UNIVERSITAT DE BARCELONA

Profilaxis de la enfermedad injerto contra receptor con altas dosis de ciclofosfamida post-trasplante: resultados en el trasplante de donante no emparentado, efecto de la dosis de progenitores hematopoyéticos y análisis de biomarcadores de daño endotelial

Alexandra Pedraza Navarrete

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ÍNDICE

1. ABREVIATURAS Y ACRÓNIMOS

Ang-2: Angiopoetina-2

AloTPH: Trasplante alogénico de progenitores hematopoyéticos

ATG: Globulina anti-timocitica

CE: Células endoteliales

CIBMTR: Centro para la Investigación Internacional de Trasplante de Medula Ósea

CMSP: Células madre de SP

CPA: Células presentadoras de antígenos

CsA: Ciclosporina

Cy: Ciclofosfamida

DAMPs: *Danger associated molecular pattern*

DE: Donante emparentado

DnE: Donante no emparentado

dsDNA: DNA de doble cadena

EASIX: Índice de Estrés y Activación Endotelial

EBMT: Sociedad Europea de Trasplante de Sangre y Medula Ósea

EICR: Enfermedad injerto contra receptor

HLA: Antígenos leucocitarios humanos

HCB: Hospital Clínic de Barcelona

ICAM-1: Molécula de adhesión intercelular (*Intercellular Adhesion Molecule*)

ICN: Inhibidor de calcineurina

IFN-γ: Interferón gama

IL: Interleuquina

IS: Inmunosupresores

MAGIC: Consorcio Internacional de EICR Aguda del Monte Sinaí

MAT-AT: Microangiopatía asociada con el trasplante

MCH: Complejo mayor de histocompatibiliad

mHA: Complejo menor de histocompatibilidad

MMF: Micofenolato de mofetil

MO: Médula ósea

MRT: Mortalidad relacionada con el trasplante

MTX: Metotrexato

NET: Trampas extracelulares de neutrófilos

NK: *Natural killers*

PAMPs: *Pathogen-associated molecular patterns*

PTCy: Altas dosis ciclofosfamida post-trasplante (*Post-Transplant Cyclophosphamide*)

REDMO: Registro Español de Donantes de Médula Ósea

REG3α: Derivado de islotes regeneradores 3-α

RIC: Acondicionamiento de intensidad reducida (*Reduced Intensity Conditioning)*

SCU: Sangre de cordón umbilical

SG: Supervivencia global

SLP: Supervivencia libre de progresión

SOS: Síndrome de obstrucción sinusoidal

SP: Sangre periférica

ST2: Supresor de tumorigenicidad 2

Tac: Tacrólimus

TCR: Receptor de los linfocitos T

TM: Trombomodulina

TNF-α: Factor de necrosis tumoral α

TNFR1: Receptor del TNF-α en las células endoteliales

VCAM-1: Molécula de adhesión de células vasculares (*Vascular Cell Adhesion Molecule*)

VWF: Factor de Von Willebrand

2. ENUMERACION DE LOS ARTICULOS DE LA TESIS

Tesis en formato de compendio de publicaciones.

La presente Tesis Doctoral consta de un objetivo principal y tres objetivos específicos y tres artículos:

Alexandra Pedraza, Sofia Jorge, María Suárez-Lledó, Arturo Pereira, Gonzalo Gutiérrez-García, Francesc Fernández-Avilés, Laura Rosiñol, Noemí Llobet, Teresa Solano, Álvaro Urbano-Ispízua, Montserrat Rovira, Carmen Martínez. High-dose Cyclophosphamide and Tacrolimus as GVHD Prophylaxis for Matched and Mismatched Unrelated Donor Transplantation. Transplant Cell Ther. 2021; 27(7):619.e1-619.e8. Impac factor 3.2. Q1. Trasplante.

Alexandra Pedraza, María Queralt Salas, Luís Gerardo Rodríguez-Lobato, Paola Charry, María Suárez-Lledo, Nuria Martínez-Cibrian, Ariadna Doménech, María Teresa Solano, Jordi Arcarons, Noemí de Llobet, Laura Rosiñol, Gonzalo Gutiérrez-García, Francesc Fernández-Avilés, Álvaro Urbano-Ispízua, Montserrat Rovira, Carmen Martínez. Effect of CD34+ cell dose on the outcomes of allogeneic stem cell transplantation with post-transplant cyclophosphamide. Transplant Cell Ther. 2023; 29 (3):181.e1-181.e10. Impac factor 3.2. Q1. Trasplante.

Alexandra Pedraza, María Queralt Salas, Luis Gerardo Rodríguez-Lobato, Silvia Escribano-Serrat, María Suárez-Lledo, Nuria Martínez-Cebrian, María Teresa Solano, Jordi Arcarons, Laura Rosiñol, Gonzalo Gutiérrez-García, Francesc Fernández-Avilés, Ana Belén Moreno-Castaño, Patricia Molina, Marc Pino, Enric Carreras, Maribel Díaz-Ricart, Montserrat Rovira, Marta Palomo, Carmen Martínez. EASIX score correlates with endothelial dysfunction biomarkers and predicts risk of acute graft-versus-host disease after allogeneic transplantation. Transplant Cell Ther. 2023; Transplant Cell Ther. 2024; 30(2):187.e1 -187.e12. Impac factor 3.2. Q1. Trasplante.

3. INTRODUCCION

3.1. TRASPLANTE ALOGÉNICO DE PROGENITORES HEMATOPOYÉTICOS

El trasplante alogénico de progenitores hematopoyéticos (aloTPH) es un procedimiento de alta complejidad indicado en el tratamiento de un gran número de enfermedades hematológicas malignas y no malignas.

En sus orígenes, el aloTPH se asoció a una elevada mortalidad derivada de las complicaciones inmunológicas e infecciosas. Gracias a las mejoras en la prevención y el tratamiento de dichas complicaciones, la mortalidad relacionada con el trasplante (MRT) y la supervivencia han mejorado de forma significativa a lo largo de los años. El aloTPH se ha convertido así en un procedimiento estándar de manera que más de un millón de pacientes han recibido un trasplante en los últimos 60 años para el tratamiento de su hemopatía [1,2]. Los avances más destacables en el aloTPH han sido, entre otros, la incorporación de los donantes no emparentados (DnE) como donantes convencionales [3 ,4], la mejora en la tipificación del HLA, la introducción de los acondicionamientos de intensidad reducida (RIC) [2, 5-8], la sustitución de la médula ósea (MO) por la sangre periférica (SP) como fuente de progenitores hematopoyéticos [9, 10] y las mejoras en la prevención, diagnóstico y tratamiento de las infecciones. [11-13]

A pesar de todos estos progresos, un número importante de pacientes siguen padeciendo la enfermedad injerto contra receptor (EICR), por lo que sigue siendo esta complicación una causa muy relevante de mortalidad y morbilidad tras el trasplante y, por lo tanto, uno de los aspectos que precisan de mejora.

3.2. ENFERMEDAD INJERTO CONTRA RECEPTOR

La EICR es la principal complicación inmunológica del aloTPH. La EICR se produce cuando las células T inmunocompetentes del donante (el injerto) reconocen al receptor (huésped) como extraño. La respuesta inmunitaria resultante activa las células T del donante que adquieren capacidad citolítica y atacan al receptor con el fin

de eliminar las células extrañas portadoras de antígenos específicos. Las dos presentaciones clínicas principales son la **EICR aguda (EICRa) y la EICR crónica (EICRc)**.

Las primeras nociones de esta alorreactividad se observaron en estudios preclínicos con ratones, donde la transferencia de células inmunitarias de un huésped a otro causaba la muerte temprana de los receptores, al generarles un cuadro clínico consistente en diarrea, lesiones cutáneas y adelgazamiento extremo patológico [14, 15].

Estos trabajos también demostraron que el proceso de reconocimiento dependía de las diferencias genéticas entre el donante y el receptor [16], y sobre todo de la disparidad antigénica [17]. Por otro lado, los investigadores observaron que estas diferencias otorgaban al trasplante más potencia antileucémica, promoviendo el efecto injerto contra tumor [18, 19].

3.2.1. Fisiopatología de la EICR aguda y crónica

Los estudios experimentales en modelos murinos describieron tres requisitos básicos que aún siguen siendo la base conceptual para poder entender la fisiopatología de la **EICR aguda** [20,21]:

1. El injerto debe contener células inmunocompetentes del donante.
2. El receptor debe contener aloantígenos que puedan ser reconocidos como extraños por las células inmunocompetentes del injerto.
3. El receptor debe ser incapaz de generar una respuesta inmune apropiada contra el injerto.

Actualmente se acepta que la EICRa se desarrolla en tres fases secuenciales [22] (Figura 1).

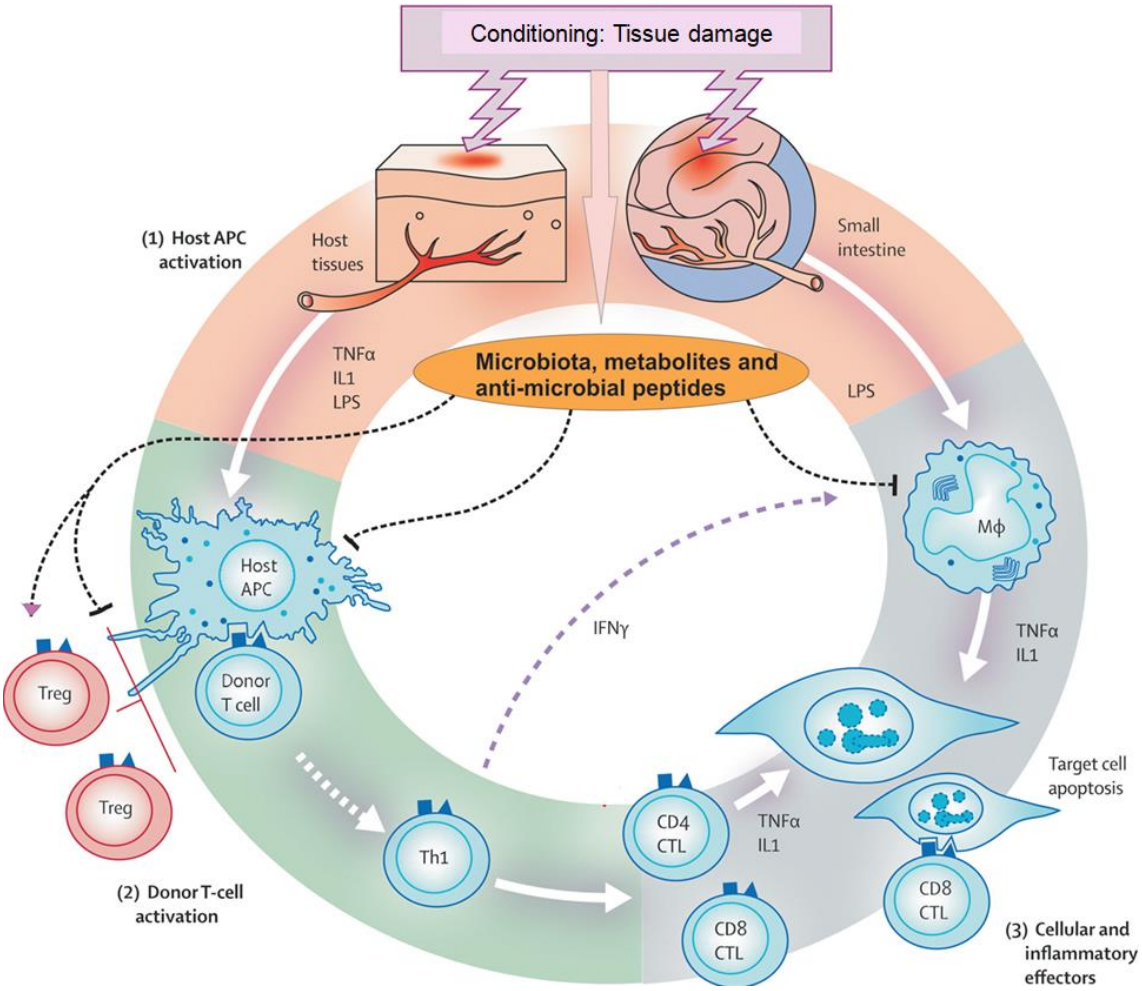

Figura 1. Fases del desarrollo de la EICRa [23].

La **primera fase** se caracteriza por la activación de las células presentadoras de antígenos (CPA) tales como las células dendríticas, los macrófagos y los linfocitos B (Figura 1). El acondicionamiento con quimioterapia/radioterapia y los procesos inflamatorios intercurrentes derivados de las infecciones producen un daño tisular importante que conduce a la liberación de múltiples antígenos, moléculas señales de peligro (*Danger Associated Molecular Pattern,* DAMPs), patrones moleculares asociados a patógenos (*Pathogen-associated molecular patterns,* PAMPs), principalmente procedentes del crecimiento bacteriano, y citoquinas proinflamatorias como el factor de necrosis tumoral α (TNF-α), la interleucina-1 (IL-1) y la IL-6 [24]. Esta exposición molecular y antigénica activa a las CPA y marca el inicio de la respuesta

inmune primaria y secundaria [25, 26]. El daño tisular a nivel gastrointestinal parece ser especialmente relevante en este proceso. Diversos trabajos experimentales han documentado la activación de las células dendríticas a partir de su interacción con PAMPs derivados de la microbiota del colon en pacientes con EICRa [27-29].

En la **segunda fase** (Figura 2), una vez activadas, las células dendríticas presentan los antígenos al receptor de los linfocitos T alorreactivos del donante provocando su activación, proliferación, diferenciación y migración [30-32]. Para que la activación linfocitaria se lleve a cabo es necesaria una segunda señal (señal co-estimuladora) como la unión entre CD40 y su ligando (CD40L) o entre CD28 y su ligando (CD80/86) [33]. En esta etapa se desencadena una cascada de señales intracelulares que inducen a la transcripción de genes para muchas proteínas, incluidas las citoquinas y sus receptores. Los linfocitos T CD4+ producen grandes cantidades de IL-2, interferón gama (IFN-γ) y TNF-α que amplifican la respuesta inmunitaria reclutando otras células inmunitarias y facilitan la presentación antigénica aumentando la expresión de moléculas de adhesión y antígenos de histocompatibilidad [22, 34, 35].

La **tercera y última fase** de la EICRa se define por la lesión tisular provocada por los efectores celulares e inflamatorios en los órganos diana (Figura 1 y 2). Una vez establecida la respuesta inflamatoria, la liberación de citoquinas promueve la migración de los linfocitos a los órganos linfoides secundarios y a los tejidos afectados [35]. Los linfocitos T citotoxicos (CD8+) y las células *natural killers* (NK) son los efectores celulares del daño tisular. En la EICR hepática, estas células utilizan preferentemente mecanismos de lisis celular mediados por la unión proteica entre FAS y FAS ligando, dado que los hepatocitos expresan grandes cantidades de FAS, mientras que en la EICR cutánea e intestinal, la muerte celular esta mediada por las vías de perforina-granzima [36-38]. La IL1, IL6, IFN-γ y el TNF-α son los efectores inflamatorios capaces de inducir apoptosis y necrosis tisular directamente, al mismo tiempo que amplifican la respuesta antigénica al activar más células dendríticas y reclutar más células proinflamatorias [37-40].

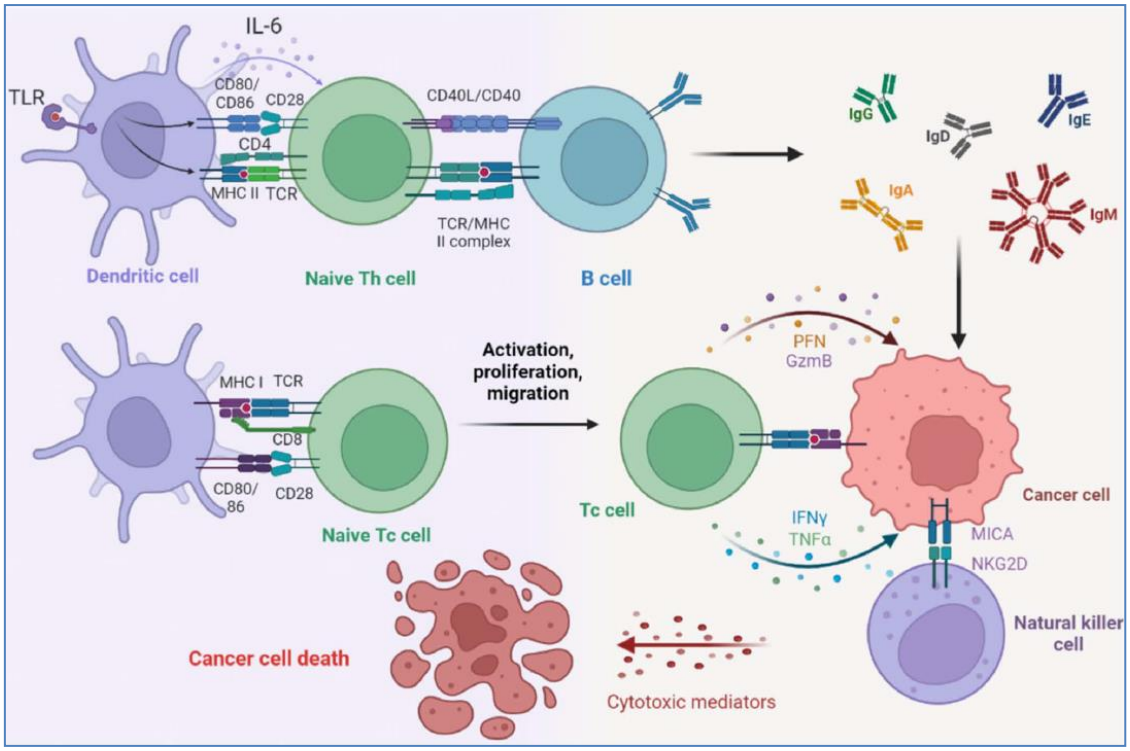

Figura 2. Activación de linfocito T y reclutamiento células proinflamatorias en la EICRa [35].

En la **EICRc**, la afectación orgánica es más heterogénea y las manifestaciones de la enfermedad son más variables que en la EICRa. La presentación clínica más frecuente se caracteriza por la inflamación y la fibrosis que afecta a uno o varios órganos de los sistemas tegumentario, musculoesquelético, cardiovascular, respiratorio, gastrointestinal, reproductor y adrenal [41].

Según estudios preclínicos en modelos murinos, la fisiopatología de la EICRc se puede entender conceptualmente en tres fases: inflamación tisular temprana, fase 1 (Figura 3), seguida de inflamación crónica, lesión tímica e inmunidad regulada por linfocitos B y T, fase 2 (Figura 4), y finalmente, reparación tisular con fibrosis, fase 3 [42] (Figura 5).

La **primera fase** de la fisiopatología de la EICRc comprende los procesos descritos en la fase inicial e intermedia de la EICRa, donde la activación de las células inmunitarias (CPA y linfocitos T) a partir de su interacción con múltiples moléculas y antígenos liberados tras el daño tisular producido por el régimen de

acondicionamiento, las infecciones o la misma EICRa, tiene un papel protagonista [42, 43].

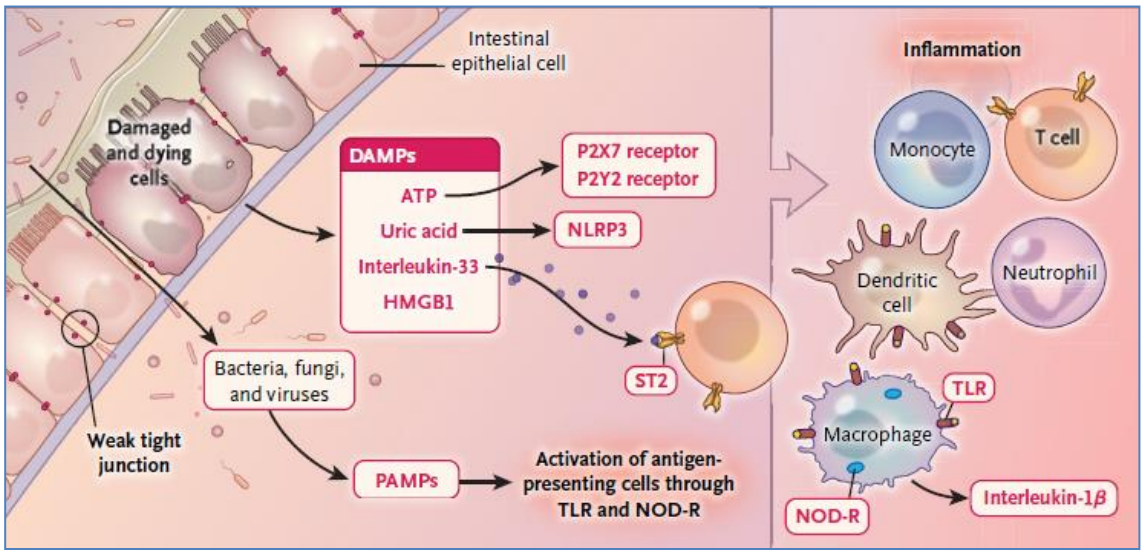

Figura 3. Fase inicial de la fisiopatología de la EICRc [24].

La **segunda fase** se caracteriza por la lesión tímica causada por los efectos tóxicos del régimen de acondicionamiento, la profilaxis farmacológica, la alorreactividad antigénica y el depósito de inmunoglobulina [24, 44, 45] (Figura 4). El daño tisular compromete a las células dendríticas y epiteliales del timo y conduce, por una parte, a la perdida de la generación de linfocitos T reguladores, y por otra, a la perdida de la regulación de los procesos de selección positiva y negativa que son esenciales para establecer la tolerancia central [46, 47]. La expresión de antígenos restringidos permite la liberación de linfocitos T CD4+ autorreactivos al torrente sanguíneo [48, 49]. La escasez de mecanismos periféricos que habitualmente controlan la proliferación de células inmunitarias autolesivas facilita el desarrollo de la EICRc [50, 51].

