PD-1), el antígeno-4 asociado al linfocito T citotóxico (CTLA-4), la inmunoglobulina de células T y el dominio 3 de mucina (TIM-3), y marcadores de senescencia, como la sobreexpresión de la molécula CD57 y la pérdida de expresión de la molécula de coactivación CD28. Todo esto desencadena una elevada apoptosis de los linfocitos T [19,24]. Los linfocitos B sufren una activación policlonal que se manifiesta por una hipergammaglobulinemia, una mayor expresión de marcadores de activación y apoptosis, un aumento en el número de células B circulantes y un mayor riesgo de transformación neoplásica [19,25].

Además de la activación del sistema inmune, la infección por VIH activa el sistema de coagulación, produciendo un estado procoagulante caracterizado por un aumento de la concentración plasmática de los dímeros-D y del factor tisular [19,26].

b. Papel en la patogénesis de la infección por VIH

La AI/Ic juega un papel fundamental en la patogénesis de la infección por el VIH y es la principal responsable de la pérdida de linfocitos T $CD4^+$ en ausencia de TAR [27,28]. Varias son las evidencias que lo respaldan. Una de ellas es la infección por el virus de la inmunodeficiencia de los simios (VIS) en sus huéspedes naturales africanos como los monos verdes (*Chlorocebus aethiops*) y los mangabeys grises (*Cercocebus atys*), nativos de África occidental tropical. Estos primates muestran bajos niveles de activación inmunológica y la infección rara vez progresa a SIDA a pesar de mantener niveles altos de viremia [29]. Por el contrario, la infección por VIS en los macacos Rhesus (*Macaca mulatta*) da lugar a unos niveles elevados de activación inmunológica, agotamiento de las células T $CD4^+$ y una rápida progresión a SIDA, a pesar de niveles más bajos o similares de viremia [30].

Por otro lado, la correlación entre la activación inmunológica y la pérdida de linfocitos T $CD4^+$ en los controladores de élite (CE), apoya esta conclusión. Los CE son un grupo minoritario (0,5%-1%) de pacientes con infección por el VIH capaces de controlar la replicación viral en ausencia de TAR [31]. Representan un fenotipo heterogéneo debido a las diferentes definiciones basadas en los límites de detección de la viremia, la presencia o ausencia de viremia de bajo nivel ocasional ("blips") o persistente, la duración del seguimiento y/o los recuentos de linfocitos T $CD4^+$. Dada esta heterogeneidad, los CE presentan diferentes características inmunológicas y virológicas, habiéndose observado que aquellos que presentan niveles más altos de activación inmune e inflamación que los pacientes bajo TAR [32,33] e individuos no

infectados por VIH [32–35] muestran una pérdida lenta pero progresiva de linfocitos T $CD4^+$ [32,35].

En la infección VIH no tratada, la activación de los linfocitos T, medida como la expresión del marcador $CD38^+$ en las células $CD8^+$, es mejor marcador del riesgo de progresión de la enfermedad a SIDA que el recuento de linfocitos $CD4^+$ o la carga viral [36–39]. En cambio, con el TAR los niveles elevados de los marcadores inflamatorios como IL-6, TNF-α, dímeros D, proteína 10 inducida por IFN-γ (IP-10), proteína C reactiva ultrasensible (hsCRP), y de activación (CD14s, CD163s, $CD38^+HLA\text{-}DR^+$) disminuyen, pero en muchos pacientes no alcanzan los valores de la población general a pesar de una supresión constante de la viremia plasmática por debajo del límite de detección de las últimas técnicas comerciales [40–44]. De hecho, los niveles altos de marcadores inflamatorios y de activación se han asociado con un mayor riesgo de ECV, neoplasias, mortalidad y una pobre recuperación de linfocitos T $CD4^+$ en individuos con TAR (Tabla 1).

Tabla 1. Asociación de marcadores de inflamación y activación con ENOS, mortalidad y pobre recuperación linfocitos $CD4^+$.

	Marcadores inflamatorios	**Marcadores activación monocitos**	**Marcadores activación linfocitos T**
ECV	IL-6 [45–47] Dímeros D [45–47] hsCRP [45–47]	CD14s [48]	$CD4^+HLA\text{-}DR^+CD38^+$ [49] $CD8^+HLA\text{-}DR^+CD38^+$ [49]
Neoplasias	IL-6 [50,51] Dímeros D [50,51] hsCRP [50,51]		
Mortalidad	IL-6 [51–54] Dímeros D [51,52,54] hsCRP [52,55]	CD14s [53,56]	
Pobre recuperación de linfocitos $CD4^+$	IL-6 [57] Dímeros D [57] hsCRP [58]	CD14s [57–59]	$CD4^+HLA\text{-}DR^+CD38^+$ [57] $CD8^+HLA\text{-}DR^+CD38^+$ [57,59,60]

c. Causas y consecuencias

Las causas subyacentes del estado de AI/Ic son multifactoriales y complejas [19,27,61] (Figura 1). Probablemente, existe una combinación de varios de los mecanismos responsables y la contribución de cada uno de estos mecanismos varíe en diferentes individuos infectados por el VIH y en las distintas fases de la infección.

Entre los diferentes factores propuestos se encuentran:

- La replicación persistente del VIH en tejidos linfoides.
- La translocación microbiana.
- La alteración en el equilibrio de subpoblaciones de linfocitos T $CD4^+$.
- Las coinfecciones con diferentes patógenos, como citomegalovirus, virus de Epstein-Barr y los virus de la B y VHC.
- El estilo de vida.

Asimismo, varios de estos mecanismos pueden retroactivarse dando lugar a un círculo vicioso incontrolado.

La AI/Ic impacta negativamente en el sistema inmunológico a través de la destrucción y/o desregulación de la arquitectura de tejidos cruciales para la homeostasis y la función de las células T, como el timo, médula ósea y los órganos linfoides secundarios. En el tejido linfoide se produce una fibrosis caracterizada por la acumulación de colágeno. El colágeno reemplaza la red reticular fibroblástica modificando la estructura y función del tejido linfoide con la consiguiente pérdida progresiva de linfocitos T naives [58,62]. La AI/Ic también da lugar a una disfunción tímica a través de la producción subóptima de linfocitos T naives, lo que contribuye a la regeneración deficiente de las células T [47,63,64]. Otra consecuencia de la AI/Ic es la desregulación del sistema inmune, tanto innato como adaptativo [47]. La AI/Ic estimula aún más la infección proporcionando dianas para la replicación del VIH debido al aumento de la expresión del receptor de quimiocinas CC tipo 5 (CCR5) haciendo que

estas células sean más susceptibles a la infección [27]. Esto conduce a una pérdida de la homeostasis de los linfocitos T que se manifiesta por una mayor proliferación, agotamiento y apoptosis de los linfocitos T, junto con un aumento en la diferenciación de las células naives [47,65]. Esta diferenciación da como resultado la generación de células que pierden la expresión de la molécula CD28 y ganan la expresión de CD57. Estas células presentan una disminución de su capacidad proliferativa asociada a un acortamiento de la longitud de sus telómeros [47,65]. Del mismo modo, la AI/Ic inhibe la función de los linfocitos NK y B, células dendríticas y monocitos, lo que conduce a una mayor replicación viral [27,47]. La persistencia de este proceso desencadena en el colapso del sistema inmune en ausencia de TAR y aparición de ENOS.

En la presente Tesis Doctoral se presentan los resultados de tres trabajos que analizan la activación inmunológica e inflamación crónica en distintos escenarios de la infección por el VIH.

- **Coinfección VIH/VHC.**

Como se ha comentado, se hipotetiza que las coinfecciones, entre ellas la producida por el VHC, son factores que aumentan la AI/Ic en pacientes con infección por el VIH.

La infección por el VHC es muy frecuente en personas que viven con el VIH, debido a que ambos virus comparten vías de transmisión. Se calcula que de los 37,7 millones de personas con infección por el VIH, aproximadamente un 6% están coinfectadas por el VHC, siendo el porcentaje más elevado en los usuarios de drogas por vía parenteral [66].

La infección crónica por VHC no sólo produce enfermedad hepática, sino que también se asocia con enfermedades cardiovasculares, renales, metabólicas y del

sistema nervioso central [67]. La hepatitis crónica/cirrosis por el VHC continúa siendo una de las principales causas de morbilidad y mortalidad en los sujetos con infección por el VIH en ausencia de tratamiento con AAD frente al VHC [68,69].

El VHC en pacientes con infección por el VIH sin TAR suele cursar con viremias elevadas, una evolución más rápida de la fibrosis, y un mayor riesgo de cirrosis y carcinoma hepatocelular comparado con pacientes monoinfectados por el VHC [70]. Sin embargo, en nuestro país su prevalencia y sus consecuencias han disminuido considerablemente tras la introducción de los AAD frente al VHC.

El tratamiento de la hepatitis C tiene como objetivo alcanzar la erradicación o curación del VHC evaluada como respuesta virológica sostenida (RVS), definida como ARN plasmático del VHC indetectable entre 3 y 6 meses después de completar la terapia, y que, actualmente, se consigue en la mayoría de los pacientes (RVS >95%) [71]. Dicha erradicación habitualmente conduce a una mejoría del grado de fibrosis y una disminución en la morbi-mortalidad asociada a la hepatopatía crónica por VHC [72–74], mejorando la supervivencia y calidad de vida tanto en pacientes monoinfectados por el VHC como coinfectados por el VIH.

No obstante, no existía evidencia clara sobre la influencia que tiene el VHC en la AI/Ic en pacientes coinfectados por el VIH y VHC (VIH/VHC). Mientras algunos estudios demuestran un mayor nivel de activación de linfocitos T en sujetos coinfectados por el VIH/VHC [75–77], otros autores encuentran un nivel similar de activación inmunológica entre pacientes infectados por el VIH y pacientes coinfectados por el VIH/VHC [78,79]. En este contexto, surge el primer objetivo de la presente Tesis Doctoral abordado en la publicación "**Eradication of Hepatitis C Virus (HCV) Reduces Immune Activation, Microbial Translocation, and the HIV DNA Level in HIV/HCV-Coinfected Patients. J Infect Dis. 2018 Jul 13;218(4):624-632. doi: 10.1093/infdis/jiy136. PMID: 29986086**"

en el que evaluamos si la erradicación del VHC disminuye la AI/Ic y facilita la recuperación inmunológica en pacientes coinfectados por el VIH/VHC.

- **Respuesta inmune discordante.**

Como se ha comentado previamente, tras el inicio del TAR la carga viral plasmática desciende a niveles indetectables mediante las técnicas comerciales habituales con un límite de detección de 20 copias/ml, y el recuento de linfocitos T $CD4^+$ aumenta en la mayoría de los pacientes. En cambio, entre el 15 y 30% de los pacientes con infección por el VIH sin tratamiento previo que comienzan TAR experimentan un aumento limitado del recuento de linfocitos $CD4^+$ a pesar de la supresión virológica continua, más frecuente en aquellos con un nadir bajo de recuentos de linfocitos $CD4^+$ [80,81]. Este fenómeno es conocido en la literatura como respuesta inmune discordante (RID). Aunque no existe consenso para tal definición, ésta siempre se ha basado en la incapacidad de aumentar los recuentos absolutos de linfocitos T $CD4^+$ por encima de diferentes umbrales durante un período de tiempo determinado o alcanzar un valor absoluto de linfocitos T $CD4^+$ en un punto de tiempo predefinido [81,82].

El recuento de linfocitos T $CD4^+$ se mantiene mediante un equilibrio entre la producción de novo, su destrucción y la redistribución tisular. Una alteración de este equilibrio conduce a una reconstitución inmune incompleta [83]. La RID se caracteriza por una producción disminuida y una mayor destrucción de los linfocitos T $CD4^+$.

Varios factores se han asociado con este fenómeno, pero ninguno de ellos explica completamente esta reconstitución inmune incompleta [82], siendo muy probable que múltiples mecanismos desempeñen un papel relevante. En los individuos con infección por el VIH y RID se han descrito graves alteraciones homeostáticas en los linfocitos T $CD4^+$ [84–86], una disminución en la función tímica [84,87], una mayor replicación viral residual [88] y niveles aumentados de activación [89–91], senescencia [84,85],

agotamiento [91,92] y apoptosis [84,90] de linfocitos T $CD4^+$. Adicionalmente, desde el punto de vista clínico, presentan una mayor tasa de morbi-mortalidad asociada a neoplasias, ECV y eventos SIDA que aquellos individuos con una adecuada recuperación inmunológica [93,94], siendo el riesgo mayor cuanto menor sea el recuento de linfocitos $CD4^+$ [95].

A lo largo de estos años, se han ensayado múltiples intervenciones terapéuticas [96] con el objetivo de reducir dicho estado de AI/Ic y, por consiguiente, incrementar los recuentos de linfocitos T $CD4^+$. Estas estrategias han incluido i) intensificación del TAR con maraviroc o raltegravir; ii) agentes que restauran la mucosa intestinal (calostro sérico bovino, probióticos y prebióticos); iii) tratamiento de coinfecciones con valganciclovir, interferón-α y ribavirina, y AAD; iv) agentes que reducen la activación de las células dendríticas plasmocitoides como la cloroquina e hidroxicloroquina y v) agentes inmunomoduladores (estatinas, mesalamina). Desafortunadamente, todas las estrategias han mostrado resultados desalentadores y sin beneficios claros, por lo que actualmente no se dispone de terapias efectivas para su aplicación clínica. Sin embargo, existe un ensayo clínico que sugiere que la infusión de células madre mesenquimales (CMM) puede ser útil para reducir la AI/Ic y mejorar la recuperación inmunológica [97].

Las CMM pueden modular la función de los linfocitos T, linfocitos B, células NK y células dendríticas, a la vez que estimulan a los linfocitos T reguladores dando lugar a un estado antiinflamatorio [98]. Estas propiedades se han comprobado en múltiples modelos animales de enfermedad y se han empleado con éxito en humanos en diversas patologías tales como enfermedad injerto contra huésped [99,100], lupus eritematoso sistémico refractario a tratamiento [101,102], artritis reumatoide [103,104], enfermedad de Crohn [105,106] y otras enfermedades autoinmunes [107]. Además, su baja inmunogenicidad permite tanto el uso de CMM autólogas [108] como alogénicas [108] siendo sus efectos similares independientemente de su procedencia (médula ósea,

tejido adiposo o cordón umbilical) [106,107,109,110]. Por todo ello, en el momento actual es ampliamente aceptado que pueden constituir una herramienta de alto potencial en la medicina regenerativa y terapia celular [97,111]. Los autores de dicho ensayo clínico observaron un aumento considerable de linfocitos T $CD4^+$ circulantes, tanto de las subpoblaciones naive como memoria, tras la infusión (x3) de 0,5 x 10^6/kg CMM obtenidas a partir de cordón umbilical. Estos efectos estuvieron asociados a una disminución sustancial de la activación inmune celular y de los distintos mediadores solubles que participan en ella [97].

Ante este resultado esperanzador, la no disponibilidad de terapias efectivas, y la escasez de estudios sobre el uso de CMM para reducir la AI/Ic en pacientes con infección por el VIH y RID, en nuestro grupo nos planteamos un ensayo clínico con el objetivo de evaluar si las CMM procedentes de una fuente más accesible, como es el tejido adiposo, son seguras y eficaces para mejorar la recuperación inmunológica y disminuir la AI/Ic en este tipo de pacientes. Este estudio forma parte del segundo objetivo de la presente Tesis Doctoral y fue abordado en el trabajo: **"Mesenchymal stromal cells in human immunodeficiency virus-infected patients with discordant immune response: Early results of a phase I/II clinical trial. Stem Cells Transl Med. 2021 Apr;10(4):534-541. doi: 10.1002/sctm.20-0213. Epub 2020 Dec 2. PMID: 33264515; PMCID: PMC7980217"**.

- **Simplificación del tratamiento antirretroviral.**

El TAR ha sido uno de los grandes éxitos en el manejo de los pacientes con infección por el VIH. En 1987 se empezó a utilizar la zidovudina, azidotimidina o AZT, un inhibidor de la transcriptasa inversa análogo de nucleósido (ITIAN) como primer fármaco antirretroviral contra el VIH. El tratamiento con AZT inicialmente demostró cierta eficacia al disminuir las infecciones oportunistas y la mortalidad en pacientes con SIDA [112], pero, debido a su escasa actividad antiviral utilizada en monoterapia, rápidamente

comenzaron a aparecer cepas resistentes y el tratamiento se convirtió en ineficaz. El mayor avance se produjo en 1996 con la aparición del primer inhibidor de la proteasa (IP) [113], comenzando así la era del denominado tratamiento antiviral de gran actividad (TARGA) [114] basada en la combinación de tres fármacos antirretrovirales. Desde entonces, la terapia estándar se ha basado en dos ITIAN junto con un tercer fármaco, bien un IP, un inhibidor de la transcriptasa inversa no análogos de nucleótidos (ITINAN) y, en los últimos años, un inhibidor de la integrasa (INI). No obstante, el entusiasmo inicial que ocasionó el TARGA se vio matizado por los efectos adversos tales como la lipodistrofia, el aumento de los factores de riesgo cardiovascular, la necesidad de un elevado cumplimiento y la aparición de mutaciones de resistencia. Al igual que con algunas enfermedades infecciosas y neoplásicas, apareció el concepto de inducción-mantenimiento en el que, tras un periodo de máxima supresión viral, ésta podría mantenerse con un número menor de fármacos. Sin embargo, inicialmente esto no se consiguió, debido probablemente a una baja barrera genética y actividad antiviral de los fármacos utilizados [115,116]. La idea resurgió años después en forma de monoterapia con la aparición de los nuevos IP, pero tras varios ensayos clínicos y estudios observacionales, se observó que, aunque estos regímenes eran capaces de mantener el control de la viremia en la mayoría de los pacientes, éstos mostraban un mayor número de blips, episodios de viremia transitoria y de fracasos virológicos comparado con la triple terapia (TT) [117,118]. A lo largo de estos últimos años se ha estudiado la doble terapia (DT) como estrategia de simplificación, basada fundamentalmente en un INI o un IP potenciado combinados con lamivudina (3TC). Estos tratamientos han mostrado una eficacia virológica similar a la TT, menos toxicidad y un coste bastante inferior [119]. Sin embargo, existe cierta preocupación sobre si la DT es capaz de controlar la replicación viral en los reservorios tisulares igual que la TT, y por lo tanto pueda influir negativamente en la AI/Ic, la recuperación inmunológica y en el reservorio del VIH.

Por ello, y dado que los datos sobre estos aspectos con la DT eran escasos y los estudios existentes habían evaluado tan sólo aspectos parciales de estos temas, diseñamos un ensayo clínico aleatorizado con la finalidad de evaluar el efecto en la recuperación inmunológica, inflamación y activación inmunológica del mantenimiento de una TT basada en INI versus simplificación a DT [darunavir/cobicistat (DRVc) o dolutegravir (DTG) más 3TC] tras 48 y 96 semanas en pacientes con infección por el VIH y viremia indetectable de forma mantenida antes de su inclusión. Este trabajo se abordó en el objetivo 3 y dio lugar a la publicación **"Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a pilot randomized trial. Clin Microbiol Infect. 2022 Mar 11:S1198-743X(22)00118-5. doi: 10.1016/j.cmi.2022.02.041. Epub ahead of print. PMID: 35289296."**

HIPÓTESIS Y OBJETIVOS

3. Hipótesis y Objetivos

Hipótesis 1. La erradicación del VHC reduce la activación inmunológica y la inflamación y facilita la reconstitución inmunológica en pacientes coinfectados por el VIH/VHC.

Objetivo 1. Estudiar el efecto de la eliminación del VHC sobre la activación y recuperación inmunológica en pacientes coinfectados por el VIH/VHC. Este objetivo se abordó en el trabajo "**Eradication of Hepatitis C Virus (HCV) Reduces Immune Activation, Microbial Translocation, and the HIV DNA Level in HIV/HCV-Coinfected Patients. J Infect Dis. 2018 Jul 13;218(4):624-632. doi: 10.1093/infdis/jiy136. PMID: 29986086".**

Hipótesis 2. La infusión de células mesenquimales troncales alogénicas de tejido adiposo (CMTATAd) a pacientes con respuesta inmune discordante disminuye la activación inmunológica y da lugar a un aumento significativo de linfocitos T $CD4^+$.

Objetivo 2. Analizar si la infusión intravenosa de cuatro dosis de CMTATAd es segura y eficaz para mejorar la recuperación inmunológica y disminuir la inflamación y activación en pacientes con respuesta inmune discordante. Este objetivo se abordó en la publicación **"Mesenchymal stromal cells in human immunodeficiency virus-infected patients with discordant immune response: Early results of a phase I/II clinical trial. Stem Cells Transl Med. 2021 Apr;10(4):534-541. doi: 10.1002/sctm.20-0213. Epub 2020 Dec 2. PMID: 33264515; PMCID: PMC7980217".**

Hipótesis 3. El mantenimiento de una tiple terapia basada en inhibidores de la integrasa junto con dos análogos en pacientes con infección por el VIH y viremia indetectable durante al menos un año da lugar a un aumento mayor del cociente $CD4^+/CD8^+$ comparado con la simplificación una doble terapia basada en DRVc o DTG más 3TC.

Objetivo 3. Evaluar el efecto en la recuperación inmunológica, inflamación y activación inmunológica del mantenimiento de una terapia antirretroviral triple basada en

inhibidores de la integrasa versus simplificación a doble terapia (DTG o DRVc más 3TC) tras 48 y 96 semanas de tratamiento en pacientes con infección por el VIH con viremia indetectable de forma mantenida. Este objetivo se abordó en el trabajo **"Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a pilot randomized trial. Clin Microbiol Infect. 2022 Mar 11:S1198-743X(22)00118-5. doi: 10.1016/j.cmi.2022.02.041. Epub ahead of print. PMID: 35289296".**

MATERIAL, MÉTODOS Y RESULTADOS

4. Material, Métodos y Resultados

4.1 Primer artículo

López-Cortés LF, **Trujillo-Rodríguez M**, Báez-Palomo A, Benmarzouk-Hidalgo OJ, Dominguez-Molina B, Milanés-Guisado Y, Espinosa N, Viciana P, Gutiérrez-Valencia A.

Eradication of Hepatitis C Virus (HCV) Reduces Immune Activation, Microbial Translocation, and the HIV DNA Level in HIV/HCV-Coinfected Patients

J Infect Dis. 2018; 218(4):624-632

doi: 10.1093/infdis/jiy136

The Journal of Infectious Diseases

Eradication of Hepatitis C Virus (HCV) Reduces Immune Activation, Microbial Translocation, and the HIV DNA Level in HIV/HCV-Coinfected Patients

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Background. There are contradictory data about the influence that hepatitis C virus (HCV) has on immune activation and inflammation in patients coinfected with human immunodeficiency virus (HIV) and HCV.

Methods. HIV/HCV-coinfected patients receiving antiretroviral treatment who achieved a sustained virological response with interferon-free regimens were consecutively enrolled in a prospective study. The following factors were assessed before, immediately after the end of, and 1 month after the end of therapy: expression of HLA-DR/CD38, PD-1, and CD57 on $CD4^+$ and $CD8^+$ T-cells; measurement of the total HIV DNA load in peripheral blood mononuclear cells; and determination of plasma levels of soluble CD14 (sCD14), lipopolysaccharide (LPS), 16S ribosomal DNA (rDNA), interleukin 6 (IL-6), D-dimers, and high-sensitivity C-reactive protein (hsCRP).

Results. Ninety-seven patients were consecutively included. At the end of therapy and 1 month later, there were significant reductions in the expression of HLA-DR and CD38 in $CD4^+$ and $CD8^+$ T cells, as well as levels of proviral HIV DNA, sCD14, LPS, 16S rDNA, and D-dimer ($P < .001$). By contrast, the expression of PD-1 and CD57 in $CD4^+$ and $CD8^+$ T cells and levels of IL-6 and hsCRP did not change. The improvement in levels of immune activation markers, proviral HIV DNA, and microbial translocation markers did not translate into an increased $CD4^+$ T-cell count or increased ratio of the $CD4^+$ T-cell count to the $CD8^+$ T-cell count.

Conclusions. HCV eradication in HIV/HCV-coinfected patients results in significant decreases in levels of immune activation markers, proviral HIV DNA load, microbial translocation markers, and D-dimers. These findings support the use of HCV treatment for all HIV/HCV-coinfected patients, even those with low-grade fibrosis.

Keywords. HIV/HCV coinfection; immune activation; anti-HCV therapy; sustained virological response; proviral HIV DNA; CD4; CD4/CD8 ratio.

Morbidity and mortality due to hepatitis C virus (HCV) infection have exceeded mortality due to human immunodeficiency virus (HIV) infection in recent years among HIV/HCV-coinfected patients [1, 2]. Sustained virological response (SVR) is associated with a >70% reduction in the risk of hepatocellular carcinoma and a 90% reduction in the risk of liver-related mortality and has substantially improved the quality of life in both HCV-monoinfected and HIV-coinfected patients [3–5]. However, because of the high cost of novel treatments for HCV infection, therapy continues to focus on fibrosis and is recommended only for patients with liver fibrosis stage ≥2 [6].

On the other hand, immune activation and inflammation due to HIV infection is currently considered to be the driving force of $CD4^+$ T-cell depletion and of the functional impairment of the immune system that eventually leads to AIDS in patients who do not receive antiretroviral therapy (ART) [7, 8]. Although ART reduces levels of both cellular and soluble activation markers, it fails to completely control immune activation and inflammation, despite consistent plasma viral load suppression [9]. This immune activation and inflammation also plays a pivotal role in the emergence of serious non–AIDS-defining events (including cardiovascular, renal, liver, neurologic, and bone diseases; non–AIDS-related cancer; and frailty), which have higher rates of morbidity and mortality among HIV-infected patients, compared with the general population [10–14]. Coinfections with different pathogens, including cytomegalovirus, Epstein-Barr virus, hepatitis B virus, or HCV, has been suggested as contributing to immune activation during HIV infection [15]. Regarding the influence of chronic hepatitis C on immune activation and inflammation in HIV/HCV-coinfected patients, we previously reported a higher level of immune activation on $CD4^+$ and $CD8^+$ T-cells in coinfected subjects [16]; similar findings were observed

Received 25 September 2017; editorial decision 7 March 2018; accepted 1June 2018.

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The Journal of Infectious Diseases® 2018;218:624–32

DOI: 10.1093/infdis/jiy136

in other studies [17–20], which compared HIV-monoinfected patients to HIV/HCV-coinfected patients. However, 2 more-recent studies found a similar level immune activation in both groups of patients [21, 22]. In this study, we aimed to evaluate the impact that eradication of HCV has on immune activation and immune recovery in HIV/HCV-coinfected patients.

MATERIALS AND METHODS

Study Population

In this prospective study, we consecutively enrolled HIV/HCV-coinfected patients who maintained an undetectable HIV RNA level during stable ART and achieved sustained virological response (SVR) with interferon-free hepatitis C treatments at the Infectious Diseases Department of the Virgen del Rocío University Hospital (Seville, Spain) from April to December 2015. SVR was defined as a negative plasma HVC RNA test result 3–6 months after ending anti-HCV therapy. Patients were treated under routine clinical care conditions, according to the prevalent HCV treatment guidelines at the time and the best medical judgment, based on HCV genotype, past history of HCV treatment, drug interactions with ART, and liver fibrosis assessed by transient elastography (FibroScan, Echosens, Paris, France). Cirrhosis was defined as a liver stiffness value of ≥14.5 kPa [23]. The exclusion criteria were hepatitis B virus coinfection, other concomitant causes of liver disease, active infections, and past or present treatment with steroids or immunosuppressive drugs before starting anti-HCV therapy. The study was approved by the Ethics Committee of the Virgen del Rocio University Hospital (protocol code: UCE-VHC-2015-1; internal code: 1050-N-15) and all of the patients provided informed consent.

End Points, Follow-up, and Laboratory Methods

The primary end point was the change in immune activation, measured by the expression of HLA-DR and CD38 in both $CD4^+$ and $CD8^+$ T cells, 1 month after completing anti-HCV therapy. Secondary end points were changes in expression of PD-1 and CD57 in both $CD4^+$ and $CD8^+$ T cells, activation of monocytes-macrophages, levels of soluble markers of inflammation, levels of markers of microbial translocation, and the level of proviral HIV DNA in peripheral blood mononuclear cells (PBMCs). Additionally, we measured changes in the $CD4^+$ T-cell count and the ratio of the $CD4^+$ T-cell count to the $CD8^+$ T-cell count (hereafter, the $CD4^+/CD8^+$ T-cell ratio) 1 and 6 months after ending anti-HCV therapy. Additionally, a control group of healthy volunteers was included in the analysis as reference.

Patients were assessed at baseline, after 1 month of anti-HCV therapy, at the end of treatment, and 1 month later, and data including adverse events, biochemical profiles, and hematologic counts were recorded. The HCV RNA level was measured at baseline, at the end of anti-HCV therapy, and 3–6 months later, using a quantitative polymerase chain reaction (PCR) assay (the Cobas TaqMan test; Roche Diagnostic Systems, Pleasanton, CA; detection limit, 15 IU/mL). The HIV RNA level (determined by the Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, version 2.0; lower detection limit, 15 copies/mL) and $CD4^+$ and $CD8^+$ T-cell counts (determined by standard flow cytometry) were measured at baseline, at the end of anti-HCV therapy, and 1 and 6 months later.

The activation was assessed in both $CD4^+$ and $CD8^+$ T cells by flow cytometry, using the expression markers 7AAD PerCp-Cy5.5 (NA), CD3 BV786 (UCHT1), CD4 APC-Cy7 (RPA-T4), CD8 BV510 (SK1), HLADR FITC (G46-6), and CD38 BV605 (HB7), and senescence and cell dysfunction were measured using the antibodies CD57 APC (NK-1) and PD-1 PE (EH12.1), respectively (all from BD Biosciences, San Diego, CA). The stained samples were acquired on a Fortessa LSR II instrument (BD Biosciences, Madrid, Spain) and analyzed with the software FACSDiva (BD Biosciences). All analyses were performed on cryopreserved PBMCs. The cell viability was >80% in all samples, and dead cells were discarded from the analysis. Plasma samples were assessed by commercially available enzyme-linked immunosorbent assay (ELISA) kits for determination of interleukin 6 (IL-6) levels (using the QuantiGlo IL-6 immunoassay; R&D Systems, Minneapolis, MN), high-sensitivity C-reactive protein (hs-CRP) levels (using the High Sensitive C Reactive Protein ELISA kit [catalog no. SK00080-02]; Aviscera Bioscience, Santa Clara, CA), D-dimer levels (using the Human D-Dimer test; Thermo Fisher Scientific, Waltham, MA), and soluble CD14 (sCD14) levels (using the Human sCD14 Quantikine ELISA kit; R&D Systems, Abingdon, United Kingdom) according to the manufacturers' instructions. Microbial translocation was assessed by measuring 2 different markers: bacterial lipopolysaccharide (LPS) levels, by using the QCL-1000 Limulus Amebocyte Lysate kit (Lonza, Basel, Switzerland); and plasma 16S ribosomal DNA concentrations, by means of quantitative PCR analysis with degenerate forward and reverse primers (8F: 5′-AGAGTTTGATYMTGGCTCAG-3′; and 361R: 5′-CGYCCATTGBGBAADATTCC-3′) and a TaqMan probe (338P: 5′-FAM-TACGGGAGGCAGCAGT-BHQ1-3′) [24].