Una vez activados, los linfocitos T alorreactivos se expanden y polarizan hacia la proliferación de células colaboradoras de tipo 1, 2 y 17 (Th1, Th2 y Th17). Los linfocitos T CD4+ autorreactivos y alorreactivos que han escapado a la regulación inmunitaria, especialmente los de tipo Th17, producen IL-17A que se encarga de mantener en el

tiempo la inflamación tisular [52, 53]. También se ha observado una producción importante de IL21 que conduce a la formación de centros germinales, donde los linfocitos B sufren hipermutaciones somáticas y producen anticuerpos con cambios en el isotipo de las inmunoglobulinas, procesos especialmente relacionados con las manifestaciones cutáneas, pulmonares y hepáticas crónicas [54, 55].

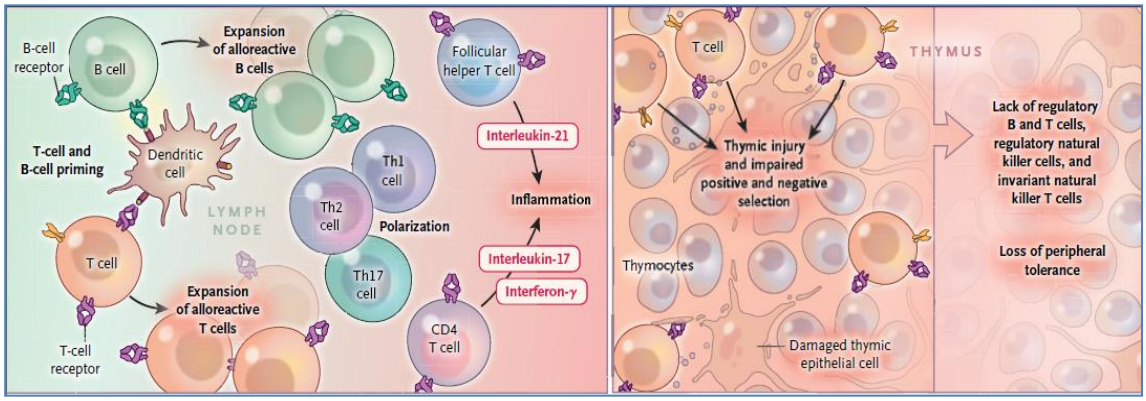

Figura 4. Fase intermedia de la fisiopatología de la EICRc [24].

En la **tercera fase** tiene lugar una reparación tisular aberrante promovida por los macrófagos activados que producen factor de crecimiento transformante β (TGF-β) y factor de crecimiento derivado de plaquetas α (PDGF-α) y terminan activando a los fibroblastos [24] (Figure 5). En respuesta a estos y otros mediadores profibróticos, los fibroblastos activados producen colágeno de matriz extracelular y biglicano, que entrecruzan el colágeno y aumentan la rigidez del tejido dando lugar al fenotipo esclerótico [56]. El daño orgánico y la fibrosis tisular también se ven facilitados por el depósito patológico de las inmunoglobulinas anómalas producidas por los linfocitos B en respuesta al factor activador de las células B (BAFF) [57, 58].

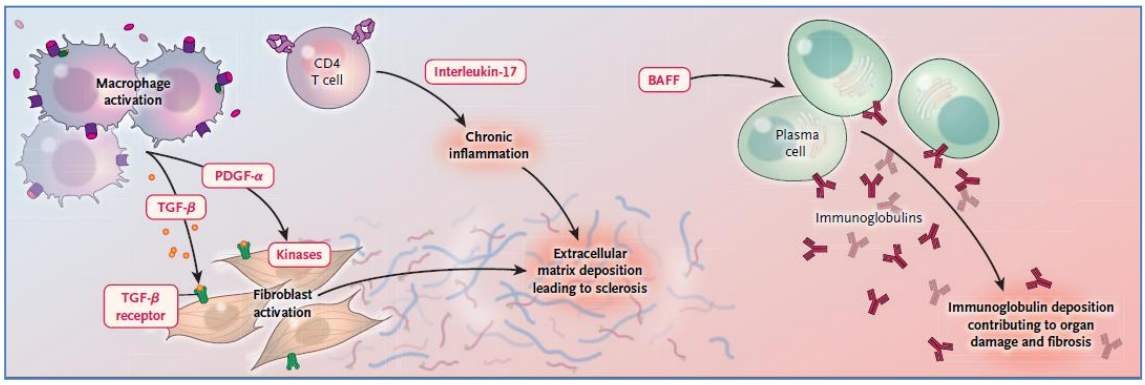

Figura 5. Última fase de la fisiopatología de la EICRc [24].

3.2.2. Compatibilidad HLA y elección del donante

La alorreactividad en el aloTPH está determinada principalmente por las diferencias que existen entre las moléculas del **complejo mayor de histocompatibilidad (MCH)** del donante y del receptor. Así, los linfocitos T activados del donante reconocerán como extraños en el receptor, los antígenos de histocompatibilidad que sean dispares a los suyos.

El MCH está constituido por una serie de glicoproteínas altamente polimórficas, también llamadas **antígenos leucocitarios humanos (HLA)**, codificadas en más de 200 genes ubicados en el brazo corto del cromosoma 6 [35] (Figura 6).

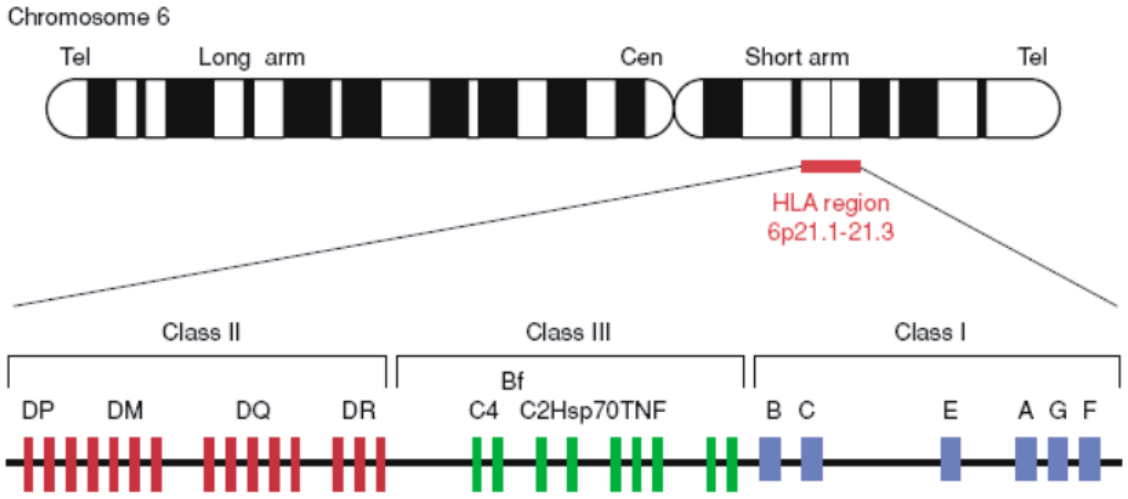

Figura 6. Representación esquemática del HLA en el cromosoma 6 [59].

Cada combinación particular de genes en los alelos presentes en un cromosoma individual se conoce como haplotipo HLA. Los haplotipos HLA se heredan de manera codominante, es decir un haplotipo completo de la madre y otro del padre, de manera que hay 4 combinaciones posibles [60]. La expresión de los genes es simultánea por lo que se maximiza el número de moléculas HLA capaces de intervenir en la respuesta inmune. La variabilidad resultante es muy útil cuando el organismo se enfrenta a infecciones por patógenos extraños, pero supone una dificultad, si se quiere encontrar dos individuos que compartan identidad HLA.

Estas proteínas transmembrana forman una cavidad a partir de dos cadenas pesadas donde se ubica un antígeno particular [35]. Según el patrón de alta afinidad, este complejo se une a un receptor especifico de los linfocitos T (TCR) para iniciar la activación celular. La interacción entre HLA y TCR, además de ser esencial en la defensa contra microorganismos, es fundamental para la diferenciación de los linfocitos T en el timo, ya que dirige los procesos de selección positiva y negativa que dan lugar a la autotolerancia [61]. Tras el aloTPH, este mecanismo de diferenciación permite que nuevos linfocitos T surjan a partir de los progenitores hematopoyéticos del donante dando lugar a la reconstitución inmune [62].

Existen **dos clases principales de HLA** [60]:

- Las **moléculas HLA de clase I**, codificadas por los genes ubicados en los locus A, B y C. Están presentes en todas las células nucleadas del organismo y se encargan de la presentación antigénica a los linfocitos T citotóxicos (CD8+).

- Las **moléculas HLA clase II**, codificadas por los genes ubicados en los locus DR, DQ y DP. Se encuentran solo en la superficie de las CPA. Contribuyen a la presentación de antígenos a los linfocitos T colaboradores (CD4+).

Hay una tercera clase de moléculas HLA, menos polimórficas, en la que se reconocen diferentes tipos de proteína que también pueden generar una respuesta aloinmune, entre ellas se encuentra diferentes componentes del complemento y citoquinas inflamatorias como el TNF [35].

Aunque las condiciones de identidad sean óptimas entre el donante y el receptor, la expresión de otra variedad de péptidos endógenos localizados en diferentes genes que conforman el **complejo menor de histocompatibilidad (mHA)** puede provocar una respuesta alorreactiva de los linfocitos T del donante y, en consecuencia, la EICR en los pacientes trasplantados con progenitores de donantes emparentados (DE) o DnE HLA idénticos [63, 64].

El descubrimiento de los HLA cambió drásticamente los resultados del aloTPH [65, 66]. La elección del donante óptimo se basa en la identidad antigénica, considerando que la identidad completa se tiene cuando el donante y el receptor comparten los locus HLA-A, B, C y DR (identidad 8/8) o HLA-A, B, C, DR, DP y DQ (identidad 10/10). Bajo esta premisa y conociendo la baja disparidad genética entre familiares y su proximidad física, los hermanos HLA idénticos se convirtieron en la mejor opción en el momento de elegir el donante ideal. Sin embargo, según las leyes de herencia mendeliana con carácter codominante, la posibilidad de que un individuo tenga un hermano HLA idéntico no supera el 30% [67].

La baja probabilidad de tener un DE HLA idéntico, motivó la creación de los registros internacionales de donantes de médula ósea. Actualmente, estas organizaciones tienen información referente al HLA de más de 36 millones de donantes voluntarios [68]. Estos datos han permitido realizar trasplantes de DnE con identidad completa o parcial (diferencia en uno de los locus del HLA [7/8]). Más recientemente, la disparidad en el 50% de los locus (haploidentidad) se ha incorporado al grupo de donantes opcionales (trasplante haploidéntico familiar). La sangre de cordón umbilical (SCU) también surgió como una fuente alternativa de progenitores hematopoyéticos, fácilmente extraíble tras el parto y sin riesgos para las madres ni los recién nacidos, pero con una compatibilidad en el HLA menos probable (6/6) [69].

Según el Registro Español de Donantes de Medula Ósea (REDMO), la probabilidad de encontrar un DnE HLA 10/10 o 9/10 en el primer mes de búsqueda está alrededor del 60% y en el segundo mes del 90% [70] (Figura 7). Los pacientes que no puedan esperar ese tiempo para recibir el aloTPH o estén en el 10% restante deberán optar por donantes alternativos [71].

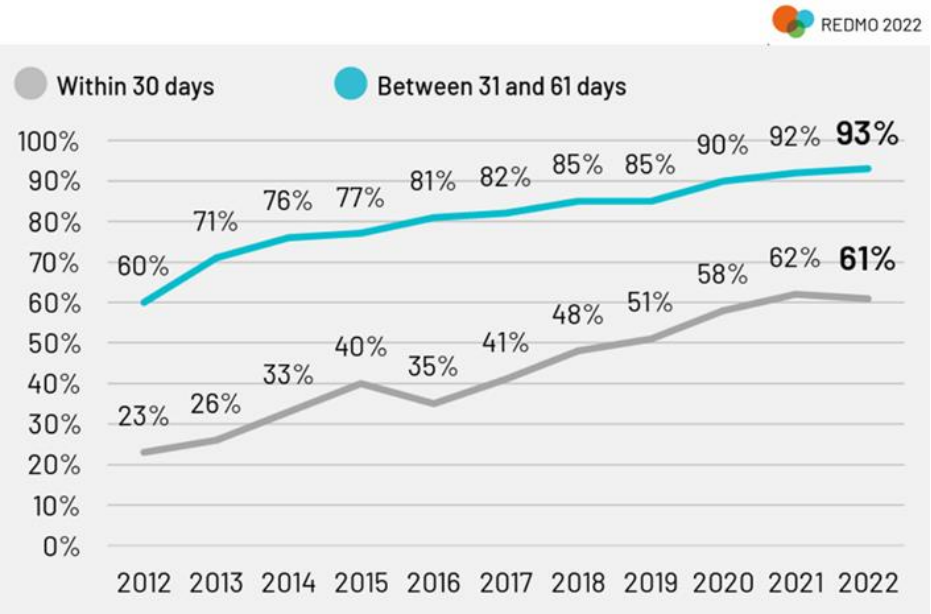

Figura 7. Probabilidad actual de encontrar un DnE HLA 10/10 o 9/10 [70].

Varios estudios comparativos han demostrado que los resultados del trasplante son peores según aumenta el número de incompatibilidades en el HLA entre el donante y el receptor [72-74]. El aloTPH a partir de donantes no HLA idénticos sigue siendo un reto terapéutico. Los trasplantes de DnE HLA 7/8 se han asociado históricamente a una menor supervivencia global (SG) y libre de progresión (SLP), así como a una mayor MRT y mayor incidencia de EICRa que los trasplantes de DnE HLA 8/8 [72] (Figura 8). Cada disparidad adicional se asocia a una disminución de la supervivencia entorno a un 10% [75, 76].

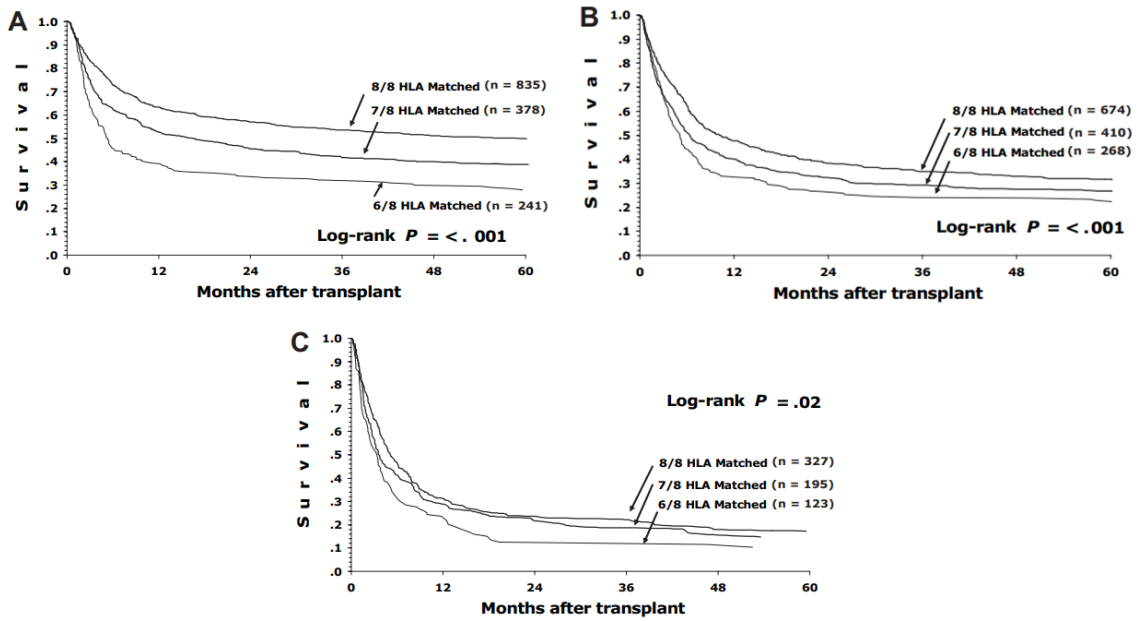

Figura 8. Supervivencia de pacientes con enfermedad incipiente, intermedia y avanzada según la disparidad HLA entre donante y receptor [72].

Los avances relacionados con la tipificación del HLA, la reducción del daño orgánico con los acondicionamientos no-mieloablativos, el tratamiento y prevención de las infecciones, y la profilaxis de la EICR se han traducido en menores tasas de MRT y en consecuencia mejores resultados para los pacientes trasplantados, incluso cuando existen diferencias en el HLA [77, 78]. Estas mejoras han conducido a un cambio en la estrategia de elección de los donantes con un uso cada vez más frecuente de los DnE y haploidénticos [79] (Figura 9).

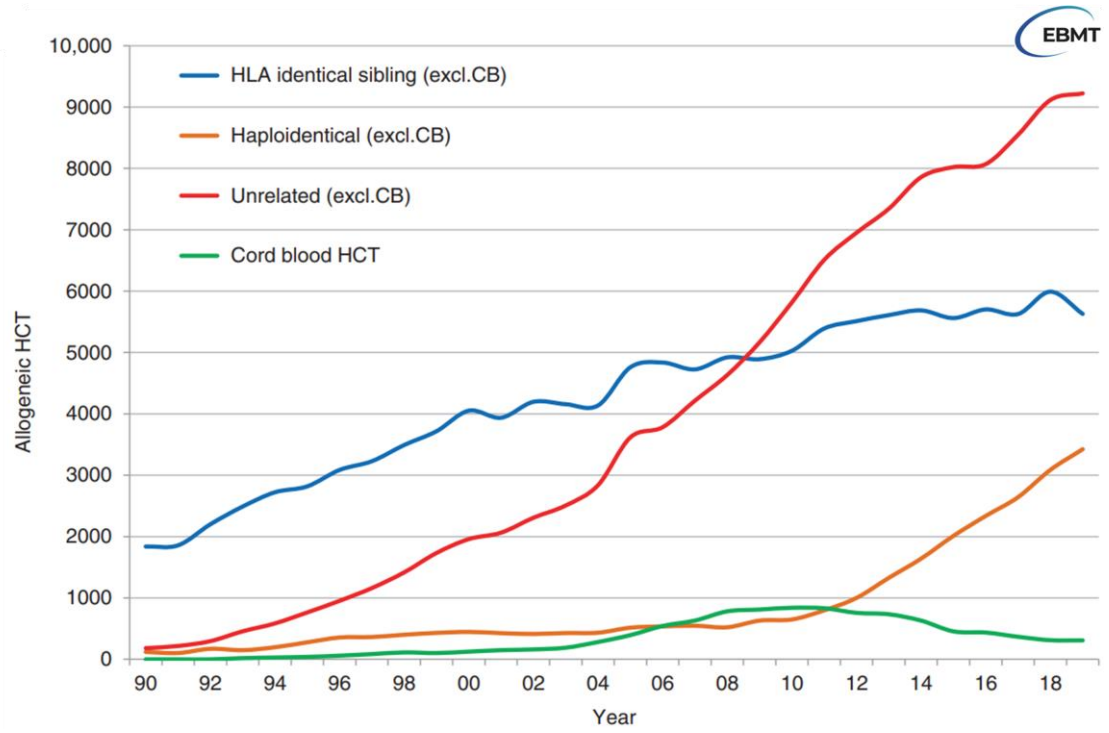

Figura 9. Evolución de la elección del tipo de donante en Europa de 1990 a 2021 según la EBMT [79].

3.2.3. Otros factores de riesgo para EICR: fuente y dosis de progenitores hematopoyéticos

La incidencia y gravedad de la EICR dependen de múltiples factores. La compatibilidad HLA mencionada anteriormente es el más importante, pero también se han estudiado otros como la diferencia de sexo o etnia entre donante y receptor, la edad avanzada de los pacientes y donantes, la intensidad del régimen de acondicionamiento, la profilaxis ineficaz de la EICR, la fuente de progenitores hematopoyéticos (MO vs. SP) y la cantidad de células hematopoyéticas contenidas en el injerto [80, 81].

En la actualidad, la SP ha sustituido casi por completo a la MO como **fuente progenitores hematopoyéticos** [70]. La aféresis de las células madre de SP (CMSP) permite la obtención de una mayor cantidad de células CD34+ y evita las dificultades logísticas que supone la punción de la MO.

Una de las principales preocupaciones relacionadas con el trasplante de SP es el elevado número de células T del donante presentes en los injertos y el riesgo mayor de

EICR. En estudios retrospectivos y ensayos clínicos con trasplantes de DnE con acondicionamientos mieloablativos y profilaxis estándar de la EICR, la SP en comparación con la MO se asoció con un aumento de la incidencia de EICRa grado II-IV y crónica, pero también demostró una recuperación hematopoyética más rápida y una menor tasa de fallo de implante [82, 83]. En los trasplantes de DE, la asociación entre la SP como fuente de progenitores y una mayor incidencia de EICR ha sido significativa solo para las formas crónicas [84].

La **dosis de células CD34+** en el injerto se ha asociado con la estabilidad del implante, la supervivencia de los pacientes, pero también el riesgo de desarrollar EICR [85, 86].

Múltiples trabajos se han publicado al respecto, con diferentes categorías de dosis y en distintos tipos de trasplante usando como fuente de progenitores tanto MO como SP, administrando esquemas de profilaxis clásicos para la EICR. Las conclusiones, aunque con cierta disparidad, coinciden en que un número bajo de progenitores en el injerto afecta negativamente a la SG y aumenta la MRT [87-89]. En concreto, las dosis bajas de células CD34+ se han asociado especialmente y de manera significativa con el desarrollo de infecciones fúngicas [90].

Por otro lado, los estudios con diferentes dinteles demuestran que las dosis altas de progenitores hematopoyéticos favorecen una recuperación más rápida de neutrófilos y plaquetas en cualquier contexto, y se asocian con una disminución en la mortalidad relacionada con el procedimiento, así como con una mayor SG y SLP [91-94].

A pesar de todo ello, los extremos del rango óptimo de la dosis de células madre hematopoyéticas del injerto en el aloTPH no están del todo establecidos. Se ha reportado que cantidades superiores a 8 x 10^6/Kg de células CD34+ predisponen al desarrollo de EICRc clínicamente significativa [85-97]. De hecho, las dosis excesivamente altas de CD34+ pueden empeorar los resultados del trasplante, como es el caso de los pacientes que reciben injertos con más de 11 x 10^6/Kg de progenitores que presentan menores tasas de SG [98].

3.2.4. Impacto de la EICR en los resultados del aloTPH

La EICR tiene un impacto tanto positivo como negativo en los resultados del aloTPH.

Varios estudios han demostrado la correlación entre la EICR y un posible efecto antileucémico [99, 100]. Los pacientes que desarrollan EICRa y/o crónica presentan una menor incidencia de recaída, especialmente cuando la enfermedad está en estadios avanzados [101, 102]. De hecho, la depleción de linfocitos T en el injerto como estrategia para controlar la EICR, favorece la recidiva de la enfermedad [103]. Esta asociación constituye la justificación principal para el uso de la infusión de leucocitos del donante en las recaídas post-trasplante [104] y se ha relacionado con el control de la enfermedad mínima residual [105, 106]. Sin embargo, dicho control puede deberse a factores independientes de la presencia o ausencia de EICR clínicamente evidente [107].

A pesar de esta menor incidencia de recaída, el posible beneficio en la SG esta contrarrestado por una mayor MRT. La EICR puede ser una causa de muerte directa por insuficiencia orgánica o indirecta, al predisponer al receptor a infecciones potencialmente mortales y al desarrollo de cánceres [108, 109].

Según el informe epidemiológico del Centro para la Investigación Internacional de Trasplante de Medula Ósea (CIBMTR) del 2022, la EICR es la principal causa de MRT después del fallo orgánico específico y de las infecciones, independientemente del tipo de donante utilizado en el trasplante (Figura 10).

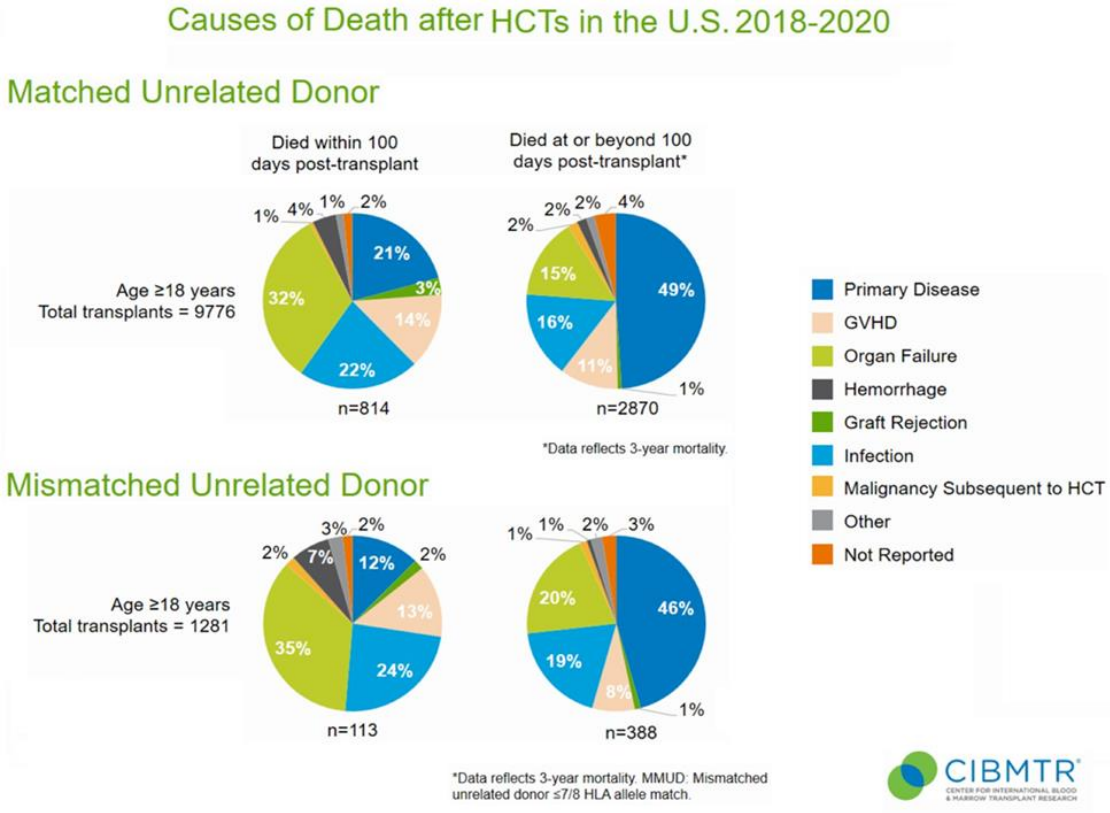

Figura 10. Causas de muerte en trasplante de DnE 2018-2020 según la CIBMTR [110].

Aunque los avances en el procedimiento de aloTPH han supuesto una mejoría de la SG, la mortalidad en los pacientes con EICR sigue siendo alta y se asocia directamente con la gravedad de las manifestaciones clínicas. Para los pacientes con EICRa, a mayor grado de compromiso tisular o cuantos más órganos se encuentren comprometidos a la vez, menor será la probabilidad de alcanzar una respuesta completa al tratamiento instaurado [111] y peor será la supervivencia post-trasplante [112, 113]. Los pacientes que presentan una EICRa grado I tienen una probabilidad de sobrevivir mayor al 90%, mientras que con grados II y III-IV la SG a 1 año es del 70% y 40%, respectivamente [114, 115].

La EICRc constituye la principal causa de pérdida auto-percibida de calidad de vida a largo plazo en el aloTPH y de muerte tardía relacionada con el tratamiento [116]. El incremento de la mortalidad de los pacientes con EICRc es especialmente significativo en aquellos con compromisos tisulares extensos, en los que se reconoce como un factor de riesgo independiente de muerte [117, 118].

3.3. Prevención de la EICR

Además de la cuidadosa elección del donante, la **inmunosupresión farmacológica** ha sido el pilar fundamental en la prevención de la EICR.

Los avances farmacológicos basados en un conocimiento más profundo de la fisiopatología de la EICR y la realización de múltiples ensayos clínicos comparativos han permitido diseñar esquemas profilácticos más eficaces y menos tóxicos [119, 120] (Figura 11). Según la Sociedad Europea de Trasplante de Sangre y Medula Ósea (EBMT), la incidencia de EICRa grado II-IV y grado III-IV durante 2011-2015 fue significativamente inferior a la del periodo 1990-1995 (28% y 11% vs. 40% y 19%, respectivamente, p<0.001) con una mortalidad también significativamente menor (36% vs. 47%) [119].

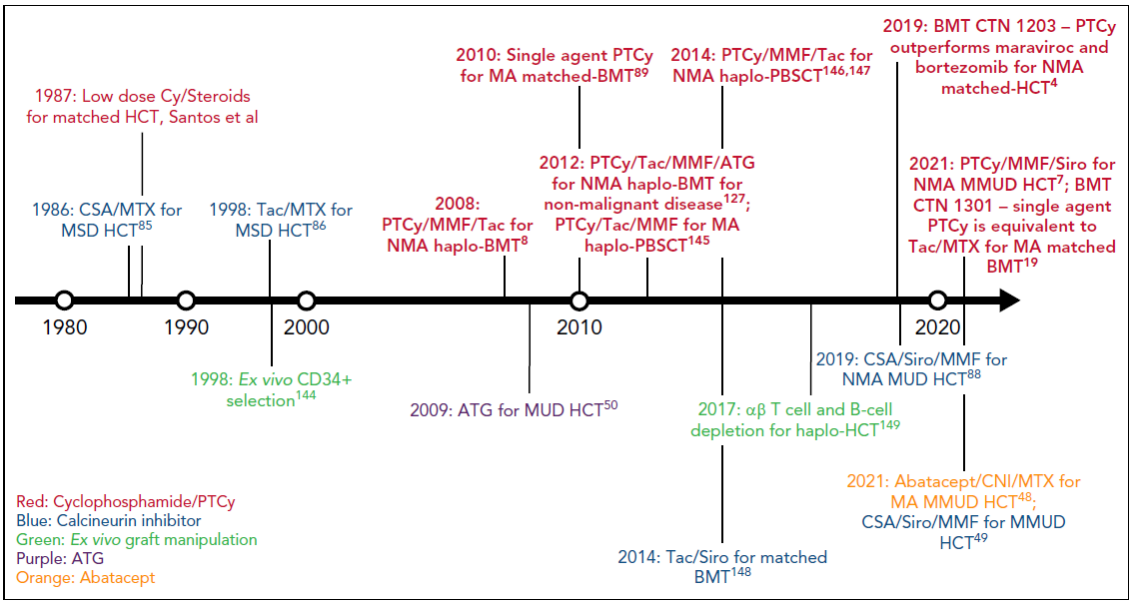

Figura 11. Línea temporal de los cambios en las estrategias profilácticas de la EICR [121].

3.3.1. Profilaxis convencional

Tradicionalmente, los mejores resultados en el aloTPH se han conseguido con la combinación de un inhibidor de calcineurina (ICN) como ciclosporina (CsA) o tacrólimus (Tac) con un medicamento antiproliferativo como el metotrexato (MTX), más que con el uso de cada uno de ellos en monoterapia [122-124]. La elección entre uno u otro tipo de ICN es controvertida. Sin embargo, ensayos clínicos aleatorizados

han demostrado que la incidencia de EICRa en pacientes que recibieron Tac/MTX es significativamente menor que en los que reciben CsA/MTX, aunque en términos de SG no se encontraron diferencias entre ambos grupos [125, 126].

Tras la instauración de los RIC, el uso de micofenolato de mofetilo (MMF) se extendió en la práctica clínica habitual, con un perfil de toxicidad menor que el MTX y una recuperación hemoperiférica más rápida [127,128]. Estas ventajas han tenido especial interés en el contexto de los trasplantes no mieloablativos, de cordón umbilical y haploidéntico [129-131].

La **eliminación de los linfocitos T alorreactivos** del injerto ha sido otra de las estrategias estudiadas para prevenir la EICR. La depleción ex-vivo de linfocitos T mediante la selección negativa de células T en el injerto ha sido eficaz para disminuir la incidencia de EICR, sin embargo, se asocia a un aumento en el riesgo de infecciones graves, fracaso del injerto y una elevada tasa de recaída de la enfermedad [132-134]. Este incremento en el riesgo de recaída y MRT es menor cuando la depleción linfocitaria se realiza a través de la selección positiva de células CD34+ [135-137].

La administración de **globulina anti-timocítica (ATG)** es la estrategia más utilizada para la linfodepleción in-vivo. Al ser policlonal, el anticuerpo reconoce los antígenos presentes en los linfocitos T y también aquellos que están en la superficie de otras células como los linfocitos B, células dendríticas, *natural killer* o células epiteliales, por lo que su acción se hace extensiva a todo el sistema inmune [138]. Ensayos clínicos aleatorizados han demostrado una reducción significativa del riesgo de EICRa y crónica con mejor SLP en los trasplantes de SP con DE o DnE HLA idéntico cuando se administra ATG junto con un ICN y MTX o MMF [139-141]. Sin embargo, los beneficios no se han traducido en una mejor SG a largo plazo y sí en un aumento de la toxicidad relacionada con el procedimiento [138, 142, 143].

Otra estrategia de depleción linfocitaria in vivo es la administración de **altas dosis ciclofosfamida post-trasplante (*Post-Transplant Cyclophosphamide,* PTCy)** con resultados muy favorables en los trasplantes de donantes haploidénticos [144]. A continuación, describimos de forma más detallada este tipo de profilaxis.

3.3.2. Profilaxis con ciclofosfamida post-trasplante

En los inicios del aloTPH en los años 60, la ciclofosfamida (Cy) fue utilizada como la base del acondicionamiento por su potente capacidad antitumoral e inmunosupresora. Santos y Owens demostraron además que, usándola a altas dosis en los días 2-5 post-trasplante en modelos murinos, era altamente eficaz en la prevención de la alorreactividad [145, 146, 147]. Debido a la preocupación sobre el efecto que Cy podría tener a esas dosis sobre el injerto hematopoyético, los estudios posteriores la utilizaron en bajas dosis hasta que finalmente fue desplazada por la CsA [148].

La Cy tiene una actividad antiproliferativa secundaria a la creación de enlaces cruzados irreversibles entre las cadenas de DNA que impiden la replicación celular [149]. La investigación sobre el efecto de la Cy en el control de la EICR ha mostrado que el fármaco actúa con mayor intensidad en la fase G1 y S del ciclo celular. Cuando se administra tras la infusión de los progenitores hematopoyéticos, su acción se dirige principalmente hacia los linfocitos del donante que se están replicando tras la interacción con el antígeno desconocido del receptor, controlando así la alorreactividad inicial post-trasplante [150, 151] (Figura 12). Este efecto no perjudica el injerto porque los progenitores hematopoyéticos tienen concentraciones altas de aldheído-deshidrogenasa (ALDH), enzima que inactiva a la mostaza fosforamida, uno de los metabolitos efectores del fármaco, al convertirla en carboxiciclofosfamida [149, 152]. Los linfocitos T reguladores CD4+ también cuentan con elevadas concentraciones de ALDH, especialmente los primeros días tras la infusión, lo que les hace resistentes a la Cy y les permite una expansión inicial que contribuye al control de los linfocitos alorreactivos residuales y a la instauración de la inmunotolerancia periférica [153, 154] (Figura 13).

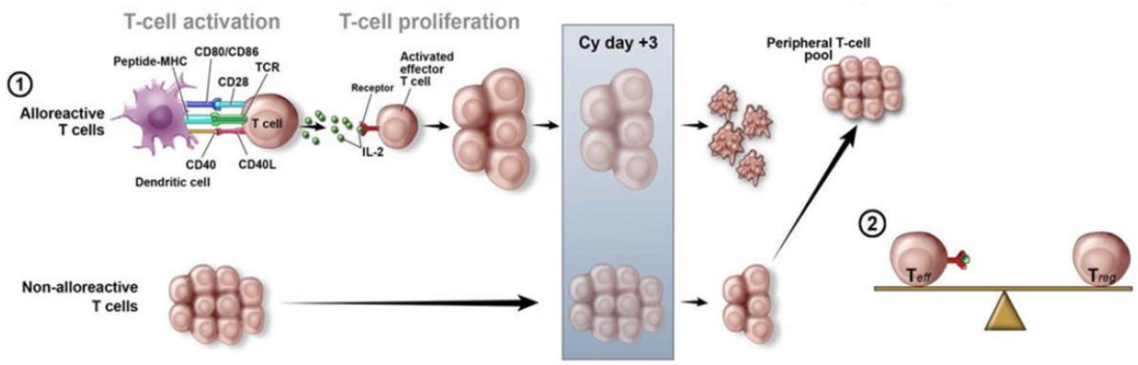

Figura 12. Mecanismo de acción PTCy. Adaptación de Luznik y cols [151].

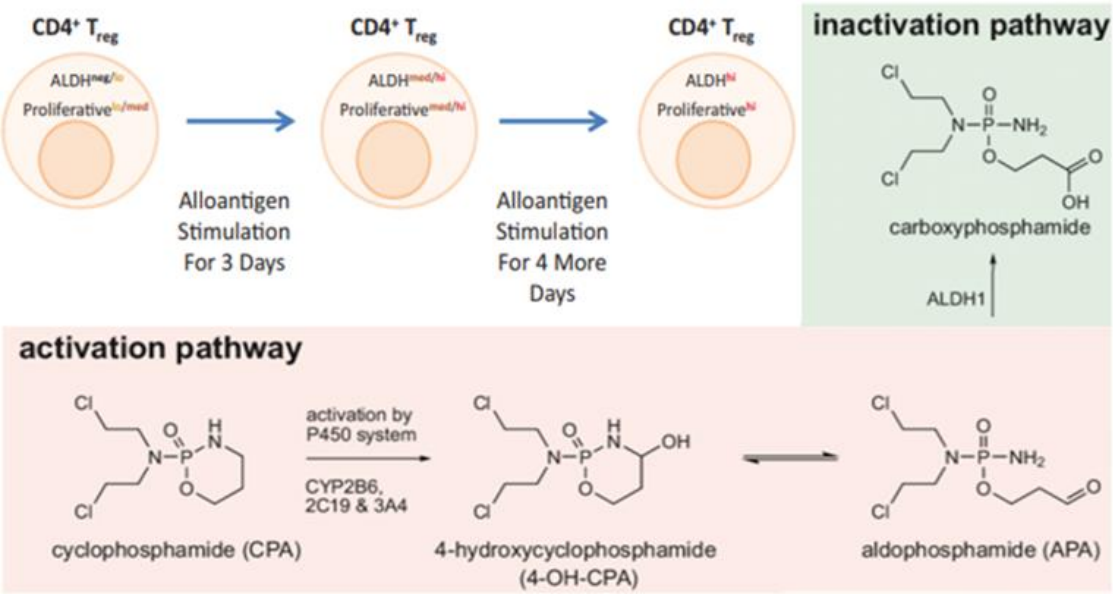

Figura 13. Inactivación de la Cy tras la acción de la ALDH en los linfocitos T CD4+. Adaptación de Kanakry y cols [154] y Jonge y cols 2005 [155].

La depleción selectiva de linfocitos T probablemente se acompaña de un deterioro funcional inducido por el fármaco sobre las células T alorreactivas supervivientes. Este efecto podría contribuir al control de la alorreactividad en el post-trasplante tardío [156, 157].

La depleción clonal intratímica de los precursores de células T alogénicas del donante es otro efecto sobre el control de la EICR que se le reconoce a la Cy, y que se ha relacionado con una tolerancia inmunológica a largo plazo que respeta el injerto hematopoyético [151, 158, 159].

Durante el inicio de la década de los dos mil, estudios preclínicos en modelos murinos realizados por L. Luznik y cols. en Baltimore demostraron que la administración de Cy a altas dosis en el post-trasplante precoz (PTCy) promueve la tolerancia de los linfocitos T alorreactivos del receptor y el donante en un contexto de disparidad parcial del HLA, lo que podría llevar a disminuir el fallo del injerto y la EICR en los trasplantes haploidénticos [160, 161].

Ensayos clínicos posteriores con trasplantes no-mieloablativos de donantes haploidénticos de MO y profilaxis contra la EICR con 50mg/kg de PTCy los días +3 y +4 post-trasplante seguidas de Tac y MMF demostraron bajas incidencias de EICRa grados II-IV (34%) y III-IV (6%) a los 200 días de la infusión, y una menor tendencia al desarrollo de EICRc extensa (5%), con aceptables tasas de fallo de injerto primario (13%), MRT (15%) y recaída al año (51%). La SG y SLP a los 2 años fueron del 36% y el 26%, respectivamente [144, 162].

Estos resultados se confirmaron en ensayos clínicos multicéntricos en cohortes más amplias, donde la SG para los trasplantes haploidénticos de MO con RIC y regímenes profilácticos para la EICR basados en PTCy superó el 60% y la incidencia acumulada de EICRa grado II-IV a los 100 días fue de 32% [163]. Los beneficios de la PTCy sobre la MRT y la SG también se constataron en cohortes de trasplantes haploidénticos de SP acondicionados con regímenes mieloablativos [164-166].

El éxito de la tolerancia inmunológica inducida por la PTCy en el contexto del trasplante haploidéntico ha hecho que los donantes con identidad parcial se conviertan en una alternativa segura y asequible, con resultados comparativamente similares a los obtenidos en los trasplantes de DE y DnE HLA idénticos con profilaxis estándar de la EICR [167-170].

En los primeros estudios dentro del entorno del trasplante de MO con DE o DnE HLA idéntico y regímenes mieloablativos, la PTCy demostró ser eficaz en monoterapia con tasas de SG y SLP a los 2 años de 55% y 39%, respectivamente. Aunque los beneficios de PTCy se vieron reflejados, espacialmente, en la baja incidencia de EICRc (10%), la incidencia de EICRa II-IV fue alta (43%) [171-173]. Los resultados de la PTCy en monoterapia fueron peores en el contexto de los trasplantes de SP por lo que la

recomendación de asociar el agente alquilante con otros inmunosupresores, siguiendo el esquema propuesto en el trasplante haploidéntico, ha seguido vigente [174].

Por otro lado, la estrategia de profilaxis basada en PTCy demostró ser significativamente superior a la combinación de un ICN con MTX en trasplantes de SP de DE o DnE con o sin diferencias en el HLA en cuanto a la prevención de EICRa y crónica. Los trasplantes con PTCy experimentaron un tiempo de recuperación de neutrófilos y plaquetas más prolongado y una mayor incidencia de infecciones, pero sin impacto en la SG. PTCy logró una supervivencia sin recaída libre de EICR superior que la observada en la rama de profilaxis estándar [175-177]. Ensayos clínicos prospectivos multicéntricos también concluyeron que la triada PTCy, Tac y MMF es superior a MTX, Tac y bortezomib o maraviroc cuando se comparan con la profilaxis estándar [178].

En trasplantes de DnE con diferencias en el HLA, también se han reportado resultados prometedores cuando se administra PTCy más un ICN o sirólimus y MMF o MTX [179-182]. En estos estudios la incidencia de EICRa grado II-IV varió del 15 al 37% y la de EICRc del 17 al 30%. Comparativamente, los esquemas basados en PTCy mostraron menores incidencias de EICRa II-IV y III-IV que los esquemas basados en ATG [179, 180].