Total cellular HIV DNA (integrated and unintegrated viral DNA) from PBMCs was assayed by using a custom TaqMan-based real-time PCR assay for the amplification of nucleotides 761–861 in the long terminal repeat of the HIV *gag* region, which is highly conserved (91%) among all HIV-1 group M subtypes, using 2 specific primers (forward: 5′-TAGCGGAGGCTAGAAGGAGA-3′; and reverse: 5′-CCCTGGCCTTAACCGAATT-3′) and a probe within the selected *gag* long terminal repeat region (5′-TACCGACGCTCTCGCACCCA-3′) labeled with FAM, as previously described [25].

Statistical Analysis

Results are expressed as median values, with interquartile ranges (IQRs) or ranges, for continuous variables, and as numbers and percentages of cases, for categorical variables. The χ^2 and Fisher exact tests were used to compare categorical variables, and the

Mann-Whitney *U* test was used to compare quantitative variables. The relationships between continuous variables were assessed by Spearman rank correlation coefficients (determined using the ρ test). The Wilcoxon signed rank test was performed to compare changes in continuous variables over time. The variables that were statistically significant in the univariate analysis were included in a logistic regression model. Differences were considered statistically significant if the *P* value was < .05, after adjustment for multiple comparisons when applicable. The statistical analyses were performed using IBM software (SPSS, version 23.0; SPSS, Chicago, IL).

RESULTS

Patients' Characteristics

A total of 105 white HIV-1/HCV-coinfected patients completed a 12-week course of treatment with anti-HCV direct-acting antiviral agents from April through December 2015. Three subjects did not achieve SVR, 2 were lost to follow-up, and 3 did not maintain an undetectable HIV RNA level. Thus, 97 subjects were included in the analysis, with main baseline characteristics are shown in Table 1. At baseline, there were no differences among patients with liver fibrosis stages 0–3, and therefore they were grouped together; however, patients with liver cirrhosis had a somewhat lower $CD4^+$ T-cell count, higher expression of CD38 in both $CD4^+$ and $CD8^+$ T cells, and higher levels of LPS and IL-6 than patients without cirrhosis. Forty-five subjects were naive to HCV treatment, and 52 were treated previously (40 received pegylated interferon alfa-2a plus ribavirin, 3 received pegylated interferon alfa-2a plus ribavirin plus telaprevir, 1 received ombitasvir/paritaprevir/ritonavir plus ribavirin, and 1 received sofosbuvir plus ribavirin), with no significant differences in baseline characteristics between the 2 groups (Supplementary Table 1). The direct-acting antiviral agents received by the patients were heterogeneous: 36 received sofosbuvir plus ledipasvir, with or without ribavirin; 26 received ombitasvir, paritaprevir/ritonavir, and dasabuvir, with or without ribavirin; 12 received ombitasvir and paritaprevir/ritonavir plus ribavirin; 17 received sofosbuvir plus daclatasvir, with or without ribavirin; and 6 received sofosbuvir plus simeprevir. The 33 healthy volunteers (male sex, 60.6%) had a median age of 42 years (range, 25–62 years) and a body mass index of 22.5 (range, 20.0–24.1).

Relationship Between T-Cell Activation and Other Variables at Baseline

$CD4^+$ T-cell counts, $CD4^+$ T-cell percentages, and $CD4^+/CD8^+$ T-cell ratios were negatively correlated with the expression of both HLA-DR and CD38 in $CD4^+$ T cells but not with other variables (Supplementary Table 2). However, plasma HCV RNA levels were not correlated with levels of activation markers in T cells or monocytes, microbial translocation markers, or inflammatory parameters overall (Supplementary Table 3) and among patients with and those without cirrhosis (data not shown).

Evolution of Immune Activation and Inflammation After HCV Eradication

Both at the end and 1 month after completing of anti-HCV therapy, there were significant decreases in the median expression of HLA-DR and CD38 on both $CD4^+$ and $CD8^+$ T cells. Among $CD4^+$ T cells, median frequencies of HLA-DR expression were 3.2% (range, 0.70%–15.3%) at baseline and 2.5% (range, 0.5%–8.0%) 1 month after completion of HCV treatment; median frequencies of CD38 expression were 8.7% (range, 6.0%–40.7%) and 5.6% (range, 0.3%–6.9%), respectively; and median frequencies of both HLA-DR and CD38 expression were 2.4% (range, 0.5%–16.7%) and 0.7% (range, 0.1%–3.0%), respectively ($P \leq .001$ for all comparisons). Among $CD8^+$ T cells, median frequencies of HLA-DR expression were 3.5% (range, 1.0%–12.0%) at baseline and 2.0% (range, 0.3%–6.4%) 1 month after completion of HCV treatment; median frequencies of CD38 expression were 3.9% (range, 0.4%–48.9%) and 3.1% (range, 0.5%–13.5%), respectively, and median frequencies of both HLA-DR and CD38 expression were 2.7% (range, 0.1%–26.0%) and 0.9% (range, 0.1%–5.9%), respectively ($P \leq .001$ for all comparisons). Differences in the expression of PD-1 or CD57 were not significantly different between baseline and 1 month after completion of HCV treatment. However, except for the expression of HLA-DR on $CD4^+$ and $CD8^+$ T cells, these values remained significantly higher than those found in healthy volunteers (Figure 1 and Table 2).

Furthermore, there were significant reductions in the median proviral HIV DNA load (from 3.16 $\log_{10}$ copies/10^6 PBMCs at baseline [range, 0.41–7.57 $\log_{10}$ copies/10^6 PBMCs] to 2.27 $\log_{10}$ copies/10^6 PBMCs [range, 0.24–5.66 $\log_{10}$ copies/10^6 PBMCs] 1 month after HCV treatment completion), the median sCD14 level (from 4649 pg/mL [range, 106–67 010 pg/mL] to 2769 pg/mL [range, 12–2516 pg/mL]), the median LPS level (from 50.1 pg/mL [range, 11.0–96.0 pg/mL] to 44.2 pg/mL [range, 5.0–63.0 pg/mL]), the 16S rDNA level (from 5.11 $\log_{10}$ copies/mL [range, 4.39–6.35 $\log_{10}$ copies/mL] to 4.75 $\log_{10}$ copies/mL [range, 2.50–6.06 $\log_{10}$ copies/mL]), and the D-dimer level (from 0.53 μg/mL [range, 0.01–5.63 μg/mL] to 0.15 μg/mL [range, 0.01–5.61 μg/mL]; $P < .001$ for all comparisons). Differences in IL-6 and hs-CRP plasma levels between baseline and 1 month after HCV treatment completion were not significantly different. Although there were no differences in the plasma levels of 16S rDNA, IL-6, and D-dimers between patients who completed HCV treatment and healthy volunteers, the concentrations of sCD14, LPS, and hs-CPR in the former group remained well above those in the latter group (Table 2). These changes were similar in patients without and those with cirrhosis (Figure 2 and Supplementary Table 4).

Evolution of $CD4^+$ T-Cell Counts and $CD4^+/CD8^+$ T-Cell Ratios After HCV Eradication

Among all patients, improvements in immune activation and other parameters did not translate into a significant increase in the median $CD4^+$ T-cell count (719 cells/μL [range, 120–1311

Table 1. Baseline Characteristics of 97 White Patients Coinfected With Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV), Overall and by Cirrhosis Status

Characteristic	Overall (n = 97)	No Cirrhosis (n = 68)	Cirrhosis (n = 29)	*P*[a]
Male sex	83 (85.6)	56 (82.4)	27 (93.1)	.171
Age, y	49 (46–53)	49 (46–52)	51 (46–53)	.544
Body mass index[b]	23.8 (21.3–27.5)	23.6 (21.1–26.5)	24.5 (21.9–28.8)	.097
Risk factor for HIV/HCV coinfection				
Previous injection drug use	68 (70.1)	46 (67.6)	22 (75.9)	
Other	29 (29.9)	22 (32.49)	7 (24.1)	.611
HCV characteristic				
Infection duration, y	21 (17–25)	21 (15–26)	23 (18–25)	.204
Subtype, %				.084
1a	39	28	8	
1b	19	15	4	
1ab	5	3	2	
3	14	8	6	
4	23	14	9	
RNA level, $\log_{10}$ IU/mL	6.23 (5.76–6.64)	6.22 (5.75–6.70)	6.28 (5.66–6.55)	.565
Fibrosis stage(s), %				
0–1	29	29	0	
2	16	16	0	
3	23	23	0	
4	29	0	29	
$CD4^+$ T-cell count, cells/μL				
Nadir	181 (67–289)	197 (83–292)	122 (23–288)	.115
Overall	719 (503–921)	791 (554–950)	621 (318–794)	.006
T cells expressing CD4, % per μL	32.8 (27.1–39.4)	33.3 (28.1–39.7)	31.4 (23.0–38.6)	.186
$CD4^+/CD8^+$ T-cell ratio[c]	0.83 (0.56–1.18)	0.83 (0.62–1.19)	0.74 (0.53–1.12)	.577
Duration of HIV RNA undetectability, mo	58 (43–90)	69 (39–109)	41 (14–67)	.019
HIV DNA load, $\log_{10}$ copies/10^6 PBMCs	3.16 (2.00–5.35)	3.15 (1.86–5.35)	3.17 (2.43–5.61)	.409
$CD4^+$ T-cell marker(s), % of $CD4^+$ T cells				
HLA-DR	3.2 (1.9–4.5)	3.0 (1.9–4.3)	3.4 (1.8–4.7)	.662
CD38	8.7 (6.1–12.4)	7.8 (5.6–10.5)	11.1 (7.2–17.3)	.006
HLA-DR and CD38	2.4 (1.3–4.00)	2.2 (1.2–3.9)	3.0 (1.5–4.2)	.162
PD-1	1.9 (1.1–3.1)	1.9 (1.1–3.1)	1.9 (0.9–3.6)	.860
CD57	2.1 (1.3–4.1)	2.6 (1.3–4.5)	1.4 (0.8–2.2)	.076
$CD8^+$ T-cell marker(s), % of $CD8^+$ T cells				
HLA-DR	3.5 (2.5–5.8)	3.2 (2.1–4.5)	5.6 (2.8–7.2)	.050
CD38	3.9 (2.4–6.5)	3.5 (2.3–5.3)	5.2 (3.1–11.1)	.060
HLA-DR and CD38	2.7 (1.5–4.45)	2.7 (1.5–4.3)	2.8 (1.5–5.7)	.645
PD-1	1.9 (1.0–3.3)	1.8 (1.0–3.2)	1.9 (0.9–5.1)	.543
CD57	11.6 (7.2–18.6)	11.3 (7.4–19.5)	12.3 (6.3–18.1)	.538
Monocyte activation marker				
sCD14 level, pg/mL	4649 (2262–6894)	4682 (2406–7931)	4300 (1657–5967)	.582
Microbial translocation marker				
LPS level, pg/mL	50.1 (28.2–61.3)	50.0 (27.2–60.5)	58.1 (49.2–63.0)	.028
16S rDNA level, $\log_{10}$ copies/mL	5.11 (4.39–6.35)	5.07 (4.85–5.45)	5.22 (4.88–5.65)	.362
IL-6 level, pg/mL	6.34 (4.69–8.51)	5.73 (4.50–7.49)	7.98 (6.30–10.14)	.011
D-dimer level, μg/mL	0.53 (0.28–1.04)	0.55 (0.29–1.06)	0.40 (0.23–1.00)	.199
hs-CRP level, mg/L	2.2 (2.0–2.7)	2.2 (1.9–2.6)	2.2 (2.0–2.7)	.953

Data are no. (%) or % of patients or median values (interquartile range).

Abbreviations: IL-6, interleukin 6; hs-CRP, high-sensitivity C-reactive protein; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; rDNA, ribosomal DNA; sCD14, soluble CD14.

[a]Values ≤.05 denote statistically significant differences.

[b]Calculated as the weight in kilograms divided by the height in meters squared.

[c]Ratio of the $CD4^+$ T-cell count to the $CD8^+$ T-cell count.

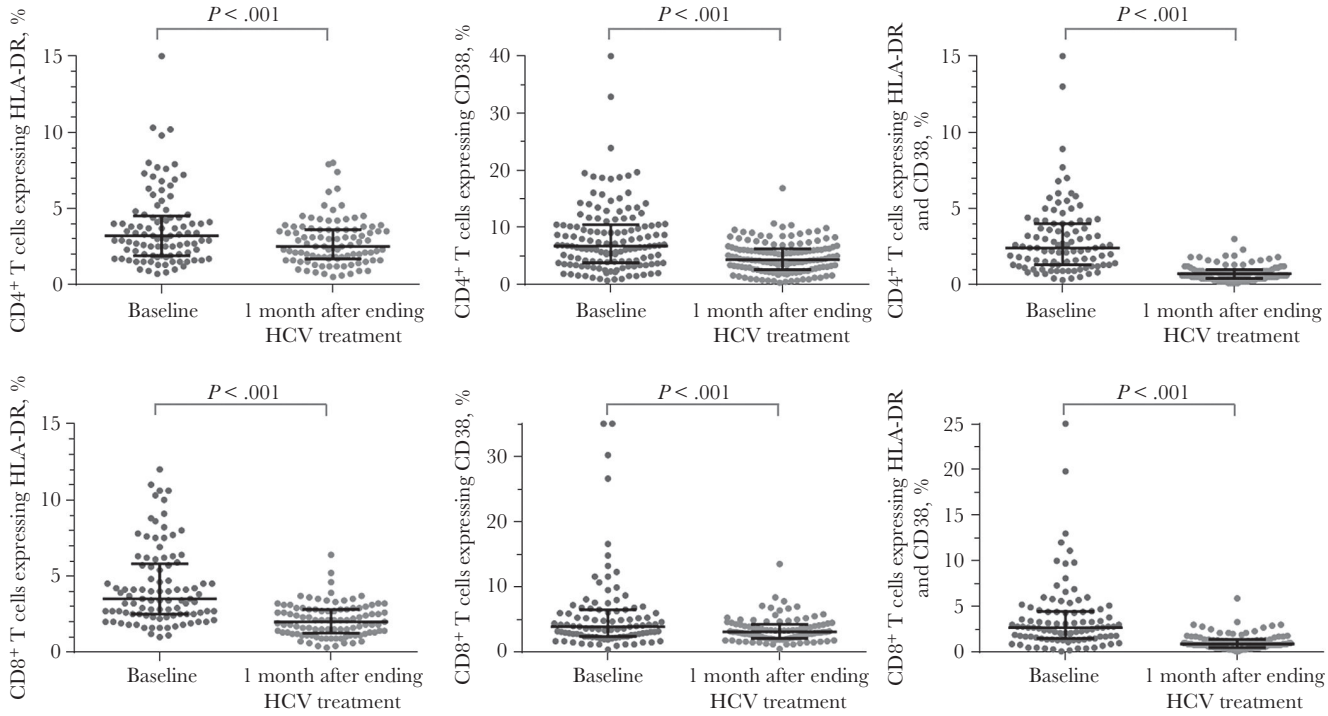

Figure 1. Changes in the percentage of CD4+ and CD8+ T cells that expressed HLA-DR and CD38 at baseline and 1 month after ending anti–hepatitis C virus (HCV) therapy in patients coinfected with human immunodeficiency virus (HIV) and HCV who achieved sustained virological response. Dots denote values for individual patients. Bars denote median values and interquartile ranges.

cells/μL] at baseline vs 736 cells/μL [range, 108–1775 cells/μL] 6 months after HCV treatment completion; $P = .125$), the median CD4+ T-cell percentage (32.8% [range, 10.7%–61.1%] vs 31.7% [range, 9.2%–63.1%]; $P = .050$), or the median CD4+/CD8+ T-cell ratio (0.83 [range, 0.16–2.53] vs 0.73 [range, 0.12–2.76; $P = .069$); similar nonsignificant differences were observed for subgroups of patients (Supplementary Table 5). At an individual level, there were 21 patients (21.6%) in whom the CD4+/CD8+ T-cell ratio increased by ≥0.1 (an arbitrarily chosen cutoff; from 0.77 [range, 0.20–2.53] at baseline to 0.96 [range, 0.33–2.76] 6 months after HCV treatment completion; $P < .001$), whereas there were 36 patients in whom the median ratio decreased by ≥0.1 (from 0.95 [range, 0.31–2.18] to 0.72 [range, 0.22–2.05]; $P < .001$). Among the analyzed variables, the only differences between them were a lower frequency of cirrhosis (19% vs 44%; $P = .024$) and a lower frequency of CD4+ T cells expressing CD38 at baseline (median, 6.2% [range, 3.0%–19.1%] vs 9.2% [range, 3.2%–40.7%]; $P = .016$). However, neither the cirrhosis frequency nor the frequency of CD38 expression among CD4+ T cells at baseline were associated with changes in the CD4+/CD8+ T-cell ratio between baseline and 6 months after the end of HCV treatment in the logistic regression model.

DISCUSSION

The fact that HCV coinfection induces a higher level of immune activation in HIV-infected patients was inferred from comparison of immune activation levels in monoinfected and coinfected patients. Only the study by Gonzalez et al [17] has shown a decreased frequency of CD38 expression on CD8+ T cells among patients (n = 14) who achieved SVR. Our study assessed the medium-term (ie, 1 month after HCV treatment cessation) impact of HCV eradication due to interferon-free regimens on several parameters related to immune activation and immune recovery in a large group of HIV/HCV-coinfected patients.

In addition to a significant reduction in CD38 and HLA-DR expression on CD4+ and CD8+ T cells, there were decreases in levels of proviral HIV DNA, sCD14, microbial translocation markers, and D-dimers after the eradication of HCV infection. Although there were not quantitative correlations between the decrease in proviral HIV DNA load and the expression of HLA-DR and CD38 on both CD4+ and CD8+ T cells, it might be hypothesized that both events might be related. In HIV/HCV-coinfected patients, HCV eradication has proven to be beneficial not only in terms of the liver-related mortality rate and fibrosis regression, but also with regard to decreases in the frequencies of HIV disease progression, non–AIDS-related events, and non–liver-related mortality [26–30]. Almost certainly, a large proportion of this benefit is due to the reduction in the level of immune activation, given its key role in driving HIV-1 disease and non–AIDS-defining events in this population [31, 32]. By contrast, the expression of immune dysfunction (PD-1) and immune senescence (CD57) markers on both CD4+ and CD8+

Table 2. Comparison of Posttreatment Characteristics of 97 White Patients Coinfected With Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) to Their Baseline Characteristics and to Characteristics of Healthy Volunteers

Characteristic	Baseline	End of HCV Treatment	1 mo After End of HCV Treatment	P^a	Healthy Volunteers	P^b
HIV DNA load, $\log_{10}$ copies/10^6 PBMCs	3.16 (0.41–7.57)	2.2 (0.31–7.2)	2.27 (0.24–5.66)	<.001	NA	
$CD4^+$ T-cell marker(s), % of $CD4^+$ T cells						
HLA-DR	3.2 (0.70–15.3)	2.3 (0.6–8.0)	2.5 (0.5–8.0)	<.001	2.2 (0.9–8.0)	.362
CD38	8.7 (6.0–40.7)	4.7 (1.5–15.8)	5.6 (0.3–16.9)	<.001	0.7 (0.1–2.9)	<.001
HLA-DR and CD38	2.4 (0.3–16.7)	0.5 (0.1–3.1)	0.7 (0.1–3.0)	<.001	0.06 (0.01–0.8)	<.001
PD-1	1.9 (0.2–12.9)	2.1 (0.2–7.0)	2.0 (0.5–10.4)	.478	0.9 (0.2–2.1)	<.001
CD57	2.1 (0.1–19.1)	1.7 (0.2–11.8)	1.9 (0.1–16.4)	.464	1.5 (0.1–55)	.067
$CD8^+$ T-cell marker(s), % of $CD8^+$ T cells						
HLA-DR	3.5 (1.0–12.0)	2.0 (0.2–7.0)	2.0 (0.3–6.4)	<.001	2.2 (0.6–5.4)	.082
CD38	3.9 (0.4–48.9)	3.2 (0.7–8.5)	3.1 (0.5–13.5)	.001	1.5 (0.4–8.7)	<.001
HLA-DR and CD38	2.7 (0.1–26.0)	0.8 (01–7.5)	0.9 (0.1–5.9)	<.001	0.45 (0.1–3.7)	.011
PD-1	1.9 (0.6–11.9)	1.8 (0.1–11.8)	2.1 (0.1–12.4)	.218	0.9 (0.08–4.58)	<.001
CD57	11.6 (0.3–41.0)	10.4 (0.3–37.1)	12.5 (0.6–30.0)	.843	11.0 (2.9–29.7)	.197
Monocyte activation marker						
sCD14 level, pg/mL	4649 (106–67 010)	4723 (35–62 661)	2769 (12–42 516)	<.001	1404 (783.4–3328)	.001
Microbial translocation marker						
LPS level, pg/mL	50.1 (11.0–96.0)	48.6 (5.0–63.0)	44.2 (5.0–63.0)	<.001	29.1 (16.18–72.74)	.003
16S rDNA level, $\log_{10}$ copies/mL	5.11 (4.39–6.35)	4.90 (3.91–6.06)	4.75 (2.50–6.06)	<.001	4.62 (2.05–7.04)	.211
IL-6 level, pg/mL	6.34 (2.46–53.16)	6.30 (2.47–33.98)	5.63 (2.35–27.48)	.573	3.8 (3.21–60.9)	.666
D-dimer level, μg/mL	0.53 (0.01–5.63)	0.24 (0.1–4.38)	0.15 (0.01–4.80)	<.001	0.2 (0.02–3.3)	.802
hs-CRP level, mg/L	2.25 (1.85–8.25)	2.25 (1.85–7.45)	2.30 (1.75–7.85)	.611	0.9 (0.3–8.10)	.002

Data are median values (ranges).

Abbreviations: IL-6, interleukin 6; hs-CRP, high-sensitivity C-reactive protein; LPS, lipopolysaccharide; NA, not applicable; PBMC, peripheral blood mononuclear cell; rDNA, ribosomal DNA; sCD14, soluble CD14.

[a]For comparisons between values at baseline and those 1 month after the end of HCV treatment. Values ≤.05 denote statistically significant differences.

[b]For comparisons between values 1 month after the end of HCV treatment and values for healthy volunteers. Values ≤.05 denote statistically significant differences.

T cells, as well as the IL-6 and hs-CRP plasma levels, did not change after SVR, suggesting that HCV has a limited or null influence on them in HIV/HCV-coinfected patients.

On the other hand, based on the adverse effect that HCV has on immune recovery among HIV-infected patients initiating ART [33–35], we hoped that the eradication of HCV would contribute to restoring immunologic parameters in these patients. However, the achievement of SVR did not translate into an increased $CD4^+$ T-cell count, $CD4^+$ T-cell percentage, or $CD4^+/CD8^+$ T-cell ratio. In this regard, a limitation of our study may be the limited number of patients with very low $CD4^+$ T-cell counts and $CD4^+/CD8^+$ T-cell ratios included in the study. It would be necessary to evaluate a larger sample of such patients to state with confidence that improvement in these parameters should not be expected in very immunosuppressed HIV-infected patients after the eradication of HCV. However, this is in concordance with results of a previous retrospective study by Saracino et al [29], in which SVR did not seem to alter the rate of long-term improvements in the $CD4^+$ T-cell count and $CD4^+/CD8^+$ T-cell ratio in HIV/HCV-coinfected patients receiving ART. This is particularly worrying in HIV-infected patients who did not achieve significant immune recovery despite consistent HIV suppression, since they have shown higher rates of morbidity and mortality due to AIDS-defining and non–AIDS-defining events [36].

Our study has other limitations, one of which is that the follow-up period was limited to 1 month after finishing HCV treatment. Data from a longer follow-up period would have reinforced the conclusions. However, the concordance between values at the end of treatment and those 1 month later allows us to be confident that these findings are immunologically relevant. Likewise, the inclusion of patients who did not achieve SVR would be very informative, but it would currently be very difficult to do so because of the elevated rate of response to the direct-acting antiviral agents.

In conclusion, our data suggest that the eradication of HCV infection in HIV/HCV-coinfected patients results in significant decreases not only in immune activation of $CD4^+$ and $CD8^+$ T cells, but also in levels of proviral HIV DNA, microbial translocation markers, sCD14, and D-dimers. It supports the use of HCV treatment in all HIV/HCV-coinfected patients regardless of fibrosis stage, with the sole exception of decompensated cirrhosis.

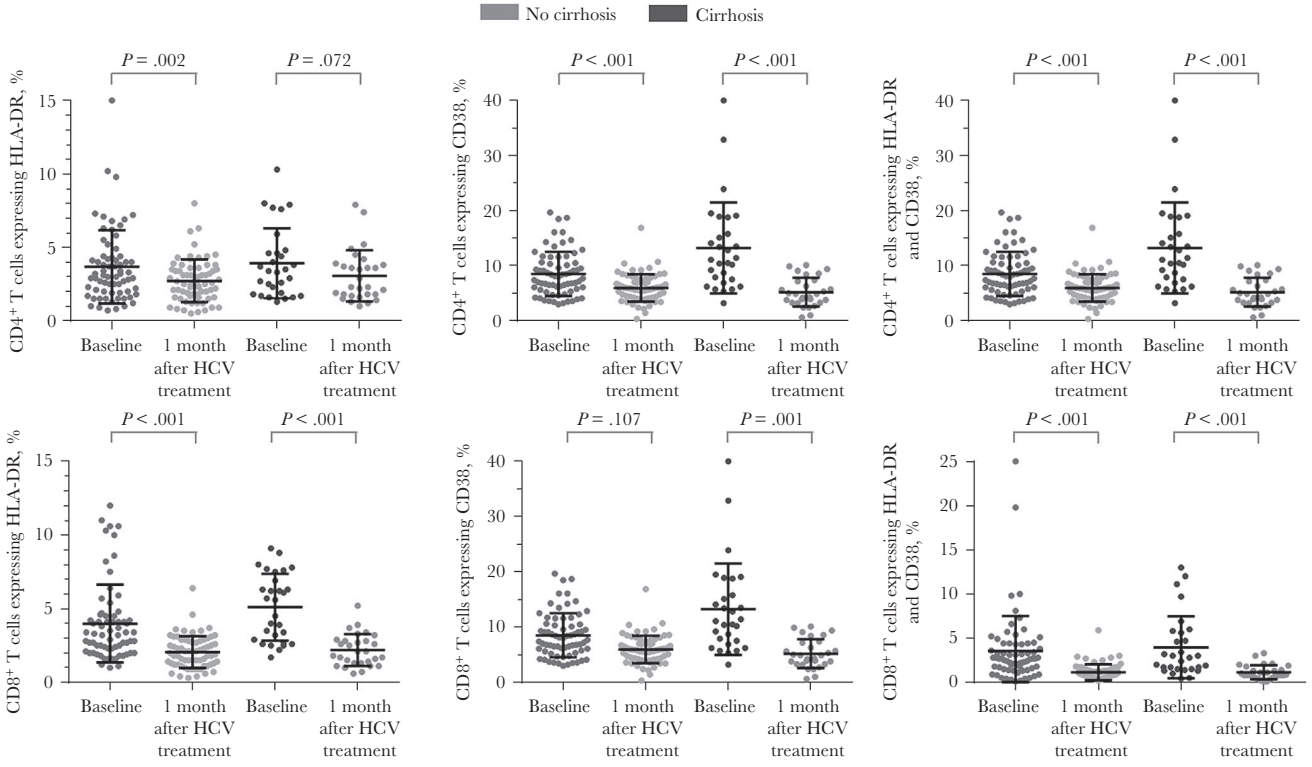

Figure 2. Changes in the percentage of CD4+ and CD8+ T cells that expressed HLA-DR and CD38 at baseline and 1 month after ending anti–hepatitis C virus (HCV) therapy in patients coinfected with human immunodeficiency virus (HIV) and HCV who achieved sustained virological response, by cirrhosis status. Dots denote values for individual patients. Bars denote median values and interquartile ranges.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank the patients, for their involvement in this study, and Tamara Fernández-Magdaleno, for her technical work.

L. F. L.-.C conceived, designed, and supervised the study. A. G.-V. was responsible for measuring drug concentrations. O. J. B.-H. and B. D.-M. were responsible for flow cytometry. M. T.-R. and A. B.-P. were responsible for determining IL-6, D-dimer, sCD14, LPS, 16S rDNA, and cellular HIV DNA levels. Y. M.-G. was responsible for acquiring and ensuring the quality of data. L. F. L.-C., N. E., and P. V. acted as clinical site investigators. All authors were involved in interpreting the data and participated in writing the manuscript. L. F. L.-C. and A. G.-V. analyzed the data and wrote the first and final drafts.

Financial support. This work was supported by Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla and Red de Investigación en SIDA (RD16/0025/0020-ISCIII-FEDER).

Potential conflicts of interest. L. F. L.-C. and P. V. have received unrestricted research funding, consultancy fees, and lecture fees from and have served on the advisory boards of Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Merck Sharp and Dohme, and ViiV Healthcare. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Supplementary tables

	Naïve patients n= 45	**Experienced patients** n= 52	**p**
Male sex	42 (80.8)	41 (91.1)	0.151
Age (years)	49 (45–52)	50 (47–53)	0.707
BMI (kg/m^2)	24.0 (21.3–27.3)	23.4 (21.0–27.9)	0.557
Risk factor for HIV/HCV Previous intravenous drug use Other	 35 (67.3) 10 (32.6)	 33 (77.3) 10 (22.7)	0.728
HCV infection duration (years)	21 (12—25)	21 (18—25)	0.320
HCV subtype 1a/1b/1ab/3/4	23/9/5/5/10	13/10/–/9/13	0.056
Fibrosis stage F0-1/F2/F3/F4	21/8/15/8	8/8/8/21	N/A
HCV-RNA $\log_{10}$ IU/ml	6.17 (5.89–6.61)	6.33 (5.56–6.66)	0.736
Nadir CD4$^+$/µl	176 (66–330)	81 (71–253)	0.229
CD4$^+$/µl	673 (495–936)	767 (592–902)	0.964
% CD4$^+$/µl	31.5 (26.8–39.5)	33.5 (27.6–39.4)	0.855
CD4$^+$/CD8$^+$ ratio	0.81 (0.52–1.19)	0.85 (0.58–1.18)	0.928
Undetectable HIV-RNA (months)	65 (38–116)	49 (29–84)	0.107
HIV-DNA, $\log_{10}$ copies/10^6 PBMCs	4.08 (2.34–5.40)	2.69 (1.82–5.30)	0.136
CD4$^+$HLA-DR$^+$ (%)	3.40 (2.35–5.12)	2.70 (1.70–4.25)	0.258
CD4$^+$CD38$^+$ (%)	7.35 (5.8–10.5)	10.2 (6.35–14.9)	0.007
CD4$^+$HLA-DR$^+$CD38$^+$ (%)	2.40 (1.42–3.92)	2.40 (1.25–4.20)	0.394
CD8$^+$HLA-DR$^+$ (%)	3.75 (2.15–5.67)	3.4 (2.5–6.25)	0.752
CD8$^+$CD38$^+$ (%)	3.55 (2.45-6.45)	4.30 (2.35–6.85)	0.345
CD8$^+$HLA-DR$^+$CD38$^+$ (%)	2.70 (1.40–4.40)	2.50 (1.65 –4.65)	0.275
CD4$^+$PD-1$^+$ (%)	2.10 (1.10–3.40)	1.40 (1.05–3.10)	0.526
CD4$^+$CD57$^+$ (%)	2.40 (1.35 – 5.10)	1.60 (1.10–2.95)	0.071
CD8$^+$PD-1$^+$ (%)	1.95 (0.92–4.57)	1.70 (1.10–3.15)	0.222
CD8$^+$CD57$^+$ (%)	10.70 (7.05–18.70)	14.10 (7.45–18.40)	0.739
sCD14 (pg/ml)	4378 (2189–6752)	4852 (2875–8714)	0.275
LPS (pg/ml)	50.03 (26.77–60.80)	50.95 (49.04–61.91)	0.066
16S rDNA ($\log_{10}$ copies/ml)	5.05 (4.84–5.32)	5.26 (4.92–5.68)	0.079
IL-6 (pg/ml)	5.82 (4.54–7.78)	6.66 (4.94–9.86)	0.782
D-dimers (µg/ml)	0.64 (0.33–1.18)	0.48 (0.27–0.97)	0.180
hs-CRP (mg/l)	2.25 (2.05–2.55)	2.35 (1.95–2.85)	0.731

Supplementary table 1. Patients` characteristics at baseline as function of previous anti-HCV treatment. Data are expressed as number (%) or median (IQR).