Son muy pocos los estudios que proponen el uso de la PTCy en combinación con un solo inmunosupresor. En el contexto del trasplante de SP de DnE HLA 7/8, nuestro grupo de trabajo reportó que PTCy más Tac, se asocia con resultados comparables en términos de incidencia de EICRa, MRT, SG y SLP con la de los trasplantes de DnE HLA idénticos y profilaxis convencional [183]. Estos resultados han inspirado a nuestro grupo a realizar investigaciones posteriores y la presente Tesis Doctoral.

3.4. EICR y endotelio

El endotelio es un órgano activo y extenso que regula la homeostasis vascular y la respuesta inflamatoria del organismo. Entre muchas otras funciones, las células endoteliales (CE) intervienen en la regulación de la adhesión y migración celular, la coagulación y la permeabilidad vascular [184, 185]. En condiciones inflamatorias, el endotelio experimenta cambios estructurales que dan lugar a un incremento en la entrada y salida de células inmunitarias desde y hacia los tejidos afectados [186, 187].

Antes, durante y después del aloTPH, el endotelio puede activarse por diferentes motivos. Los regímenes de acondicionamiento, la reactividad alogénica del injerto, el uso de inmunosupresores profilácticos, las citoquinas liberadas por los tejidos lesionados, la traslocación de endotoxinas del tracto gastrointestinal o las infecciones son causas reconocidas de activación endotelial [188-192] (Figura 14). Un estímulo intenso o mantenido puede llevar a un estado irreversible de disfunción del endotelio que puede progresar hasta el proceso proinflamatorio, procoagulante y proapoptótico característico de las complicaciones post-trasplante de origen endotelial. [193].

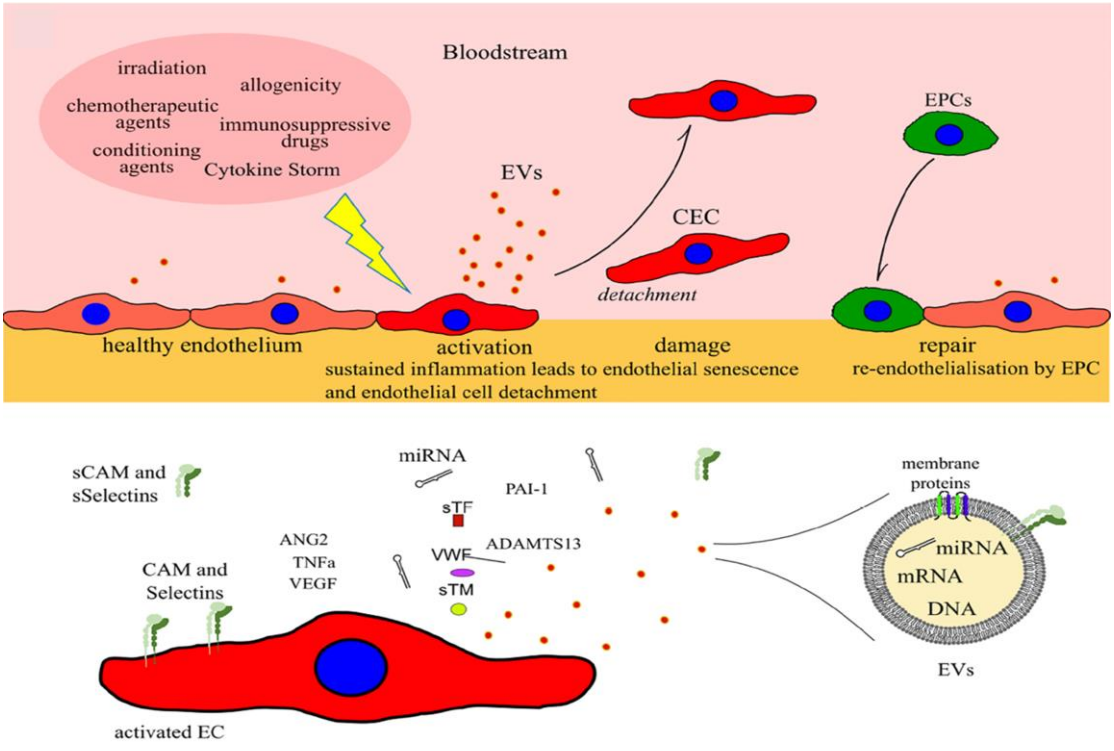

Figura 14. Activación y daño endotelial [194].

Los linfocitos T alorreactivos del injerto pueden reconocer como extraños a los antígenos HLA en la superficie de las CE del receptor e iniciar la lesión endotelial en los órganos diana de la EICR [192, 195, 196]. Sin embargo, el daño endotelial no es sólo una consecuencia de la EICR, sino que también se reconoce como un posible desencadenante [197]. Las CE dañadas y los procesos inflamatorios mediados por el endotelio vascular constituyen parte del inicio de la expresión antigénica incompatible que interviene en las primeras fases de esta complicación del post-trasplante.

La primera evidencia de lesión vascular inmunomediada se obtuvo en las biopsias de piel de pacientes con EICRa cutánea, donde se comprobaron depósitos perivasculares de factor de Von Willebrand (VWF) [198, 199]. En otras muestras tisulares de pacientes con EICRa se demostró la pérdida de trombomodulina (TM) endotelial y el aumento de la expresión de moléculas de adhesión como ICAM-1 y VCAM-1 [200-202]. Más recientemente, análisis histológicos en modelos murinos han evidenciado alteraciones graves en la microestructura endotelial y una angiogénesis temprana que favorece la transmigración leucocitaria hacia los órganos afectados por la EICR [26, 203].

Las citoquinas proinflamatorias circulantes como TNF-α, IFNγ, IL-1 e IL-6 también están elevadas durante la EICRa como consecuencia de la activación endotelial [204, 205] (Figura 15). La unión de TNF-α a su receptor en las CE (TNFR1) activa una cascada compleja de eventos de señalización donde intervienen VCAM-1, E-selectina e ICAM-1 y que facilita el tránsito de leucocitos a las zonas afectadas [206]. A su vez, esta señalización produce una elevación de la angiopoetina-2 (Ang-2), que aumenta la permeabilidad vascular y la neovascularización [192, 207].

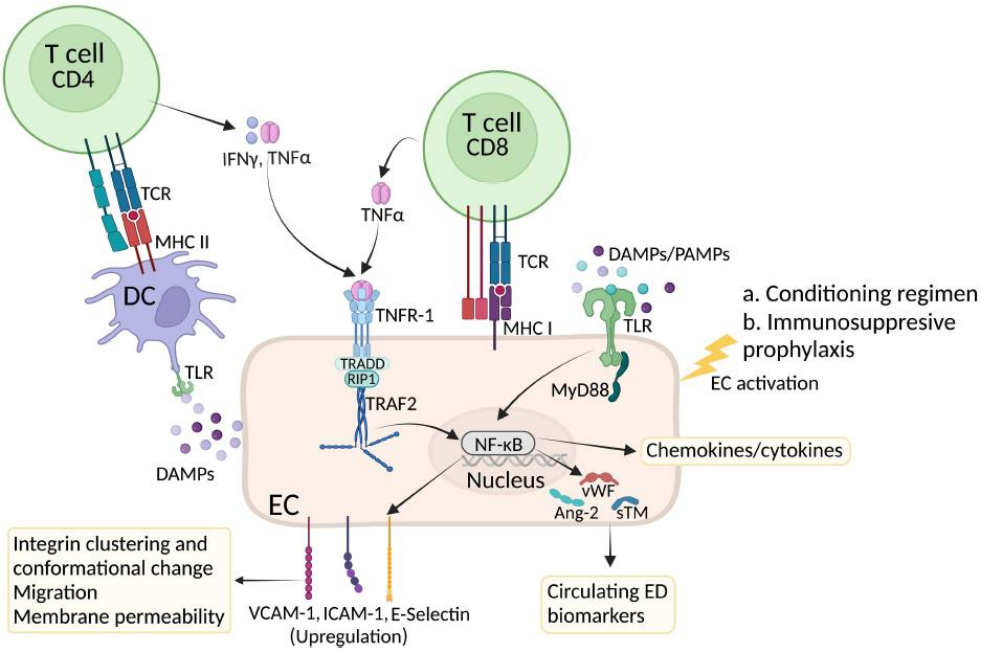

Figura 15. Factores que intervienen en la activación del endotelio en la EICR [208].

Los niveles de CE circulantes (CEC), micropartículas y microvesículas endoteliales aumentan cuando se produce una lesión endotelial [209, 210]. Su presencia en sangre se ha asociado con el diagnóstico, seguimiento y predicción de EICRa [211, 192].

Varios de estos biomarcadores plasmáticos de activación y daño endotelial se han relacionado con el diagnóstico, el pronóstico y la respuesta al tratamiento de las principales complicaciones de origen endotelial en el aloTPH (Tabla 1). Ejemplo de ello son los factores procoagulantes como el VWF o la TM soluble, las moléculas de adhesión como VCAM-1, ICAM-1, P selectina o E selectina, las moléculas angiogénicas como la Ang-2, los factores de crecimiento del endotelio vascular como el VEGF, los mediadores proinflamatorios como el TNFR1 soluble, las IL2, IL8 o L-ficolina, las micropartículas o microvesículas endoteliales (VEs), y las CEC [194, 212, 213].

Complicaciones endoteliales	Diagnóstico	Predicción y pronóstico
SOS	ST2, Ang-2, L-Ficolina, Ac H, VCAM-1, PAI-1 Ag, VEs, MiRNA	L-Ficolina, Ac H, VCAM-1, ICAM-1, E-selectina, EASIX
Síndrome de implante	TNFR1	VWF, TNFR1
Síndrome de fuga capilar	VEGF, Ang-2	
MAT-AT	NETs, sC5b-9 Haptoglobina decreciente	ST2, NETs, sC5-b9, VCAM-1, E-selectina, Factor Ba
EICRa	VWF, TNFR1, IL-2, IL-8, HGF, CEC, TIM3, VEs, miR155	Ang-2, TM, HGF, IL-8, IL-2, TNFR1, ICAM-1, VCAM-1, E-selectina, MAGIC (ST2, REG3a), ST2, NETs, VEs, miR155
SNI / DAD	ICAM-1, VCAM-1, eNOS, Ang-2	

SNI, Síndrome de neumonía idiopática; DAD, Daño alveolar difuso; Ac H, Ácido hialurónico; HGF, factor de crecimiento de hepatocitos; eNOS, óxido nítrico sintasa endotelial

Tabla 1. Biomarcadores diagnósticos y pronósticos de las principales complicaciones de origen endotelial. Adaptación de Moreno y cols [213] y Lia y cols [194].

Estudios experimentales in-vivo han demostrado que algunos de estos biomarcadores tienen un papel relevante en la predicción del síndrome de obstrucción sinusoidal (SOS), la microangiopatía asociada con el trasplante (MAT-AT) y la EICR. Incluso se han propuesto como potenciales dianas terapéuticas [194, 212, 213].

En concreto, los biomarcadores que han demostrado una capacidad predictiva para la EICRa se muestran en la Tabla 1. Entre los más destacados se encuentran el VWF y el TNFR1, cuyos valores altos a día +7 post-trasplante han demostrado tener un valor predictivo positivo para EICRa hasta del 90% [214]. Los niveles altos de Ang-2 medidos el día +21 del trasplante también se han relacionado con un mayor riesgo de EICRa, así mismo los niveles persistentemente altos de Ang-2 y TM soluble se han propuesto como marcadores de EICRa refractaria a corticoides [215, 216].

El aumento de otras moléculas como las fibras extracelulares formadas a partir del DNA de doble cadena (dsDNA) liberado por los neutrófilos tras su activación en el contexto del daño endotelial, conocidas como trampas extracelulares de neutrófilos (NET), también se han asociado con un posible aumento de la mortalidad y el

desarrollo de EICRa gastrointestinal al medirlas en los días +30, +60 y +100 del trasplante [217].

La identificación de diferentes biomarcadores solubles ha permitido el diseño de paneles para el diagnóstico y la estratificación pronóstica. Los investigadores del Consorcio Internacional de EICR Aguda del Monte Sinaí (MAGIC) desarrollaron y validaron un algoritmo basado en un modelo compuesto por dos biomarcadores de daño endotelial del tracto gastrointestinal, el supresor de tumorigenicidad 2 (ST2) y el derivado de islotes regeneradores 3-α (REG3α). Estos biomarcadores demostraron ser capaces de identificar a los pacientes con alto riesgo de EICRa grave y mayor MRT antes de la aparición de los síntomas, al inicio de los mismos y después del tratamiento, especialmente cuando existe refractariedad a corticoides [218, 219, 220]. En solitario, los niveles elevados de ST2 el día +28 pueden ser útiles para predecir la probabilidad de EICRa, la MRT y la SG [221].

Otros biomarcadores más asequibles se han estudiado también para predecir la evolución de los pacientes con EICR o el riesgo de desarrollar otras complicaciones relacionadas con el endotelio vascular. Algunos estudios sugieren que la trombopenia inferior a 100 x 10^9/L podría ser un factor predictivo de mortalidad en pacientes con EICRc [222]. El **Índice de Estrés y Activación Endotelial (EASIX)**, definido por la fórmula LDH (U/L) x creatinina (mg/dL)/plaquetas (x 10^9/L), se desarrolló como un biomarcador indirecto de disfunción endotelial. Estudios recientes demuestran que EASIX, cuando se mide en diferentes momentos antes y después del trasplante, es útil para predecir la mortalidad en pacientes con EICRa, el ingreso en UCI y el desarrollo de MAT-AT o SOS [223-227]. Las ventajas demostradas por EASIX, nos motivaron a estudiar su potencial capacidad predictiva para la EICRa en uno de los trabajos de la presente Tesis Doctoral.

3.5. Programa de AloTPH en el Hospital Clínic de Barcelona

El programa de aloTPH en el Hospital Clínic de Barcelona (HCB) se inició a mediados de los años 70. Durante la última década, más de 500 pacientes han recibido un aloTPH en nuestro hospital. La leucemia aguda y los síndromes mielodisplásicos son las principales indicaciones del procedimiento. El uso de DnE con o sin compatibilidad HLA ha ido aumentado hasta llegar a representar un 74% en el periodo comprendido entre 2019 a 2021. La proporción de trasplantes haploidénticos se ha duplicado, pasando de un 12% en el 2012 a un 24% en el 2023 (Figura 16).

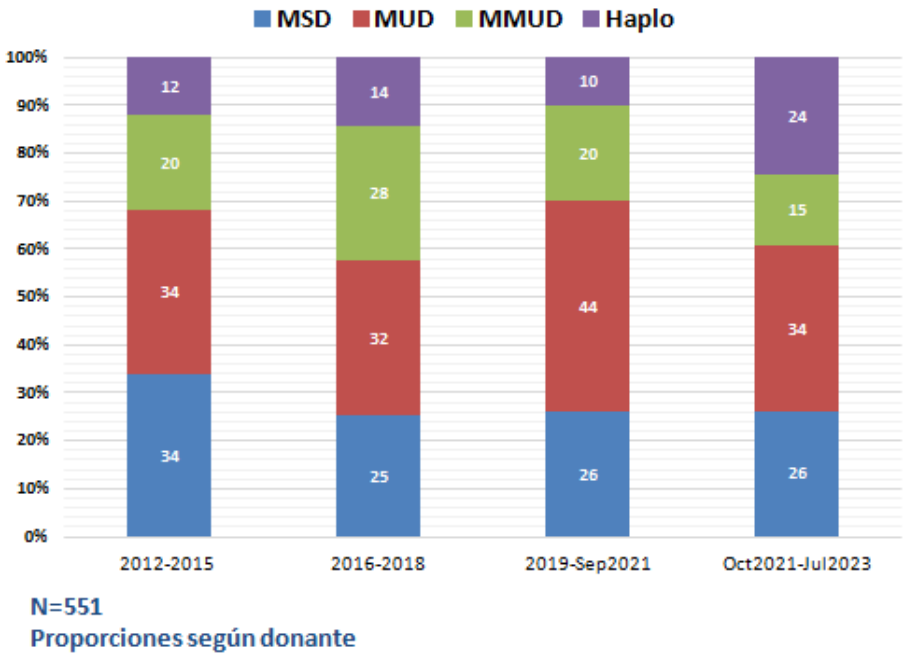

Figura 16. Cambios en la elección del donante del 2012 al 2023 en la Unidad de aloTPH del HCB.

El protocolo institucional de profilaxis para la EICR ha variado a lo largo del tiempo. Antes de la introducción de la PTCy, el esquema de profilaxis estándar consistía en CsA más MTX para los trasplantes con acondicionamientos mieloablativos y Tac más MMF para los trasplantes con RIC. La PTCy más Tac y MMF se administró por primera vez el 2013 únicamente en trasplantes haploidénticos. Desde 2014, el esquema se extendió al aloTPH de DnE con una diferencia en el HLA (7/8). Con la intención de reducir la toxicidad y promover la reconstitución inmune más temprana

se eliminó el MMF del esquema profiláctico. PTCy más Tac (PTCy/Tac) se implementó posteriormente en los aloTPH de DnE HLA 8/8 (2016) y DE HLA 8/8 (2019). Actualmente más del 90% de los trasplantes realizados en nuestro centro reciben profilaxis para la EICR con PTCy 50mg/kg/d administrada los días +3 y +4 del trasplante, seguidos de Tac 0,03mg/kg/d desde el día +4 hasta el día +90 a dosis terapéutica y con una reducción progresiva de la dosis en ausencia de EICR hasta el día +180 (Figura 17).

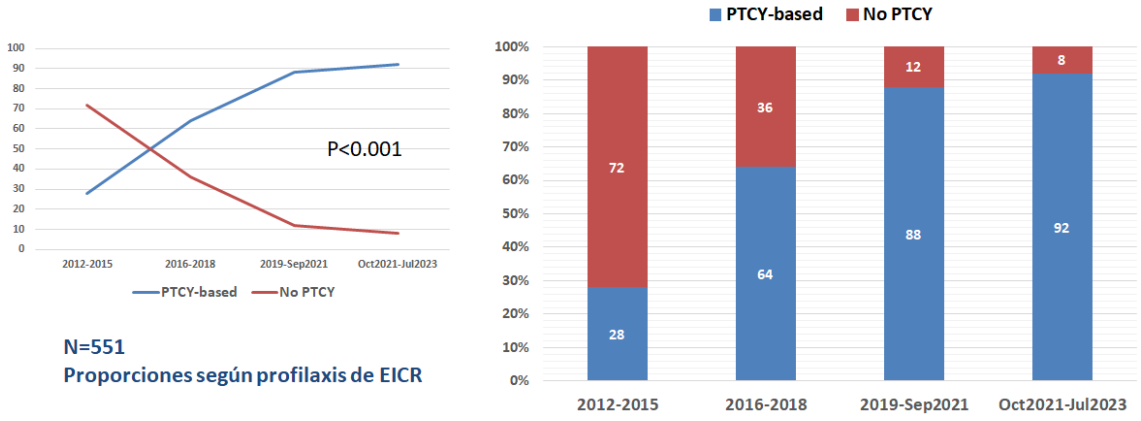

Figura 17. Cronología de la introducción de la PTCy en la Unidad de aloTPH del HCB.

Globalmente, con esta estrategia de prevención de la EICR en nuestra institución, la incidencia de EICRa grado II-IV a los 100 días y EICRc moderada-grave al año son del 25,3% y 2,8%, respectivamente (Figura 18). La SG, SLP y supervivencia sin recaída libre de EICR al año son del 84,2%, 78,6% y 71,6%, respectivamente, mientras que la MRT y la incidencia de recaída al año son del 12,1% y 9,3%. Todo ello ha supuesto una mejoría significativa en comparación con la experiencia previa.

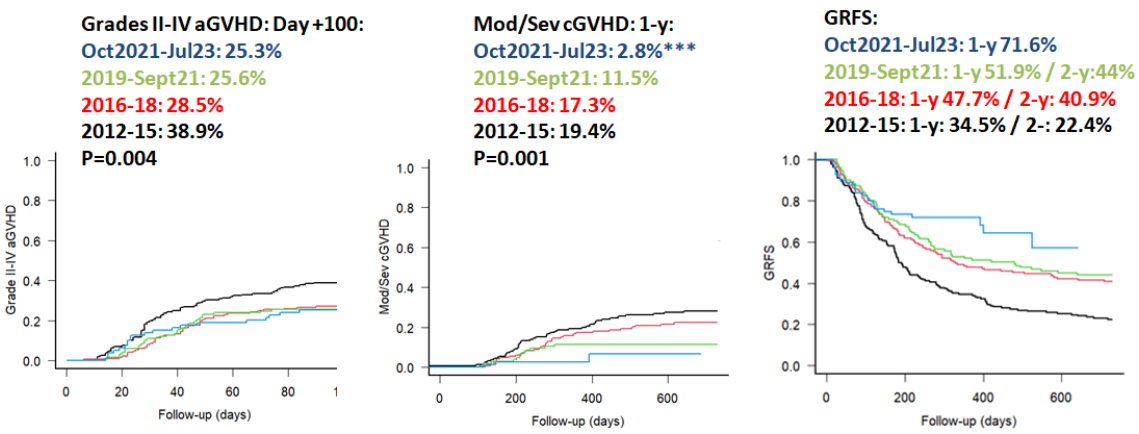

Figura 18. Datos sobre EICRa grado II-IV, EICRc moderada-severa y supervivencia libre de progresión y EICR significativa del 2012 al 2023 en el HCB.

Sin duda alguna, la prevención y el tratamiento de la EICR son aspectos de gran relevancia en la investigación de cara a mejorar los resultados del aloTPH. Los esfuerzos científicos se han dirigido a mejorar los esquemas profilácticos y al estudio de biomarcadores con alto valor predictivo que permitan identificar de manera precoz los pacientes a riesgo de desarrollar esta complicación. La presente Tesis Doctoral aspira a contribuir al conocimiento en este campo.