	CD4⁺ T cells/µl		**CD4⁺ T cells** (%)		**CD4⁺/CD8⁺**	
	ρ	p-value	ρ	p-value	ρ	p-value
CD4⁺HLA-DR⁺ (%)	-0.388	**<0.001**	-0.382	**<0.001**	-0.354	**<0.001**
CD4⁺CD38⁺ (%)	-0.255	**0.012**	-0.301	**0.003**	-0-235	**0.022**
CD4⁺HLA-DR⁺CD38⁺ (%)	-0.119	0.245	-0.068	0.507	-0.05	0.958
CD8⁺HLA-DR⁺ (%)	-0.240	**0.018**	-0.013	0.900	-0.70	0.496
CD8⁺CD38⁺ (%)	-0.143	0.162	-0.150	0.144	-0.058	0.572
CD8⁺HLA-DR⁺CD38⁺ (%)	-0.047	0.644	0.030	0.830	0.053	0.605
HCV-RNA $\log_{10}$ IU/ml	-0.143	0.164	-0.072	0.482	-0.030	0.773
HIV-DNA, $\log_{10}$ copies/10^6 PBMCs	-0.024	0.814	-0.012	0.814	-0.088	0.814
sCD14 (pg/ml)	-0.028	0.791	-0.028	0.791	0.111	0.284
LPS (pg/ml)	-0.115	0.239	-0.015	0.279	-0.134	0.208
16S rDNA ($\log_{10}$ cop/ml)	-0.073	0.492	-0.073	0.492	0.002	0.983
IL-6 (pg/ml)	-0.010	0.927	-0.049	0.927	0.004	0.973
D-dimers (µg/ml)	0.079	0.464	0.000	0.997	-0.034	0744
hs-CRP (mg/l)	0.030	0.388	0.046	0.464	0.034	0.754

Supplementary table 2. Correlations between CD4⁺ T cells, CD4% and CD4⁺/CD8⁺ T cells ratio with other variables at baseline.

	HCV-RNA $\log_{10}$ IU/ml	
	ρ	p-value
sCD14 (pg/ml)	0.059	0.569
LPS (pg/ml)	0.083	0.438
16S rDNA ($\log_{10}$ copies/ml)	0.002	0.987
IL-6 (pg/ml)	-0.049	0.646
D-dimers (μg/ml)	-0.019	0.854
hs-CRP (mg/l)	-0.125	0.246

Supplementary table 3. Correlations between HCV viremia and monocyte activation (sCD14), microbial translocation, and inflammatory markers.

	No cirrhosis (n= 68)			**Cirrhosis** (n= 29)		
	Basal	**1 month after end of treatment**	**p**	**Basal**	**1 month after end of treatment**	**p**
$CD4^+HLA\text{-}DR^+$ (%)	3.0 (0.70–15.3)	2.5 (0.5–8.0)	**0.002**	3.4 (1.3–10.3)	2.3 (1.0–7.0)	0.072
$CD4^+CD38^+$ (%)	7.8 (3.0–19.7)	5.8 (0.3–16.9)	**<0.001**	11.1 (3.2–40.7)	4.9 (0.6–10.1)	**<0.001**
$CD4^+HLA\text{-}DR^+CD38^+$ (%)	2.2 (0.3–8.9)	0.6 (0.1–3.0)	**<0.001**	3. (0.7–16.7)	0.7 (0.1–2.3)	**<0.001**
$CD8^+HLA\text{-}DR^+$ (%)	3.2 (1.0–12.0)	1.9 (0.3–6.4)	**<0.001**	5.6 (1.7–9.1)	2.1 (0.6–5.2)	**<0.001**
CD8 $CD38^+$ (%)	3.5 (0.4-43.1)	3.1 (0.5–13.5)	0.107	5.2 (1.2–48.9)	3.1 (1.4–5.8)	**0.001**
$CD8^+HLA\text{-}DR^+CD38^+$ (%)	0.9 (0.1–26.0)	0.9 (0.1–5.9)	**<0.001**	2.8 (0.4–13.0)	0.8 (0.1–3.3)	**<0.001**
$CD4^+PD\text{-}1^+$ T cells (%)	1.9 (0.2–12.9)	2.1 (0.5–10.4)	0.752	1.9 (0.3–11.7)	2.0 (0.3–7.3)	0.411
$CD4^+CD57^+$ T cells (%)	2.6 (0.1–19.1)	2.0 (0.5–16.4)	0.480	1.4 (0.6–6.9)	1.1 (0.1–9-.6)	0.850
$CD8^+PD\text{-}1^+$ T cells (%)	1.9 (0.7–10.8)	2.1 (0.4-6.1)	0.794	1.9 (0.6–11.9)	2.2 (0.1–12.4)	0.767
$CD8^+CD57^+$ T cells (%)	11.3 (0.4–41.0)	13.7 (0.8–30.0)	0.382	12.3 (0.3–29.3)	8.1 (0.6–28.4)	0.248
sCD14 (pg/ml)	4682 (106–67010)	2889 (15–42516)	**0.005**	4300 (245–44213)	2676 (12–22123)	**0.012**
HIV-DNA, $\log_{10}$ copies/10^6 PBMCs	3.15 (0.41–7.57)	2.08 (0.24–5.65)	**<0.001**	3.17 (0.78–7.16)	2.57 (0.61–5.66)	**0.011**
LPS (pg/ml)	50.0 (11.0–72.0)	42.9 (5.0–63.0)	**<0.001**	58.1 (21.0–96.0)	45.4 (13.0–62.0)	**<0.001**
16S rDNA ($\log_{10}$ copies/ml)	5.07 (4.39–6.07)	4.70 (2.50–6.06)	**<0.001**	5.22 (4.48–6.35)	4.82 (3.62–5.81)	**<0.001**
IL-6 (pg/ml)	5.73 (2.46–22.66)	5.45 (2.35–27.48)	0.538	7.98 (4.09–53.16)	7.01 (3.90–25.97)	0.158
D-dimers (μg/ml)	0.55 (0.07–5.63)	0.13 (0.01–4.80)	**<0.001**	0.40 (0.01–2.94)	0.19 (0.01–1.61)	**0.018**
hs-CRP (mg/l)	2.25 (1.85–8.25)	2.25 (1.85–7.85)	0.795	2.25 (1.85–6.95)	2.45 (1.85–4.55)	0.584

Supplementary table 4. Changes in the percentage of $CD4^+$ and $CD8^+$ T cells that expressed HLA-DR, CD38, PD-1, and CD57, monocytes activation (sCD14), HIV proviral DNA, microbial translocation (LPS and 16S rDNA), IL-6, hs-CRP, and D-dimer levels after the end of HCV treatment in patients without and with cirrhosis. Data are expressed as median (range).

All patients (n= 97)	**Basal**	**End of HCV treatment**	**6 months after end of treatment**	**p**
$CD4^+$ T cells/µl	719 (120─1311)	655 (105–1517)	736 (108–1775)	0.125
$CD4^+$ T cells (%)	32.8 (10.7─61.1)	32.9 (9.4–66.0)	31.7 (9.2–63.1)	0.050
$CD4^+/CD8^+$ ratio	0.83 (0.16 - 2.53)	0.76 (0.14–2.94)	0.73 (0.12–2.76)	0.069
$CD4^+$ <350/µl (n= 14)				
$CD4^+$ T cells/µl	242 (120–346)	287 (105–505)	263 (108–696)	0.074
$CD4^+$ T cells (%)	25.1 (10.7–38.4)	25.0 (9.4–43.8)	23.7 (9.1–46.1)	0.064
$CD4^+/CD8^+$ ratio	0.54 (0.16–1.13)	0.52 (0.14–1.10)	0.55 (0.12–1.13)	0.109
CD4/CD8 ≤0.4 (n= 11)				
$CD4^+$ T cells/µl.	410 (144–966)	387 (105–579)	421 (108–819)	0.182
$CD4^+$ T cells (%)	17.7 (10.7–22.6)	19.6 (9.4–31.6)	18.3 (9.2–23.1)	0.859
$CD4^+/CD8^+$ ratio	0.31 (0.16–0.40)	0.31 (0.14–0.56)	0.30 (0.12–0.43)	0.790
No cirrhosis (n= 68)				
$CD4^+$ T cells/µl	791 (146–1311)	749 (137–1517)	756 (108–1775)	0.340
$CD4^+$ T cells (%)	33.3 (10.7–61.1)	33.7 (9.4–66.0)	32.1 (9.2–63.1)	0.363
$CD4^+/CD8^+$ ratio	0.83 (0.16–2.53)	0.83 (0.14–2.94)	0.77 (0.12–2.76)	0.557
Cirrhosis (n= 29)				
$CD4^+$ T cells/µl	621 (120─1123)	497 (105–1058)	643 (235–1186)	0.150
$CD4^+$ T cells (%)	31.4 (11.8─48.5)	32.0 (14.3–54.0)	29.5 (12.3–49.9)	0.041
$CD4^+/CD8^+$ ratio	0.74 (0.17─2.16)	0.68 (0.21–2.12)	0.67 (0.21–2.05)	0.033

Supplementary table 5. Changes in the $CD4^+$ T cell count, percentage and $CD4^+/CD8^+$ ratio in different groups of patients. Data are expressed as median (range).

4.2 Segundo artículo

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Stem Cells Transl Med. 2021; 10(4):534-541

doi: 10.1002/sctm.20-0213

Received: 21 May 2020 | Accepted: 10 October 2020

DOI: 10.1002/sctm.20-0213

HUMAN CLINICAL ARTICLES

STEM CELLS TRANSLATIONAL MEDICINE

Mesenchymal stromal cells in Human Immunodeficiency Virus-infected patients with discordant immune response: Early results of a phase I/II clinical trial

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Funding information
Andalusian Network for the Design and Translation of Advanced Therapies; Andalusian Regional Ministry of Health and Families, Grant/Award Number: 201600073585-tra; Red de Investigación en SIDA, Grant/Award Number: RD16/0025/0020-ISCIII-FEDER; Instituto de Salud Carlos III, Grant/Award Number: PI15/01041

Abstract

Between 15% and 30% of HIV-infected subjects fail to increase their $CD4^+$ T-cell counts despite continuous viral suppression (immunological nonresponders [INRs]). These subjects have a higher morbidity and mortality rate, but there are no effective treatments to reverse this situation so far. This study used data from an interrupted phase I/II clinical trial to evaluate safety and immune recovery after INRs were given four infusions, at baseline and at weeks 4, 8, and 20, with human allogeneic mesenchymal stromal cells from adipose tissue (Ad-MSCs). Based on the study design, the first 5 out of 15 INRs recruited received unblinded Ad-MSC infusions. They had a median $CD4^+$ nadir count of 16/μL (range, 2-180) and $CD4^+$ count of 253 cells per microliter (171-412) at baseline after 109 (54-237) months on antiretroviral treatment and 69 (52-91) months of continuous undetectable plasma HIV-RNA. After a year of follow-up, an independent committee recommended the suspension of the study because no increase of $CD4^+$ T-cell counts or $CD4^+/CD8^+$ ratios was observed. There were also no significant changes in the phenotype of different immunological lymphocyte subsets, percentages of natural killer cells, regulatory T cells, and dendritic cells, the inflammatory parameters analyzed, and cellular associated HIV-DNA in peripheral blood mononuclear cells. Furthermore, three subjects suffered venous thrombosis events directly related to the Ad-MSC infusions in the arms where the infusions were performed. Although the current study is based on a small sample of participants, the findings suggest that allogeneic Ad-MSC infusions are not effective to improve immune recovery in INR patients or to reduce immune activation or inflammation. ClinicalTrials.gov identifier: NCT0229004. EudraCT number: 2014-000307-26.

KEYWORDS

clinical trial, HIV infection, immunological nonresponders, mesenchymal stromal cells

1 | INTRODUCTION

Chronic immune activation and inflammation is considered today as the main driving force of $CD4^+$ T-cell depletion and the functional impairment of the immune system caused by HIV infection.[1] Antiretroviral therapy (ART) achieves the control of viremia in most subjects and reduces both cellular and soluble activation markers, leading to immune recovery in a high proportion of subjects.[2] However, between 15% and 30% of subjects exhibit a poor $CD4^+$ T-cell recovery despite successful viral suppression; these subjects are known as immunological nonresponders (INRs).[3] Although the definition of immunological nonresponse lacks consensus, it has always been based in the increase of $CD4^+$ T-cell counts above different thresholds in a given time period.[4] These subjects show severe homeostatic alterations in $CD4^+$ T cells, with a disturbed maturational profile, a reduced thymic function, and increased levels of activation and apoptosis, among other characteristics.[5] From a clinical point of view, INRs show higher rates of morbidity and mortality associated with AIDS and non-AIDS events such as cardiovascular events, neurocognitive impairment, non-AIDS malignancies, end-stage liver and renal diseases, bone disorders, and frailty than those with a good immune response.[6-8] Moreover, when these subjects grow old, this situation will be aggravated by age-associated immunosenescence.[9] In these subjects, many strategies have been evaluated, such as ART intensification, immunomodulators, immunosuppressive agents, and probiotics, though with disappointing results; thus, no current effective therapies are available.[10]

On the other hand, several studies, both in vitro and in vivo, have shown that mesenchymal stromal cells (MSCs) can modulate the function of T helper cells and B lymphocytes, natural killer (NK) cells, and dendritic cells, whereas stimulating regulatory T (T_{reg}) cells results in a change from a proinflammatory state to an anti-inflammatory state.[11-13] These properties have been demonstrated in multiple animal models of disease and have been used successfully in humans with graft vs host disease and several autoimmune and nonimmune diseases.[14,15] Up to now, only one study has been carried out with MSCs from cord blood in INRs, which resulted in a significant increase in circulating $CD4^+$ T lymphocytes and a decrease of the activation of T lymphocytes and soluble inflammation mediator levels without significant adverse effects or loss of viremia control,[16] but this study has not been replicated. Thus, our aim was to evaluate, for the first time, whether MSCs coming from a more accessible source, such as adipose tissue, are safe and effective in improving the immune recovery in INRs.

Lessons learned

- Adipose tissue allogeneic adult mesenchymal stromal cells infusions are not effective to improve immune recovery or to reduce immune overactivation or inflammation state in immunologically nonresponding HIV-infected patients.
- Donor-associated factors and manufacturing procedure could affect efficacy and safety.

Significance statement

HIV infection is characterized by a progressive $CD4^+$ T-cell depletion and an immune overactivation and inflammation state. Antiretroviral therapy suppresses viral replication and reduces this aberrant state, leading to an immune recovery in a high proportion of subjects. However, between 15% and 30% of patients fail to achieve sufficient immune reconstitution, triggering a rise in the rate of morbidity associated with both AIDS and non-AIDS events. These patients are called immune nonresponders (INRs), and no current therapies are available to treat them. Mesenchymal stromal cells have been shown to have immunomodulatory properties because they interact and modulate the function of multiple immune cells. For that reason, we planned this clinical trial to evaluate the efficacy and safety of infusions of human allogeneic mesenchymal stromal cells from adipose tissue (Ad-MSCs) in INRs. However, our findings suggested that Ad-MSC infusions are not effective to improve immune recovery in INRs or to reduce immune overactivation or inflammation state.

2 | MATERIALS AND METHODS

2.1 | Study design

This was originally planned as a phase I/II, randomized, placebo-controlled, clinical trial designed to evaluate the safety and efficacy of adipose tissue allogeneic adult MCSs (Ad-MSCs) in INRs. The design was carried out jointly with the Andalusian Network for the Design and Translation of Advanced Therapies (http://terapiasavanzadas.juntadeandalucia.es), which also acted as sponsor.

Because of security concerns, in the first phase, five eligible INRs received unblinded Ad-MSCs with a safety minimum period of 15 days between patients. Once all five subjects had completed the four Ad-MSC infusions, an independent data-monitoring committee (IDMC) performed a preliminary analysis of safety and efficacy data. In the second phase, (permanently suspended), 10 additional subjects would have been randomized to receive Ad-MSC infusions or placebo.

2.2 | Study approval

The clinical trial was approved by the National Health Authority and the Ethics Committee for Clinical Research of the participating site.

All of the subjects provided informed consent. It was registered at the European Medicine Agency with EudraCT number 2014-000307-26 and Clinical trials.gov number NCT0229004, and it was conducted according to the principles of Good Clinical Practice (GCP) at Virgen del Rocío University Hospital in Seville (Spain).

2.3 | Adipose tissue allogeneic adult mesenchymal stromal cells

Ad-MSCs were provided by the Cell Production and Tissue Engineering Unit of Virgen de las Nieves University Hospital (Granada). Ad-MSCs were obtained from subcutaneous adipose tissue samples from four donors obtained by surgical exeresis in aseptic conditions fulfilling the provisions of the Spanish Royal Decree 1301/2006.

The donors were younger than 60 years and negative for hepatitis B virus, hepatitis C virus, and HIV infection and *Treponema pallidum*. Only one donor was diabetic and a smoker (Table S1). Once the adipose tissue was obtained, a mechanical disintegration of the tissue was performed, followed by an enzymatic digestion with collagenase type A. The cell fraction was separated by centrifugation and seeded in plates, and after two culture-expansion passages Ad-MSCs were isolated. The formulation of medium for Ad-MSC expansion was as follows: Dulbecco's modified Eagle's medium with 10% of fetal bovine serum, 2% of L-alanine and L-glutamine, 0.1 mg/mL of gentamicin, and 100 UI/mL of penicillin. After the expansion Ad-MSCs were frozen and put into quarantine until quality controls were performed (Table S2). When a patient was included in the clinical trial, Ad-MSCs were thawed and expanded for 1 week, and new quality controls were performed before delivery (Table S3). The finished product was a cell suspension containing allogeneic expanded Ad-MSCs at a concentration of 2 000 000 cells per milliliter in Ringer's lactate solution containing 1% human albumin. The volume was adjusted according to the patient's weight and packaged in sterile bags preserved at 2°C to 8°C until their intravenous infusion at a dose of 1×10^6 Ad-MSCs per kilogram of body weight (Figures S1 and S2).

2.4 | Cell infusion procedure

Ad-MSCs were administered through a peripheral venous catheter over 1 to 2 hours using an infusion pump (infusion rate 2 mL/min) at a dose of 1×10^6 Ad-MSCs per kilogram of body weight at baseline and at weeks 4, 8, and 20 (Figure 1). Before administration, the cell suspension was tempered and stirred manually or using an electric stirrer to dissolve any cell aggregates that could have occurred during transport, and subjects received premedication with methyl prednisolone (0.5 mg/kg i.v.), dexchlorpheniramine (5 mg, i.v.), and oral acetaminophen.

2.5 | Study subjects

The subjects were selected among the HIV-infected adults followed at the Virgen del Rocío University Hospital. An INR was defined as an HIV-infected subject with a basal $CD4^+$ T-cell count ≤350 cells per microliter whose $CD4^+$ T-cell count increased <75 or <150 cells per microliter after 1 or 2 years with undetectable viral load, respectively, and/or a $CD4^+$ T-cell count increase <350 cells per microliter after 3 years on treatment. Exclusion criteria included opportunistic infections in the previous 12 months, active coinfections with hepatitis B virus or hepatitis C virus, Child-Pugh class C liver cirrhosis, portal hypertension or hypersplenism, malignant tumors, or treatment in the previous 12 months with immunomodulators, interferon, chemotherapy, or any other drug that might alter $CD4^+$ T-cell count. Pregnant or breastfeeding women and subjects refusing to use accepted contraceptive methods throughout follow-up were also excluded from participation in the trial. All subjects agreeing to participate in the clinical trial provided written informed consent before undergoing any study-related procedure.

2.6 | Endpoints, follow-up, and assessments

The main aims of the study were to assess the safety and efficacy of four infusions of Ad-MSCs. In accordance with GCP, all adverse events (AEs) observed by the investigator or reported by the subjects, whether or not attributed to the investigational medicinal product (IMP), were carefully monitored and recorded. AEs were summarized and classified on the basis of MedDRA terminology and their relationship to Ad-MSC administration and categorized via the standardized toxicity-grade scale used by the AIDS Clinical Trials Group.[17] The causality of AEs with the IMP was assessed by the principal investigator and reevaluated by a qualified person responsible for pharmacovigilance appointed by the trial's sponsor.

Efficacy was measured as the changes in $CD4^+$ T-cell counts, percentage of $CD4^+$ T cells, and $CD4^+/CD8^+$ ratios after infusions and throughout 96 weeks after the first Ad-MSC dose. The subjects had a total of 12 visits: at baseline and on weeks 3, 4, 7, 8, 12, 19, 20, 24, 36, 48, and 96, when adverse events, adherence to ART (subjects' self-report and pharmacy records), hematology and chemistry tests, $CD4^+$ and $CD8^+$ T-cell counts, and plasma HIV-RNA levels were assessed. The $CD4^+$ and $CD8^+$ T-cell counts were determined in fresh blood with an FC 500 flow cytometer (Beckman Coulter, Brea, CA). Plasma HIV-1 RNA levels were measured by quantitative polymerase chain reaction (Cobas AmpliPrep-Cobas TaqMan HIV-1 test, version 2.0; Roche Diagnostics, Basel, Switzerland) with a lower detection limit of 20 copies per milliliter, according to the manufacturer's instructions.

2.7 | Laboratory measurements

2.7.1 | Cell surface staining for immune profile

Peripheral blood mononuclear cells (PBMCs) were isolated using a gradient technique with Ficoll and stored with fetal bovine serum and 10% dimethyl sulfoxide in liquid nitrogen until assay time. On the day

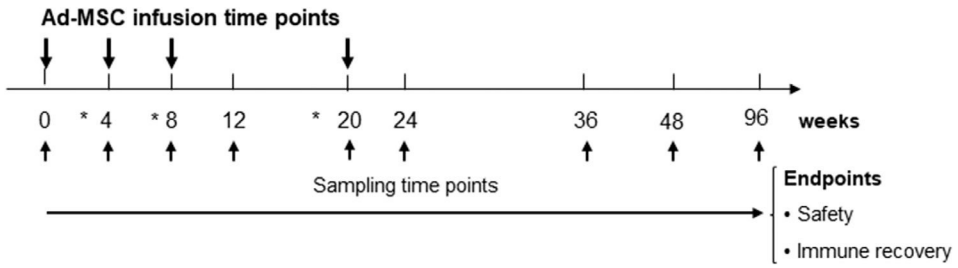

FIGURE 1 Scheme of visits, infusions of Ad-MSCs, and sampling time points. *, safety visits, 1 week before the next Ad-MSC infusion. Ad-MSC, adipose tissue allogeneic adult mesenchymal stromal cells

of the assay, frozen PBMCs were thawed at 37°C and washed with phosphate-buffered saline. Afterward, cells were stained with different fluorochrome-conjugated antibodies to assess the expression of surface markers, including CD3-BV786, CD4-APC-Cy7, CD8-BV510, CD45RA-Pe-Cy7, CD27-BV421, HLA-DR-PE-CF594, Lineage Cocktail 1-FITC, CD56-PE-CF594, CD16-AF700, CD25-BV421, Foxp3-PE, PD1-BV605, Ki67-FITC, CD38-BV605, HLA-DR-FITC, CD57-APC, CD28-PE, Annexin V-APC, and CD31-PE (all from BD Biosciences, San Jose, CA) and CD11c-PE and CD123-APC (BioLegend, San Diego, CA). Viable cells were identified using 7AAD-PerCP-Cy5.5 (BD Biosciences) or LIVE/DEAD fixable Yellow Dead Cell Stain (Invitrogen, Carlsbad, CA) for intracellular staining. The cellular markers were analyzed by multicolor flow cytometry on total $CD4^+$ ($CD3^+$ $CD4^+$) and $CD8^+$ ($CD3^+$ $CD8^+$) T cells, and the different subsets were defined as follows: naïve (T_N) $CD45RA^+$ $CD27^+$, $CD4^+$ recent thymic emigrants (RTEs) $CD45RA^+$ $CD27^+$ $CD31^+$, central memory (T_{CM}) $CD45RA^-$ $CD27^+$, effector memory (T_{EM}) $CD45RA^-$ $CD27^-$, and terminally differentiated (T_{EMRA}) $CD45RA^+$ $CD27^-$. Other cellular subsets were defined as follows: myeloid dendritic cells (mDCs; Lin-1^- $HLA\text{-}DR^+$ $CD11c^+$ $CD123^-$), plasmacytoid dendritic cells (pDCs; Lin-1^- $HLA\text{-}DR^+$ $CD11c^-$ $CD123^+$), NK cells ($CD3^-$ $CD56^+$ $CD16^+$), and T_{reg} cells ($CD3^+$ $CD4^+$ $CD25^{++}$ $Foxp3^+$). Moreover, the frequency of T cells expressing markers of cellular proliferation (Ki67), apoptosis (Annexin V), exhaustion (PD-1), activation (HLA-DR/CD38), and replicative senescence ($CD28^-$ $CD57^+$) were also quantified in both total $CD4^+$ and $CD8^+$ T cells. Samples were acquired on a Fortessa LSR II instrument (BD Biosciences, Madrid, Spain), discarding dead cells, and analyzed using FlowJo 9.3.2 software.

2.7.2 | Cellular associated HIV-DNA

Total cellular associated HIV-DNA (integrated and unintegrated viral DNA) extracted from PBMCs using Blood DNA Mini Kit (Omega Bio-Tek, Norcross, GA) was assayed by real-time polymerase chain reaction using specific primers (forward: 5′-TAGCGGAGGCTAGAAGGAGA-3′; reverse: 5′-CCTGGCCTTAACCGAATT-3′) and a probe within the selected gag long terminal repeat region (5′-TACCGACGCTCTCGCACCCA-3′) labeled with FAM.

2.7.3 | Enzyme-linked immunosorbent assays

Plasma samples were aliquoted and stored at −20°C until subsequent analysis of the following biomarkers: high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, TNF-α, and soluble CD14 (sCD14). The levels of hsCRP were determined with an immunoturbidimetric serum assay using Cobas 701 (Roche Diagnostics, Mannheim, Germany). Commercially available enzyme-linked immunosorbent assays were used for the assay of IL-6 (Quantikine HS Human IL-6 immunoassay kit; R&D Systems, Minneapolis, MN), TNF-α (using Quantikine HS Human TNF-α immunoassay kit; R&D Systems), and sCD14 (Human CD14 ELISA Kit; Thermo Fisher Scientific, Waltham, MA) following the manufacturers' instructions.

2.8 | Statistical analysis

Results were expressed as median values with interquartile ranges (IQRs) for continuous variables and as numbers and percentages of cases for categorical variables. The Wilcoxon signed rank test was performed to compare changes in continuous variables over time. Differences were considered statistically significant if the *P* value was <.05. The statistical analyses were performed using IBM software (SPSS, version 23.0; SPSS Inc., Chicago, IL).

3 | RESULTS

3.1 | Characteristics of the participants

Five White INRs were included in the initial phase of the study. After evaluating the results at week 48, an IDMC recommended the suspension of the clinical trial. Therefore, here we report the results of these five subjects. All of them were men and completed the study with an ART adherence of 100%. Overall, the median (IQR) basal age was 53 (45-58) years, $CD4^+$ T-cell nadir 16 (2-108) cells per microliter, $CD4^+$ T-cell count 253 (211-340) cells per microliter, $CD4^+/CD8^+$ T-cell ratio 0.42 (0.19-0.48), percentage of $CD4^+$ T cells 24.1% (11.4-z25.5), months on ART 109 (73-210), and 69 (53-84) months of continuous undetectable plasma HIV-RNA levels before their inclusion in the study (Table 1).

3.2 | Safety and tolerability of Ad-MSC infusions in HIV-infected subjects

In total, 10 venous thrombosis events, 24 to 48 hours after Ad-MSC infusion, were observed in three out of the five subjects in the arms

where Ad-MSCs were infused, which required low molecular weight heparin treatment. A decrease in the emergence of thrombotic events was observed when low molecular weight heparin (80 mg of enoxaparin per day) was given 1 day before, on the day of the infusion, and during the following 2 days, together with a retraining course on the cell infusion procedure, as it was detected that the infusion rate was significantly higher than that established by protocol in one case. All other adverse events (n = 5) were considered unrelated to the Ad-MSCs (Table 2). Furthermore, no subjects presented alterations in the biochemical and hematological parameters or increases in HIV viral load (data not shown).

3.3 | Efficacy of Ad-MSC infusions in HIV-infected subjects

Overall, no significant changes were observed in the $CD4^+$ T-cell counts, percentage of $CD4^+$, or $CD4^+/CD8^+$ ratios after infusions and throughout follow-up (Figure 2). Likewise, there were no significant changes in the different subsets of $CD4^+$ and $CD8^+$ T lymphocytes (T_N, RTE, T_{CM}, T_{EM}, and T_{EMRA}) except for an increase in the T_{EM} $CD8^+$ T subset (Figures S3 and S4); nor were there changes in the percentage of NK cells, T_{reg} cells, mDCs, and pDCs (Figure S5). Furthermore, we did not observe changes in the activation, proliferation, senescence and apoptosis, or exhaustion markers of $CD4^+$ or $CD8^+$ T cells. We observed a decrease in the percentage of $PD1^+$ $CD4^+$ T cells (5.2 vs 1.6; $P = .043$) and a trend in $PD1^+$ $CD8^+$ T cells (0.9 vs 0.4; $P = .080$) at week 96 (Figures S6 and S7). On the other hand, the sCD14 plasma levels, measured with monocyte activation markers and the different proinflammatory proteins (IL-6, TNF-α, hsCRP) showed no significant changes (Figure S8). Likewise, the viral reservoir, measured as total cell-associated HIV-DNA, remained stable throughout the follow-up (Figure S9).