4. HIPOTESIS

La enfermedad injerto contra receptor (EICR) es una de las complicaciones más frecuentes y graves del trasplante alogénico de progenitores hematopoyéticos. La investigación dirigida a mejorar las estrategias de profilaxis e identificar biomarcadores predictivos de EICR es una prioridad para los equipos de trasplante.

En los últimos años, hemos asistido a una importante mejoría en la prevención de la EICR gracias al uso de altas dosis de ciclofosfamida post-trasplante (PTCy). En un contexto tan complejo como el trasplante haploidéntico, donde la disparidad HLA es máxima, PTCy ha reducido de forma significativa la incidencia de las formas graves de EICR, tanto aguda como crónica, convirtiendo este tipo de trasplante en un estándar en multitud de centros a lo largo del mundo. La experiencia de PTCy con donantes convencionales (emparentados y no emparentados) es mucho menor. En este escenario nos planteamos las siguientes hipótesis:

1. Al igual que sucede en el trasplante haploidéntico, PTCy también es eficaz y segura en la prevención de EICR en los receptores de trasplantes de donantes no emparentados HLA idénticos o con una diferencia antigénica. Esta eficacia se mantiene incluso con un esquema reducido de inmunosupresión consistente en PTCy con tacrólimus en lugar de PTCy más tacrólimus y micofenolato de mofetilo.

2. En el ámbito de la profilaxis de la EICR con PTCy, la dosis de células CD34+ infundida a los pacientes afecta a los resultados del trasplante.

3. El Índice de Activación y Estrés Endotelial (EASIX) es equiparable a algunos biomarcadores plasmáticos de actividad y daño endotelial post-trasplante. Ambos son capaces de predecir del riesgo de desarrollar EICR aguda.

5. OBJETIVOS

El **objetivo principal** de la presente Tesis Doctoral ha sido evaluar los resultados del uso de altas dosis de ciclofosfamida post-trasplante (PTCy) más tacrólimus como profilaxis de la EICR en el trasplante alogénico de progenitores hematopoyéticos de donantes no emparentados en nuestro centro, en este contexto, analizar el impacto de la dosis de progenitores hematopoyéticos sobre los resultados del trasplante y la capacidad predictiva para EICR del Índice de Estrés y Activación Endotelial (EASIX) y de algunos biomarcadores de lesión endotelial.

Los **objetivos específicos** planteados fueron:

1. Evaluar si PTCy más tacrólimus equipara los resultados del trasplante a partir de donantes no emparentados con una diferencia antigénica en el HLA con los del trasplante de donantes no emparentados HLA idéntico. En concreto, comparar entre ambos grupos la recuperación hematopoyética y reconstitución inmune, la incidencia acumulada de EICR aguda y crónica, las incidencias acumuladas de recaída y de mortalidad no relacionada con la recaída, y las probabilidades de supervivencia libre de progresión y de supervivencia global.

2. Calcular el punto de corte óptimo de la dosis de células CD34+ infundidas para mejorar los resultados del trasplante en una cohorte de pacientes cuya profilaxis para la EICR se realizó con esquemas basados en PTCy.

3. Investigar la dinámica post-trasplante de los biomarcadores plasmáticos de actividad endotelial: antígeno del factor Von Willebrand (VWF:Ag), molécula de adhesión de células vasculares (VCAM-1) y receptor del factor de necrosis tumoral α (TNFR1), y del EASIX, así como la correlación entre dichos parámetros y su capacidad predictiva de EICR aguda.

6. MATERIAL METODOS Y RESULTADOS

6.1. Profilaxis de la EICR con altas dosis de ciclofosfamida y tacrólimus en el trasplante de donante no emparentado con y sin identidad HLA.

"High-dose Cyclophosphamide and Tacrolimus as GVHD Prophylaxis for Matched and Mismatched Unrelated Donor Transplantation."

El régimen profiláctico óptimo para la EICR en el aloTPH de DnE con disparidad en un solo locus del HLA no está establecido. El uso de PTCy tras el trasplante haploidéntico es capaz superar el impacto negativo que tienen las diferencias en el HLA sobre la supervivencia, por lo que podría ser también una opción eficaz en el aloTPH con una sola disparidad antigénica. Sin embargo, la información disponible en este contexto es limitada. Por otro lado, la mayoría de los estudios publicados hasta la fecha de nuestra investigación han utilizado el modelo original del trasplante haploidéntico que combina tres inmunosupresores: PTCy, ICN y MMF. En este trabajo, analizamos los resultados del aloTPH de DnE HLA 7/8 con la combinación PTCy y Tac y los comparamos con los DnE HLA 8/8 usando el mismo esquema de profilaxis para la EICR.

Se analizaron retrospectivamente los datos clínicos de 109 pacientes, receptores de un aloTPH de DnE HLA 7/8 (n=55) y DnE HLA 8/8 (n=54) en nuestro centro. La fuente de progenitores hematopoyéticos fue principalmente la SP (98%). No se observaron diferencias entre los aloTPH de DnE HLA 7/8 y DnE HLA 8/8 con respecto a la incidencia acumulada de EICRa grado II-IV (31% vs 32%, p=0,9) y III-IV (9% vs 7%, p=0,7) a los 100 días, ni diferencia en la EICRc moderada-grave a los 2 años (18% vs 14%, p=0,8).

Ambos grupos mostraron una incidencia acumulada similar de MRT al 1 año (13% vs 9%, p=0,5) y tasas de recaída a los 3 años (24% vs 25%, p=0,7). La SLP y la SG a los 3 años para los aloTPH de DnE HLA 7/8 y DnE HLA 8/8 fueron del 56% y 57% (p=0,9) y del 64% y 65% (p=0,6), respectivamente. La probabilidad de sobrevivir a los 3 años sin EICRc moderada-grave ni recaída fue del 56% y del 55%, respectivamente.

En conclusión, la profilaxis de la EICR con PTCy y Tac consigue tasas bajas de EICRa grave y EICRc, así como buenos resultados en la supervivencia de los pacientes que reciben un aloTPH de SP tanto DnE HLA 7/8 como de DnE HLA 8/8. Esta estrategia profiláctica es capaz de superar el impacto negativo que supone la disparidad del HLA en un solo locus.

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Full Length Article
Analysis

High-Dose Cyclophosphamide and Tacrolimus as Graft-versus-Host Disease Prophylaxis for Matched and Mismatched Unrelated Donor Transplantation

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A B S T R A C T

The optimal prophylaxis regimen for graft-versus-host disease (GVHD) in the setting of single-locus mismatched unrelated donor (MMUD) allogeneic hematopoietic stem cell transplantation (alloHSCT) is unclear. The use of high-dose post-transplant cyclophosphamide (PTCy) after haploidentical transplantation is effective at overcoming the negative impact of HLA disparity on survival. Limited information is available regarding the efficacy of this strategy in alloHSCT from MMUDs. Most of the published studies have used the triple immunosuppressant model of haploidentical transplant combining PTCy with calcineurin inhibitors and mycophenolate mofetil or methotrexate. In our study, we propose the use of a simpler GVHD prophylaxis protocol comprising PTCy in combination with tacrolimus for MMUD and matched unrelated donor (MUD) alloHSCT. We performed a retrospective analysis of 109 consecutive recipients of alloHSCT from unrelated donors (MMUD, n = 55; MUD, n = 54) in a single center. Graft source was primarily peripheral blood (98%). No differences were observed between the MMUD and MUD groups with respect to 100-day cumulative incidence of grade II to IV acute GVHD (aGVHD; 31% versus 32%, respectively, $P = .9$), grade III to IV aGVHD (9% versus 7%, $P = .7$), and moderate/severe chronic GVHD (cGVHD) at 2 years (18% versus 14%, $P = .6$). Both groups showed similar cumulative incidence of 1 year nonrelapse mortality (13% versus 9%; $P = .5$) and 3-year relapse rates (24% versus 25%, $P = .7$). Progression-free survival and overall survival at 3 years for MMUD and MUD were 56% and 57% ($P = .9$) and 64% and 65% ($P = .6$), respectively. The 3-year probability of survival free of moderate/severe cGVHD and relapse was 56% and 55%, respectively. GVHD prophylaxis with PTCy and tacrolimus achieves low rates of severe aGVHD and cGVHD, as well as good survival outcomes, in recipients of both MMUD and MUD peripheral blood alloHSCT. This strategy overcomes the negative impact of single-locus HLA disparity.

The optimal prophylaxis regimen for graft-versus-host disease (GVHD) in the setting of unrelated donor allogeneic hematopoietic stem cell transplantation (alloHSCT) remains challenging, especially in the case of single-locus mismatched HLA unrelated donors (MMUDs). Historically, donor-recipient HLA compatibility has been the leading factor that predicts the outcome of alloHSCT; indeed, multiple studies report that overall results get worse as the degree of HLA mismatch increases, which is due to higher rates of GVHD, graft failure, and nonrelapse mortality (NRM) [1-6]. Unfortunately, a significant proportion of patients have no available HLA-matched related donor or unrelated donor (MUD). In this situation, the alternative donor sources are haploidentical donors, cord blood progenitor cells, or MMUDs; for all of these cases, new and improved GVHD prophylaxis regimens are needed.

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Several strategies aiming to improve the results of MMUD alloHSCT have been explored, including in vivo T cell depletion (TCD) with anti-thymocyte globulin (ATG), which has become the standard of care at many centers [7-9]. Although TCD reduces the incidence of GVHD, it is associated with delayed immune reconstitution, resulting in increased relapse risk and serious infections after transplant. GVHD prophylaxis with high-dose post-transplant cyclophosphamide (PTCy) plus tacrolimus and mycophenolate mofetil (MMF), pioneered by Luznik et al. [10] in 2008, is currently a successful and widely utilized method for haploidentical alloHSCT that significantly reduces the incidence of severe acute GVHD (aGVHD) and chronic GVHD (cGVHD) and increases survival. More recently, some studies report promising results after using triple GVHD prophylaxis based on the haploidentical model with PTCy, calcineurin inhibitors, and methotrexate (MTX) or MMF for MMUD transplants [11-14]. According to these studies, the incidence of grade II to IV aGVHD ranges from 15% to 37% and that of cGVHD from 17% to 30%. The optimal combination of immunosuppressant (IS) drugs to add to PTCy is not yet defined, and most published studies are based in the triplet haplo-schedule. A previous study from our center reports similar results using a model of MMUD transplantation based on peripheral blood stem cell (PBSC) grafts and a GVHD prophylaxis protocol comprising double therapy with PTCy and tacrolimus rather than triple IS drugs [15]. Based on these results, we established PTCy plus tacrolimus as the standard of care at our institution for both MMUD and MUD.

Here, we performed a retrospective analysis of the safety and efficacy of PTCy plus tacrolimus for unrelated alloHSCT and compared the outcomes of MMUD and MUD in a single institution.

MATERIALS AND METHODS

Patients and Donors

This study, performed at the Hospital Clínic in Barcelona (Spain) between February 2015 and December 2019, analyzed data from 109 consecutive patients who underwent unrelated alloHSCT for malignant hematologic diseases using PTCy-based GVHD prophylaxis. Eligibility criteria for transplant were as follows: age, 18 to 69 years; an Eastern Cooperative Oncology Group performance status $\leq$2; left ventricular ejection fraction $\geq$35%; forced expiratory volume in 1 second and forced vital capacity $\geq$40% of predicted; adequate hepatic function (total bilirubin $\leq$3.0 mg/dL or absence of clinically significant liver disease); and lack of a familiar HLA-identical donor. Patients with previous alloHSCT were excluded.

All unrelated donor/recipient pairs were typed by high resolution for allelic level for HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. For patients who did not have a 10/10 HLA-matched related or MUD, a search was performed based on a single HLA mismatch at HLA-A, HLA-B, HLA-Cw, or HLA-DRB1 (7/8 HLA mismatch). Standard methodology was used to detect donor-specific anti-HLA antibodies. Other donor selection criteria, in order of priority, were as follows: matched cytomegalovirus (CMV) IgG serologic status, a male donor for a male recipient, major ABO compatibility, and minor ABO compatibility. The protocol received institutional review board approval, and all participants provided signed informed consent.

Treatment Protocol and Supportive Care

Specific conditioning regimens used were based on the type of hematologic disease and patient characteristics in accordance with institutional protocols. Patients aged >50 years or previously submitted to an autologous HSCT received a reduced-intensity conditioning (RIC) regimen; otherwise, myeloablative conditioning (MAC) regimens were administered. All patients received fludarabine-based conditioning schemes (fludarabine 30 to 40 mg/m^2/d for 4 days) in combination with busulfan (3.2 mg/kg/d for 4 days for MAC or 3 days for RIC), total body irradiation (12 Gy for MAC or 8 Gy for RIC), melphalan (70 mg/m^2 for 2 days), cyclophosphamide (14.5 mg/kg for 2 days) plus 2 Gy total body irradiation, or a sequential conditioning regimen consisting of fludarabine 30 mg/m^2/d for 5 days, cytosine arabinoside 2 g/m^2 for 5 days, idarubicin 12 mg/m^2 for 3 days, and melphalan 70 mg/m^2 for 2 days (Table 1).

All patients received GVHD prophylaxis with high-dose PTCy (50 mg/kg i.v. once daily on days +3 and +4), along with Mesna at 80% of the cyclophosphamide dose (divided into 4 doses), followed by tacrolimus (0.03 mg/kg as a 24-hour i.v. perfusion) from day +5. Serum levels of tacrolimus were maintained between 5 and 15 ng/mL. No adjustments of tacrolimus doses were performed according to the Disease Risk Index (DRI). Tacrolimus was continued until day +90 and then tapered if GVHD grades II to IV were absent. ATG or alemtuzumab was not administered. Colony-stimulating factors or CMV prophylaxis were not given routinely.

Antimicrobial prophylaxis was administered according to our institutional practice guidelines. Standard prophylaxis included levofloxacin from day 0 until neutrophil engraftment, fluconazole until day +60, and acyclovir until day +365 (for patients who were seropositive for herpes simplex virus). Standard *Pneumocystis jirovecii* prophylaxis was used until CD4$^+$ T cell recovery (>200 cells/μL) and/or until immunosuppression was discontinued. CMV quantitative PCR was performed weekly through to at least day +60, and preemptive therapy was initiated if viral reactivation was detected (a PCR result above 1000 UI/mL or 2 consecutive rising values), according to standard recommendations.

Definitions

Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) of $>0.5 \times 10^9$/L after transplantation. Platelet recovery was defined as a platelet count of $>20 \times 10^9$/L without transfusion in the 7 preceding days. Donor chimerism was assessed by PCR-based amplification of polymorphic short tandem repeat regions in peripheral blood samples taken on days +30, +60, and +90. Chimerism analysis was performed using separated myeloid and T cell lymphoid fractions whenever possible. Complete donor chimerism was defined as detection of >95% donor DNA in a sample.

Primary graft failure was defined as the absence of ANC recovery ($>0.5 \times 10^9$/L) before day +28, which was maintained for 3 consecutive days, with a platelet count of $<20 \times 10^9$/L, hemoglobin level of <80 g/L, and the need for transfusion support.

Toxicity was scored using Common Terminology Criteria for Adverse Events version 5. Veno-occlusive disease was graded using the Baltimore criteria, and hemorrhagic cystitis was considered significant when macroscopic hematuria was present in the absence of other clinical conditions. aGVHD was scored using the Glucksberg criteria, and cGVHD was defined and scored according to the current National Institutes of Health consensus criteria [16].

Statistical Analysis

Categorical variables related to patients, disease, and transplant procedure were represented as frequencies and proportions and statistically compared by the χ^2 or Fisher exact test. Quantitative variables were summarized as median and interquartile range (IQR) and compared by the Mann-Whitney test. NRM was defined as the time from alloHSCT to death in the absence of prior relapse/progression. The relapse rate was calculated as the time from alloHSCT to relapse/progression. NRM and relapse rate events were considered competing risks. Progression-free survival (PFS) was defined as the time from alloHSCT to relapse/progression or death from any cause, and overall survival (OS) was defined as the time from alloHSCT to death from any cause. A composite endpoint, defined as survival-free moderate/severe cGVHD and relapse (cGRFS), was also studied. This endpoint was calculated as the time from alloHSCT to the onset of moderate/severe cGVHD, to relapse/progression, or to death from any cause.

Estimates of NRM, aGVHD, cGVHD, and disease relapse were calculated using cumulative incidence curves to accommodate competing risks. Death in remission was considered a competing risk for relapse, disease progression as a competing risk for NRM, and death from all causes as a competing risk for GVHD. The probabilities of OS, PFS, and cGRFS were estimated using the Kaplan-Meier method and compared by univariate Cox regression, which was also used to analyze the prognostic impact of all variables on PFS and OS. Median follow-up was estimated by the reverse Kaplan-Meier method. Statistical significance was defined at the .05 level. Analysis was performed using the SPSS statistical software (IBM SPSS statistics version 22.0; SPSS, Inc., Chicago, IL, USA) and STATA 11 (StataCorp LP, College Station, TX, USA).

RESULTS

Patient, Transplants, and Graft Characteristics

The characteristics of patients and transplants in the MMUD (n = 55) and MUD (n = 54) groups were comparable (Table 1). The median age of the total study population was 53 years. Acute leukemia and myelodysplastic syndrome were the most frequent transplant indications (65%). Most patients (88%) had intermediate- and high-risk disease, as by the refined DRI [17]. PBSC was the predominant graft source (98%). The median follow-up for all patients in the MMUD and the MUD groups was 3.0 (IQR, 1.9 to 3.1) years and 2.0 (IQR, 1.7 to 4.5) years, respectively.

Table 1
Patient and Transplant Characteristics According to the Type of Donor

Variable	All Patients (N = 109)	7/8 MMUD (n = 55)	8/8 MUD (n = 54)	*P* Value
Age, median (IQR), yr	53 (41-62)	51 (40-60)	53 (42-63)	.3
Donor age, median (IQR), yr	31 (25-37)	29 (23-36)	32 (27-38)	.2
Female/male, No.	46 / 63	23 / 32	23 / 31	.9
Diagnosis				.4
Acute leukemia/myelodysplastic syndrome	71 (65)	37 (67)	34 (63)	
Chronic lymphoproliferative syndromes	18 (17)	7 (13)	11 (20)	
Chronic myeloproliferative syndromes	18 (17)	9 (16)	9 (17)	
Multiple myeloma	2 (2)	2 (4)	–	
Disease status				.5
Complete response	69 (63)	34 (62)	35 (65)	
Partial response	29 (27)	15 (27)	14 (26)	
Active disease	11 (10)	6 (11)	5 (9)	
DRI				.7
Low	13 (12)	7 (13)	6 (10)	
Intermediate	52 (48)	25 (45)	27 (50)	
High	44 (40)	23 (42)	21 (39)	
Conditioning regimen				.7
Myeloablative	50 (45)	25 (49)	25 (46)	
FLUBU4	32 (29)	16 (29)	16 (30)	
FLUTBI 12 Gy	18 (17)	9 (16)	9 (17)	
Reduced intensity	59 (54)	30 (53)	29 (55)	
FLUBU3	44 (40)	19 (34)	25 (46)	
FLUTBI 8 Gy	5 (5)	3 (5)	2 (4)	
FLUMEL	1 (1)	1 (2)	0	
IDA/FLAG/MEL	8 (7)	7 (13)	1 (2)	
FLU/CFM/TBI2Gy	1 (1)	0	1 (2)	
Comorbidity score (HCT-CI)				.3
≤3	86 (79)	48 (87)	38 (70)	
>3	23 (21)	7 (13)	16 (30)	
Donor/recipient sex				.2
Female/female	17 (16)	8 (15)	9 (17)	
Female/male	19 (17)	12 (22)	7 (13)	
Male/female	29 (27)	15 (27)	14 (26)	
Male/male	44 (40)	20 (36)	24 (44)	
Donor/recipient CMV status				.5
Negative/negative	20 (18)	6 (11)	14 (26)	
Negative/positive	36 (33)	22 (40)	14 (26)	
Positive/negative	7 (6)	2 (4)	5 (9)	
Positive/positive	46 (42)	25 (45)	21 (39)	
HLA mismatch				
HLA A	21(38)	21(38)	–	
HLA B	15 (27)	15 (27)	–	
HLA C	13 (23)	13 (23)	–	
HLA DR	6 (11)	6 (11)	–	
Graft source				.6
Bone marrow	2 (4)	2 (4)	–	
Peripheral blood	107 (98)	53 (96)	54 (100)	
CD34 × 10^6/kg, median (IQR)	5.7 (4.2-7.1)	6.0 (4.2-7.2)	5.6 (4.2-6.6)	.5
CD3 × 10^6/kg, median (IQR)	257 (170-344)	231 (163-343)	276 (170-359)	.4
Follow-up, median (IQR), yr	2.2 (1.7-3.6)	3.0 (1.9-3.1)	2.0 (1.7-4.5)	<.001

Values are presented as number (%) unless otherwise indicated.
HCT-CI indicates hematopoietic cell transplantation comorbidity index.