4 | DISCUSSION

Among the different properties of MSCs, their immunoregulatory potential is noteworthy because they can interact with cells of both the innate and adaptive immune systems, leading to the modulation of several effector functions.[18,19]

MSCs are able to suppress T lymphocyte activation and proliferation by decreasing the production of TNF-α and IFN-γ while inducing IL-10 and IL-4 expression by $CD4^+$ T cells. In addition, they inhibit the dendritic cell maturation and natural killer cell activation, induce T_{reg} cell differentiation, and promote a shift of macrophage toward anti-inflammatory phenotype, as well as secrete anti-inflammatory cytokines such as IL-1Ra, IL-10, TGF-β, and hepatocyte growth factor, among others.[14,18,20]

Moreover, given their low immunogenicity, both autologous and allogeneic cells can safely be administered.[21-24]

Zhang *et al.*[16] have reported the only study carried out with MSCs in HIV-infected subjects in which they transfused umbilical cord MSCs at a dose of 0.5×10^6 cells per kilogram body weight on day 0, month 1, and month 2 to seven INRs. They observed neither short-term clinical adverse effects nor HIV-1 load rebound, and six of the seven subjects displayed a sharp increase in $CD4^+$ T-cell counts of more than 50% compared with baseline values. In this study the umbilical cord MSCs preferentially expanded naïve and central memory $CD4^+$ T-cell counts, whereas the effector memory and the terminally differentiated subsets were gradually decreased. Moreover, Zheng *et al.* observed a significant decrease in the percentages of $CD38^+$ and $CD38^+$ $HLA\text{-}DR^+$ CD8 T cells, decreased PD-1 expression on total $CD4^+$ and $CD8^+$ T cells, and a significant reduction in plasma levels of several proinflammatory cytokines and chemokines.

In our study, the subjects received four doses (1×10^6/kg) of Ad-MSCs, but no changes were observed in the $CD4^+$ T-cell counts, percentages, or $CD4^+/CD8^+$ ratios, and no consistent changes were observed in the different subsets and phenotypes of the $CD4^+$ and $CD8^+$ T cells or percentage of mDCs, pDCs, NK cells, or T_{reg} cells. Likewise, the infusions of Ad-MSCs had no effects on sCD14, IL-6, TNF-α, and hsCRP plasma levels. Overall, we did not find a decrease in activation, exhaustion, apoptosis, and senescence at week 96. Only a significant decrease in the $PD1^+$ $CD4^+$ T cells was found, but its meaning is uncertain, and this change did not influence $CD4^+$ T-cell recovery.

The principal differences with our study are the origin of the MSCs and the doses administered. Whereas Zhang *et al.*[16] obtained MSCs from umbilical cord, specifically Wharton's jelly, we used adipose tissue, which is a more accessible source for obtaining MSCs. Although MSC immunoregulatory properties are unrelated to their

TABLE 1 Baseline characteristic of the subjects

Patient number	Age (years)	BMI (kg/m^2)	HIV transmission route	Smoker	CDC stage	Months on treatment	HIV-RNA <50 copies/mL (months)	Nadir $CD4^+$ count/μL	$CD4^+$ count/μL	% $CD4^+$	$CD4^+/CD8^+$
1	47	31.5	IVDU	Yes	A3	109	52	3	269	24.10	0.46
2	56	33.7	HTS	Yes	C3	184	69	16	251	11.44	0.20
3	53	18.0	IVDU	Yes	C3	237	77	2	171	11.49	0.19
4	61	27.4	HTS	No	C3	93	91	37	412	24.30	0.42
5	43	30.8	HTS	Yes	A3	54	53	180	253	26.70	0.50

Abbreviations: BMI, body mass index; CDC, Centers for Disease Control and Prevention; HTS, heterosexual contact; IVDU, previous intravenous drug use.

TABLE 2 MedDRA coded (version 22.1) adverse events occurred within 96 weeks of follow-up

MedDRA: System organ class/preferred term	Number of episodes	Number of cases	Relation with IMP
Administration site reactions	10	3	Y
Infusion site thrombosis	10		
Infections and infestations	6	3	N
Nasopharyngitis	1		
Conjunctivitis	1		
Herpes ophthalmic	1		
Pneumonia	1		
Diarrhea	1		
Tonsillitis	1		
General disorders and administration site conditions	2	2	N
Pyrexia	2		
Injury, poisoning and procedural complications	2	2	N
Trauma	1		
Accident	1		
Psychiatric disorders	1	1	N
Anxiety	1		
Gastrointestinal disorders	1	1	N
Hiatal hernia	1		
Respiratory, thoracic, and mediastinal disorders	1	1	N
Dyspnea	1		
Skin and subcutaneous tissue disorders	1	1	N
Pruritus	1		
Nervous system disorders	1	1	N
Syncope	1		
Renal and urinary disorders	1	1	N
Microalbuminuria	1		

Abbreviations: IMP, investigational medicinal product; N, no; Y, yes.

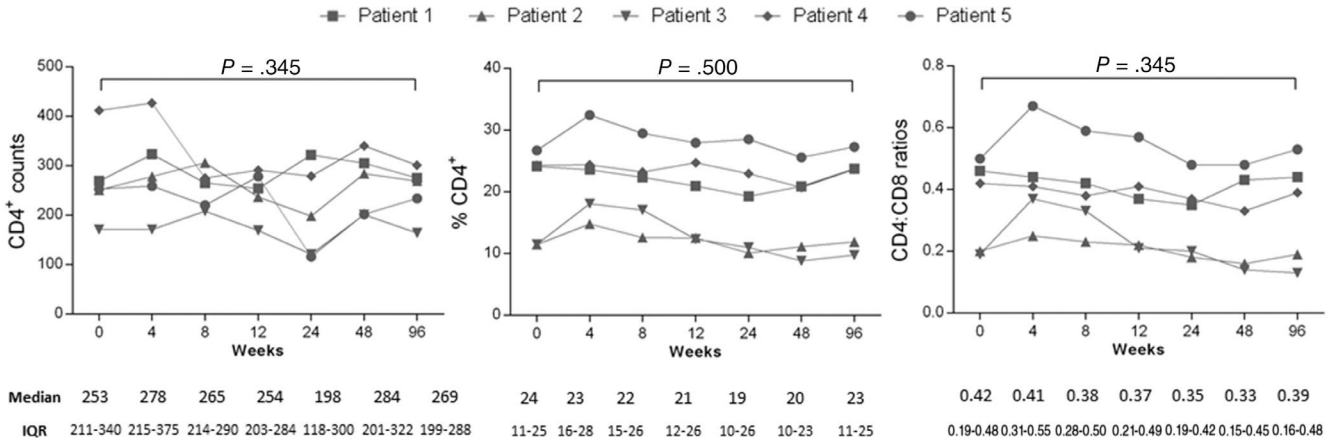

FIGURE 2 Evolution of the $CD4^+$ T-cell counts, percentages, and $CD4^+/CD8^+$ ratios after 96 weeks of follow-up. IQR, interquartile range

origin (bone marrow, adipose tissue, or umbilical cord),[25,26] we are not certain if the different cellular origin could be responsible for the discrepant results. Adult MSCs offer numerous advantages, but it has been reported that the yield, proliferative potential, and plasticity of MSCs decreases progressively with the advancing age of the donor compared with embryonic stem cells.[27] Also, Donders *et al*. showed

that genes associated with cell adhesion, proliferation, and modulation of the immune system are enriched in Wharton's jelly-derived MSCs.[28]

Likewise, recently, it has been proposed that other factors, such as tobacco, diabetes, or morbid obesity, can influence not only cell performance during manufacturing but also their pharmacological action.[29,30] All this could have influenced the lack of efficacy of MSCs in our study.

The high number of venous thrombosis events (VTEs) observed could have a complex multifactorial causality. On one side, the study subjects may be at increased risk of VTEs because of the patients' history of parenteral drug use (2/5), higher risk of recurrence in cases of previous thrombotic episodes,[31,32] or a high infusion rate (>3 mL/min) recorded in some infusions. In fact, the total of 10 events occurred in only three subjects, all having received four doses of the Ad-MSC suspension. Furthermore, in one patient, the VTEs could have been caused by the use of small-caliber veins on the back of the hands for the infusions, as this patient could not be infused in the arm. In addition, it has been reported that MSCs express tissue factor and have procoagulant activity, being observed more frequently in Ad-MSCs than in those of other sources. Furthermore, cell dose, handling conditions, growth media, and donor-associated factors might also influence procoagulant activity.[33-36]

Our study has several limitations. Regarding the safety of Ad-MSCs, it would have been desirable to determine their tissue factor expression before their administration, although in more than 150 patients treated with this same product in different clinical trials sponsored by the Andalusian Network for the Design and Translation of Advanced Therapies, thrombotic events occurred exceptionally. Furthermore, a full characterization of the infused MSCs was carried out, but neither in vitro functional assay to determine their immunomodulatory potency nor biodistribution analysis was performed. However, previous studies have shown that after intravenous infusion, cells were accumulated in lung, spleen, liver, and bone marrow.[37]

On the other hand, a higher number of patients and a control group, planned in the second phase of the trial, would have allowed us to better assess the fluctuations that some patients presented in several immunological parameters. Furthermore, the analyses are based on PBMC samples, which may not always reliably reflect tissue-related processes during HIV infection. The use of lymph node samples instead of PBMCs would have needed periodical biopsies, lowering the feasibility of the study.

5 | CONCLUSION

The Ad-MSC infusions have not proven to be effective to improve the immune recovery, nor have they succeeded in reducing immune activation or levels of inflammatory markers in INRs, at least with the dosage schedule selected.

ACKNOWLEDGMENTS

The authors are indebted to all involved in the trial, the Biobank of the Andalusian Public Health System, and particularly the subjects. The authors also thank to Dr José Alcamí Pertejo (Unidad de Inmunopatologia del SIDA, Centro Nacional de Microbiología, Spain) for his comments on the manuscript. This study was funded by the Instituto de Salud Carlos III (AES 2015; grant PI15/01041), Red de Investigación en SIDA (Spanish HIV/AIDS Research Network; RD16/0025/0020-ISCIII-FEDER), the Andalusian Regional Ministry of Health and Families (grant salud-201600073585-tra), and the Andalusian Network for the Design and Translation of Advanced Therapies through the Andalusian Progress and Health Public Foundation.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

M.T.-R.: collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; P.V., A.R.-G., O.E.-I., and S.A.-S.: provision of study material or patients, final approval of manuscript; I.R.-J.: administrative support, collection and/or assembly of data, final approval of manuscript; A.I.Á.-R.: data analysis and interpretation, final approval of manuscript; J.M.-A., R.M., and O.F.-L.: financial support, administrative support, final approval of manuscript; E.R.-M.: data analysis and interpretation, final approval of manuscript; A.G.-V.: conception/design, data analysis and interpretation, manuscript writing, final approval of manuscript; L.F.L.-C.: conception/design, provision of study material or patients, final approval of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Trujillo-Rodríguez M, Viciana P, Rivas-Jeremías I, et al. Mesenchymal stromal cells in Human Immunodeficiency Virus-infected patients with discordant immune response: Early results of a phase I/II clinical trial. *STEM CELLS Transl Med*. 2020;1–8. https://doi.org/10.1002/sctm.20-0213

Supplementary Table 1. Clinical information about donors

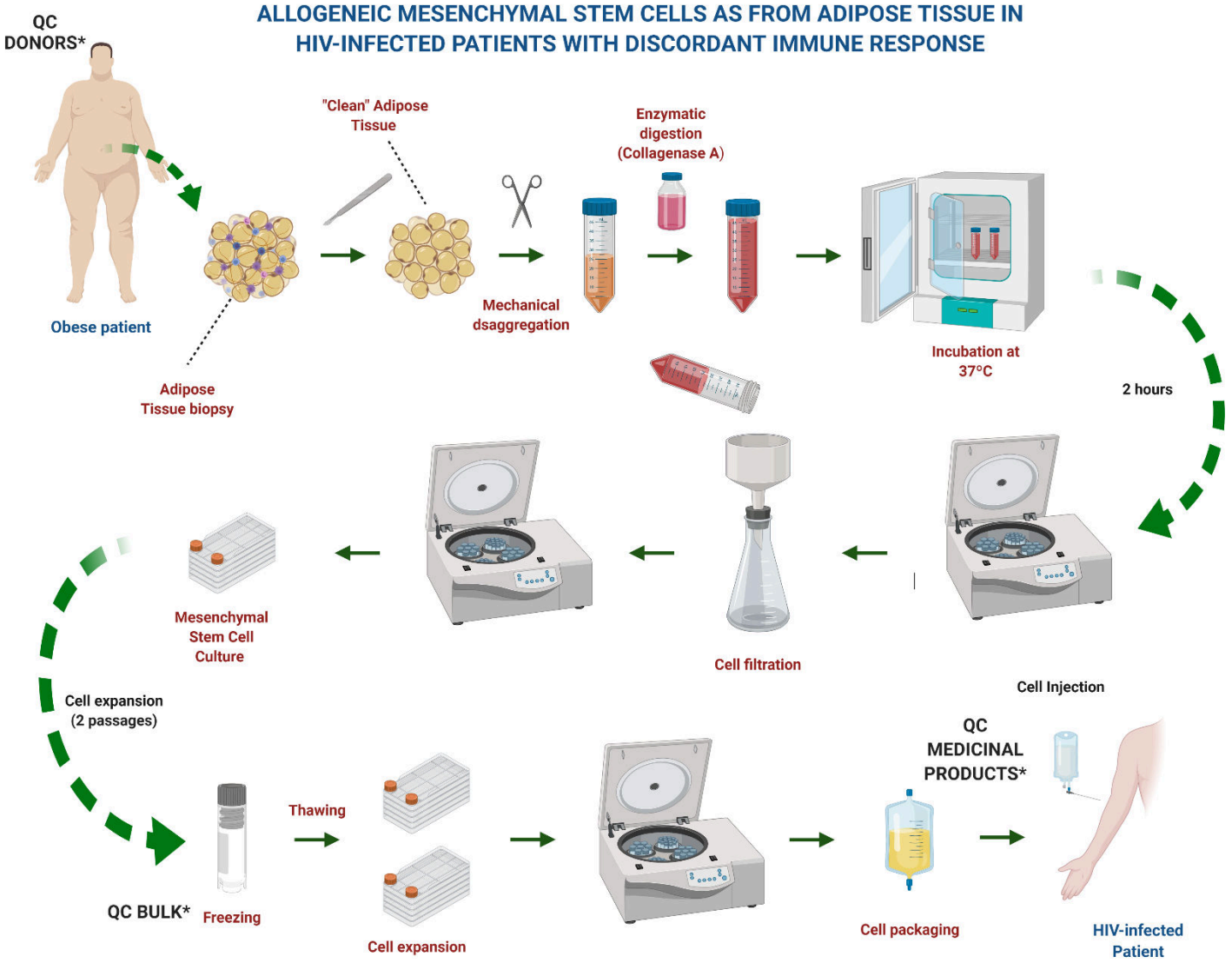
PATIENT	DOSE	BATCH	DONOR	DIABETES	HYPERTENSION	SMOKER	IMC
	1	1605D05	1605	YES	NO	YES	37,5
	2	1605D06					
	3	1605D09					
EC19/D/01	4	1701D10	1701	NO	NO	NO	45,88
	1	1605D07	1605	YES	NO	YES	37,5
	2	1607D04	1607	NO	NO	NO	32,71
	3	1607D08					
EC19/D/02	4	1701D13	1701	NO	NO	NO	
	1	1607D03	1607	NO	NO	NO	32,71
	2	1701D03	1701	NO	NO	NO	45,88
	3	1701D06					
EC19/D/03	4	1701D16					
	1	1701D08	1701	NO	NO	NO	45,88
	2	1701D11					
	3	1701D12					
EC19/D/04	4	1701D18					
	1	1701D17	1701	NO	NO	NO	45,88
	2	1702 D03	1702	NO	NO	NO	Not available
	3	1702 D05					
EC19/D/05	4	1702 D06					

Supplementary Table 2. Quality controls after the expansion Ad-MSC.

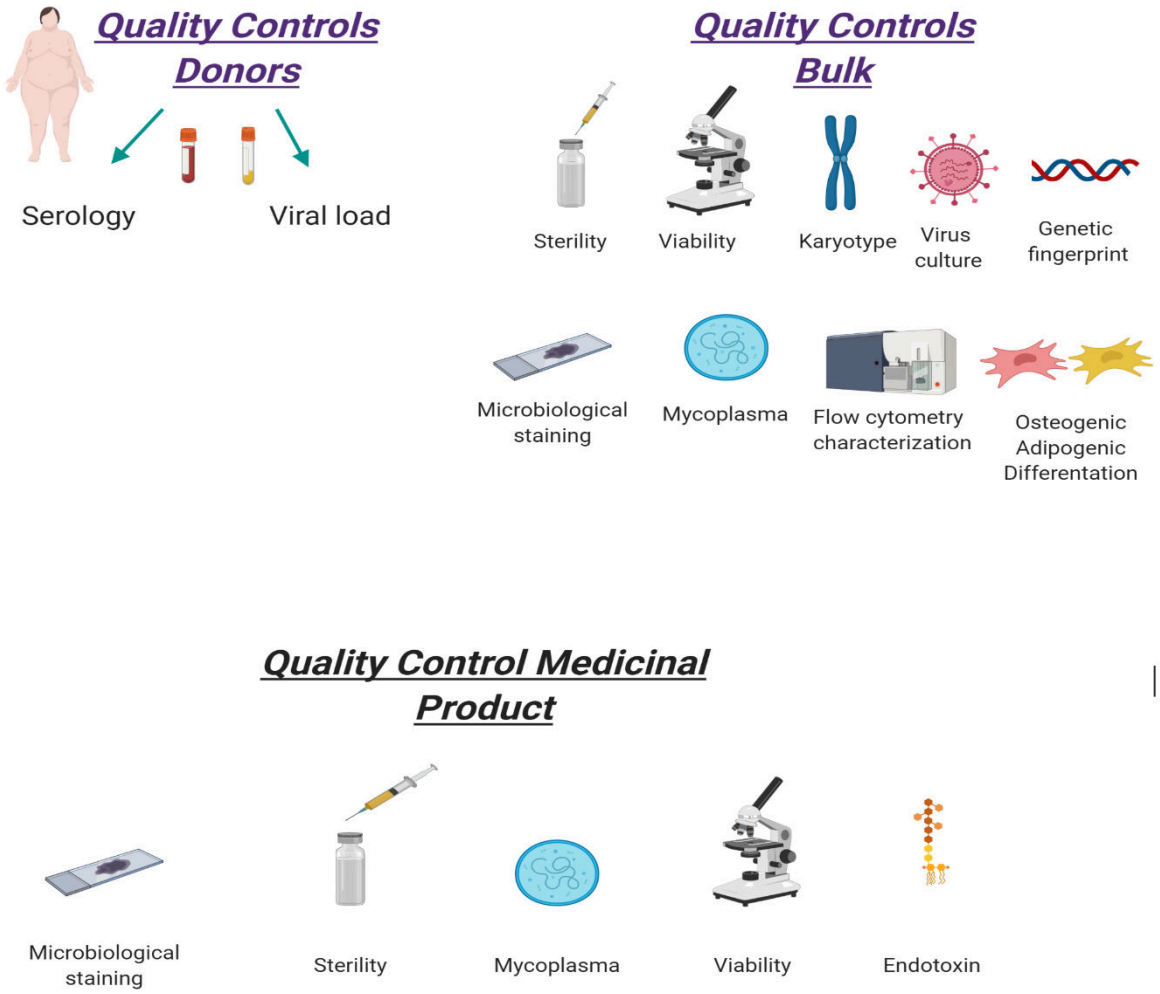
		BACTH			
		1605	**1607**	**1701**	**1702**
Viability (%)		97,0	92,4	97,7	95,2
Sterility test		Absence of microorganisms	Absence of microorganisms	Absence of microorganisms	Absence of microorganisms
Gram stain		Absence of microorganisms	Absence of microorganisms	Absence of microorganisms	Absence of microorganisms
Calcofluor stain		Absence of fungal structures	Absence of fungal structures	Absence of fungal structures	Absence of fungal structures
Mycoplasma		Absence of mycoplasma	Absence of mycoplasma	Absence of mycoplasma	Absence of mycoplasma
Cariotype		Absence of chromosomal abnormalities	Absence of chromosomal abnormalities	Absence of chromosomal abnormalities	Absence of chromosomal abnormalities
Differentiation	**Adipocytes**	Positive differentiation	Positive differentiation	Positive differentiation	Positive differentiation
	Osteocytes	Positive differentiation	Positive differentiation	Positive differentiation	Positive differentiation
Phenotype	**CD90**	99,50	98,98	100,00	98,60
	CD73	99,60	96,40	100,00	99,50
	CD105	100,00	98,98	100,00	98,60
	CD 44	100,00	98,05	97,00	97,55
	CD45	0,00	0,00	0,00	0,00
	CD19	0,00	0,00	0,00	0,00
	CD11b	0,00	0,00	0,00	0,00
	HLA DR	0,00	0,00	0,00	0,00
DNA Fingerprint		Matching donor	Matching donor	Matching donor	Matching donor
Adventitious virus		Absence of virus	Absence of virus	Absence of virus	Absence of virus

Supplementary Table 3. Quality control before delivery

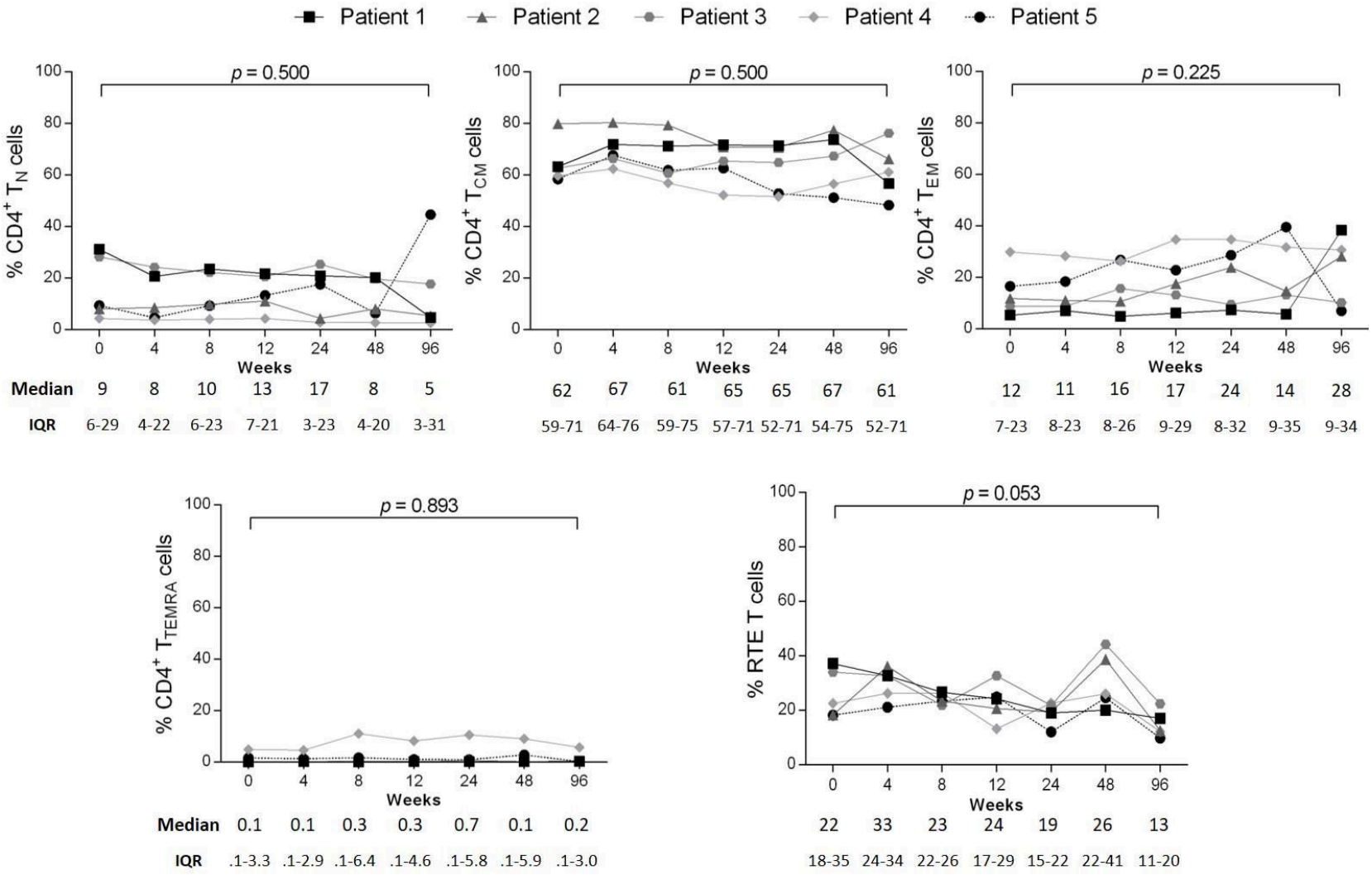
PATIENT	DOSE	BATCH	VIABILITY	STERILITY	ENDOTOXINS	MYCOPLASMA
EC19/D/01	1	1605D05	96,50	Absence of microorganisms	<1,00	Absence of mycoplasma
	2	1605D06	95,20	Absence of microorganisms	<1,00	Absence of mycoplasma
	3	1605D09	98,00	Absence of microorganisms	<1,00	Absence of mycoplasma
	4	1701D10	95,91	Absence of microorganisms	<1,00	Absence of mycoplasma
EC19/D/02	1	1605D07	97,20	Absence of microorganisms	<1,00	Absence of mycoplasma
	2	1607D04-D05	96,20	Absence of microorganisms	<1,00	Absence of mycoplasma
	3	1607D08	97,80	Absence of microorganisms	<1,00	Absence of mycoplasma
	4	1701D13	96,10	Absence of microorganisms	<1,00	Absence of mycoplasma
EC19/D/03	1	1607D03	98,00	Absence of microorganisms	<1,00	Absence of mycoplasma
	2	1701D03	97,80	Absence of microorganisms	<1,00	Absence of mycoplasma
	3	1701D06	95,48	Absence of microorganisms	<1,00	Absence of mycoplasma
	4	1701D16	99,10	Absence of microorganisms	<1,00	Absence of mycoplasma
EC19/D/04	1	1701D08	98,70	Absence of microorganisms	<1,00	Absence of mycoplasma
	2	1701D11	98,75	Absence of microorganisms	<1,00	Absence of mycoplasma
	3	1701D12	95,80	Absence of microorganisms	<1,00	Absence of mycoplasma
	4	1701D18	98,40	Absence of microorganisms	<1,00	Absence of mycoplasma
EC19/D/05	1	1701D17	97,50	Absence of microorganisms	<1,00	Absence of mycoplasma
	2	1702 D03	98,40	Absence of microorganisms	<1,00	Absence of mycoplasma
	3	1702 D05	97,60	Absence of microorganisms	<1,00	Absence of mycoplasma
	4	1702 D06	96,70	Absence of microorganisms	<1,00	Absence of mycoplasma



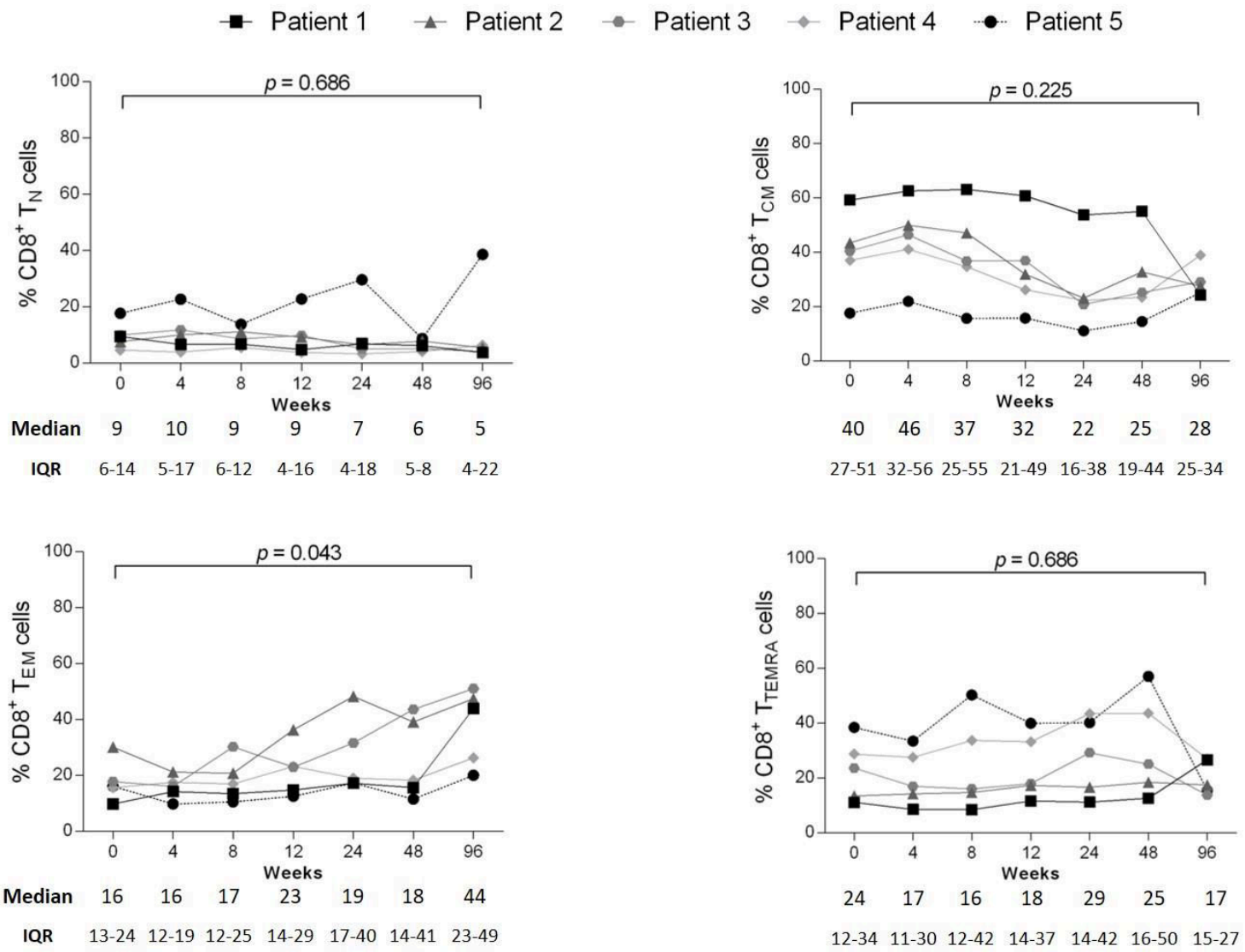
Supplementary Figure 1. An overview of the manufacturing process of allogeneic mesenchymal stem cells.