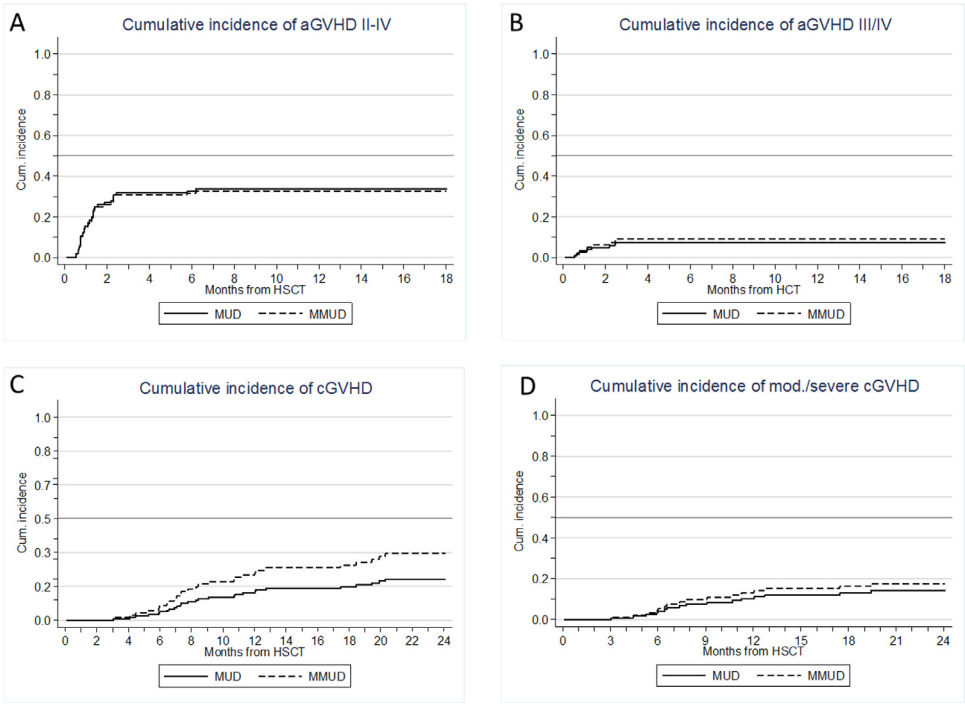

Figure 1. Cumulative incidences of acute and chronic GVHD according to type of donor (MMUD versus MUD). (A) aGVHD grade II to IV, (B) aGVHD grade III to IV, (C) global cGVHD, and (D) moderate to severe cGVHD. Death and relapse were competing events for GVHD.

Engraftment and Chimerism

Time to neutrophil recovery was similar for both groups of patients (median, 18 days [IQR, 15 to 21 days] for MMUD and 20 days [IQR, 18 to 22 days] for MUD, $P = .2$). No significant differences were observed between groups in platelet engraftment (median, 14 days [IQR, 12 to 19 days] for MMD and 14 days [IQR, 12 to 22 days] for MUD, $P = .6$). Primary graft failure occurred in 1 patient in the MMUD group and in no patients in the MUD group. Full donor chimerism in either $CD3^+$ or unsorted cells was achieved in 40 of 49 (82%) and 40 of 50 (80%) evaluable patients in the MMUD and MUD groups, respectively, by day 60.

Immune Reconstitution

Recovery of $CD3^+/CD4^+$ and $CD3^+/CD8^+$ lymphocytes was similar between the MMUD and MUD groups, with a median time to achieve >200 $CD3^+/CD4^+$ cells/μL of 240 days (IQR, 208 to 333) for MMUD and 241 days (IQR, 210 to 313) for MUD ($P = .7$).

aGVHD and cGVHD

The cumulative incidence of aGVHD by day +100 was similar between the MMUD and MUD groups: grades II to IV (31% versus 32%; Standardized Hazard Ratio, 0.9; 95% CI, 0.5 to 1.8; $P = .9$) and grades III to IV (9% versus 7%; SHR, 1.3; 95% CI, 0.3 to 4.7; $P = .7$) (Figure 1A,B). Skin was the most affected organ in both groups (51% of the aGVHD cases; grades II to IV, 30%), followed by the gastrointestinal tract (23%; grades II to IV, 17%), and hepatic aGVHD (6%; grades II to IV, 4%). Twenty-four percent of patients developed multiple-organ involvement. Steroid-refractory aGVHD was observed in 10 and 7 patients in MMUD and MUD groups, respectively (18% versus 13%, $P = .3$).

There was no difference between the groups with respect to the cumulative incidence of 2-year global cGVHD (33% versus 19%; SHR, 1.8; 95% CI, 0.8 to 3.8; $P = .1$) or moderate to severe cGVHD (18% versus 14%; SHR; 1.3; 95% CI, 0.5 to 3.4; $P = .6$) (Figure 1C,D). Of the patients who were alive at 12 and 24 months after transplantation, the percentage of patients without relapse

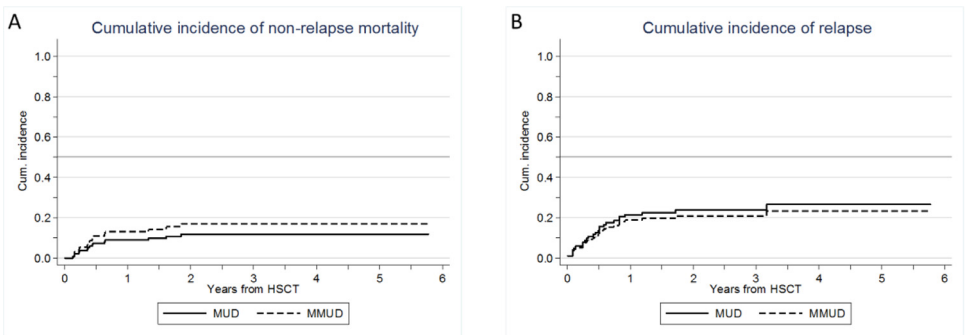

Figure 2. Probability of NRM (A) and cumulative incidence of relapse (B) according to type of donor (MMUD versus MUD). Death was a competing event for relapse and relapse for NRM.

and free of IS drugs was high, without significant differences between MMUD and MUD groups (80% versus 88% at 12 months, $P = .4$, and 84% versus 94% at 24 months, $P = .3$, respectively).

NRM, Cause of Death, Toxicity, and Relapse

There were no significant differences in the cumulative incidence of NRM at 1 year between the MMUD and MUD groups (13% versus 9%; SHR = 1.5; 95% CI, 0.5 to 4.1; $P = .4$) (Figure 2A). In the MMUD group, the causes of nonrelapse death were systemic and pulmonary toxoplasmosis (n = 2), multimicrobial septic shock (n = 2), aGVHD (n = 2), thrombotic microangiopathy (n = 1), melanoma (n = 1), and lung neoplasia (n = 1). In the MUD group, 4 nonrelapse deaths were recorded (septic shock, n = 2; cryptogenic pneumonia, n = 1; and severe intestinal aGVHD, n = 3).

No differences were found between groups regarding the incidence of moderate to severe oral and/or gastrointestinal mucositis (31% for MMUD versus 35% for MUD; $P = .2$). The incidence of sinusoidal obstruction syndrome was 4%. The incidence of hemorrhagic cystitis was 22% in the MMUD group and 15% in the MUD group ($P = .3$). All cases of hemorrhagic cystitis were associated with poliomavirus BK infection. All patients were managed with hyperhydration; 6 patients required continuous bladder irrigation, but no severe forms of hemorrhagic cystitis were observed.

Five (9%) and 2 (4%) patients in the MMUD and MUD groups, respectively, developed proven or probable invasive pulmonary aspergillosis ($P = .2$). There were also no significant differences between the groups regarding the rate of severe bacterial infection (15% in the MMUD group versus 13% in the MUD group; $P = .8$). CMV reactivation occurred in 31 of 47 (66%) of seropositive MMUD patients and in 20 of 35 (57%) of seropositive MUD patients ($P = .41$). There were no significant differences between the MMUD and MUD groups with respect to the median time to first CMV reactivation (37 versus 38 days after transplant; $P = .2$) or duration of first CMV reactivation treatment (15 versus 19 days; $P = .14$). The incidence of CMV disease was higher in the MMUD than in the MUD group (16% and 3%, respectively; $P = .03$). Six patients had gastrointestinal CMV disease (5 colitis, 1 gastritis), and 2 had CMV retinitis in the MMUD group; only 1 patient in MUD group had CMV disease (pneumonitis). Thirty-eight percent of CMV reactivations and 78% of CMV disease occurred in the context of GVHD and steroid treatment. No differences between groups were found in the type of conditioning or in the incidence of aGVHD or steroid-refractory aGVHD. The higher incidence of CMV disease in the MMUD group could be partially explained by the fact that, although not statistically different, the proportion of high-risk CMV seropositive patients (donor negative/recipient positive) was higher in the MMUD (40%) in comparison to MUD group (26%) (Table 1).

The cumulative incidence of relapse at 3 years was similar between the MMUD and MUD groups (24% and 25%, respectively; SHR, 0.8; 95% CI, 0.4 to 1.9; $P = .7$) (Figure 2B).

Survival

Seventy-four (70%) of the 109 patients are currently alive, with a median follow-up for all patients of 2.2 years (IQR, 1.7 to 3.6). OS at 3 years was 61% for the whole cohort, with no significant differences between the MMUD and MUD groups (64% versus 65%; hazard ratio [HR], 1.2; 95% CI, 0.6 to 2.3; $P = .6$) (Figure 3A). Estimated PFS at 3 years was 56%, with comparable results for MMUD and MUD (56% versus 57%; HR, 1.0; 95% CI, 0.5 to 1.8; $P = .9$) (Figure 3B). Survival free of moderate/severe cGVHD and relapse (cGRFS) at 3 years was 56% for MMUD and 55% for MUD (HR, 1.0; 95% CI, 0.5 to 1.8; $P = 1.0$) (Figure 3C). Univariate analysis of other variables that could affect OS, PFS, and cGRFS, including patient age, the hematopoietic cell transplantation specific comorbidity index, DRI, and conditioning type did not identify any statistical differences.

DISCUSSION

The results of our single-institution retrospective study suggest that use of a simpler GVHD prophylaxis protocol with PTCy in combination with tacrolimus is effective at preventing GVHD after MMUD PBSC alloHSCT. Moreover, the incidence of GVHD, NRM, and relapse is comparable to that of MUD

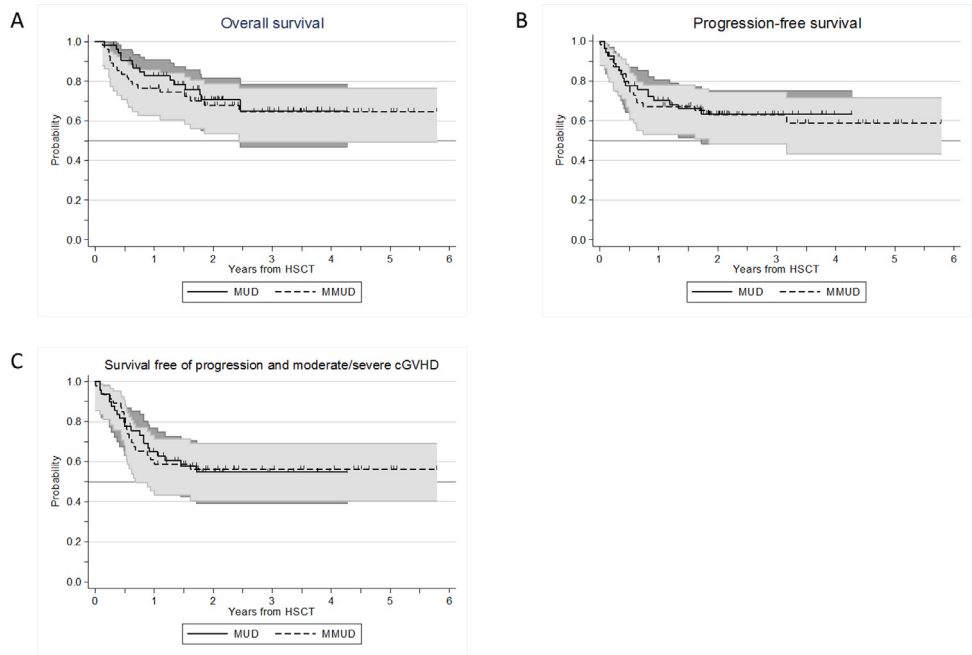

Figure 3. Kaplan-Meyer estimates (±90%) of (A) OS, (B) PFS, and (C) cGRFS, according to the type of donor MMUD versus MUD.

Table 2
Summary of the Results of Recent Studies in AlloHSCT Using MMUD and PTCy-Based GVHD Prophylaxis

Series	Patient Population	GVHD Prophylaxis	Conditioning Regimen	NRM	Relapse Rate	Acute GVHD	Chronic GVHD	OS	PFS
Pedraza et al. Retrospective study	MMUD N = 55 versus 7/8 MUD N = 54	PTCy + TCR	MAC 48% RIC 52%	1-year 13% versus 9% (P = .4)	3-year 24% versus 25% (P = .7)	II-IV: 31% versus 32% (P = .9) III-IV: 9% versus 7% (P = .7)	1-year Global 33% versus 19% (P = .1) Moderate/severe 18% versus 14% (P = .6)	3-year 64% versus 65% (P = .6)	3-year (P = .9)
Battipaglia et al.[11] Retrospective study	9/10 MMUD N = 272	PTCy + CSP/ TCR + MMF/MTX, N = 93 versus ATG + CSP/ TCR + MMF/MTX, N = 179	MAC 50% RIC 50%	2-year 16% versus 29% (P = .06)	2-year 29% versus 37% (P = .31)	II-IV: 30% versus 32% (P = .39) III-IV: 9% versus 19% (P<0.04)	2-year extensive 17% versus 20% (P = .31)	2-year 56% versus 38% (P = .07)	2-year 55% versus 34% (P<0.05)
Soltermann et al.[12] Retrospective study	MMUD N = 80	PTCy + CSP + MMF, N = 22 versus ATG + CSP/ TCR + MMF/MTX, N = 58	MAC 56% RIC 44%	1-year 5% versus 28% (P = .07)	1-year 18% versus 15% (P = .74)	II-IV: 15% versus 50% (P = .006)	26% versus 35% (P = .14)	1-year 91% versus 64% (P = .008)	1-year 77% versus 57% (P = .11)
Mehta et al.[13] Prospective study	9/10 MMUD N = 113 (7/8 MMUD N = 84)	PTCy + TCR + MMF, N = 41 versus ATG + TCR + MTX, N = 72	MAC 48% RIC 52%	2-year 35% vs 25% (P > .5)	2-year 20% vs 31% (P > .5)	II-IV: 37% versus 36% (P = .8) III-IV: 17% versus 12% (P = .5)	2-year 30% versus 42% (P = .6)	2-year 52% vs 40% (P > .5)	2-year 42% vs 38% (P > .5)
Gaballa et al.[14] Prospective study	9/10 MMUD N = 46	PTCy + TCR + MMF	RIC 100%	1-year 31%	1-year 19%	II-IV: 33% III-IV: 13%	2-year 19%	1-year 60%	1-year 47%
Kasamon et al.[18] Prospective study	9/10 MMUD N = 11 8/10 MMUD N = 4 >7/10 MMUD N = 5	PTCy + MMF + SIR	RIC 100%	1-year 0% 2-year 6%	1-year 35%	II-IV: 20% III-IV: 0%	1-year 16%	3-year 62%	3-year 52%
Lorentino et al.[19] Retrospective study	MUD N = 305 9/10 MMUD N = 159	PTCy alone or in combination with other drugs	MAC 54% RIC 46%	2-year 20% 2-year 16% (P = .15)	2-year 24% 2-year 28% (P = .41)	II-IV: 28% versus 28% (P = .84) III-IV: 10% versus 8% (P = .51)	2-year 35% versus 44% (P = .21)	2-year 62% versus 59% (P = .86)	2-year 56% versus 56% (P = .64)

TCR indicates tacrolimus; CSP, cyclosporine; SIR, sirolimus.

transplants using the same GVHD prophylaxis regimen, which translates into similar survival outcomes.

Several strategies, including ATG and, more recently, PTCy-based GVHD prophylaxis, have been used in an attempt to improve the historically unfavorable outcomes of MMUD alloHSCT [11-15,18]. ATG has been considered the standard of care for MMUD alloHSCT for years at many centers [7,8]. Some recent studies have retrospectively compared the outcomes of MMUD transplantation using ATG with that using PTCy, both in combination with calcineurin inhibitors plus MMF or MTX (Table 2) [11-13]. The European Society for Blood and Marrow Transplantation (EBMT) performed a matched-pair analysis comparing patients receiving PTCy with those receiving ATG [11]. The authors observed lower rates of grade III to IV aGVHD and similar rates of grade II to IV aGVHD and cGVHD using PTCy compared with ATG. Furthermore, PTCy was associated with significantly superior survival outcomes in terms of PFS and GRFS. Incidences of aGVHD and cGVHD, PFS, and OS observed in our study using PTCy plus tacrolimus were similar to that reported in the PTCy arm of the EBMT study. Recently, Soltermann et al. [12] explored the efficacy of a modified PTCy protocol using a reduced dose (40 mg/kg) of PTCy combined with 2 IS drugs and compared the results with those receiving ATG. In that study, the incidence of aGVHD grades II to IV was significantly lower in the PTCy group, and a trend for lower incidence of cGVHD and NRM was observed. PTCy patients had a better prognosis in terms of 1-year OS, with similar PFS, than ATG patients. Finally, Mehta et al. [13], in a prospective study using mostly bone marrow grafts, did not find differences between PTCy-based and ATG groups with respect to the incidence of aGVHD, cGVHD, NRM, relapse rates, PFS, or OS.

Our results are in agreement with studies using PTCy in combination with calcineurin inhibitors plus MMF or MTX (Table 2) [11-14,19]. The question of what is the optimal combination of IS added to PTCy remains a matter of debate. Although single-agent PTCy after MAC HLA-matched transplant and bone marrow stem cells has proved feasible, an excessive aGVHD and NRM have been reported with the use of PBSCs, suggesting that additional IS drugs are needed in this context [20-23]. In the setting of MUD and MMUD alloHSCT, most studies have been performed using GVHD prophylaxis schedules based on the triplet haploidentical GVHD prophylaxis schedule (PTCy combined with a calcineurin inhibitor plus MMF, MTX, or sirolimus) (Table 2) [11-14,18,19]. In a retrospective study from the EBMT, Ruggeri et al. [24] analyzed 423 patients who received HLA-matched related and unrelated alloHSCT and PTCy alone or in combination with other IS drugs as GVHD prophylaxis. In a multivariate analysis, in comparison to PTCy alone, the addition of 2 IS drugs was associated with significant reduced risk of NRM and extensive cGVHD, as well as with higher OS. It should be noted that, in contrast to the group of PTCy alone, a significant proportion of patients in the groups of 1 (36%) or 2 (49%) IS drugs also received TCD. On the contrary, in a recently published study comparing patients receiving MUD versus MMUD with PTCy alone or in combination with 1 or 2 ISs, any outcome differences between groups stratifying for the total number of IS drugs were observed [19]. Cumulative incidence of aGVHD at 100 days (grades II to IV, 28% for MUD and 28% for MMUD; grades III to IV, 10% and 8%, respectively) and cGVHD at 2 years (35% and 44%) was very similar to that observed in our study using PTCy plus tacrolimus. It is also worth mentioning that in our study, most patients alive and without relapse were free of IS drugs at 12 and 24 months post-transplant in both groups.

The present study has the same inherent limitations as other retrospective analyses. However, it also has some strengths. These include the fact that all patients were treated consecutively at a single institution using the same donor selection criteria, conditioning regimen protocols, and supportive care protocols.

In summary, our data suggest that a GVHD prophylaxis platform comprising PTCy plus tacrolimus results in outcomes for 7/8 HLA-MMUD similar to those of MUD. Although these data require confirmation in larger and randomized prospective trials, the results are promising and may help to expand the donor pool for patients who lack an HLA-matched or unrelated donor.

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6.2. Efecto de la dosis de células CD34+ en los resultados del trasplante alogénico con el uso de altas dosis de ciclofosfamida.

"Effect of CD34+ cell dose on the outcomes of allogeneic stem cell transplantation with post-transplant cyclophosphamide."

La influencia de la dosis de progenitores hematopoyéticos infundidos sobre los resultados del aloTPH con profilaxis estándar de la EICR sigue generando controversia. La información referente a este tema es escasa en el caso de los trasplantes alogénicos con esquemas profilácticos para la EICR basados en PTCy. En este trabajo nos propusimos evaluar el efecto que tiene la cantidad de células CD34+ contenidas en los injertos de SP sobre los resultados del trasplante con PTCy.

Realizamos un análisis retrospectivo de los datos clínicos y analíticos de 221 pacientes, receptores de un aloTPH con PTCy de DE (n=22), DnE HLA idéntico 8/8 (n=83), DnE con una disparidad antigénica HLA 7/8 (n=73) y donantes haploidénticos (n=43) en nuestro centro. Basándonos en el método de partición binaria y tomando en cuenta la SG como variable principal, se propuso 5 x 10^6/kg como valor de corte óptimo para la dosis de células CD34+. De acuerdo con nuestro protocolo institucional, la dosis máxima de células CD34+ infundida se limitó a 8 x 10^6/kg. De esta manera, la cohorte de estudio se dividió en 2 grupos: dosis alta (de 5 a 8 x 10^6/kg células CD34+) y dosis baja (≤ 5 x 10^6/kg células CD34+).

Los pacientes que recibieron injertos con dosis altas de células CD34+ tuvieron una mediana de tiempo hasta el injerto de neutrófilos y plaquetas significativamente más corta que los que recibieron injertos con dosis bajas (19 vs. 21 días, *p*=0,002; 16 vs. 22 días, *p*=0,04, respectivamente). No hubo diferencias entre los grupos de dosis altas y bajas en las incidencias acumuladas de EICRa grado II-IV (25% vs. 23%, *p*=0,7) y III-IV (5% vs 4%, *p*=0,4) a los 100 días, o EICRc moderada-grave a los 2 años (9% vs 6%, *p*=0,5). La dosis de células CD34+ no tuvo ningún efecto sobre la supervivencia de los trasplantes de DE, DnE HLA 8/8 o DnE HLA 7/8. Sin embargo, en los aloTPH de donantes haploidénticos con dosis bajas de células CD34+, la SG y la SLP fueron significativamente inferiores (HR 6,01, *p*=0,004 y HR 4,57, *p*=0,004, respectivamente).