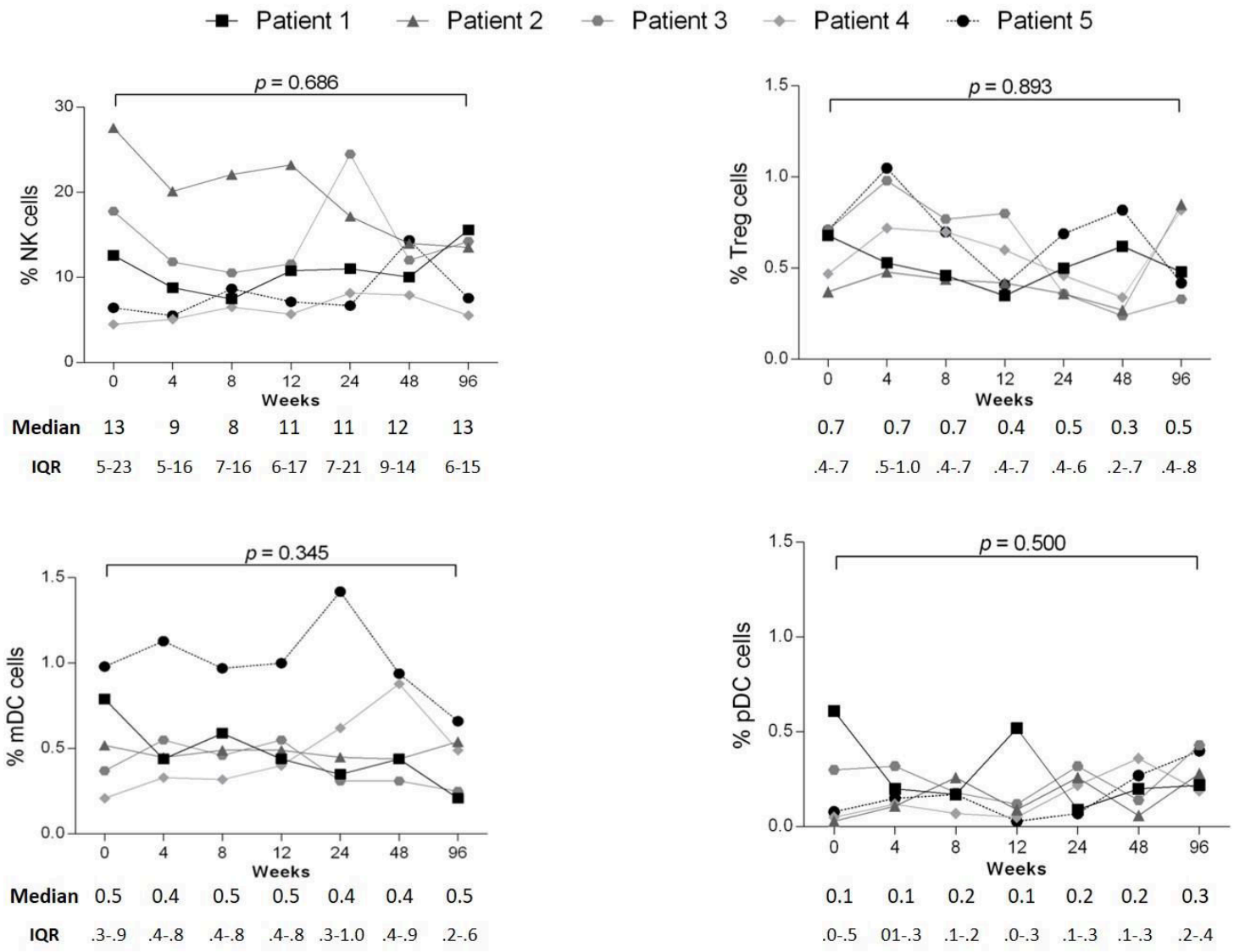
Supplementary Figure 2. Quality controls of mesenchymal stem cells.



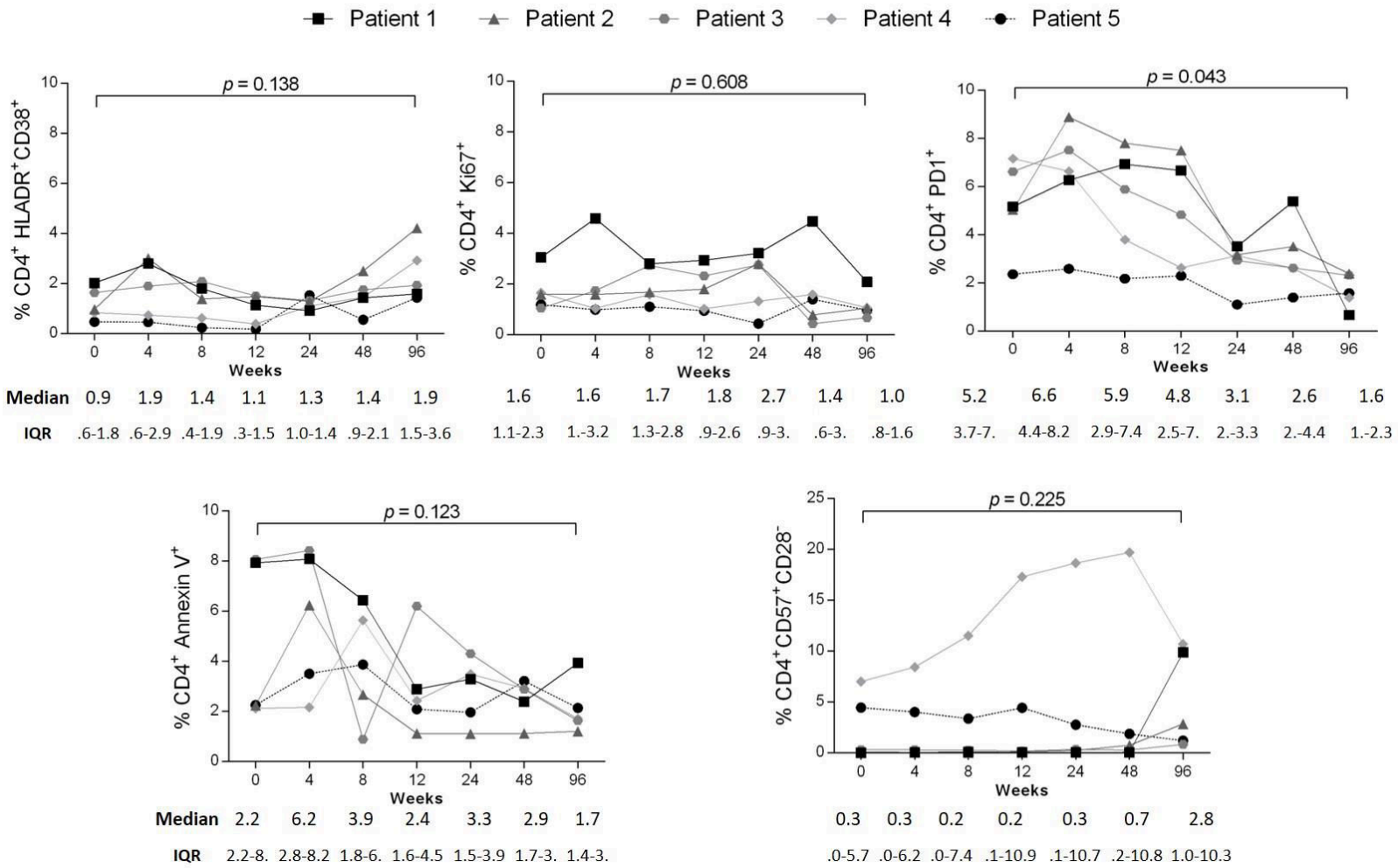
Supplementary Figure 3. Evolution of the percentage of $CD4^+$ T cell subsets through 96 weeks of follow-up. N, naïves. CM, central memory. EM, effector memory. TEMRA, terminally differentiated effector memory cells re-expressing CD45RA. RTE, recent thymic emigrants.



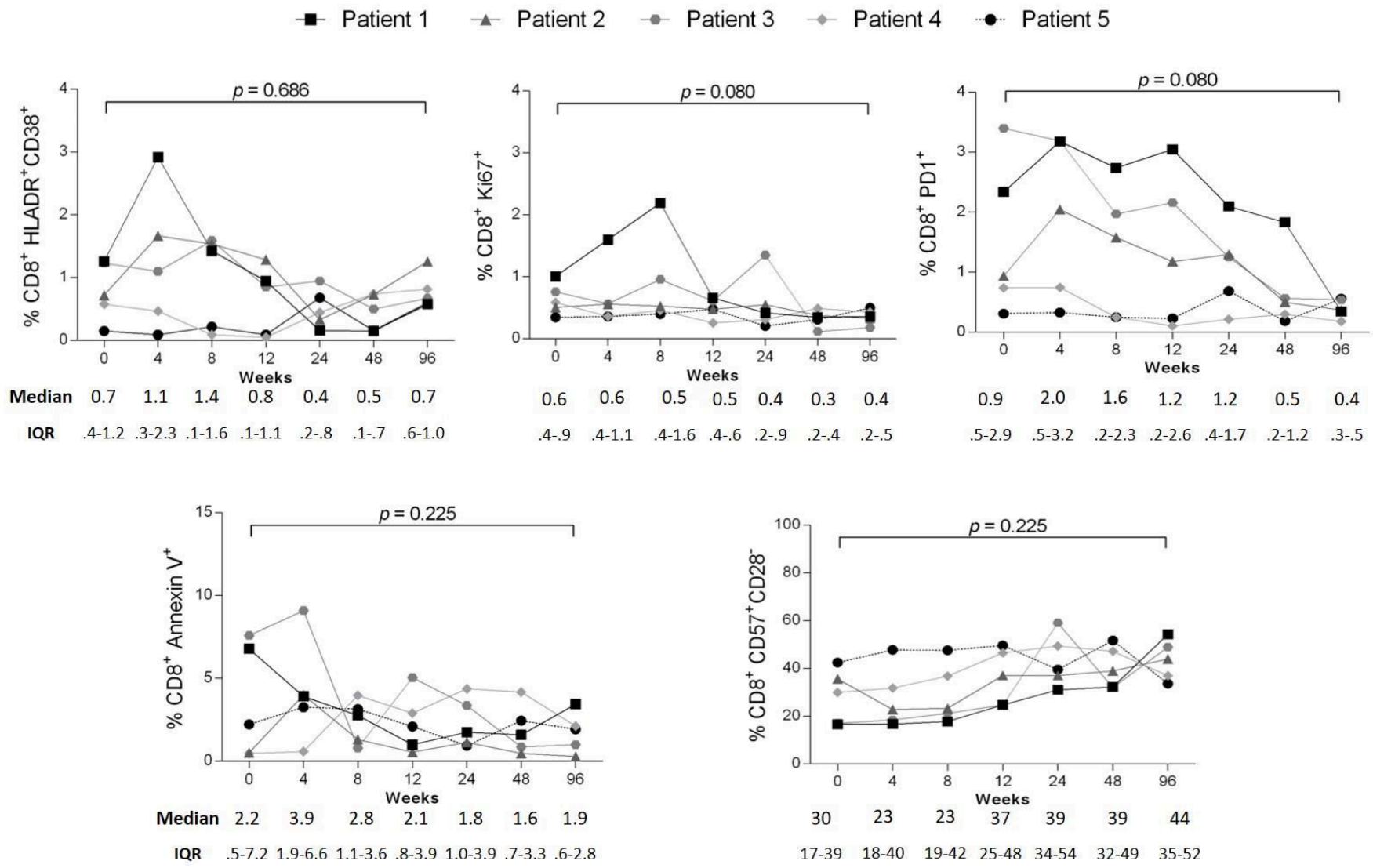
Supplementary Figure 4. Evolution of the percentage of $CD8^+$ T cell subsets through 96 weeks of follow-up. N, naïves. CM, central memory. EM, effector memory. TEMRA, terminally differentiated effector memory cells re-expressing CD45RA.



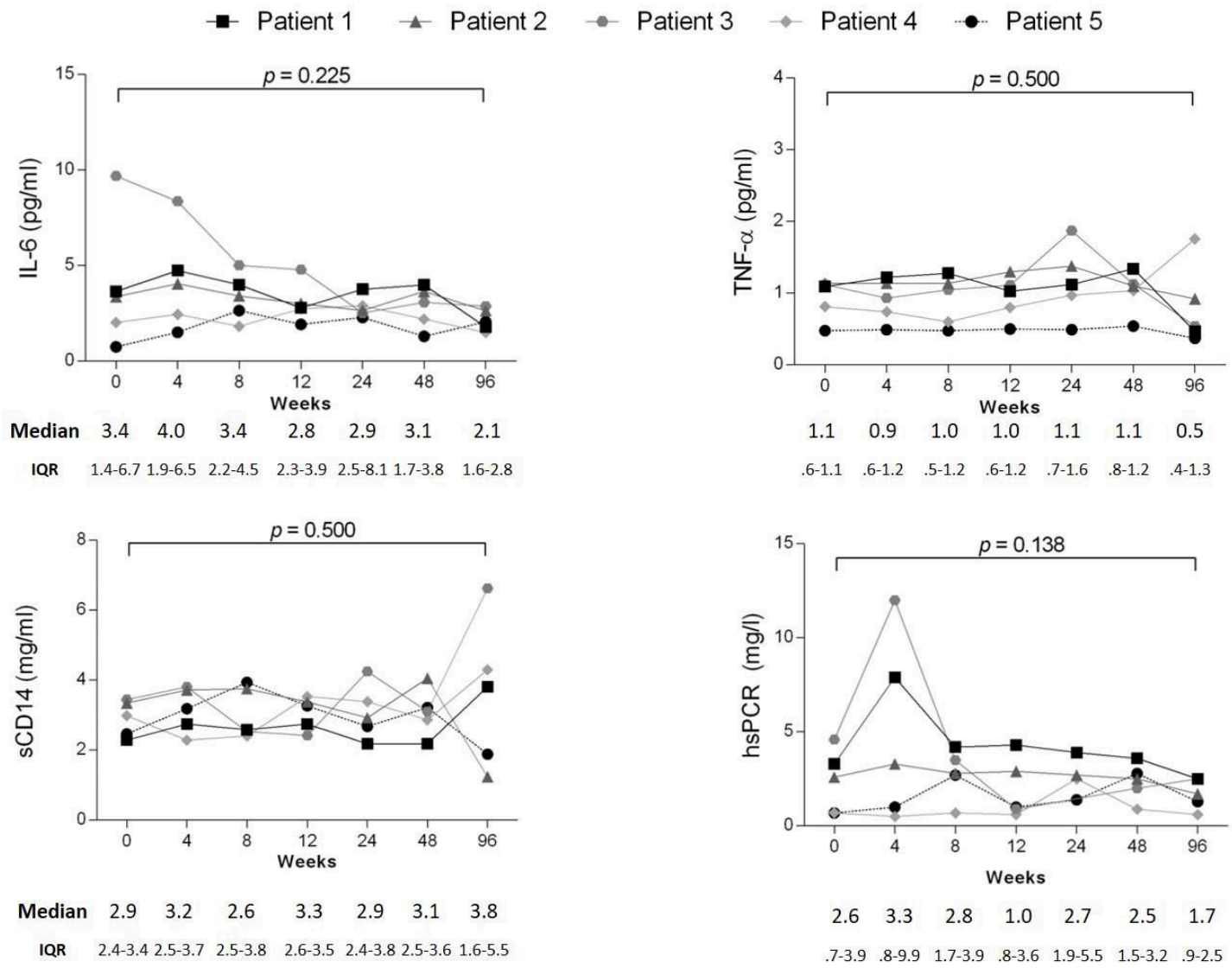
Supplementary Figure 5. Evolution of the percentage of Natural Killer Cells (NK), regulatory T-Cells (T_{regs}), myeloid (mDC) and plasmacytoid dendritic cells (pDC) through 96 weeks of follow-up.



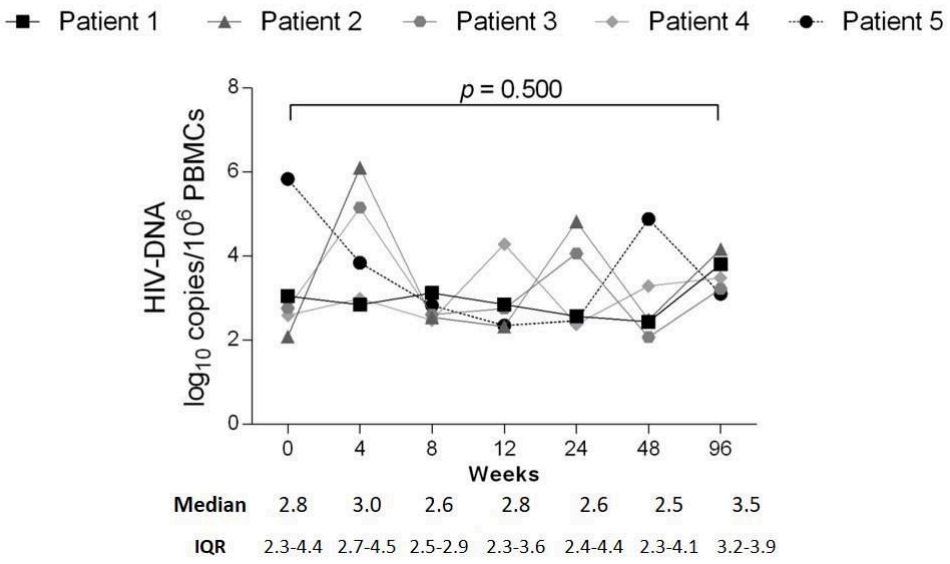
Supplementary Figure 6. Effect of mesenchymal stem cell transfusions in activation (HLA-DR$^+$CD38$^+$), proliferation (Ki67$^+$), exhaustion (PD1$^+$), apoptosis (annexin V$^+$) and senescence (CD57$^+$CD28$^-$) markers on CD4$^+$ T cells through 96 weeks of follow-up.



Supplementary Figure 7. Effect of mesenchymal stem cell transfusions in activation (HLA-DR$^+$CD38$^+$), proliferation (Ki67$^+$), exhaustion (PD1$^+$), apoptosis (annexin V$^+$) and senescence (CD57$^+$CD28$^-$) markers on CD8$^+$ T cells through 96 weeks of follow-up.



Supplementary Figure 8. Evolution of the plasma levels of interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), soluble CD14 (sCD14) and high sensitivity C-reactive protein (hsCRP) through 96 weeks of follow-up.



Supplementary Figure 9. Evolution of cellular-associated HIV-DNA from PBMCs through 96 weeks of follow-up.

4.3 Tercer artículo

Trujillo-Rodríguez M, Muñoz-Muela E, Serna-Gallego A, Milanés-Guisado Y, Praena-Fernández JM, Álvarez-Ríos AI, Herrera-Hidalgo L, Domínguez M, Lozano C, Romero-Vazquez G, Roca C, Espinosa N, Gutiérrez-Valencia A, López-Cortés LF

Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a randomized trial

Clin Microbiol Infect. 2022; S1198-743X(22)00118-5

doi: 10.1016/j.cmi.2022.02.041

Clinical Microbiology and Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a randomized trial

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ARTICLE INFO

Article history:
Received 8 October 2021
Received in revised form
24 February 2022
Accepted 27 February 2022
Available online xxx

Editor: A. Huttner

Keywords:
Dual therapy
HIV reservoir
HIV treatment
Immune activation and inflammation
Immune recovery
Simplification strategy
Triple therapy

ABSTRACT

Objectives: To evaluate whether simplification of antiretroviral treatment to dual therapy (DT) negatively impacts immune recovery (IR), immune activation and inflammation (IA/I), and HIV reservoir.
Methods: An open-label, single-centre, randomized controlled trial conducted in adult virologically suppressed HIV-infected patients on triple therapy (TT) with elvitegravir-cobicistat, emtricitabine and tenofovir alafenamide or dolutegravir (DTG), abacavir, and lamivudine (3TC). Participants were randomized to continue TT or switch to DTG, or darunavir/cobicistat (DRVc) plus 3TC. IR was assessed by $CD4^+/CD8^+$ ratio at 48 and 96 weeks. Changes in immune activation, proliferation, exhaustion, senescence, and apoptosis in $CD4^+$ and $CD8^+$ T cells, plasma sCD14, hsCRP, D-dimers, β2-microglobulin, IL-6, TNF-α and IP-10 levels, cell-associated HIV-DNA (CA-DNA), and unspliced HIV-RNA (usRNA) were also analysed.
Results: One hundred and fifty-one participants were enrolled. Fourteen patients did not complete the follow up. In the ITT and PP analysis, the IR was similar between the treatment arms. In the ITT analysis, the median increase in $CD4^+/CD8^+$ ratio was 0.10, 0.04, and 0.07 at week 48, and 0.09, 0.05, and 0.08 at week 96 for TT, DTG/3TC, and DRVc/3TC, respectively. After adjusting for confounding factors, the slopes of changes in $CD4^+/CD8^+$ ratio over time were independent of treatment ($F = 1.699$; $p = 0.436$) and related only to baseline values ($F = 756.871$; $p = 0.000$). There were no differences in IA/I, CA-DNA, or usRNA between treatment arms.
Discussion: Both IR and IA/I, CA-DNA, and usRNA were similar in the three treatment groups, regardless of maintaining TT or simplifying to DTG/3TC or DRVc/3TC in virologically suppressed HIV-infected patients. **María Trujillo-Rodríguez, Clin Microbiol Infect 2022;▪:1**

Introduction

The gold standard for antiretroviral treatment (ART) has been based on two nucleos(t)ide reverse transcriptase inhibitors (NRTI) combined with a protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or an integrase strand transfer

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https://doi.org/10.1016/j.cmi.2022.02.041
1198-743X/

inhibitor. The idea of simplifying ART came up because of the short- and long-term toxicity and frequent resistance-associated mutations. However, early simplification strategies failed to maintain viral suppression, due to a low genetic barrier and antiviral activity of drugs [1]. The idea re-emerged in the form of ritonavir-boosted PI monotherapy, although those regimens resulted in more low-level viral replication episodes and virological failures than triple therapy (TT) [2]. Recently, simplification strategies based on a boosted PI or dolutegravir (DTG) plus lamivudine (3TC) have shown similar virological efficacy to TT in HIV-suppressed patients [3–7] and even in treatment-naïve patients [8], with lower cost [9].

On the other hand, although ART reduces the immune activation and inflammation (IA/I), it fails to completely normalize them, despite plasma viral load suppression, playing a pivotal role in non-AIDS events [10]. Incomplete suppression of viral replication in lymphatic tissues, not reflected in plasma, could contribute to it due to lower drug concentrations in tissues [11]. Consequently, there is concern that simplification to dual therapy (DT) could not control viral replication as much as TT, impacting immune recovery (IR), IA/I, or HIV reservoir. To date, there are scarce data on DT that have evaluated partial aspects of these issues, sometimes with contradictory results, and most without control groups [7,12–18]. Moreover, expert opinions believe that DT could negatively affect patients' prognosis [19]. Thus, this randomized clinical trial aimed to provide insights into this controversy through a comprehensive evaluation of IR, IA/I, cell-associated HIV-DNA (CA-DNA), and unspliced HIV-RNA (usRNA) up to 96 weeks of follow up.

Methods

Study design and participants

The TRIDUAL (TRIple versus DUAL therapy) study is an open-label, single-centre, noninferiority, randomized clinical trial (Clinical trials.gov: NCT03447873) carried out in Virgen del Rocío University Hospital in Seville, Spain.

Eligible participants were adults who started ART later than 01 January 2012, had undetectable plasma HIV RNA for at least 1 year, and were ≥6 months on FTC/TAF/EVGc or ABC/3TC/DTG. Exclusion criteria included HIV resistance to the study drugs, pregnancy, active opportunistic infection, HBV or HCV coinfection, cirrhosis, portal hypertension, previous or current malignancies, treatment with immunomodulatory agents, and use of drugs with potential interactions with the prescribed drugs.

Participants who met the conditions were centrally randomized via phone interface at the clinical trials unit of our hospital based on time with undetectable viral load (1–2, 2–3, 3–4, or >4 years), to continue with the previous TT or switch to DTG/3TC (50/300 mg) or DRVcobicistat (DRVc)/3TC (800_{150}/300 mg), using random numbers and making the relative sizes of the groups in each stratum similar. The randomization list was constructed using the separate strata balanced randomization module of the WinPepi Etcetera program (Epidemiologic Perspectives & Innovations) (see Table S1).

Patients were excluded in case of virological failure, defined as two consecutive plasma viral loads ≥200 copies/mL, treatment change or interruption, loss of follow up, a diagnosis of malignancy, or death. We selected an arbitrary cut-off point of ≥200 copies/mL to define virological failure because of possible measurement errors in HIV RNA assays close to their limit of quantification [20].

Ethics

The study was conducted according to Good Clinical Practice principles after being approved by the Ethics Committee for Clinical Research of the Virgen Macarena and Virgen del Rocío University Hospitals (03/2017) and the National Health Authority. All participants provided signed informed consent.

Endpoints, follow up, and assessments

Patients were assessed at baseline, at the third and sixth month, and then every 6 months, including adverse effects, haematology and biochemistry tests, $CD4^+$ and $CD8^+$ T cell counts in fresh blood with an FC 500 flow cytometer (Beckman Coulter, Brea, CA), and plasma HIV RNA levels (Cobas AmpliPrep-Cobas TaqMan HIV-1 test, version 2.0; Roche Diagnostics) with a lower detection limit of 20 copies per milliliter.

The primary endpoint was to evaluate IR at 48 weeks after switching to DT compared with continuing on TT. Secondary endpoints were IR at 96 weeks, changes in IA/I markers, CA-DNA, and usRNA at 48 and 96 weeks. IR was assessed by $CD4^+/CD8^+$ ratio. Activated ($CD38^+HLA\text{-}DR^+$), proliferating ($Ki67^+$), exhausted ($PD\text{-}1^+$), senescent ($CD57^+CD28^-$), and apoptotic (annexin V^+) $CD4^+$ and $CD8^+$ were also identified. We measured plasma sCD14 as monocyte activation marker, hsCRP, D-dimers, β2M, IL-6, TNF-α, and IP-10 as markers of inflammation and coagulation, and CA-DNA and usRNA as HIV reservoir and transcription markers.

Methods for cell surface staining for immune profile, quantitation of cell-associated HIV-DNA and usHIV-RNA, and enzyme-linked immunosorbent assays are described in the Supplementary material.

Statistical analysis

Given that we had not found clear references in the literature to calculate the sample size and the proof-of-concept nature of this study, no formal sample size was calculated. It was based on a retrospective analysis of our database and feasibility resources. We aimed to include 50 participants per treatment arm, which could provide enough information. In addition, a post-hoc analysis was performed to calculate statistical power [21].

Results were expressed as medians and IQRs for continuous variables and numbers and percentages for categorical variables. The Kruskal-Wallis test and the Friedman test was used to compare changes in quantitative and continuous variables over time in each treatment group, respectively. Correlations were assessed by Spearman's correlation coefficient.

A linear mixed model with random intercepts and slopes for repeated measurements was used to compare the time courses of the $CD4^+/CD8^+$ ratio according to regimen strategy, adjusted for possible confounders, including age, sex, baseline values, nadir $CD4^+$, and time with undetectable viral load. The overall effect of each explanatory variable on the outcome variable $CD4^+/CD8^+$ ratio was tested with the F-test and CI_{95}. A missing data analysis was conducted. Expectation maximization algorithms were applied to identify the nature of missingness (MCAR [Missing completely at random] or MAR [Missing at random]), by Little's Missing MCAR Test. The multiple imputation method was used for dealing with missing data [22].

Analyses were performed in the intention-to-treat (ITT) population and per-protocol (PP). The ITT population included all participants who underwent randomization. In the PP analysis, only

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those participants who completed the follow up of the study were analysed.

SPSS v. 26.0 and R software were used for statistical analyses; p values < 0.05 were considered significant.

Results

Study population

One hundred and fifty-one participants were enrolled between May 2017 and September 2018 (ITT population); demographics and HIV infection data are detailed in Table 1. Fifty-three patients were randomized to continue TT, 50 to DTG/3TC, and 48 to DRVc/3TC. Regarding the time with undetectable plasma viral load, 41 (27.2%) patients were between 1 and 2 years, 40 (26.5%) between 2 and 3, 21 (13.9%) between 3 and 4, and 49 (32.5%) >4 years without differences among groups (p = 0.346) (see Fig. S1).

Eight (5.3%) patients were withdrawn from the study before completing 48 weeks, and a further six patients during the second year. Therefore, 137 (90.7%) participants formed the per-protocol population (Fig. 1). Only one virological failure (2370 copies/mL) was observed in the DRVc/3TC arm, with a self-reported full ART adherence, and without resistance mutations detected.

No patient had adverse effects or significant laboratory abnormalities (data not shown).

Immune recovery

In the ITT analysis, there were no differences in the $CD4^+/CD8^+$ ratio baseline between groups (TT, 1.0 [0.7–1.4]; DTG/3TC, 0.9 [0.6–1.2]; DRVc/3TC, 0.9 [0.6–1.2]) (p = 0.173). According to the treatment arm, the increase in the $CD4^+/CD8^+$ ratio at week 48 were 0.10 (−0.01 to 0.25), 0.04 (−0.03 to 0.19), and 0.07 (0–0.23) for TT, DTG/3TC, and DRVc/3TC (p = 0.318), respectively. From baseline to week 96, these increases were 0.09 (−0.02 to 0.25), 0.05 (−0.03 to 0.15), and 0.08 (0–0.21) (p = 0.378), respectively. In PP analysis, these figures were 0.09 (−0.01 to 0.25), 0.04 (−0.03 to 0.20), and 0.07 (0–0.24) for TT, DTG/3TC, and DRVc/3TC (p = 0.435), respectively, at week 48 and 0.09 (−0.02 to 0.25), 0.05 (−0.03 to 0.15), and 0.08 (0–0.21) for TT, DTG/3TC, and DRVc/3TC (p = 0.378), respectively, at week 96. Baseline and evolutive data for each treatment arm are shown in Table 2.

A lineal mixed model adjusted for age, sex, baseline $CD4^+/CD8^+$ ratio, time with undetectable viral load, and treatment × time interaction showed that the slopes of change over time for $CD4^+/CD8^+$ ratio were independent of treatment and related only to baseline values both by ITT (Table 3 and Fig. 2) and PP analysis (see Fig. S2 and Table S2), respectively.

The baseline $CD4^+/CD8^+$ ratio correlated inversely with activation ($\rho = -0.406$), proliferation ($\rho = -0.380$) and exhaustion ($\rho = -0.352$) of $CD4^+$ T cells (all, p < 0.0001), but most of them disappeared throughout follow up. Likewise, a negative relationship between $CD4^+/CD8^+$ ratio and usRNA levels was observed during the follow up ($\rho = -0.288$ at week 48 and −0.282 at week 96; all p < 0.0001). Nevertheless, inconsistent associations were detected between $CD4^+/CD8^+$ ratio with soluble factors and CA-DNA (see S3–S5).

Immune activation and inflammation

During the follow up there were no differences between the treatment arms in activation, proliferation, exhaustion, senescence, and apoptosis of $CD4^+$ and $CD8^+$ T cells. Detailed data by treatment arm are described in Table 2.

On the other hand, the sCD14 decreased (p ≤ 0.002) and IL-6 had a small increase (p < 0.0001) regardless of the treatment arm and without differences among them (Table 2). Besides, β2M, hsCRP, D-dimers, TNF-α, and IP-10 levels showed small fluctuations without differences between groups.

HIV reservoir and transcriptional activity

CA-DNA did not change during the first year (p = 0.474) but decreased during the second year a median of 0.11 $\log_{10}$ (p = 0.02), without differences in treatment groups (p = 0.659).

Table 1
Baseline patient characteristics of ITT population

	INSTI +2 NRTIs (*n* = 53)	DTG/3TC (*n* = 50)	DRVc/3TC (*n* = 48)
Male sex	51 (96.2)	44 (88.0)	44 (91.7)
Age, years	33 (28–44)	29 (26–37)	34 (25–41)
Age group			
<30 years	15 (28.3)	26 (52.0)	16 (33.0)
30–45 years	29 (54.7)	18 (36.0)	25 (52.1)
>45 years	9 (17.0)	6 (12.0)	7 (14.6)
Risk factor for HIV			
MSM	44 (83.0)	38 (76.0)	36 (75.0)
Heterosexual	9 (17.0)	7 (14.0)	7 (14.6)
Other/Unknown		2 (4.0)	4 (8.3)
IVDU		3 (6.0)	1 (2.1)
Previous CDC C stage	2 (3.8)	3 (6.0)	4 (8.3)
Nadir CD4 count, cells/μL	342 (266–453)	282 (191–416)	284 (144–403)
Nadir $CD4^+/CD8^+$ ratio	0.4 (0.2–0.5)	0.3 (0.2–0.5)	0.2 (0.2–0.5)
Months on treatment	37 (23–62)	38 (26–61)	39 (29–73)
HIV-RNA <50 copies/mL, months	31 (18–56)	34 (23–57)	34 (24–70)
HIV-RNA <50 copies/mL group			
1–2 years	17 (32.1)	12 (24.0)	12 (25.0)
2–3 years	14 (26.4)	14 (28.0)	12 (25.0)
3–4 years	5 (9.4)	9 (18.0)	7 (14.6)
>4 years	17 (32.1)	15 (30.0)	17 (35.4)
Baseline CD4 count, cells/μL	794 (596–1123)	750 (590–917)	711 (542–976)
Baseline $CD4^+/CD8^+$ ratio	1.0 (0.7–1.4)	0.9 (0.6–1.1)	0.8 (0.6–1.2)

Data are expressed as median (interquartile range) or n (%). INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine; MSM, men who have sex with men; IVDU, previous intravenous drug use.

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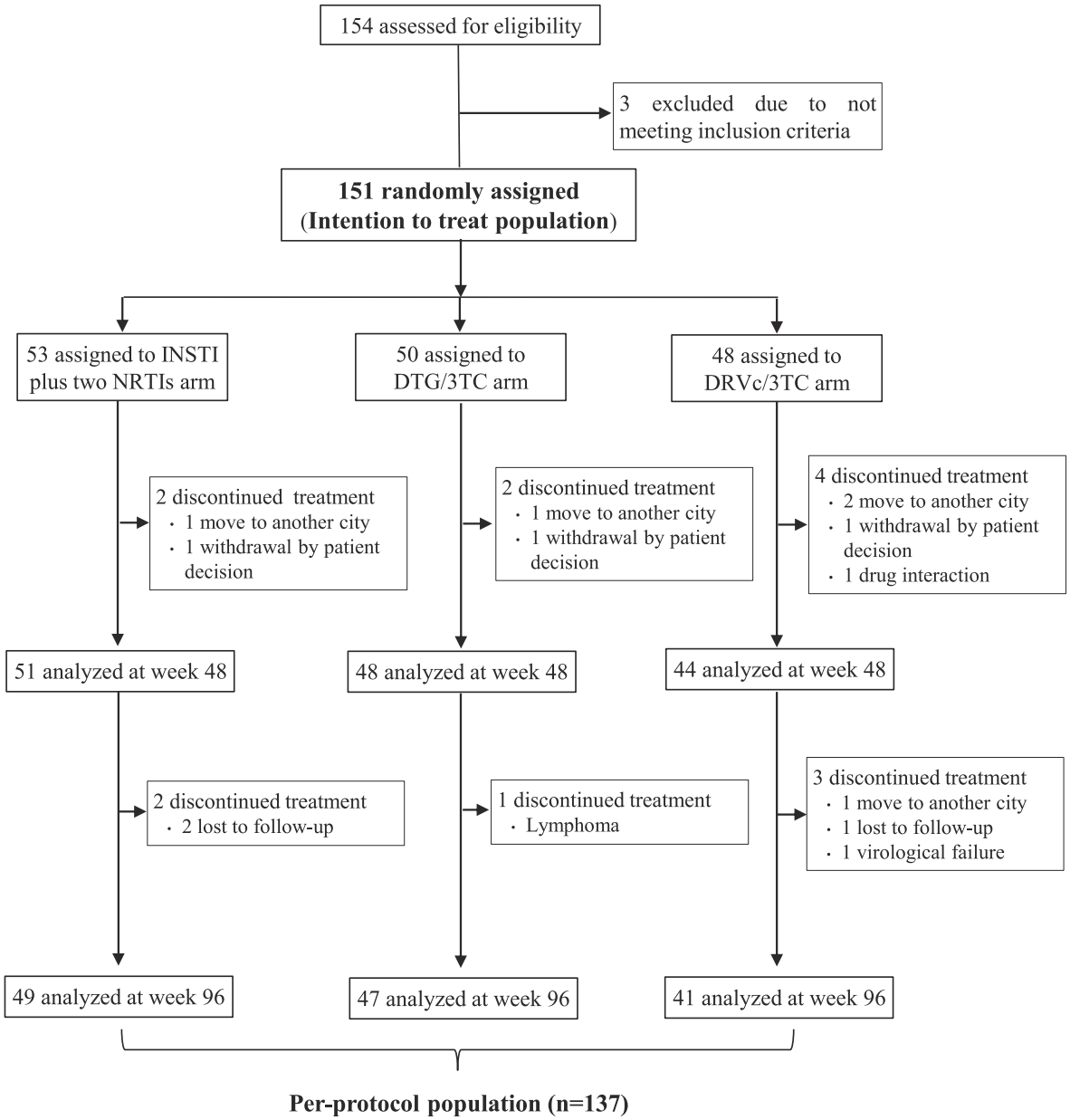

Fig. 1. Study flow diagram. INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine; ART, antiretroviral treatment.

Baseline and evolutive data for each treatment arm are shown in Table 2.

The most consistent association found with CA-DNA was the $CD4^+$ nadir at baseline and week 48 ($\rho = -0.292$, and -0.328, respectively; both $p < 0.0001$) (see Figs. S3–S5). Conversely, usRNA increased a median of 0.3 $\log_{10}$ during the second year, equally in all three groups ($p = 0.223$). CA-DNA and usRNA levels were strongly correlated throughout follow up ($\rho = 0.332$ at baseline, 0.521 at week 48, and 0.362 at week 96; all $p < 0.0001$). At baseline and 48 weeks, the usRNA levels were associated positively with activation, proliferation, exhaustion, and apoptosis of $CD4^+$ T cells, although this relationship disappeared at week 96. In addition, correlations between usRNA levels with activation, proliferation, exhaustion, and senescence of $CD8^+$ T cells were observed at baseline, although there were not long-term associations (see Figs. S3–S5).

Discussion

This study has not detected differences in IR, IA/I, HIV reservoir, nor the transcriptional activity in virologically suppressed patients regardless of maintaining TT or switching to DT.

To date, only partial aspects of simplification's immunological or inflammatory consequences of DT have been evaluated in virologically suppressed patients. Quirós-Roldan et al. observed that $CD4^+/CD8^+$ ratio continued to rise after simplification to boosted atazanavir or DRV plus 3TC [12]. Monsalvo et al. observed that the $CD4^+/CD8^+$ ratio increase of 245 patients who switched to boosted DRV or DTG based NRTI-sparing therapies over 48 weeks was only relative to baseline values [18]. These data are similar to our results after 48 and 96 weeks by ITT and PP.

Maggiolo et al. have explored $CD8^+$ activation after switching to boosted DRV plus rilpivirine versus maintaining a boosted PI plus

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Table 2
Evolution of immune activation, inflammation, and HIV reservoir parameters over follow up in patients with INSTI + 2 NRTIs, DTG/3TC, and DRVc/3TC of ITT population

	INSTI + 2 NRTIs			DTG/3TC		DRVc/3TC			[a] p	[b] p
	Basal (n = 53)	Week 48 (n = 51)	Week 96 (n = 49)	Basal (n = 50)	Week 48 (n = 48)	Week 96 (n = 47)	Basal (n = 48)	Week 48 (n = 44)	Week 96 (n = 41)		
$CD4^+/CD8^+$ ratio	1.0 (0.7–1.4)	1.1 (0.7–1.6)	1.1 (0.8-1.6)	0.9 (0.6–1.1)	1.0 (0.7–1.3)	0.9 (0.6–1.2)	0.8 (0.6–1.2)	1.1 (0.8–1.3)	1.0 (0.7–1.3)	0.414	0.218
$CD4^+$ T cells											
HLA-DR^+CD38^+ (%)	1.2 (1.0–1.8)	1.2 (0.9–1.7)	1.1 (0.6–1.9)	1.5 (1.1–1.8)	1.4 (1.1–2.4)	1.5 (1.0–2.4)	1.7 (1.2–2.2)	1.4 (1.2–2.0)	1.7 (1.0–2.9)	0.086	0.333
$Ki67^+$ (%)	0.7 (0.5–1.2)	0.9 (0.6–1.4)	1.2 (0.8–2.3)	0.8 (0.6–1.3)	0.9 (0.6–1.5)	1.7 (1.2–2.6)	0.9 (0.5–1.5)	0.9 (0.6–1.1)	2.0 (1.2–2.5)	0.307	0.446
PD-1^+ (%)	7.1 (5.0–12.7)	6.7 (4.4–9.9)	9.2 (5.9–17.3)	8.7 (6.8–12.9)	6.8 (5.2–10.9)	12.9 (6.4–18.2)	7.2 (5.4–11.9)	5.8 (4.1–8.3)	10.5 (5.5–15.8)	0.605	0.314
$CD28^-CD57^+$ (%)	5.6 (1.8–12.1)	6.8 (2.8–19.1)	3.4 (1.2–7.5)	3.1 (1.5–8.9)	8.1 (1.4–13.9)	2.6 (1.4–7.8)	4.7 (2.5–8.9)	5.7 (2.9–9.1)	4.9 (2.8–7.4)	0.236	0.863
Annexin V^+ (%)	0.6 (0.4–1.2)	0.9 (0.5–1.5)	2.2 (1.7–2.9)	0.8 (0.5–1.5)	0.9 (0.7–1.5)	2.0 (1.3–2.4)	0.7 (0.4–1.3)	1.2 (0.7–1.6)	2.1 (1.7–2.9)	0.529	0.316
$CD8^+$ T cells											
HLA-DR^+CD38^+ (%)	1.7 (1.1–2.6)	1.4 (0.8–2.2)	1.1 (0.6–2.0)	1.7 (1.0–2.9)	1.5 (0.9–2.4)	1.5 (0.9–2.3)	2.2 (1.6–3.9)	1.5 (1.1–2.5)	1.6 (0.9–2.4)	0.782	0.573
$Ki67^+$ (%)	0.7 (0.4–1.0)	0.6 (0.3–0.9)	1.4 (0.9–1.9)	0.6 (0.4–0.9)	0.6 (0.4–0.9)	1.6 (1.0–2.1)	0.7 (0.4–1.0)	0.6 (0.4–0.8)	1.6 (1.4–2.0)	0.471	0.426
PD-1^+ (%)	8.2 (4.3–12.9)	7.9 (4.1–10.6)	13.6 (6.4–19.7)	8.2 (5.1–13.1)	8.0 (4.9–11.1)	12.9 (8.7–18.9)	7.8 (3.9–10.6)	6.2 (3.9–9.9)	9.9 (5.6–16.4)	0.801	0.297
$CD28^-CD57^+$ (%)	41.5 (30.6–51.9)	47.9 (36.1–57.9)	37.5 (24.8–49.7)	39.2 (33.2–46.6)	48.7 (33.5–59.7)	33.9 (25.7–40.9)	40.6 (32.9–51.9)	40.5 (29.5–53.9)	36.7 (28.2–49.6)	0.122	0.874
Annexin V^+ (%)	0.9 (0.7–1.8)	1.0 (0.6–1.7)	2.4 (1.7–2.8)	1.1 (0.7–2.2)	1.0 (0.7–1.7)	2.1 (1.4–2.6)	0.9 (0.5–1.6)	1.1 (0.8–1.6)	2.2 (1.5–2.7)	0.071	0.525
β2 microglobulin (mg/L)	1.9 (1.7–2.2)	1.9 (1.7–2.2)	2.0 (1.8–2.3)	1.9 (1.7–2.3)	1.9 (1.7–2.3)	2.0 (1.7–2.3)	2.1 (1.8–2.3)	1.9 (1.8–2.2)	2.1 (1.8–2.4)	0.886	0.744
sCD14 (μg/mL)	2.7 (1.9–3.6)	2.5 (1.7–3.5)	1.5 (1.3–1.9)	3.0 (2.1–3.6)	2.3 (1.6–3.5)	1.5 (1.2–1.8)	2.7 (1.8–3.6)	2.2 (1.8–3.1)	1.7 (1.2–1.9)	0.832	0.506
hsCRP (mg/L)	1.8 (0.9–3.6)	1.9 (0.9–3.9)	1.3 (0.7–3.2)	1.1 (0.7–2.9)	1.2 (0.7–2.0)	1.2 (0.6–3.4)	1.3 (0.7–2.6)	1.2 (0.7–1.9)	1.7 (0.9–2.9)	0.468	0.438
IL-6 (pg/mL)	1.7 (1.3–2.3)	1.7 (1.1–2.8)	2.5 (1.6–3.9)	1.9 (1.2–2.7)	1.9 (1.2–3.3)	2.5 (1.6–4.7)	1.7 (1.1–2.9)	1.7 (1.3–2.9)	2.5 (1.7–4.7)	0.390	0.982
TNF-α (pg/mL)	0.9 (0.6–1.2)	0.9 (0.6–1.3)	0.7 (0.6–0.9)	0.8 (0.7–1.2)	0.9 (0.7–1.2)	0.8 (0.6–1.1)	0.9 (0.8–1.2)	0.9 (0.8–1.2)	0.8 (0.7–1.2)	0.535	0.988
IP-10 (pg/mL)	81.4 (56.4–126.4)	76.2 (57.0–113.5)	77.2 (54.7–90.6)	81.2 (55.9–114.5)	86.3 (55.3–113.5)	80.3 (57.5–110.3)	100.7 (73.3–134.4)	94.1 (51.8–136.5)	81.7 (60.1–112.9)	0.553	0.195
D-Dimers (μg/L)	255.0 (200.0-372.5)	225.0 (170.0-305.0)	240.0 (185.0-305.0)	280.0 (220.0-350.0)	220.0 (170.0–370.0)	270.0 (220.0–382.5)	260.0 (205.0–367.5)	245.0 (170.0–347.5)	220.0 (190.0–295.0)	0.915	0.161
HIV-DNA log_{10} copies $/10^6$ PBMC	2.6 (2.3–3.0)	2.6 (2.4–2.9)	2.4 (2.3–2.6)	2.6 (2.4–2.9)	2.7 (2.6–2.9)	2.5 (2.2-2.7)	2.6 (2.4-2.9)	2.6 (2.3–2.9)	2.5 (2.2–2.9)	0.715	0.659
usHIV-RNA log_{10} copies $/10^6$ TBP	2.8 (2.5–3.1)	2.9 (2.6–3.3)	3.1 (2.8–3.6)	2.9 (2.7–3.3)	3.0 (2.7–3.2)	3.3 (3.0–3.6)	3.0 (2.4–3.3)	3.0 (2.5–3.5)	3.2 (2.6–3.7)	0.204	0.223

Data are expressed as median (IQR). INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DTG, dolutegravir; 3TC, lamivudine; DRVc, darunavir/cobicistat.
[a] p value for differences between baseline and week 48 among TT, DTG/3TC, and DRVc/3TC treatment arms.
[b] p value for differences between baseline and week 96 among TT, DTG/3TC, and DRVc/3TC treatment arms.

Table 3
Fixed effects on the $CD4^+/CD8^+$ ratio by ITT analysis

Evolution of the $CD4^+/CD8^+$ ratio					
Fixed effects	Estimate	95% CI	F	p	Post-hoc power (%)
Intercept	0.1024	[-0.1124–0.3173]	3.472	0.064	45.77
Time with plasma HIV-RNA <50 copies/mL (years)			1.755	0.158	41.12
1–2	0.0789	[-0.0046–0.1626]		0.064	
2–3	-0.0088	[-0.0923–0.0747]		0.835	
3–4	0.0483	[-0.0538–0.1506]		0.351	
>4	ref.				
Sex			0.057	0.811	5.65
Men	0.0147	[-0.1069–0.1364]		0.811	
Women	ref.				
Age	-0.0004	[-0.0038–0.0029]	0.063	0.801	5.72
Baseline $CD4^+/CD8^+$ ratio	0.9456	[0.8779–1.0134]	756.871	0.000	100
Time (months)	0.0193	[-0.0022–0.0409]	0.608	0.436	12.17
ART regimen			1.699	0.184	35.73
INSTI + 2 NRTIs	ref.				
DTG/3TC	-0.0409	[-0.1519–0.0699]		0.468	
DRVc/3TC	0.0630	[-0.0483–0.1744]		0.267	
ART regimen x time			1.552	0.213	32.94
INSTI + 2 NRTIs x time	ref.				
DTG/3TC x time	-0.0147	[-0.0457–0.0162]		0.350	
DRVc/3TC x time	-0.0280	[-0.0593–0.0032]		0.079	

ref., reference category; INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

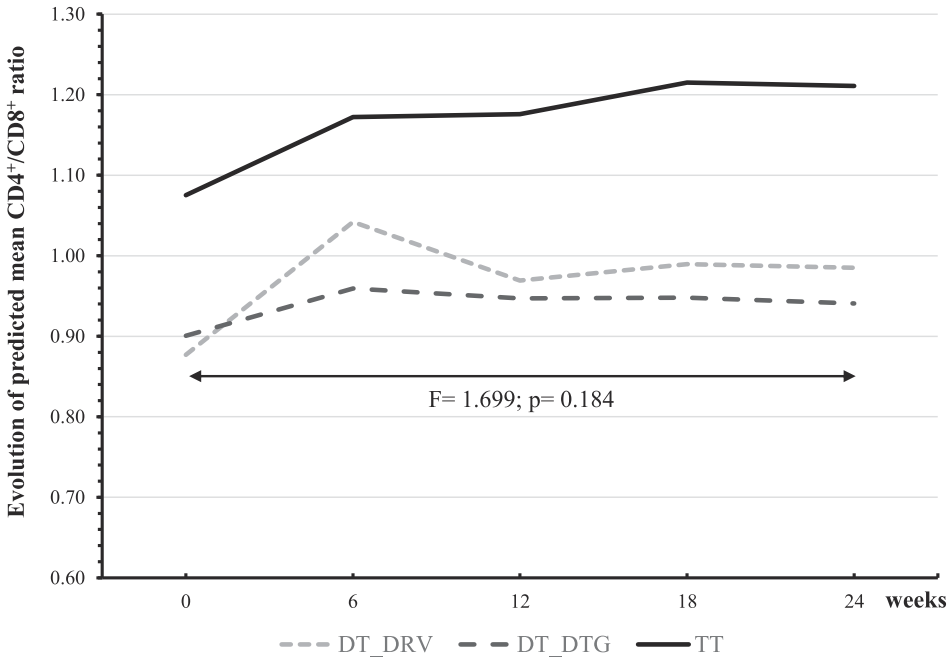

Fig. 2. Evolution of the slopes of change over time for the $CD4^+/CD8^+$ ratio in the three treatments arms by ITT analysis. The slopes are adjusted by the estimated beta coefficients of the specific confounders and covariates for the model: age, sex, baseline values, nadir $CD4^+$, time with undetectable viral load, and ART regimen × time interaction. INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

two NRTIs, with a decrease in $CD8^+CD38^+HLA\text{-}DR^+$ cells in both arms [23]. Our results showed slight fluctuations in IA, exhaustion, proliferation, senescence, and apoptosis on $CD4^+$ and $CD8^+$ T cells over 96 weeks of follow up, but without differences between the three regimens.

Regarding the plasma inflammation and coagulation markers, Vallejo et al. in a cross-study observed that sCD14 and IL-6 levels in DT participants were lower than those on TT [24]. Likewise, Belmonti et al. in a substudy of the ATLAS-M trial reported that simplification to boosted atazanavir plus 3TC did not affect inflammation markers compared with maintaining TT [16]. Finally, in the phase 3 SWORD-1&2 trial, Aboud et al. did not find a consistent pattern of change in inflammation and coagulation markers between patients on DTG/rilpivirine and those on TT after 48 weeks [17], consistent with our results, except that we detected a significant decrease in sCD14 in all treatment arms. However, Serrano-Villar et al. suggested that reducing ART to less than three drugs may lead to a less favorable long-term anti-inflammatory profile (Serrano-Villar et al., 23rd International Aids Conference 2020). In our study, we observed a small increase in IL-6 levels in all treatment groups. The mechanisms involved in the increase of IL-6 may be complex, although we have not been able to identify which

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factor or factors may be responsible for this. In any case, it is reassuring that it occurred equally in all three treatment groups and is a further reason to continue to follow these patients in the longer term.

HIV reservoir has only been evaluated in two studies by Lombardi et al., who observed, just like us, a similar decline in HIV-DNA levels with DT and TT [15,25].

Finally, we evaluated for the first time the impact of simplification to DT on HIV transcription. We detected a slight increase in usRNA, with no differences between groups. At both baseline and week 48, usRNA levels were associated with the activation, proliferation, exhaustion, and apoptosis of $CD4^+$ T cells. Based on our results, we hypothesize that usRNA may be one of the causes of the persistent phenotypic alterations of $CD4^+$ T cells.

Our study has some limitations. First, the analyses are based on PBMC, (peripheral blood mononuclear cells), which may not always reflect tissue-related processes during HIV infection. Second, the assumption made for sample size estimate may not be entirely accurate.

Since we have not detected differences in IR, IA/I, or HIV reservoir between maintaining an integrase strand transfer inhibitor–based TT or simplifying to DTG or DRVc plus 3TC in virologically suppressed HIV-infected patients, we can conclude that dual therapy is a suitable option for antiretroviral treatment simplification.

Transparency declaration

LFLC has received unrestricted research funding from Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, and ViiV Healthcare, and consultancy fees and lecture fees from Gilead Sciences, Janssen-Cilag, and ViiV Healthcare, outside the submitted work. All other authors declare no competing interests. This work was supported by Instituto de Salud Carlos III through the project (PI18/01298) to LFLC; Subprogram Miguel Servet (CP19/00159) to AGV; PFIS contracts (FI19/00304) to EMM; and Subprograma Rio Hortega (CM19/00152) to LHH; and co-financed by the European Regional Development Fund “a way to make Europe” and The SPANISH AIDS Research Network (RD16/0025/0020-ISCIII-FEDER).

Author contributions

LFLC was head of the project and AGV developed the protocol. LFLC, MD, GRV, CR, and NE contributed to the recruitment and management of patients. MTR, EMM, ASG, AIAR, LHH, and CL performed the laboratory determinations. LFLC, AGV, MTR, YMG, and JMPF analysed the data. LFLC, AGV, and MTR verified the underlying data and wrote the final manuscript. AGV and LFLC contributed equally to the work. All authors reviewed the manuscript, suggested edits, and approved the final version.

Acknowledgements

This study would not have been possible without the collaboration of all patients, medical and nursing staff, and data managers who have taken part in this project. The findings and conclusions in this paper are those of the authors and do not necessarily represent the funding agencies' official position.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.02.041.

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Please cite this article as: Trujillo-Rodríguez M et al., Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a randomized trial, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.041

Supplementary Methods

Cell surface staining for immune profile

PBMCs were isolated using Ficoll gradient centrifugation and stored with FBS and 10% DMSO in liquid nitrogen. On the day of the assay, the PBMCs were thawed and washed with PBS. Cells were then stained with different monoclonal antibodies (CD3-BV786, CD4-APC/Cy7, CD8-BV510, PD-1-PE, Ki-67-FITC, CD38-BV605, HLA-DR-FITC, CD57-APC, CD28-PE, and APC Annexin V) (BD Biosciences, USA). Viable cells were identified using 7-AAD (BD Biosciences, USA) or LIVE/DEAD fixable Yellow Dead Cell Stain (Invitrogen) for intracellular staining. Samples were acquired on a Fortessa LSR II instrument (BD Biosciences, Spain) and analyzed using FlowJo 9.3.2 software.

Quantitation of cell-associated HIV-DNA and usHIV-RNA

Total CA-DNA and usRNA were quantified by droplet digital PCR (ddPCR) using the BIO-RAD QX200 Droplet Reader. CA-DNA was extracted from PBMCs using the Blood DNA Mini Kit (Omega, Bio-Tek), and HIV RNA with the miRNeasy kit (Qiagen, Bio-Tek). The extracted genomic DNA was treated with the BsaJI restriction enzyme (New England Biolab, NEB), according to the manufacturer's protocol. Total RNA was treated with DNAse I (Takara) to eliminate DNA contamination. DNA and RNA concentrations were measured using the Qubit Assay kit (ThermoFisher Scientific) brought to a concentration of 30 ng/µL. The One-Step RT-ddPCR kit was used for RNA reverse transcription and to run the ddPCR. For both CA-DNA and usRNA, we used a mixture of two primers in the viral 5-LTR and gag regions. Forward primer 1: `5-TGTGTGCCCGTCTGTTGTGT-3' and reverse primer:`5-GCCGAGRCCTGCGTCGAGAG-3' with a specific probe: `5-FAM-

CAGTGGCGCCCGAACAGGGA-BHQ1-3', and forward primer 2: `5-CATGTTTTCAGCATTATCAGAAGGA-3', and reverse primer: `5-TGCTTGATGTCCCCCCACT-3' with a specific probe: `5-VIC-CCACCCCACAAGATTTAAACACCATGCTAA-BHQ1-3'. The RPP30 and TBP housekeeping genes were quantified to normalize the input DNA and RNA samples respectively. Copy numbers were calculated using Bio-Rad QuantaSoft software v.1.7.4.

Enzyme-linked immunosorbent assays

Plasma samples were aliquoted and stored at -80ºC until subsequent analysis. hsCRP and β2-M were determined by an immunoturbidimetric method (Cobas 701; Roche Diagnostics) and D-dimers by an automated latex-enhanced immunoassay (HemosIL D-Dimer HS 500, Instrumentation Laboratory, USA). IL-6, TNF-α, IP-10, and sCD14 were measured by immunoassay kits, following the manufacturers' instructions (Quantikine HS Human IL-6 and TNF- α, R&D Systems, USA; Human CXCL10 ELISA kit, Abcam, UK; and Human CD14 ELISA Kit; Thermo Fisher Scientific, MA, respectively).

Supplementary Tables

Table S1. Balanced randomization list for three treatment groups with four strata based on time with undetectable viral load.

Stratum 1 (1-2 years with undetectable viral load)			
1	DTG/3TC	16	INSTI-based TT
2	DTG/3TC	17	DTG/3TC
3	INSTI-based TT	18	DRVc/3TC
4	DRVc/3TC	19	DRVc/3TC
5	DTG/3TC	20	DTG/3TC
6	INSTI-based TT	21	INSTI-based TT
7	DTG/3TC	22	DTG/3TC
8	DRVc/3TC	23	INSTI-based TT
9	DRVc/3TC	24	DRVc/3TC
10	DRVc/3TC	25	INSTI-based TT
11	DRVc/3TC	26	DTG/3TC
12	INSTI-based TT	27	INSTI-based TT
13	DRVc/3TC	28	INSTI-based TT
14	INSTI-based TT	29	DRVc/3TC
15	DTG/3TC	30	DTG/3TC
Stratum 2 (2-3 years with undetectable viral load)			
1	DTG/3TC	16	DTG/3TC
2	DRVc/3TC	17	INSTI-based TT
3	INSTI-based TT	18	DRVc/3TC
4	DRVc/3TC	19	INSTI-based TT
5	INSTI-based TT	20	DRVc/3TC
6	DRVc/3TC	21	INSTI-based TT
7	DTG/3TC	22	DTG/3TC
8	DRVc/3TC	23	INSTI-based TT
9	DTG/3TC	24	INSTI-based TT
10	DTG/3TC	25	DTG/3TC
11	INSTI-based TT	26	DRVc/3TC

12	DRVc/3TC	27	DRVc/3TC
13	INSTI-based TT	28	DRVc/3TC
14	DTG/3TC	29	INSTI-based TT
15	DTG/3TC	30	DTG/3TC
Stratum 3 (3-4 years with undetectable viral load)			
1	DTG/3TC	26	DTG/3TC
2	INSTI-based TT	27	DTG/3TC
3	DRVc/3TC	28	INSTI-based TT
4	DTG/3TC	29	INSTI-based TT
5	DTG/3TC	30	INSTI-based TT
6	INSTI-based TT	31	DRVc/3TC
7	DTG/3TC	32	INSTI-based TT
8	INSTI-based TT	33	DRVc/3TC
9	DRVc/3TC	34	INSTI-based TT
10	INSTI-based TT	35	INSTI-based TT
11	DRVc/3TC	36	INSTI-based TT
12	DTG/3TC	37	INSTI-based TT
13	INSTI-based TT	38	DTG/3TC
14	INSTI-based TT	39	DRVc/3TC
15	DTG/3TC	40	DTG/3TC
16	DRVc/3TC	41	DRVc/3TC
17	DRVc/3TC	42	DRVc/3TC
18	DTG/3TC	43	DTG/3TC
19	INSTI-based TT	44	INSTI-based TT
20	DRVc/3TC	45	DRVc/3TC
21	DRVc/3TC	46	DTG/3TC
22	DTG/3TC	47	INSTI-based TT
23	DTG/3TC	48	DRVc/3TC
24	DTG/3TC	49	DRVc/3TC
25	DRVc/3TC	50	DRVc/3TC
Stratum 4 (>4 years with undetectable viral load)			
1	DRVc/3TC	26	DTG/3TC
2	DRVc/3TC	27	DRVc/3TC

3	**DRVc/3TC**	**28**	**DTG/3TC**
4	**INSTI-based TT**	**29**	**INSTI-based TT**
5	**DTG/3TC**	**30**	**DRVc/3TC**
6	**DRVc/3TC**	**31**	**DTG/3TC**
7	**INSTI-based TT**	**32**	**DRVc/3TC**
8	**DTG/3TC**	**33**	**INSTI-based TT**
9	**DTG/3TC**	**34**	**DTG/3TC**
10	**DTG/3TC**	**35**	**INSTI-based TT**
11	**DRVc/3TC**	**36**	**INSTI-based TT**
12	**DRVc/3TC**	**37**	**DTG/3TC**
13	**INSTI-based TT**	**38**	**DTG/3TC**
14	**DRVc/3TC**	**39**	**DTG/3TC**
15	**DRVc/3TC**	**40**	**DRVc/3TC**
16	**DRVc/3TC**	**41**	**DRVc/3TC**
17	**DTG/3TC**	**42**	**INSTI-based TT**
18	**INSTI-based TT**	**43**	**INSTI-based TT**
19	**DRVc/3TC**	**44**	**INSTI-based TT**
20	**DTG/3TC**	**45**	**DRVc/3TC**
21	**INSTI-based TT**	**46**	**INSTI-based TT**
22	**DTG/3TC**	**47**	**INSTI-based TT**
23	**DTG/3TC**	**48**	**DRVc/3TC**
24	**DTG/3TC**	**49**	**DTG/3TC**
25	**INSTI-based TT**	**50**	**INSTI-based TT**

Abbreviations: INSTI, integrase inhibitor; TT, triple therapy; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

Table S2. Fixed effects on the $CD4^+/CD8^+$ ratio by PP analysis.