Estos resultados indican que en los aloTPH de SP con PTCy, la infusión de 5 a 8 x 10^6/kg células CD34+ se asoció con un injerto de neutrófilos y plaquetas más rápido, independientemente del tipo de donante utilizado. La dosis de células CD34+ sólo impactó en los resultados de supervivencia de los trasplantes haploidénticos, donde la administración de ≤ 5 x 10^6/kg progenitores afectó negativamente la supervivencia de los pacientes.

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Full Length Article
Allogeneic – Adult

Effect of CD34$^+$ Cell Dose on the Outcomes of Allogeneic Stem Cell Transplantation with Post-Transplantation Cyclophosphamide

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A B S T R A C T

The impact of infused CD34$^+$ cell dose on outcomes after allogeneic hematopoietic stem cell transplantation (alloHSCT) using standard graft-versus-host disease (GVHD) prophylaxis remains controversial. Information on this subject is scarce for alloHSCT using high-dose post-transplantation cyclophosphamide (PTCy). We aimed to assess the effect of CD34$^+$ cell dose in peripheral blood stem cell (PBSC) grafts on the outcome of alloHSCT using PTCy-based GVHD prophylaxis. To do so, we conducted a single-center retrospective analysis of 221 consecutive adult patients who underwent PTCy alloHSCT from HLA-matched sibling donors (MSDs; n = 22), HLA-matched unrelated donors (MUDs; n = 83), mismatched unrelated donors (MMUDs; n = 73), and haploidentical donors (n = 43). Based on the binary partitioning method, 5 × 10^6/kg was used as the optimal cutoff for CD34$^+$ cell dose. According to our institutional protocol, the maximum CD34$^+$ cell dose was capped at 8 × 10^6/kg. The study cohort was divided into 2 groups based on CD34$^+$ cell dose: high dose (>5 to 8 × 10^6/kg) and low dose (≤5 × 10^6/kg). Patients receiving high-dose CD34$^+$-containing grafts had significantly shorter median times to neutrophil engraftment and platelet engraftment compared to those who received low-dose CD34+ (19 days versus 21 days [*P* = .002] and 16 days versus 22 days [*P* = .04], respectively). There were no differences between the high-dose and low-dose groups in the cumulative incidence of day +100 acute GVHD (grade II-IV: 25% versus 23% [*P* = .7]; grade III-IV: 5% versus 4% [*P* = .4], respectively) or 2-year chronic GVHD (moderate/severe GVHD: 9% versus 6%; *P* = .5). There was no impact of CD34$^+$ cell dose on survival outcomes with the use of MSDs, MUDs, or MMUDs. Recipients of haploidentical alloHSCT using low-dose CD34$^+$ cells had significantly worse overall survival (hazard ratio [HR], 6.01; *P* = .004) and relapse-free survival (HR, 4.57; *P* = .004). In recipients of PBSC PTCy alloHSCT, infused CD34$^+$ cell doses >5 to 8 × 10^6/kg were associated with faster neutrophil and platelet engraftment, independent of donor type. Our study suggests an impact of CD34$^+$ cell dose on survival outcomes only with haploidentical donors, for whom the administration of a CD34$^+$ cell dose ≤5 × 10^6/kg significantly decreased survival outcomes.

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INTRODUCTION

Post-transplantation high-dose cyclophosphamide (PTCy) has become an increasingly popular approach for graft-versus-host disease (GVHD) prophylaxis in allogeneic hematopoietic stem cell transplantation (alloHSCT), not only in the context of haploidentical alloHSCT [1], but also in transplants from HLA-matched sibling donors (MSD), matched unrelated donors (MUDs), and mismatched unrelated donors (MMUDs) [2–6]. As in alloHSCT using other GVHD prophylaxis strategies, peripheral blood stem cells (PBSCs) are considered as the graft source of choice in many PTCy alloHSCTs because of their ease of collection and richness in CD34$^+$ cells. However, the impact of graft cell dose on clinical outcomes after transplantation has not been evaluated in detail in the PTCy setting.

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The vast majority of analyses reported to date evaluating the influence of graft cell dose on survival outcomes after transplantation has been performed in patients undergoing alloHSCT using standard (ie, non-PTCy) GVHD prophylaxis. These studies have reported inconsistent results that can be explained by differences in disease categories, donor type, conditioning regimen, and GVHD prophylaxis. Low $CD34^+$ cell dose, defined by different thresholds depending on the study, has been associated with higher rates of nonrelapse mortality (NRM) and inferior overall survival (OS) [7,8]. On the other hand, high $CD34^+$ cell dose has been associated with faster neutrophil and platelet engraftment but a higher incidence of clinically significant chronic GVHD and relapse [9–13]. In any case, these findings may not be applied to the PTCy platform, which is associated with a significantly lower risk of GVHD.

The aim of this study was to assess the impact of infused $CD34^+$ cell dose on PBSC PTCy alloHSCT outcomes.

METHODS

Patients and Donors

This study, performed at the Hospital Clínic in Barcelona, Spain between March 2013 and July 2021, analyzed data from 221 consecutive patients who underwent their first peripheral blood alloHSCT for malignant hematologic disease using PTCy-based GVHD prophylaxis. Eligibility criteria for transplantation were age 18 to 69 years, Eastern Cooperative Oncology Group Performance Status ≤2, left ventricular ejection fraction ≥35%, forced expiratory volume in 1 second and forced vital capacity ≥40% of predicted, and adequate hepatic function (defined as total bilirubin ≤3.0 mg/dL or absence of clinically significant liver disease). Patients with previous alloHSCT and indeterminate Disease Risk Index (DRI) were excluded.

All donor-recipient pairs underwent high-resolution typing by allelic level for HLA-A, -B, -Cw, -DRB1, and -DQB1. For patients who did not have a 10/10 HLA MSD or MUD, a search was performed based on a single HLA mismatch at HLA-A, -B, -Cw, or -DRB1 (7/8 HLA mismatch). In the absence of a 10/10 or 9/10 HLA-matched donor, an MMUD was selected. Haploidentical transplantation was performed when an MSD, MUD, or MMUD could not be found. According to our institutional protocol, the absence of anti-HLA antibodies was systematically verified in all patients.

The study protocol received Institutional Review Board approval, and all participants provided signed informed consent. Clinical information was collected retrospectively and updated in April 2022. This study was approved by the local Ethics Committee and was conducted following standards set forth by the Declaration of Helsinki.

Graft Information

T cell-replete PBSC grafts were infused in all cases. The PBSC doses were calculated based on patients' actual body weight. According to our institutional protocol, the maximum $CD34^+$ cell dose was capped at 8×10^6/kg. Stem cells were mobilized with s.c. infused granulocyte colony-stimulating factor daily for 5 days. After progenitor stem cell collection, the $CD34^+$ and $CD3^+$ cell counts were determined. The products were cryopreserved in all cases after March 2020 because of the Coronavirus disease 2019 pandemic; cellularity was not reevaluated after thawing.

Based on the binary partitioning method, 4.96×10^6/kg was the optimal cutoff of $CD34^+$ cell dose to separate patients in 2 groups in terms of our main outcome variable (OS). To make the cutoff applicable to regular clinical practice, high dose was defined as >5 to 8×10^6/kg $CD34^+$ cells, and low dose was defined as ≤5 $\times 10^6$/kg $CD34^+$ cells. We evaluated the transplantation outcomes for the complete cohort as well as separately according to type of donor selected.

Conditioning Regimen, GVHD Prophylaxis, and Supportive Care

The specific conditioning regimens used were based on the type of hematologic disease and patient characteristics in accordance with institutional protocols. Patients age >50 years or who had undergone previous autologous HSCT received a reduced-intensity conditioning (RIC) regimen; otherwise, a myeloablative conditioning (MAC) regimen was administered. All patients received fludarabine-based conditioning (fludarabine 30 to 40 mg/m^2/day for 4 days) in combination with busulfan (3.2 mg/kg/day for 4 days for MAC or 3 days for RIC), total body irradiation (TBI) (12 Gy for MAC or 8 Gy for RIC), melphalan (70 mg/m^2 for 2 days), cyclophosphamide (14.5 mg/kg for 2 days) plus 2 Gy TBI, or a sequential conditioning regimen consisting of fludarabine 30 mg/m^2/day for 5 days, cytosine arabinoside 2 g/m^2 for 5 days, idarubicin 12 mg/m^2 for 3 days, and melphalan 70 mg/m^2 for 2 days.

The institutional standard GVHD prophylaxis consisted of PTCy 50 mg/kg/day administered on days +3 and +4, followed by tacrolimus started on day +5 at a dose of .04/kg/day i.v., titrated to a therapeutic level of 5 to 15 ng/L, maintained until day +90 and tapered progressively up to day +180 in the absence of GVHD. In addition, mycophenolate mofetil was administered from day +5 to day +28 for haploidentical transplant recipients. Antithymocyte globulin (ATG) and alemtuzumab were not administered. Colony-stimulating factor and cytomegalovirus prophylaxis were not provided routinely. Supportive care and infectious prophylaxis have been described previously [6].

Definitions

Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count >.5 $\times 10^9$/L after transplantation. Platelet recovery was defined as a platelet count >20 $\times 10^9$/L without transfusion in the 7 preceding days. Primary graft failure was defined as the absence of absolute neutrophil count recovery (>.5 $\times 10^9$/L) before day +28, which was maintained for 3 consecutive days with a platelet count <20 $\times 10^9$/L, hemoglobin level <80 g/L, and the need for transfusion support. Immune reconstitution was defined as $CD3^+/CD4^+$ and $CD3^+/CD8^+$ lymphocyte counts >200 cells/L.

Toxicity was scored using Common Terminology Criteria for Adverse Events version 5. Acute GVHD (aGVHD) was scored using the Glucksberg criteria, and chronic GVHD (cGVHD) was defined and scored according to the current National Institutes of Health consensus criteria [14].

Statistical Analysis

$CD34^+$ cell dose was considered the primary explanatory variable of interest, and OS and NRM were the primary outcome variables of the study. The primary variable of interest was treated as continuous variable and transformed into a dichotomous variable after calculating the optimal cutoff value (5×10^6/kg $CD34^+$ cells) for OS prediction. This cutoff value was calculated based on the binary partitioning method [15], which uses the likelihood ratio statistic to evaluate the performance of individual splits. This test permits partitioning of a set of longitudinal data into 2 mutually exclusive groups based on an optimal split of a continuous prognostic variable. Relapse-free survival (RFS), cumulative incidence of relapse (CIR), and cumulative indices of acute and chronic GVHD and primary GF were considered relevant variables of interest for exploring the study's secondary endpoints.

Time to event was calculated from the date of transplantation to the date of the event or last follow-up. OS and RFS were calculated using the Kaplan-Meier method. NRM and CIR were estimated using competing-risk analysis and considering relapse as a competing event for NRM and death without relapse as a competing event for CIR. The cumulative incidence of GVHD was calculated by accounting for death and relapse as competing events, and the cumulative incidence of primary GF was estimated considering death as the competing event. Univariate and multivariate regression analyses were conducted to explore the impact of $CD34^+$ cell dose on post-transplantation outcomes. Those variables found to be significant in the univariate model or considered clinically relevant were included in the multivariate analysis. The primary explanatory variable was always included in the multivariate model. All P values were 2-sided, and $P <$ .05 was considered to indicate statistical significance. Statistical analyses were performed using EZR software [16].

RESULTS

Patient and Donor Characteristics

Baseline characteristics of the entire cohort and of 2 groups stratified according to $CD34^+$ cell dose are provided in Table 1. Acute leukemia and myelodysplastic syndrome were the most frequent indications for transplantation (72%). Most patients (79%) had low- to intermediate-risk disease as defined by the refined DRI. The most frequently selected donor type was MUD/MMUD (71%). One hundred forty-four patients (65%) received a high $CD34^+$ cell dose, and 77 (35%) received a low $CD34^+$ dose. No differences between the 2 groups were seen in the main characteristics such as patient age, hematologic diagnosis, DRI, Karnofsky Performance Status (KPS), Hematopoietic Cell Transplantation Comorbidity Index, and conditioning regimen. The median duration of follow-up was 25 months (IQR, 11 to 55 months) for patients in the high-dose group and 21 months (IQR, 6 to 41 months) in the low-dose group (P = .06).

Engraftment and Immune Reconstitution

Engraftment information is summarized on Table 2. Overall, 217 patients (98%) achieved primary engraftment. Patients receiving high-dose $CD34^+$ cell-containing grafts had significantly

Table 1
Patient, Donor, and Transplantation Characteristics According to $CD34^+$ Cell Dose

Characteristic	All Patients (N = 221)	$CD34^+$ Cell Dose, $\leq 5 \times 10^6$ (N = 77)	$CD34^+$ Cell Dose 5-8 $\times 10^6$ (N = 144)	*P* Value
Patient information				
Age, yr, median, (range)	52 (18-70)	53 (19-69)	51 (18-70)	.20
Male sex	121 (54)	48 (62)	73 (51)	.09
Baseline diagnosis, n (%)				—
AML	81 (37)	32 (42)	49 (34)	
ALL	39 (18)	10 (13)	29 (20)	
MDS/CMML	38 (17)	17 (22)	21 (15)	
MPN	13 (6)	4 (5)	9 (6)	
NHL/CLL	30 (13)	5 (6)	25 (17)	
Other	20 (9)	9 (12)	11 (7)	
DRI high-very high, n (%)	47 (21)	18 (23)	29 (20)	.57
KPS 60-80, n (%)	51 (23)	17 (22)	34 (24)	.77
HCT-CI score >3, n (%)	49 (22)	15 (19)	34 (24)	.48
Donor information				
Age, yr, median (IQR)	34 (26-44)	34 (26-45)	35 (26-43)	.44
Male sex, n (%)	121 (55)	48 (62)	73 (51)	.03
Female donor to male recipient, n (%)	37 (17)	20 (28)	17 (12)	.007
Donor type, n (%)				
HLA MSD	22 (10)	6 (8)	16 (11)	.52
10/10 HLA MUD	83 (38)	28 (36)	55 (38)	
7/8 HLA MMUD	73 (33)	30 (39)	43 (30)	
Haploidentical	43 (19)	13 (17)	30 (21)	
Donor/recipient CMV status, n (%)				
Positive/positive	106 (48)	35 (45)	71 (49)	.59
Negative/positive	76 (34)	29 (38)	47 (32)	
Positive/negative	19 (8)	5 (6)	14 (10)	
Negative/negative	20 (9)	8 (10)	12 (8)	
Graft information				
$CD34^+$ cell dose, $\times 10^6$, median (IQR)	5.7 (4.5-7.0)	4 (3.6-4.5)	7.5 (5.8-7.5)	—
$CD3^+$ cell dose, $\times 10^6$, median (IQR)	252 (184-340)	254 (184-359)	248 (184-327)	.45
Type of stem cell product, n (%)				
Fresh	169 (76)	58 (75)	111 (77)	.76
Cryopreserved	52 (23)	19 (25)	33 (23)	
Transplantation information, n (%)				
Conditioning regimen				
MAC	90 (41)	28 (36)	62 (43)	.49
FluBu 4 days	51 (23)	21 (27)	30 (21)	
FluTBI 12 Gy	39 (18)	7 (9)	32 (22)	
RIC	131 (59)	49 (64)	82 (57)	
FluBu 2-3 days	74 (33)	30 (39)	44 (31)	
FluBu 2-8 Gy	22 (10)	7 (9)	15 (10)	
FluMel	14(6)	6 (8)	8 (6)	
IDA-FLAG/Mel (sequential)	8 (4)	4 (4)	4 (3)	
Flu/CFM/TBI 2 Gy (Baltimore)	13 (6)	2 (3)	11 (7)	
Follow-up, mo, median (IQR)	24 (9-50)	21 (6-41)	25 (11-55)	.06

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasms; NHL, non-Hodgkin lymphoma; CLL, chronic lymphoblastic leukemia; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; CMV, cytomegalovirus; Flu, fludarabine; Bu busulfan; TBI, total body irradiation; Mel, melphalan; IDA-FLAG, idarubicin, fludarabine, cytarabine, granulocyte colony-stimulating factor; CFM, cyclophosphamide.

shorter median times to neutrophil engraftment (19 days versus 21 days; $P = .002$) and platelet engraftment (16 days versus 22 days; $P = .04$) compared with those receiving low-dose grafts (Table 2, Figure 1). Four alloHSCT recipients (1.8%) experienced primary GF, including 1 patient with an HLA-matched donor, 2 patients with an MMUD, and 1 patients with a haploidentical donor. The cumulative incidence of GF was 4% for patients in the low-dose group and .7% for those in the high-dose group ($P = .08$) (Table 2).

The cumulative incidences of $CD4^+$ and $CD8^+$ lymphocyte recovery at 1 year were similar in the high-dose and low-dose groups (58% versus 55% [$P = .2$] and 64% versus 63% [$P = .4$], respectively). The infusion of $\leq 5 \times 10^6$/kg $CD34^+$ cells was not associated with worse $CD4^+$ and $CD8^+$ cell recovery (hazard ratio [HR], .83 [$P = .36$] and .88 [$P = .53$], respectively). The $CD3^+$ cell dose had no significant effect on hematopoietic recovery or immune reconstitution.

Table 2
Main Post-Transplantation Outcomes According to $CD34^+$ Cell Dose

Post-Transplantation Outcome	$CD34^+$ Cell Dose $\leq 5 \times 10^6$	$CD34^+$ Cell Dose 5-8 $\times 10^6$	*P* Value
Engraftment			
Days to neutrophil engraftment, median (IQR)	21 (18-25)	19 (16-22)	**.002**
Days to platelet engraftment, median (IQR)	22 (13-30)	16 (12-24)	**.04**
Cumulative incidence of primary graft failure, % (95% CI)	4 (1-10)	.7 (.1-3)	.08
Cumulative incidence of GVHD, % (95% CI)			
Day +100 grade II-IV aGVHD	23 (15-33)	25 (18-32)	.70
Day +100 grade III-IV aGVHD	4 (1-10)	5 (2-10)	.45
2-year moderate/severe cGVHD	6 (2-14)	9 (5-16)	.55
Infections, n (%)			
Bacterial infection	41 (53)	73 (50)	.71
CMV reactivation	43 (56)	77 (53)	.66
Other viral infections	30 (39)	66 (46)	.32
Fungal infection	12 (15)	12 (8)	.09
1-year NRM, % (95% CI)*	21 (13-31)	13 (8-20)	.18
1-year cumulative incidence of relapse, % (95% CI)	21 (13-31)	19 (13-26)	.50
Survival outcomes, % (95% CI)*			
2-year OS	64 (52-74)	71 (62-78)	**.04**
2-year RFS	52 (40-62)	59 (50-66)	.07

Significant *P* values are in bold type.
* Estimated probability.

GVHD

The cumulative incidence of aGVHD by day +100 was similar in high-dose and low-dose groups: grade II-IV, 25% versus 23% (HR, .89; *P* = .7); grade III-IV, 5% versus 4% (HR, .6; *P* = .4) (Table 2, Figure 2). There was no between-group difference in the cumulative incidence of 2 year moderate-severe cGVHD (9% versus 6%; HR, .7; *P* = .5) (Table 2, Figure 2). There was no statistically significant correlation between infused $CD3^+$ cell dose and the development of GVHD.

NRM, Relapse, and Infections

The cumulative incidence of NRM at 1 year was 13% for patients in the high-dose group compared with 21% for those in the low-dose group (*P* = .18) (Table 2, Figure 3). The analysis of NRM according to donor type and $CD34^+$ cell dose showed no differences between the high-dose and low-dose groups in MSD, MUD, and MMUD alloHSCT, whereas a trend toward a higher NRM was observed in recipients of haploidentical donor alloHSCT receiving low-dose $CD34^+$ cell grafts (39% versus 13%; *P* = .05) (Table 3, Figure 4). Only older age and a KPS of 60 to 80 were identified as significant risk factors for NRM in the univariate and multivariate analyses (Table 3). In the high-dose group, the causes of death without relapse were bacterial septic shock (n = 8), aGVHD (n = 4), infectious or toxic leukoencephalopathy (n = 2), thrombotic microangiopathy (n = 1), melanoma (n = 1), and lung neoplasia (n = 1). In the low-dose group, 18 non-relapse-related deaths were recorded due to bacterial septic shock (n = 12), neurotoxoplasmosis

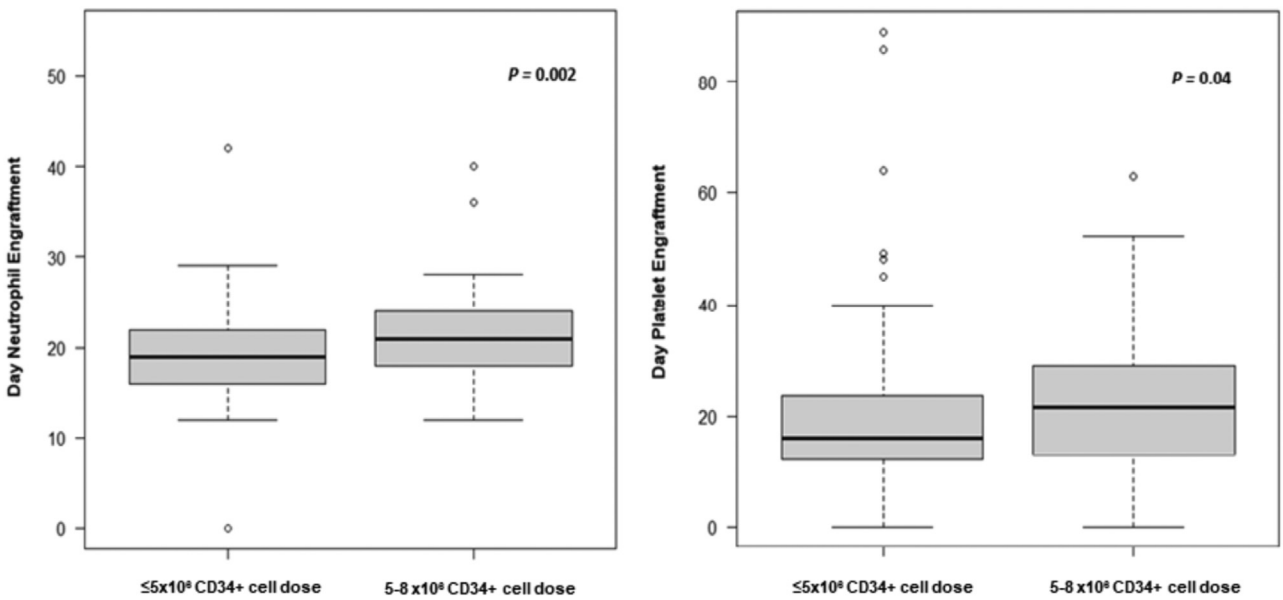

Figure 1. Times to neutrophil and platelet engraftment.