Evolution of the $CD4^+/CD8^+$ ratio

Fixed effects	Estimate	95% CI	F	p	Post-hoc Power (%)
Intercept	0.1585	[-0.0716–0.3887]	3.329	0.070	44.18
Time with plasma HIV-RNA <50 copies/mL (years)			1.139	0.336	30.14
1–2	0.0336	[-0.0559–0.1233]		0.459	
2–3	-0.0475	[-0.1378–0.0426]		0.299	
3–4	0.0291	[-0.0835–0.1419]		0.609	
> 4	ref.				
Sex			0.018	0.893	5.20
Men	0.0085	[-0.1178–0.1349]		0.893	
Women	ref.				
Age	0.0001	[-0.0034–0.0038]	0.011	0.915	5.13
Baseline $CD4^+/CD8^+$ ratio	0.9188	[0.8398–0.9978]	528.980	0.000	100
Time (months)	0.0099	[-0.0116–0.0315]	0.150	0.699	6.72
ART regimen			1.114	0.329	24.59
INSTI + 2 NRTIs	ref.				
DTG/3TC	-0.0596	[-0.1746–0.0554]		0.309	
DRVc/3TC	0.0271	[-0.0904–0.1448]		0.650	
ART regimen x time			0.516	0.597	13.50
INSTI + 2 NRTIs x time	ref.				
DTG/3TC x time	-0.0057	[-0.0367–0.0251]		0.713	
DRVc/3TC x time	-0.0164	[-0.0484–0.0156]		0.314	

Abbreviations: ref., reference category; INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

Supplementary Figures

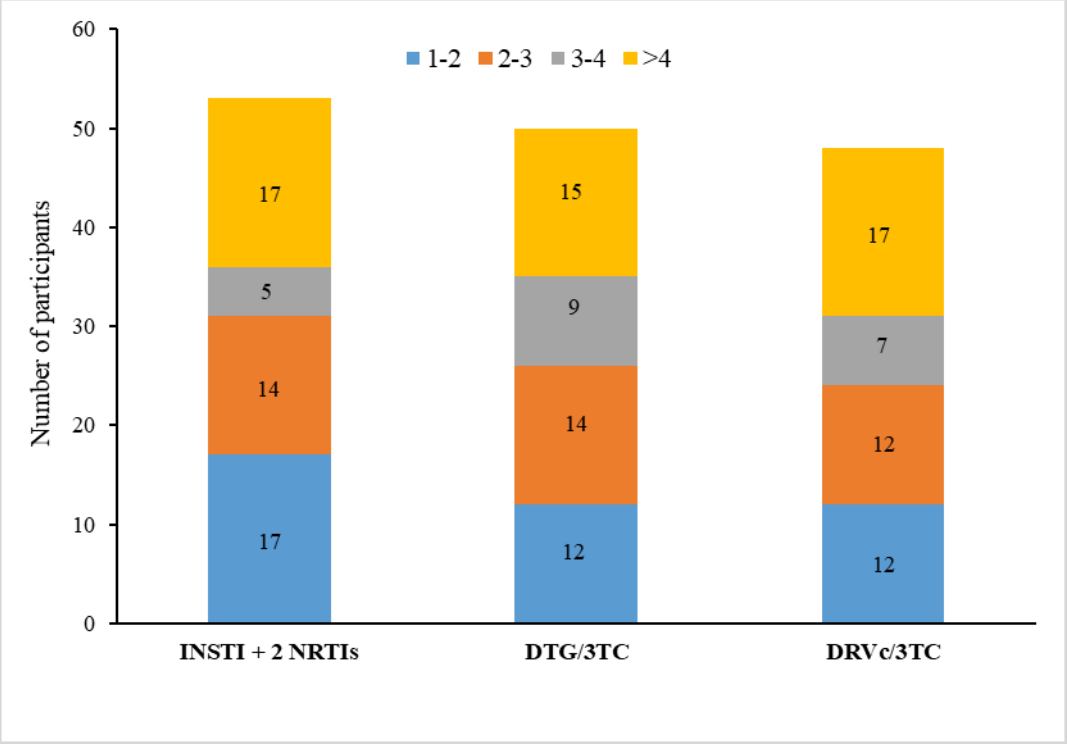

Figure S1. Number of randomized patients according to undetectable viral load time (years) by treatment arm.

Bar graphs representing the number of patients regarding the time with undetectable viral load (1-2, 2-3, 3-4, > 4 years) by treatment arm.

Abbreviations: INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

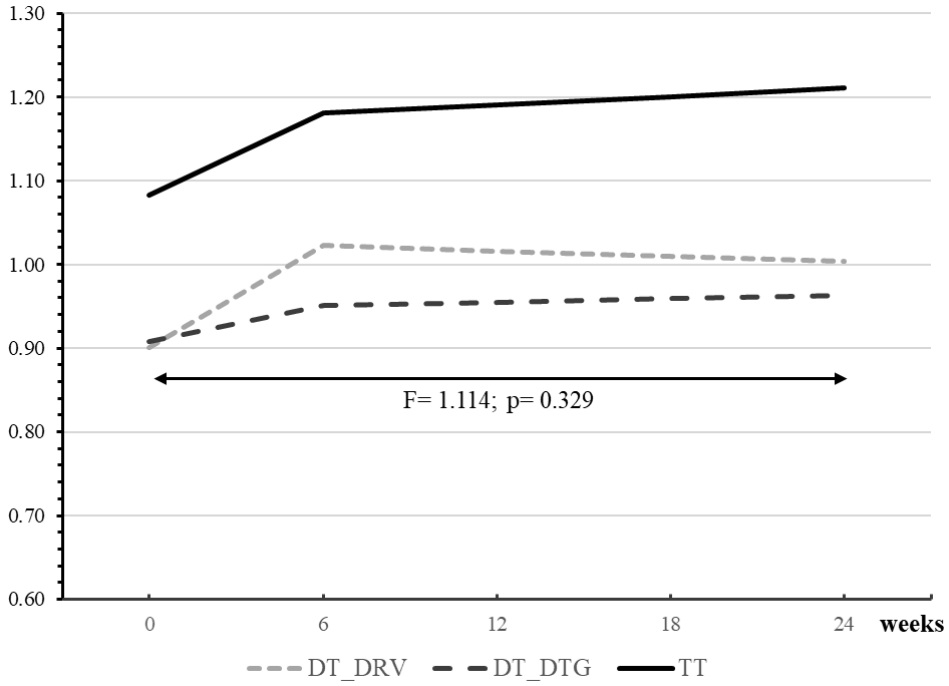

Figure S2. Evolution of the slopes of change over time for the CD4$^+$/CD8$^+$ ratio in the three treatments arms by PP analysis.

The slopes are adjusted by the estimated beta coefficients of the specific confounders and covariates for the model: age, sex, baseline values, nadir CD4$^+$, time with undetectable viral load and ART regimen x time interaction.

Abbreviations: INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

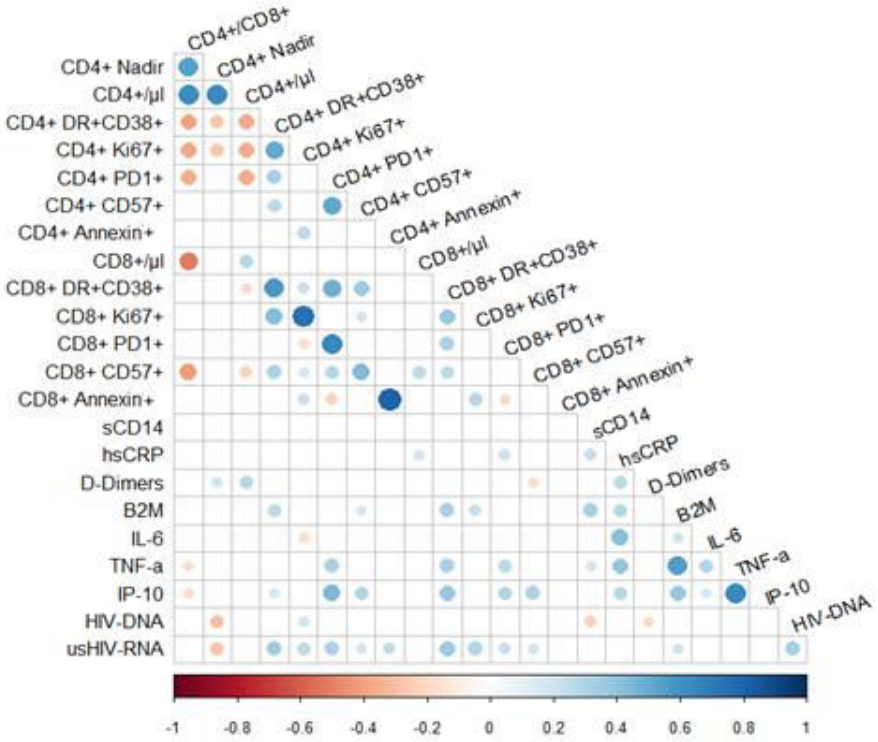

Figure S3. Correlations between immunological parameters, immune activation, proliferation, exhaustion, senescence and apoptosis markers in T cells, inflammatory and coagulation markers, and HIV reservoir at baseline.

Correlation matrix representing correlations between immunological parameters, immune activation, proliferation, exhaustion, senescence and apoptosis markers in T cells, inflammatory and coagulation markers, and HIV reservoir. Blue color represents positive correlations and red color shows negative correlations. The color intensity and the size of the circle are proportional to the R coefficient. Only statistically significant correlations are shown.

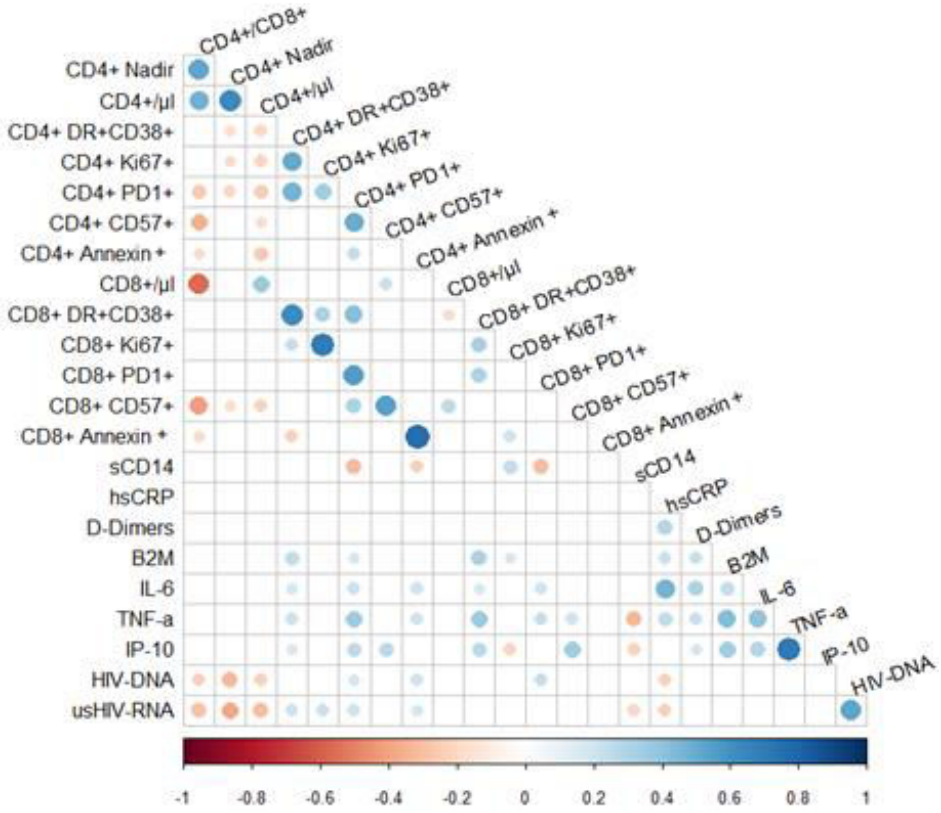

Figure S4. Correlations between immunological parameters, immune activation, proliferation, exhaustion, senescence and apoptosis markers in T cells, inflammatory and coagulation markers, and HIV reservoir at week 48.

Correlation matrix representing correlations between immunological parameters, immune activation, proliferation, exhaustion, senescence and apoptosis markers in T cells, inflammatory and coagulation markers, and HIV reservoir. Blue color represents positive correlations and red color shows negative correlations. The color intensity and the size of the circle are proportional to the R coefficient. Only statistically significant correlations are shown.

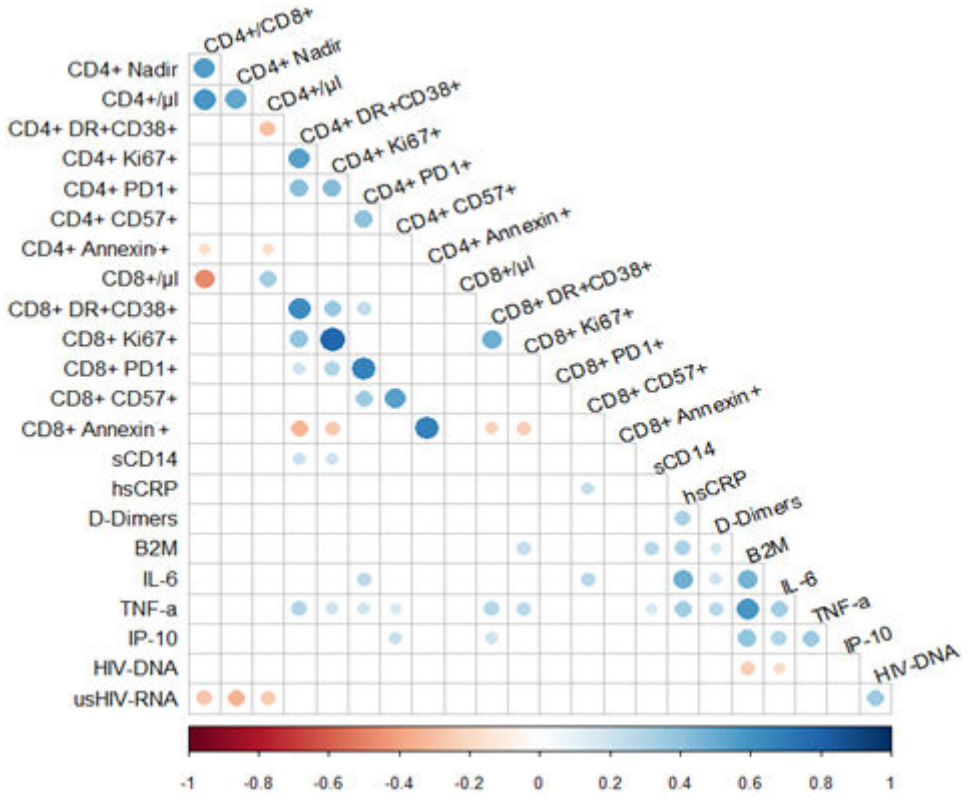

Figure S5. Correlations between immunological parameters, immune activation, proliferation, exhaustion, senescence and apoptosis markers in T cells, inflammatory and coagulation markers, and HIV reservoir at week 96.

Correlation matrix representing correlations between immunological parameters, immune activation, proliferation, exhaustion, senescence and apoptosis markers in T cells, inflammatory and coagulation markers, and HIV reservoir. Blue color represents positive correlations and red color shows negative correlations. The color intensity and the size of the circle are proportional to the R coefficient. Only statistically significant correlations are shown.

RESUMEN DE RESULTADOS

5. Resumen de Resultados

5.1 Primer artículo

López-Cortés LF, Trujillo-Rodríguez M, Báez-Palomo A, Benmarzouk-Hidalgo OJ, Dominguez-Molina B, Milanés-Guisado Y, Espinosa N, Viciana P, Gutiérrez-Valencia A. Eradication of Hepatitis C Virus (HCV) Reduces Immune Activation, Microbial Translocation, and the HIV DNA Level in HIV/HCV-Coinfected Patients. J Infect Dis. 2018 Jul 13;218(4):624-632. doi: 10.1093/infdis/jiy136. PMID: 29986086.

- Objetivo primario
  - Estudiar el cambio de la activación inmunológica, medida por la expresión de HLA-DR y CD38 en linfocitos T $CD4^+$ y $CD8^+$, tras conseguir una RVS al completar el tratamiento de 12 semanas con AAD anti-VHC y tras un mes después.

- Objetivos secundarios
  - Evaluar el cambio en la expresión de CD57 y PD-1 en linfocitos T $CD4^+$ y $CD8^+$, en la activación monocítica, los marcadores solubles de inflamación y los marcadores de translocación microbiana, así como en los niveles de ADN proviral en células mononucleares de sangre periférica (PBMCs) al completar la terapia anti-VHC y tras un mes después.
  - Analizar la evolución del recuento de linfocitos T $CD4^+$ y el cociente $CD4^+/CD8^+$ 1 y 6 meses después de finalizar el tratamiento anti-VHC.

- Materiales y métodos
  - Estudio prospectivo de pacientes coinfectados por el VIH/VHC con viremia indetectable y RVS (definida como un resultado negativo de la prueba de ARN

del VHC en plasma 3 a 6 meses después de finalizar la terapia anti-VHC) con AAD.

- Los pacientes fueron evaluados al inicio del estudio, al final del tratamiento y 1 mes después.
- Análisis de la expresión de HLA-DR/CD38, PD-1 y CD57 en linfocitos T $CD4^+$ y $CD8^+$ mediante citometría de flujo.
- La recuperación inmunológica se evaluó por el recuento y el porcentaje de linfocitos T $CD4^+$, y el cociente $CD4^+/CD8^+$.
- Se cuantificaron los niveles plasmáticos de CD14s, lipopolisacárido (LPS), IL-6, dímeros D y hsCRP mediante ensayo por inmunoadsorción ligado a enzimas (ELISA).
- El ADN ribosómico (ADNr) 16S en plasma y ADN total del VIH en PBMCs se cuantificó por PCR cuantitativa.

- Resultados
  - Al finalizar la terapia y un mes después, hubo disminuciones significativas en la expresión de HLA-DR y CD38 en los linfocitos T $CD4^+$ y $CD8^+$, así como en los niveles de ADN proviral, CD14s, LPS, ADNr 16S y dímeros D.
  - Sin embargo, la expresión de PD-1 y CD57 en linfocitos T $CD4^+$ y $CD8^+$ y los niveles de IL-6 y hsCRP no cambiaron al finalizar la terapia ni un mes después respecto a las determinaciones basales.
  - No se observó un aumento significativo del recuento de linfocitos T $CD4^+$ ni del cociente $CD4^+/CD8^+$.

- Conclusiones
  - La erradicación del VHC en pacientes coinfectados por el VIH/VHC da lugar a una disminución significativa de la activación inmunológica en linfocitos T $CD4^+$ y $CD8^+$, en monocitos, en el reservorio del VIH, en los marcadores de

translocación microbiana y en los niveles plasmáticos de dímeros D; sin embargo, ello no dio lugar a una mejoría de la recuperación inmunológica.

5.2 Segundo artículo

Trujillo-Rodríguez M, Viciana P, Rivas-Jeremías I, Álvarez-Ríos AI, Ruiz-García A, Espinosa-Ibáñez O, Arias-Santiago S, Martínez-Atienza J, Mata R, Fernández-López O, Ruiz-Mateos E, Gutiérrez-Valencia A, López-Cortés LF. Mesenchymal stromal cells in human immunodeficiency virus-infected patients with discordant immune response: Early results of a phase I/II clinical trial. Stem Cells Transl Med. 2021 Apr;10(4):534-541. doi: 10.1002/sctm.20-0213. Epub 2020 Dec 2. PMID: 33264515; PMCID: PMC7980217.

- Objetivos
  - Evaluar la seguridad y eficacia de la infusión intravenosa de 4 dosis de CMTATAd en pacientes con infección por VIH y RID.

- Materiales y métodos
  - Este estudio se planteó originalmente como un ensayo clínico de fase I/II, aleatorizado, controlado con placebo dividido en dos fases: en la fase inicial, cinco pacientes con respuesta inmune discordante recibieron la infusión de CMTATAd sin cegar. Una vez que los cinco sujetos completaron las cuatro infusiones, un Comité Independiente de Monitorización de Datos (CIMD) realizó un análisis preliminar de los datos de seguridad y eficacia. Si esta evaluación hubiera sido positiva, se continuaría con la segunda fase, reclutándose 10 sujetos adicionales asignados al azar para recibir infusiones de CMTATAd o placebo.
  - Las CMTATAd se administraron al inicio del estudio y en las semanas 4, 8 y 20.
  - La seguridad se evaluó como la incidencia de efectos adversos, incluyendo los relativos a alteraciones de parámetros de laboratorio.

- La recuperación inmunológica se evaluó por el recuento de linfocitos T $CD4^+$, el porcentaje de linfocitos T $CD4^+$ y el cociente $CD4^+/CD8^+$.
- El fenotipo de los distintos subtipos celulares (naive $CD45RA^+CD27^+$, centrales de memoria $CD45RA^-CD27^+$, efectoras de memoria $CD45RA^-CD27^-$, y terminalmente diferenciadas $CD45RA^+CD27^-$) y la activación (HLA-DR/CD38), proliferación (Ki67), agotamiento (PD-1), senescencia ($CD28^-CD57^+$) y apoptosis (Anexina V) de linfocitos T $CD4^+$ y $CD8^+$ se determinó mediante citometría de flujo.
- Se cuantificaron los niveles plasmáticos de CD14s, IL-6, TNF-α, y hsCRP mediante ELISA.
- El ADN total del VIH en PBMCs se cuantificó por PCR cuantitativa.

- Resultados
  - Después de un año de seguimiento, el CIMD recomendó la suspensión del ensayo debido a que no se observó aumento en los recuentos de linfocitos T $CD4^+$ ni en el cociente $CD4^+/CD8^+$ y tres pacientes sufrieron eventos de trombosis venosa en los brazos donde se realizaron las infusiones.
  - Tampoco hubo cambios significativos en las diferentes subpoblaciones de linfocitos T $CD4^+$ y $CD8^+$, excepto por un aumento en los $CD8^+$ efectores de memoria, ni en linfocitos NK, T reguladores, ni células dendríticas.
  - No se detectaron variaciones en la activación, proliferación, senescencia y apoptosis, ni en los marcadores de agotamiento de los linfocitos T $CD4^+$ o $CD8^+$, excepto una disminución en el porcentaje de linfocitos $CD4^+$ $PD1^+$.
  - Los niveles plasmáticos de CD14s y las diferentes proteínas inflamatorias (IL-6, TNF-α, hsCRP) no mostraron cambios significativos.
  - Asimismo, el reservorio viral, medido como el ADN-VIH asociado a PBMCs, permaneció estable durante todo el seguimiento.

- Conclusiones
  - Las infusiones de CMTATAd no son efectivas para mejorar la recuperación inmunológica ni reducir la activación inmunológica e inflamación en pacientes con infección por el VIH y RID, al menos con la pauta posológica seleccionada.

5.3 Tercer artículo

Trujillo-Rodríguez M, Muñoz-Muela E, Serna-Gallego A, Milanés-Guisado Y, Praena-Fernández JM, Álvarez-Ríos AI, Herrera-Hidalgo L, Domínguez M, Lozano C, Romero-Vazquez G, Roca C, Espinosa N, Gutiérrez-Valencia A, López-Cortés LF. Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a randomized trial. Clin Microbiol Infect. 2022 Mar 11:S1198-743X(22)00118-5. doi: 10.1016/j.cmi.2022.02.041. Epub ahead of print. PMID: 35289296.

- Objetivo primario
  - Evaluar la recuperación inmunológica tras la simplificación de una TT basada en INI a una DT (DTG o DRVc más 3TC) después de 48 semanas de tratamiento en pacientes con infección por el VIH con viremia indetectable de forma mantenida.

- Objetivos secundarios
  - Evaluar la recuperación inmunológica, la inflamación y activación inmunológica, el reservorio viral, medido como el ADN-VIH asociado a PBMCs y la transcripción viral, como el ARN asociado a PBMCs, tras la simplificación de una TT basada en INI a una DT (DTG o DRVc más 3TC) después de 48 y 96 semanas de tratamiento en pacientes con infección por el VIH con viremia indetectable de forma mantenida.

- Material y métodos
  - Ensayo clínico fase IV abierto, aleatorizado y unicéntrico.
  - Los participantes fueron randomizados en función del tiempo con viremia indetectable a continuar con TT o simplificar a DTG o DRVc más 3TC.

- La recuperación inmunológica se evaluó mediante el cociente $CD4^+/CD8^+$.
- La activación (HLA-DR/CD38), proliferación (Ki67), agotamiento (PD-1), senescencia ($CD28^-CD57^+$) y apoptosis (Anexina V) de linfocitos T $CD4^+$ y $CD8^+$ se determinó mediante citometría de flujo.
- Se determinaron los niveles plasmáticos de CD14s, IL-6, TNF-α, IP-10, dímeros D, β2 microglobulina y hsCRP mediante ELISA.
- El ADN proviral y transcritos completos de ARN viral (usRNA) asociado a células se cuantificaron mediante PCR digital en gotas (ddPCR).

- Resultados
  - Cincuenta y tres pacientes continuaron con TT, 50 pacientes cambiaron a DTG/3TC y 48 pacientes a DRVc/3TC.
  - Ocho pacientes fueron retirados del ensayo antes de completar las 48 semanas y otros seis pacientes durante el segundo año. Los motivos de exclusión fueron: fallo virológico (1), cambio de ciudad (5), pérdida de seguimiento (3), linfoma (1), retirada por decisión del paciente (3), interacción farmacológica (1).
  - El cociente $CD4^+/CD8^+$ aumentó tras 96 semanas de tratamiento, pero el incremento fue independiente del tipo de tratamiento y se relacionó solo con los valores de $CD4^+/CD8^+$ iniciales.
  - No hubo diferencias entre los brazos de tratamiento, en la activación, proliferación, agotamiento, senescencia y apoptosis de las células T $CD4^+$ y $CD8^+$ durante las 96 semanas de seguimiento.
  - Los niveles de marcadores solubles de inflamación presentaron pequeños cambios durante las 96 semanas de seguimiento sin diferencias entre los tres brazos de tratamiento.
  - Los niveles de ADN asociado a PBMCs disminuyeron tras 96 semanas de seguimiento, siendo esta disminución similar en los tres brazos de tratamiento.

En cambio, los niveles de usRNA aumentaron discretamente y por igual en los tres grupos de tratamiento tras 96 semanas.

- Conclusiones
  - La simplificación del tratamiento a DTG o DRVc más 3TC en pacientes con infección por el VIH y supresión virológica no tiene ningún impacto en la recuperación inmunológica, ni en el estado de inflamación y activación inmunológica, al igual que tampoco en el reservorio del VIH en comparación con el mantenimiento de una TT basada en INI.

DISCUSIÓN GLOBAL

6. Discusión global

Los trabajos desarrollados durante esta Tesis Doctoral se han centrado en el análisis de la activación inmunológica e inflamación crónica en tres escenarios distintos de la infección por el VIH: la coinfección por el VIH/VHC, la respuesta inmune discordante y la simplificación del TAR.

- **Coinfección VIH/VHC.**

Los resultados del primer estudio demuestran una disminución significativa en la AI/Ic y en los marcadores de translocación microbiana tras la erradicación del VHC en pacientes coinfectados por VIH/VHC. En concreto, hemos observado una reducción significativa en la expresión de los marcadores de activación CD38 y HLA-DR tanto en los linfocitos $CD4^+$ como $CD8^+$ al finalizar el tratamiento frente al VHC y un mes después. Asimismo, hemos detectado disminuciones significativas en los niveles de ADN del VIH proviral asociado a células, y marcadores plasmáticos de activación monocitaria (CD14s), de translocación microbiana y dímeros D. Por el contrario, la expresión de los marcadores de agotamiento (PD-1) y senescencia inmune (CD57) en linfocitos $CD4^+$ y $CD8^+$, así como los niveles plasmáticos de IL-6 y hsCRP, no cambiaron después de la RVS, lo que sugiere que el VHC tiene una influencia limitada o nula sobre ellos en este tipo de pacientes.

Por otro lado, debido al efecto negativo que tiene el VHC sobre la recuperación inmunológica entre los sujetos con infección por el VIH que inician TAR [120,121], esperábamos que la erradicación del VHC contribuyese a mejorar los recuentos y porcentajes de linfocitos $CD4^+$ o los cocientes $CD4^+/CD8^+$, dato que no observamos ni a corto ni a medio plazo (6 meses tras la finalización del tratamiento). Este resultado concuerda con un estudio retrospectivo de Saracino *et al*. [122], en el que la RVS no

mejoró a largo plazo el recuento de linfocitos $CD4^+$ ni el cociente $CD4^+/CD8^+$ en pacientes VIH/VHC tras la RVS.

Nuestros resultados coinciden con varios aspectos ya descritos en la literatura. Así, González *et al.* observaron una disminución en la frecuencia de células $CD8^+$ que expresaban CD38 tras 24 semanas de tratamiento y RVS con IFN más ribavirina en 14 sujetos VIH/VHC [76]. Similares conclusiones encontraron dos estudios posteriores al nuestro. Uno de ellos demostró, en una cohorte de 59 pacientes coinfectados por el VIH/VHC y 161 monoinfectados por el VHC, que el aclaramiento del VHC con AAD disminuye el porcentaje de linfocitos T activados durante la terapia y hasta un año posterior a la RVS [123]. Asimismo, Auma *et al.* encontraron que, después del inicio de la terapia anti-VHC, la coexpresión de $CD38^+HLA\text{-}DR^+$ en linfocitos $CD4^+$ y $CD8^+$ disminuyó a las 12 y 36 semanas en pacientes coinfectados por el VIH/VHC (n=40) [124]. Otro estudio publicado recientemente mostró que, tras 36 semanas después de alcanzar una RVS, los pacientes VIH/VHC (n=33) con cirrosis avanzada experimentaron una disminución en distintos marcadores plasmáticos inflamatorios y relacionados con la activación y agotamiento del sistema inmunológico (PD-1 soluble, PD-L1, CXCL10, CXCL8, IL12p70, IL-10 y TGFb), marcadores de enfermedad hepática (medición de la rigidez del hígado, gradiente de presión venosa hepática y transaminasas, entre otros) y la expresión de genes estimulados por IFN, principalmente codificantes de quimioquinas y proteínas antivirales [125]. En contraposición, otros autores no han detectado diferencias en la AI/Ic tras el tratamiento anti-VHC. Najafi Fard *et al.* observaron que la terapia aumentó significativamente el potencial de las células T colaboradoras y T citotóxicas para producir IFN-γ, IL-17 e IL-22 en pacientes VHC (n=18) y VIH/VHC (n=17), pero no tuvo ninguna influencia significativa en el estado de activación de las células T $CD4^+$ y $CD8^+$ [126]. Farcomeni *et al.* sugirieron que, aunque los pacientes lograron la erradicación del VHC con AAD, el estado de activación inmunológica de linfocitos $CD4^+$ y $CD8^+$, tanto en pacientes monoinfectados por el VHC

(n=10) como en pacientes coinfectados por el VIH/VHC (n=10), permanece elevado 36 semanas después de finalizar la terapia anti-VHC [127]. Sin embargo, una limitación de estos últimos estudios mencionados es el tamaño de muestra relativamente pequeño en cada grupo de pacientes.

En conclusión, en nuestro estudio hemos observado la influencia que tiene la erradicación del VHC sobre la disminución de la AI/Ic en pacientes coinfectados por el VIH/VHC, evaluada tanto a corto como a largo plazo.

- **Respuesta inmune discordante.**

Otro de los aspectos del presente trabajo se ha basado en el estudio de pacientes con infección por el VIH y respuesta inmune discordante. Diversos factores hacen que el estudio de este tipo de pacientes requiera especial interés. En estos pacientes, se ha descrito una mayor activación de linfocitos T $CD4^+$ [89–91] y de marcadores inflamatorios (IL-6, IP-10, hsCRP) [128–131], al igual que un mayor riesgo de morbi-mortalidad asociada a neoplasias, ECV y eventos SIDA [93,94]. Se han llevado a cabo diversas estrategias para incrementar los recuentos de linfocitos $CD4^+$ y reducir la AI/Ic en estos sujetos, pero todas sin beneficios claros.

Zhang *et al*. [97] llevaron a cabo el primer estudio realizado con CMM en sujetos infectados por el VIH y RID. Transfundieron tres dosis de CMM de cordón umbilical, a una dosis de $0{,}5\times10^6$ células por kilogramo de peso corporal, a siete pacientes. No observaron efectos adversos a corto plazo ni repunte de la carga viral, y seis de los siete pacientes mostraron un fuerte aumento en los recuentos de linfocitos $CD4^+$ de más del 50% en comparación con los valores iniciales. Las CMM del cordón umbilical incrementaron los recuentos de las subpoblaciones $CD4^+$ naives y centrales de memoria, mientras que la subpoblación efectora de memoria y terminalmente diferenciada disminuyeron gradualmente. Además, detectaron una disminución

significativa en los porcentajes de linfocitos $CD8^+$ activados, una disminución de la expresión de PD-1 en las células $CD4^+$ y $CD8^+$ y una reducción significativa en los niveles plasmáticos de marcadores y citoquinas inflamatorias (hsCRP, IL-6, IFN-α/γ, TNF-α, IL-1ra, IL-12, IL-9) y quimioquinas (MIP-1β, IP-10, IL-8, MCP-1, RANTES).

En nuestro trabajo, los individuos con infección por el VIH y RID recibieron cuatro dosis (1×10^6/kg) de CMM de tejido adiposo, pero no observamos cambios en los recuentos de linfocitos $CD4^+$, el porcentaje o el cociente $CD4^+/CD8^+$, ni tampoco en las diferentes subpoblaciones celulares ni en los marcadores de activación, agotamiento, apoptosis y senescencia. Del mismo modo, las infusiones no tuvieron efectos sobre los niveles plasmáticos de CD14s, IL-6, TNF-α y hsCRP. Solo observamos una disminución significativa de la expresión de PD-1 en los linfocitos $CD4^+$ en cuatro individuos (5,2 vs 1,6; p= 0,043), pero su significado aislado es incierto, y este cambio no influyó en la recuperación de los linfocitos $CD4^+$. Por otro lado, tres participantes sufrieron eventos de trombosis venosa en los brazos donde se realizaron las infusiones. Los episodios de trombosis venosa observados podrían tener una causalidad multifactorial. Por un lado, los participantes del estudio pueden tener un mayor riesgo de trombosis venosa debido a los antecedentes de uso de drogas por vía parenteral (2/5), un mayor riesgo de recurrencia en casos de episodios trombóticos previos [132] o se podría haber debido a una alta velocidad de infusión registrada en algunas ocasiones. Además, se ha observado que las CMM expresan factor tisular y tienen actividad procoagulante, siendo las CMM de tejido adiposo las más procoagulantes y las que expresan más factor tisular que las CMM derivadas de otras fuentes [133].

Las principales diferencias con el estudio de Zhang *et al.* son el origen de las CMM y las dosis administradas. Aunque las propiedades inmunorreguladoras de las CMM no están relacionadas con su origen [134,135], no tenemos certeza de si el origen celular diferente podría ser el responsable de los resultados discrepantes. Las CMM adultas

ofrecen numerosas ventajas, pero se ha observado que el rendimiento, el potencial proliferativo y la plasticidad de las CMM disminuyen progresivamente con la edad avanzada del donante en comparación con las células madre embrionarias [136].

Nuestro estudio tiene varias limitaciones. Entre las más importantes cabe destacar que un mayor número de pacientes y un grupo control, previsto en la segunda fase del ensayo, nos habría permitido valorar mejor las fluctuaciones que presentaban algunos pacientes en varios parámetros inmunológicos. Sin embargo, la segunda fase del ensayo no se pudo llevar a cabo porque, después de un año de seguimiento, un comité independiente recomendó la suspensión del estudio al no observarse un aumento en los recuentos de linfocitos $CD4^+$ ni en el cociente $CD4^+/CD8^+$ y la presencia de efectos adversos.

Recientemente, el mismo grupo de investigadores (Wang *et al.* [137]) ha publicado los resultados de un ensayo clínico llevado a cabo para evaluar la seguridad y la eficacia de la infusión de CMM de cordón umbilical en 72 pacientes con infección por VIH y RID. Los pacientes fueron asignados a recibir una dosis alta ($1,5\times10^6$/kg), baja ($0,5\times10^6$/kg) de CMM de cordón umbilical o placebo. Estos autores demostraron que ambas dosis fueron seguras y bien toleradas, y no ocurrieron eventos graves relacionados con las transfusiones. A pesar de que observaron tendencias significativas de aumento de los recuentos de células $CD4^+$ y los cocientes $CD4^+/CD8^+$ en los grupos de dosis alta y baja respecto al valor basal después de 96 semanas de tratamiento, no hubo una diferencia significativa en dichos parámetros entre los tres grupos, lo que indica que las CMM de cordón umbilical en ambas dosis administradas no mostraron una eficacia significativa para la recuperación inmunológica en pacientes con infección por VIH y RID. Además, no se evaluaron marcadores relacionados con la AI/Ic.

Por lo tanto, el uso de CMM parece no ser una estrategia terapéutica útil para incrementar la recuperación inmunológica y disminuir la elevada AI/Ic en los pacientes con infección por el VIH y RID, por lo que sería necesario seguir profundizando en la búsqueda de terapias efectivas para esta población especial de pacientes con infección por el VIH.

- **Simplificación del tratamiento antirretroviral.**

El último trabajo de esta Tesis Doctoral ha abordado el análisis de la AI/Ic en pacientes con infección por el VIH y viremia indetectable de forma mantenida tras la simplificación de una triple terapia a una doble terapia.

La idea de simplificación del TAR surgió con el objetivo de mejorar la toxicidad del tratamiento y la adherencia al mismo, al tratarse la infección por el VIH de una enfermedad crónica en la que los pacientes recibirán tratamiento de duración indefinida. A lo largo de estos últimos años se han llevado a cabo varios estudios de simplificación a DT, basados fundamentalmente en un INI o un IP potenciado combinados con 3TC que han mostrado una eficacia virológica similar a la TT. En cambio, las consecuencias inmunológicas que puede ocasionar la simplificación del TAR a estas DT no están del todo claras. Varios autores han evaluado el cociente $CD4^+/CD8^+$ tras la simplificación a distintos regímenes de DT, al considerarse un marcador que se correlaciona con la AI/Ic [138]. Quirós-Roldán *et al.* observaron que el cociente $CD4^+/CD8^+$ aumentó después de la simplificación a atazanavir (ATV) potenciado o DRV más 3TC, pero no después de cambiar a lopinavir potenciado más 3TC o IP potenciado más un INI [139]. Otros autores notificaron una mejora en el cociente $CD4^+/CD8^+$ en los pacientes que simplificaron a DT (basada en DRV potenciado o DTG) y que partían de un cociente basal más bajo, sin diferencias según el tipo de régimen dual [140]. En cambio, Mussini *et al.* compararon la evolución del cociente $CD4^+/CD8^+$ en pacientes que simplificaron a DT (principalmente a IP potenciados más 3TC) con aquellos que cambiaron de un régimen

de TT a otro, detectando que el aumento fue más significativo en los que cambiaron a otro régimen de TT que a DT [141].

En nuestro trabajo, los pacientes con infección por el VIH con carga viral plasmática indetectable durante al menos un año y más de 6 meses en TT basada en INI fueron randomizados a continuar con TT o simplificar a DTG o DRVc más 3TC. Hemos observado un aumento similar del cociente $CD4^+/CD8^+$ en todos los grupos de tratamiento después de 48 y 96 semanas, siendo este aumento independiente del régimen de tratamiento y sólo relacionado con los valores basales del cociente $CD4^+/CD8^+$. Además, nuestros resultados mostraron ligeras fluctuaciones en los marcadores de activación inmunológica, agotamiento, proliferación, senescencia y apoptosis de linfocitos $CD4^+$ y $CD8^+$ durante 96 semanas de seguimiento, siendo estas fluctuaciones similares en los tres regímenes de tratamiento. Hasta la fecha, solo Maggiolo *et al.* han explorado la activación inmunológica después de simplificar a DRV potenciado más rilpivirina frente a mantener un IP potenciado más dos ITIAN, encontrando una disminución en los linfocitos $CD8^+CD38^+HLA\text{-}DR^+$ en ambos brazos, siendo ligeramente superior en el brazo de DT (3,4% vs 5,2%) [142].

Respecto a los marcadores de inflamación, nuestros resultados muestran una disminución en las concentraciones plasmáticas de CD14s y un pequeño aumento de IL-6 tras 96 semanas de seguimiento, sin diferencias entre los brazos de tratamiento. El resto de marcadores inflamatorios (β2M, hsCRP, dímeros D, TNF-α e IP-10) mostraron pequeñas fluctuaciones sin diferencias entre los grupos de tratamiento. Vallejo *et al.* en un estudio transversal encontraron que los niveles plasmáticos de IL-6 y CD14s fueron más bajos en los pacientes a las 48 semanas después del cambio a DT (mayoritariamente DTG más rilpivirina) en comparación con los encontrados en los pacientes que continuaron con TT [143]. Por el contrario, en un subestudio del ensayo ATLAS-M (estudio en 266 pacientes que demostró la no inferioridad de ATV/r más 3TC

vs. TT en pacientes virológicamente suprimidos durante al menos 6 meses) observaron que la simplificación a ATV más 3TC no afectó a los marcadores de inflamación en comparación con el mantenimiento de una TT [144]. Finalmente, en los ensayos de fase 3 SWORD-1 & 2, Aboud *et al.* no encontraron un patrón consistente de cambio en los marcadores de inflamación y coagulación entre los pacientes que simplificaron a DTG más rilpivirina y los que continuaban con TT después de 48 semanas [145]. No obstante, Serrano-Villar *et al.* han sugerido que reducir el TAR a menos de tres fármacos puede conducir a un perfil antiinflamatorio menos favorable a largo plazo, con aumentos en los niveles plasmáticos de hsCRP, dímeros-D e IL-6 [Serrano-Villar *et al.*, 23ª Conferencia Internacional sobre el SIDA 2020], aunque las conclusiones están basadas en un modelo matemático.

Otra de las aportaciones de nuestro estudio fue el análisis del reservorio del VIH y la actividad transcripcional. El ADN proviral disminuyó a lo largo del seguimiento sin diferencias entre los grupos de tratamiento. Por el contrario, el ARN asociado a PBMCs aumentó discretamente durante el segundo año por igual en los tres grupos, hecho para el que no tenemos una explicación clara. En este sentido, cabe destacar que no observamos relación entre los aumentos de ARN asociado a PBMCs y los de IL-6. Sólo Lombardi *et al.* han evaluado, en dos estudios [146,147], el impacto en los niveles de ADN proviral de la simplificación a DT versus mantener TT. En el primero de ellos, 104 participantes simplificaron a ATV/r más 3TC y 97 continuaron con TT basada en ATV/r más dos ITIAN [146], mientras que en el segundo, 40 pacientes simplificaron a DTG más 3TC y otros 40 mantuvieron la TT basada en dos ITIAN junto con un IP, un ITINAN o un INI [147]. Estos autores detectaron que después de 48 semanas de tratamiento, la DT resultó en una disminución similar en los niveles de ADN proviral en comparación con el mantenimiento de una TT.

No hay referencias en la literatura que hayan evaluado la transcripción del VIH en la simplificación a DT.

Por tanto, con estos resultados se puede afirmar que la simplificación a DT (DTG o DRVc más 3TC) no tiene ningún impacto en la recuperación inmunológica, AI/Ic, el reservorio o actividad transcripcional del VIH en comparación con el mantenimiento de TT basado en INI.

Un aspecto a destacar de los estudios aquí presentados es la influencia de la recuperación inmunológica en la AI/Ic. En los tres estudios, hemos observado una correlación negativa entre la recuperación inmunológica, evaluada por el recuento o porcentaje de $CD4^+$ y el cociente $CD4^+/CD8^+$, con distintos marcadores celulares y solubles de la AI/Ic, lo que indica que un peor estado del sistema inmunológico se asocia con un mayor nivel de AI/Ic. Sin embargo, una limitación de los tres trabajos es la ausencia de análisis de asociación entre marcadores de la AI/Ic con la aparición de ENOS, dado que no ha transcurrido tiempo suficiente para realizar un seguimiento a largo plazo.

Aun así, el conjunto de estos trabajos contribuye a la comprensión del estado de activación inmunológica e inflamación crónica en estos pacientes. Sin embargo, ante las múltiples causas que la generan, la existencia de diversos marcadores y la falta de consenso sobre cuáles son los ideales para monitorizarla y su utilización en la práctica clínica, es una necesidad y objetivo primordial seguir profundizando en la caracterización de este proceso para poder reducir sus consecuencias e identificar posibles dianas terapéuticas que contribuyan a mejorar la esperanza y calidad de vida de las personas con infección por el VIH.

CONCLUSIONES

7. Conclusiones

1. La erradicación de la infección por VHC en pacientes coinfectados por el VIH/VHC da lugar a disminuciones significativas no solo en la activación inmunológica de los linfocitos $CD4^+$ y $CD8^+$, sino también en los niveles de ADN proviral, marcadores de translocación microbiana, CD14s y dímeros D.

2. Las infusiones de CMTATAd no han demostrado ser efectivas para mejorar la recuperación inmunológica, ni han logrado reducir la activación inmunológica o los niveles de marcadores inflamatorios en pacientes con infección por el VIH y respuesta inmune discordante, al menos con la pauta posológica seleccionada.

3. La simplificación del tratamiento a DT con DTG o DRVc más 3TC en pacientes con infección por el VIH y supresión virológica no tiene ningún impacto en la recuperación inmunológica, ni en la activación inmunológica e inflamación crónica, al igual que tampoco en el reservorio del VIH en comparación con el mantenimiento de una TT basada en INI.

4. La recuperación inmunológica (medida por el recuento o porcentaje de $CD4^+$ y el cociente $CD4^+/CD8^+$), se asocia inversamente con la activación inmunológica e inflamación.

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ABREVIATURAS

9. Abreviaturas

3TC: Lamivudina

AAD: Antivirales de acción directa

ADNr 16S: ADN ribosómico 16S

AI/Ic: Activación inmunológica e inflamación crónica

ATV: Atazanavir

AZT: Zidovudina o azidotimidina

CCL: Ligando de quimioquina con motivo C-C (del inglés *C-C Motif Chemokine Ligand*)

CCR5: Receptor de quimiocinas CC tipo 5 (del inglés *C-C chemokine receptor type 5*)

CD: Clúster de diferenciación (del inglés *Cluster of Differentiation)*

CD14s: CD14 soluble

CD163s: CD163 soluble

CDC: Centros para el Control y Prevención de Enfermedades (del inglés *Centers for Disease Control and Prevention*)

CE: Controladores de élite

CIMD: Comité Independiente de Monitorización de Datos

CMM: Células madre mesenquimales

CMTATAd: Células mesenquimales troncales alogénicas de tejido adiposo

CTLA-4: Antígeno-4 asociado al linfocito T citotóxico (del inglés *Cytotoxic T-Lymphocyte Antigen 4)*

ddPCR: PCR digital en gotas (del inglés *droplet digital PCR*)

DRVc: Darunavir/cobicistat

DT: Doble terapia

DTG: Dolutegravir

ECV: Enfermedad cardiovascular

ELISA: Ensayo por inmunoadsorción ligado a enzimas (del inglés *Enzyme-Linked ImmunoSorbent Assay)*

ENOS: Eventos no definitorios de SIDA

HLA: Antígeno leucocitario humano (del inglés *Human leukocyte antigen*)

hsCRP: Proteína C reactiva ultrasensible (del inglés *high-sensitivity C-reactive protein*)

IFN: interferon

IL: Interleuquina

INI: Inhibidor de la integrasa

IP: Inhibidor de la proteasa

IP-10: Proteína 10 inducida por interferón gamma (del inglés *Interferon gamma-induced protein 10*)

ITIAN: Inhibidor de la transcriptasa inversa análogo de nucleósido o nucleótido

ITINAN: Inhibidor de la transcriptasa inversa no análogos de nucleótidos

LPS: Lipopolisacárido

ONUSIDA: Programa Conjunto de las Naciones Unidas sobre el VIH/Sida

PBMCs: Células mononucleares de sangre periférica (del inglés *Peripheral blood mononuclear cells*)

PD-1: Proteína de muerte celular programada 1 (del inglés *Programmed cell death protein 1*)

PD-L1: Ligando 1 de muerte programada (del inglés *Programmed Death-ligand 1*)

RID: Respuesta inmune discordante

RVS: Respuesta virológica sostenida

SIDA: síndrome de inmunodeficiencia adquirida

TAR: Tratamiento antirretroviral

TARGA: Tratamiento antirretroviral de gran actividad

TIM-3: Inmunoglobulina de células T y el dominio 3 de mucina (del inglés *T cell immunoglobulin and mucin domain-3*)

TT: Triple terapia

TNF-α: Factor de necrosis tumoral alfa (del inglés *tumor necrosis factor α*)

usRNA: ARN no empalmado (del inglés *unspliced RNA*)

VHC: Virus de la hepatitis C

VIH: Virus de inmunodeficiencia humana

VIS: Virus de la inmunodeficiencia de los simios

FINANCIACIÓN

10. Financiación

Esta Tesis Doctoral ha sido desarrollada en el contexto de los siguientes proyectos de investigación financiados en convocatorias públicas competitivas:

Red Española de Investigación en SIDA (RD16/0025/0020-ISCIII-FEDER). Investigador principal: Luis Fernando López Cortés.

Proyecto del Fondo de Investigación Sanitaria (PI15/01041). Seguridad y eficacia del tratamiento con células mesenquimales troncales alogénicas de tejido adiposo en pacientes con infección. Investigador principal: Luis Fernando López Cortés.

Proyecto del Fondo de Investigación Sanitaria (PI18/01298). Evaluación de la recuperación inmunológica del mantenimiento de una terapia antirretrovial triple versus simplificación a biterapia en pacientes con infección por el VIH con virema indetectable. Investigador principal: Luis Fernando López Cortés.

Red Andaluza de Diseño y Traducción de Terapias Avanzadas; Consejería de Salud y Familias de la Junta de Andalucía (201600073585-tra). Investigador principal: Luis Fernando López Cortés.

ANEXOS

11. Anexos

11.1 Otros trabajados desarrollados en el periodo de tesis

a. Publicaciones

1. Pérez-Gómez A, Gasca-Capote C, Vitallé J, Ostos FJ, Serna-Gallego A, **Trujillo-Rodríguez M**, Muñoz-Muela E, Giráldez-Pérez T, Praena-Segovia J, Navarro-Amuedo MD, Paniagua-García M, García-Gutiérrez M, Aguilar-Guisado M, Rivas-Jeremías I, Jiménez-León MR, Bachiller S, Fernández-Villar A, Pérez-González A, Gutiérrez-Valencia A, Rafii-El-Idrissi Benhnia M, Weiskopf D, Sette A, López-Cortés LF, Poveda E, Ruiz-Mateos E; Virgen del Rocío Hospital COVID-19 and COHVID-GS Working Teams. Deciphering the quality of SARS-CoV-2 specific T-cell response associated with disease severity, immune memory and heterologous response. Clin Transl Med. 2022 Apr;12(4):e802. doi: 10.1002/ctm2.802. PMID: 35415890; PMCID: PMC9005926.

2. Codina H, Vieitez I, Gutierrez-Valencia A, Skouridou V, Martínez C, Patiño L, Botero-Gallego M, **Trujillo-Rodríguez M**, Serna-Gallego A, Muñoz-Muela E, Bobillo MM, Pérez A, Cabrera-Alvar JJ, Crespo M, O'Sullivan CK, Ruiz-Mateos E, Poveda E. Elevated Anti-SARS-CoV-2 Antibodies and IL-6, IL-8, MIP-1β, Early Predictors of Severe COVID-19. Microorganisms. 2021 Oct 29;9(11):2259. doi: 10.3390/microorganisms9112259. PMID: 34835384; PMCID: PMC8624589.

3. Pérez-Gómez A, Vitallé J, Gasca-Capote C, Gutierrez-Valencia A, **Trujillo-Rodriguez M**, Serna-Gallego A, Muñoz-Muela E, Jiménez-Leon MLR, Rafii-El-Idrissi Benhnia M, Rivas-Jeremias I, Sotomayor C, Roca-Oporto C, Espinosa N, Infante-Domínguez C, Crespo-Rivas JC, Fernández-Villar A, Pérez-González A, López-Cortés LF, Poveda E, Ruiz-Mateos E; Virgen del Rocío Hospital COVID-19 Working Team. Dendritic cell deficiencies persist seven months after SARS-

CoV-2 infection. Cell Mol Immunol. 2021 Jul 21:1–12. doi: 10.1038/s41423-021-00728-2. Epub ahead of print. PMID: 34290398; PMCID: PMC8294321.

4. De Pablo-Bernal RS, Jimenez-Leon MR, Tarancon-Diez L, Gutierrez-Valencia A, Serna-Gallego A, **Trujillo-Rodriguez M**, Alvarez-Rios AI, Milanes-Guisado Y, Espinosa N, Roca-Oporto C, Viciana P, Lopez-Cortes LF, Ruiz-Mateos E. Modulation of Monocyte Activation and Function during Direct Antiviral Agent Treatment in Patients Coinfected with HIV and Hepatitis C Virus. Antimicrob Agents Chemother. 2020 Aug 20;64(9):e00773-20. doi: 10.1128/AAC.00773-20. PMID: 32571815; PMCID: PMC7449156.

5. Milanés-Guisado Y, Gutiérrez-Valencia A, Muñoz-Pichardo JM, Rivero A, **Trujillo-Rodriguez M**, Ruiz-Mateos E, Espinosa N, Roca-Oporto C, Viciana P, López-Cortés LF. Is immune recovery different depending on the use of integrase strand transfer inhibitor-, non-nucleoside reverse transcriptase- or boosted protease inhibitor-based regimens in antiretroviral-naive HIV-infected patients? J Antimicrob Chemother. 2020 Jan 1;75(1):200-207. doi: 10.1093/jac/dkz421. PMID: 31617904.

6. Milanés-Guisado Y, Gutiérrez-Valencia A, **Trujillo-Rodríguez M**, Espinosa N, Viciana P, López-Cortés LF. Absolute CD4+ T cell count overstate immune recovery assessed by CD4+/CD8+ ratio in HIV-infected patients on treatment. PLoS One. 2018 Oct 22;13(10):e0205777. doi: 10.1371/journal.pone.0205777. PMID: 30346965; PMCID: PMC6197681.

7. Gutierrez-Valencia A, **Trujillo-Rodriguez M**, Fernandez-Magdaleno T, Espinosa N, Viciana P, López-Cortés LF. Darunavir/cobicistat showing similar effectiveness as darunavir/ritonavir monotherapy despite lower trough

concentrations. J Int AIDS Soc. 2018 Feb;21(2):e25072. doi: 10.1002/jia2.25072. PMID: 29430854; PMCID: PMC5808101.

8. Gutierrez-Valencia A, Benmarzouk-Hidalgo OJ, Rivas-Jeremías I, Espinosa N, **Trujillo-Rodríguez M**, Fernandez-Magdaleno T, Viciana P, López-Cortés LF. Viral Kinetics in Semen With Different Antiretroviral Families in Treatment-Naive Human Immunodeficiency Virus-Infected Patients: A Randomized Trial. Clin Infect Dis. 2017 Aug 15;65(4):551-556. doi: 10.1093/cid/cix358. PMID: 28449051.

b. Comunicaciones a congresos

1. Póster
   - Título: T-CELL RESPONSE QUALITY TO ACUTE SARS-CoV-2 INFECTION, IMMUNE MEMORY AND HCoV.
   - Autores: Alberto Pérez-Gómez, Carmen Gasca-Capote, Joana Vitallé, Francisco Ostos, **María Trujillo-Rodríguez**, Cristina Roca-Oporto, Nuria Espinosa, Alicia Gutiérrez-Valencia, Mohammed Rafii-El-Idrissi Benhnia, Daniela Weiskopf, Shane Crotty, Alessandro Sette, Luis F. López-Cortes, Eva Poveda, Ezequiel Ruiz-Mateos.
   - Congreso: Conference on Retroviruses and Opportunistic Infections 2022.
   - Lugar y fecha: 12 de Febrero a 16 de Febrero de 2022. Online.

2. Póster
   - Título: Anti-CD4 role in immunological non-responders HIV-infected patients: Cause of $CD4^+$ T cell depletion?
   - Autores: Esperanza Muñoz-Muela, **María Trujillo-Rodríguez**, Ana Serna-Gallego, Ezequiel Ruiz-Mateos, Nuria Espinosa, Critina Roca-Oporto,

Mohamed Rafii-El-Idrissi Benhnia, Luis F. López-Cortés, and Alicia Gutiérrez-Valencia.

- Congreso: XII CONGRESO NACIONAL GeSIDA
- Lugar y fecha: 29 de Noviembre a 02 de Diciembre de 2021. Málaga.

3. Comunicación oral
    - Título: Consecuencias inmunológicas de la simplificación a biterapia (DTG/3TC o DRVc/3TC) en pacientes con triple terapia basada en inhibidores de la integrasa.
    - Autores: **María Trujillo Rodríguez**; Esperanza Muñoz Muela; Ana Serna Gallego; Sofía Rubio Ponce; Montserrat Domínguez; Cristina Roca; Nuria Espinosa; Pompeyo Viciana; Alicia Gutiérrez Valencia; Luis Fernando López Cortés.
    - Congreso: XI CONGRESO NACIONAL GeSIDA.
    - Lugar y fecha: 10 a 13 de Diciembre de 2019. Toledo.

4. Comunicación oral
    - Título: Consecuencias inmunológicas de la simplificación a biterapia (DTG/3TC o DRVc/3TC) en pacientes con triple terapia basada en inhibidores de la integrasa.
    - Autores: **María Trujillo Rodríguez**; Esperanza Muñoz Muela; Ana Serna Gallego; Sofía Rubio Ponce; Montserrat Domínguez; Cristina Roca; Nuria Espinosa; Pompeyo Viciana; Alicia Gutiérrez Valencia; Luis Fernando López Cortés.
    - Congreso: XXI Congreso de la Sociedad Andaluza de Enfermedades Infecciosas.
    - Lugar y fecha: 21 a 23 de Noviembre de 2019. Sevilla.

5. Comunicación oral
    - Título: Seguridad y eficacia del tratamiento con células mesenquimales troncales adultas alogénicas de tejido adiposo expandidas en pacientes con VIH y respuesta inmunológica discordante.
    - Autores: **María Trujillo Rodríguez**; Tamara Fernández Magdaleno; Inmaculada Rivas Jeremías; Sofía Rubio Ponce; Antonio Ruiz García; Ezequiel Ruiz Mateos; Cristina Roca; Nuria Espinosa; Pompeyo Viciana; Alicia Gutiérrez Valencia; Luis Fernando López Cortés.
    - Congreso: XX Congreso de la Sociedad Andaluza de Enfermedades Infecciosas.
    - Lugar y fecha: 29 de Noviembre a 01 de Diciembre de 2018. Jerez de la Frontera.

6. Póster
    - Título: Direct antiviral agents treatment on HIV co-infected patients reduces monocyte polyfunctionality.
    - Autores: Rebeca De Pablo Bernal; Maria Reyes Jimenez León; Laura Tarancón Diez; Alicia Gutiérrez Valencia; Tamara Fernández Magdaleno; Beatriz Dominguez Molina; **María Trujillo Rodríguez**; Cristina Roca; Nuria Espinosa; Pompeyo Viciana; Luis López Cortés; Ezequiel Ruiz Mateos.
    - Congreso: X CONGRESO NACIONAL GeSIDA.
    - Lugar y fecha: 06 a 09 de Noviembre de 2018. Madrid.

7. Póster
    - Título: Seguridad y eficacia del tratamiento con células mesenquimales troncales adultas alogénicas de tejido adiposo expandidas en pacientes con VIH y respuesta inmunológica discordante.

- Autores: **María Trujillo Rodríguez**; Tamara Fernández Magdaleno; Inmaculada Rivas Jeremías; Sofía Rubio Ponce; Antonio Ruiz García; Ezequiel Ruiz Mateos; Cristina Roca; Nuria Espinosa; Pompeyo Viciana; Alicia Gutiérrez Valencia; Luis Fernando López Cortés.
- Congreso: X CONGRESO NACIONAL GeSIDA.
- Lugar y fecha: 06 a 09 de Noviembre de 2018. Madrid.

8. Póster
    - Título: Relación entre la translocación microbiana y la activación inmune persistente en pacientes con infección por el VIH y respuesta inmune discordante.
    - Autores: **María Trujillo Rodríguez**; Alicia Báez Palomo; Maria José Castro Pérez; Tamara Fernández Magdaleno; Nuria Espinosa; Pompeyo Viciana; Alicia Gutiérrez Valencia; Luis Fernando López Cortés.
    - Congreso: XIX Congreso de la Sociedad Andaluza de Enfermedades Infecciosas.
    - Lugar y fecha: 14 a 16 de Diciembre de 2017. Granada.

9. Póster
    - Título: Relación entre la translocación microbiana y la activación inmune persistente en pacientes con infección por el VIH y respuesta inmune discordante.
    - Autores: **María Trujillo Rodríguez**; Alicia Báez Palomo; Maria José Castro Pérez; Tamara Fernández Magdaleno; Nuria Espinosa; Pompeyo Viciana; Alicia Gutiérrez Valencia; Luis Fernando López Cortés.
    - Congreso: IX Congreso Nacional GeSIDA.
    - Lugar y fecha: 28 de Noviembre a 01 de Diciembre de 2017. Vigo.

10. Póster

- Título: Influencia de las Cmin de Darunavir en la eficacia de la monoterapia con DRVcobi, comparado con DRVrtv.
- Autores: **Trujillo Rodríguez M**; Gutiérrez-Valencia A; Fernández-Magdaleno T; Espinosa N; Viciana P; López-Cortés LF.
- Congreso: XVIII Congreso de la Sociedad Andaluza de Enfermedades Infecciosas.
- Lugar y fecha: 24 a 26 de Noviembre de 2016. Córdoba.