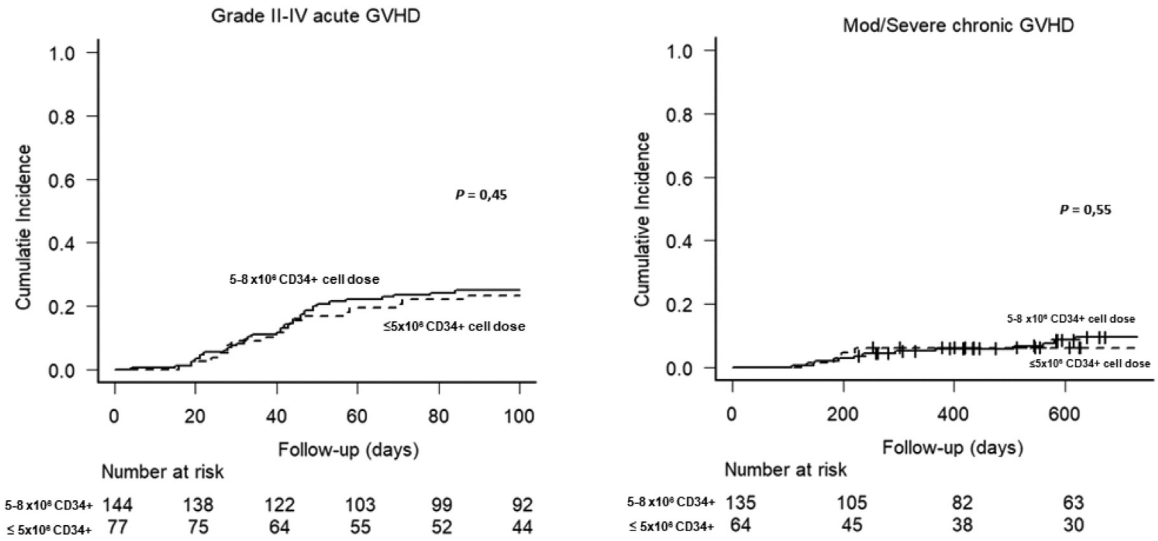

Figure 2. Cumulative incidences of acute and chronic GVHD according to CD34$^+$ cell dose.

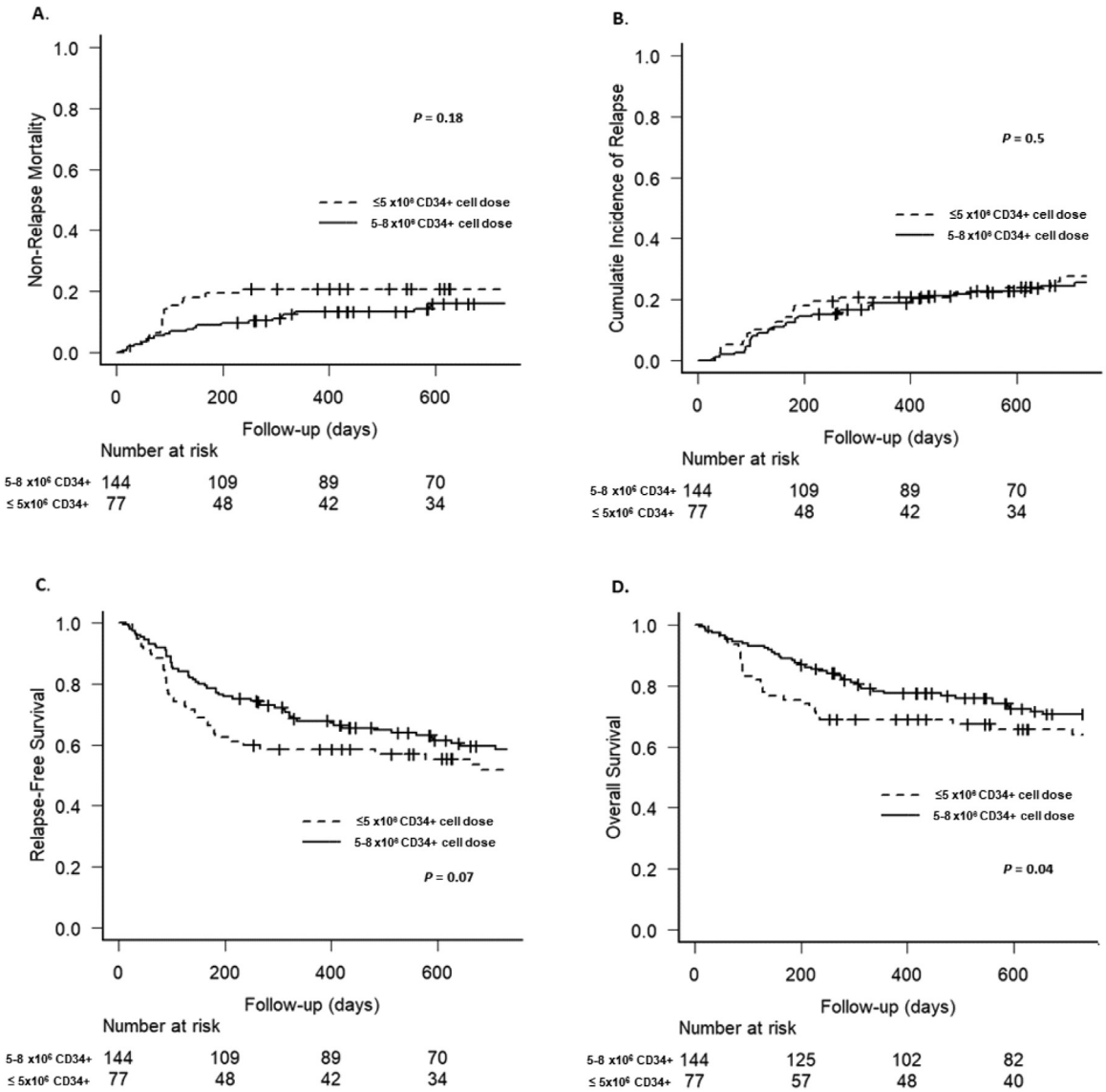

Figure 3. Main post-transplantation outcomes according to CD34$^+$ cell dose. (A) NRM; (B) CIR; (C) RFS; (D) OS.

Table 3
Main Post-Transplantation Outcomes According to Donor Type and $CD34^+$ Cell Dose

Post-Transplantation Outcomes	$CD34^+$ Cell Dose $\leq 5 \times 10^6$	$CD34^+$ Cell Dose 5-8 $\times 10^6$	P Value
MSD and MUD transplantation, % (95% CI)			
1-yr NRM	15 (5-29)	11 (5-20)	.39
1-yr relapse rate*	21 (9-36)	26 (16-36)	.45
2-yr OS	70 (50-84)	69 (56-79)	.72
2-yr RFS	57 (38-72)	52 (39-64)	.91
MMUD transplantation, % (95% CI)			
1-yr NRM	20 (8-36)	16 (7-29)	.83
1-yr relapse rate*	17 (6-32)	16 (7-29)	.56
2-yr OS	67 (47-81)	71 (55-83)	.98
2-yr RFS	63 (44-78)	67 (51-79)	.69
Haploidentical transplantation, % (95% CI)			
1-yr NRM	39 (13-64)	13 (4-28)	**.05**
1-yr relapse rate*	46 (17-72)	11 (3-25)	**.02**
2-yr OS	39 (14-63)	83 (64-92)	**<.001**
2-yr RFS	15 (3-39)	76 (56-88)	**<.001**

Significant *P* values are in bold type.
* Cumulative incidence.

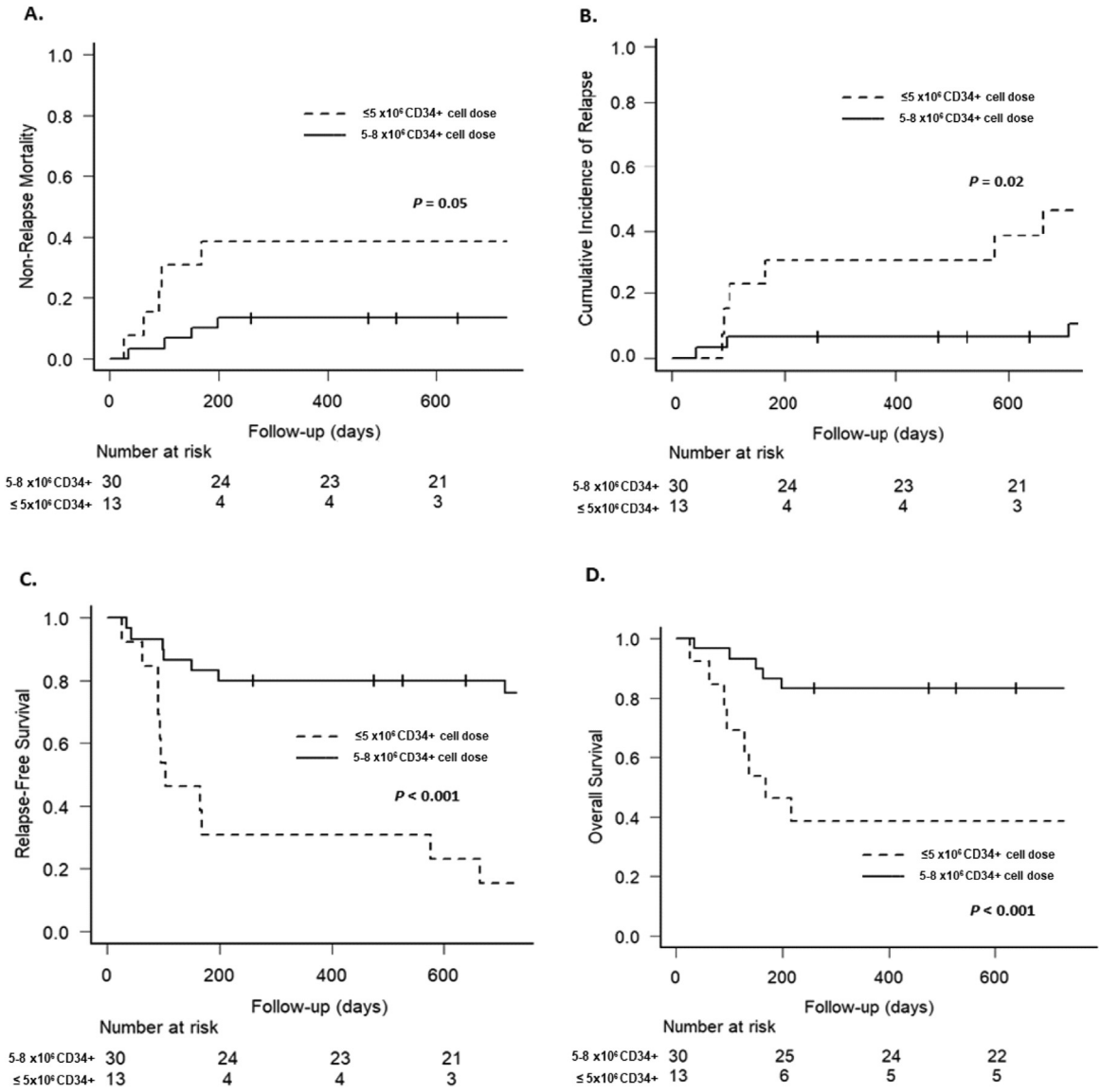

Figure 4. Main outcomes after haploidentical alloHSCT according to $CD34^+$ cell dose: (A) NRM; (B) CIR; (C) RFS; (D) OS.

(n = 1), severe aGVHD (n = 2), cryptogenic pneumonia (n = 2), and myocardial infarction (n = 1).

Cumulative incidence of relapse (CIR) rate was similar between groups (19% for high-dose vs. 21% for low-dose, P=.5) (Table 2, Figure 3). The analysis of CIR according to donor type and CD34+ cell dose showed that, on the contrary to other type of donors, the use of haploidentical donors with low-dose CD34+ cells was associated with higher CIR (46% vs. 11%, P=.02) (Table 3, Figure 4). In univariate analysis, older age, KPS of 60-80%, and high-very high DRI were associated with higher risk of relapse (Table 4). The impact of age and performance status on CIR was confirmed in multivariate analysis (Table 4).

Cytomegalovirus reactivation occurred in 40 of 83 (48%) seropositive patients in the high-dose group and in 18 of 40 (45%) seropositive patients in the low-dose group (P = .6). The incidence of other viral infections was also similar in the 2 groups (55% versus 54%; P = .5). There also were no significant differences between the groups in the rate of bacterial infections (50% versus 52%; P = .7) or fungal infections (12% versus 22%; P = .6).

OS and RFS

One hundred and forty-three patients (65%) were alive at the time of this report, with a median follow-up for all patients of 2 years (IQR, .7 to 4.1 years). At 2 years post-transplantation, OS and RFS for the entire cohort were 68% (95% CI, 61% to 74) and 56% (95% CI, 49% to 63%), respectively. OS was significantly higher in the high-dose group compared with the low-dose group (71% versus 64%; P = .04) (Table 2, Figure 3). Nevertheless, when this analysis was conducted according to donor type, the negative impact of low $CD34^+$ cell dose on post-transplantation outcomes was observed only in the haploidentical alloHSCT setting (83% versus 38%; $P < .001$) (Table 3, Figure 4). Considering these results, a multivariate regression model was created using $CD34^+$ cell dose ($\leq 5 \times 10^6$ versus >5 to 8×10^6), donor type (haploidentical versus others), and the product of the 2 ($CD34^+$ cell dose and donor type) as explanatory variables. The estimated coefficient of the product variable compared the effect on the risk of OS from receiving grafts with low cellularity between recipients of haploidentical donor grafts and recipients of grafts from other donor types. As reported in Table 4, haploidentical alloHSCT with a low $CD34^+$ cell dose resulted in a statistically significant worse OS (HR, 6.01; P = .006). However, the infusion of low $CD34^+$ cell dose-containing grafts in alloHSCT performed from MSDs, MUDs, and MMUDs did not have a statistically significant effect on OS (HR, 1.15; P = .59) (Supplementary Tables S1 and S2). In addition, older age, KPS 60 to 80, Hematopoietic Cell Transplantation Comorbidity Index >3, and high-very high DRI also were associated with lower OS.

A trend toward a better RFS also was observed in the high-dose group (59% versus 52%; P = .07) (Table 2, Figure 3). Similar to OS, the adverse effect of low doses of $CD34^+$ cells on RFS was observed only with the use of haploidentical donors (HR, 4.57; P = .004), and these differences were not statistically significant when low-dose grafts were included outside of the haploidentical alloHSCT setting (HR, 1.04; P = .85) (Table 3, Figure 4, Supplementary Figures 1 and 2). Other variables statistically significantly associated with worse RFS were old age, KPS 60 to 80, high-very high DRI, and nadir of neutropenia >19 days (Table 4). We observed no significant effect of infused $CD3^+$ cell dose on OS or RFS outcomes.

DISCUSSION

Our present results show that the impact of $CD34^+$ cell dose on PBSC PTCy alloHSCT survival outcomes depends on the type of donor. Administration of a $CD34^+$ cell dose $\leq 5 \times 10^6$/kg with haploidentical PBSC PTCy HSCT was associated with decreased OS and RFS. This negative effect was not observed when other types of donors were used. A high $CD34^+$ cell dose was associated with faster neutrophil and platelet recovery in all alloHSCT recipients independent of donor type, consistent with prior studies [17].

Several studies have analyzed the effect of $CD34^+$ cell dose on the outcome after PBSC alloHSCT using standard, non-PTCy GVHD prophylaxis and have reported inconsistent conclusions [18–25]. Some of these studies, using different $CD34^+$ cell dose thresholds, showed that higher doses are associated with higher rates of cGVHD, NRM, and relapse [9–13]. More recently, several studies evaluated the effect of $CD34^+$ cell dose on outcomes after PTCy alloHSCT, including 2 studies of haploidentical transplantation [26,27]. The European Society for Blood and Marrow Transplantation (EBMT) published a retrospective registry-based study of 414 haploidentical alloHSCT for acute myelogenous leukemia, including PTCy-based or ATG-based GVHD prophylaxis [26]. As in our study, patients were divided into 2 cohorts based on $CD34^+$ dose with a cutoff point of 4.96×10^6/kg. The median $CD34^+$ cell dose was 6.58×10^6/kg (range, 2.2 to 31.2×10^6). Patients in the high-dose group ($>4.96 \times 10^6$/kg) experienced higher rates of neutrophil and platelet engraftment and a shorter median time to engraftment compared with those in the low-dose group. $CD34^+$ cell dose did not impact the development of aGVHD or cGVHD. Patients who received $>4.96 \times 10^6$/kg $CD34^+$ cells had prolonged survival, owing mainly to a reduced NRM rate. There was no impact of the type of GVHD prophylaxis, PTCy versus ATG, on survival outcomes in this study. Our results using a virtually identical cutoff were similar to those of the EBMT study for haploidentical transplant recipients. Salas et al. [27] explored the impact of $CD34^+$ cell dose in 68 PBSC haploidentical HSCT recipients using RIC and PTCy plus ATG. Patients were divided into 2 groups using a $CD34^+$ cell dose $\geq 9 \times 10^6$/kg as a threshold. In this study, the median infused $CD34^+$ cell dose was 9.32×10^6/kg (range, 3.4 to 28.6×10^6/kg). The authors reported that the infusion of higher-cellularity grafts ($\geq 9 \times 10^6$/kg $CD34^+$ cells) had a significant adverse impact on OS and nonsignificant trends toward worse NRM and RFS. No statistically significant differences in outcomes were seen when other exploratory cutoff values of $CD34^+$ cells were considered (7×10^6/kg, 8×10^6/kg, and 10×10^6/kg); however, the impact of low $CD34^+$ cell doses ($<5 \times 10^6$/kg) was not specifically analyzed. In our study, the maximum $CD34^+$ cell dose was capped at 8×10^6/kg in accordance with our institutional protocol based on previously published data that associated high $CD34^+$ cell dose with worse post-transplantation outcomes in alloHSCT using conventional GHVD prophylaxis [9,10,13,28], and thus we could not analyze the impact of doses $>8 \times 10^6$/kg on outcomes of transplantation.

Regarding other type of donors, Elmariah et al. [17] retrospectively analyzed the effect of cell dose on outcomes of 144 adult recipients of PBSC alloHSCT with PTCy-based GVHD prophylaxis for a hematologic malignancy. Infused $CD34^+$ cell doses were stratified into low-dose ($\leq 5 \times 10^6$/kg), intermediate-dose (5 to 10×10^6/kg), and high-dose ($>10 \times 10^6$/kg) groups. Similar to our study, $CD34^+$ cell dose had no significant impact on aGVHD, cGVHD, or relapse. However, in contrast to our findings, their multivariate analysis showed worse NRM,

Table 4
Univariate and Multivariate Models: Risk Factors for OS, NRM, RFS, and CIR

Variables	OS, Cox Regression Model		NRM, Fine-Gray Proportional Hazards Regression Model		RFS, Cox Regression Model		CIR, Fine-Gray Proportional Hazards Regression Model	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Univariate regression analysis								
$CD34^+$ cell dose								
Continuous	.91 (.79-1.05)	.21	.89 (.71-1.16)	.32	.90 (.79-1.23)	.12	.89 (.72-1.12)	.32
$\leq 5 \times 10^6$ (vs 5-8 $\times 10^6$)	1.56 (.99-2.44)	**.05**	1.53 (.81-2.88)	.18	1.43 (.95-2.13)	**.08**	1.53 (.81-2.88)	.18
CD3 cell dose								
Continuous	1.01 (.99-1.01)	.46	1 (.99-1.01)	.88	1.0 (.99-1.01)	.94	1.00 (.99-1.00)	.88
Age								
Continuous	1.02 (1.01-1.03)	**.01**	1.03 (1.01-1.05)	**.02**	1.02 (1.01-1.03)	**.008**	1.03 (1.00-1.06)	**.03**
Age >60 yr	2.47 (1.58-3.86)	<**.001**		**.001**		<**.001**	2.82 (1.49-5.33)	**.001**
HCT-CI score			2.82 (1.49-5.36)		2.13 (1.43-3.16)			
>3 (vs 0-3)	1.76 (1.08-2.87)			.57			1.23 (.61-2.48)	.57
KPS		**.02**	1.22 (.60-2.48)		1.49 (.95-2.34)	.080		
80-60 (vs 100-90)	2.07 (1.29-3.33)			<**.001**			2.97 (1.58-5.60)	<**.001**
DRI		**.002**				**.002**		
High-very high (vs low-intermediate)	2.19 (1.35-3.54)	**.001**	2.97 (1.57-5.60)	**.05**	1.94 (1.26-2.97)	**.004**	1.89 (.99-3.62)	**.05**
Conditioning intensity								
RIC (vs MAC)	1.22 (.78-1.90)						1.06 (.57-1.98)	
Grouped donors		.37	1.89 (.99-3.62)	.86	1.88 (1.22-2.91)	.20		.86
Haplo (vs others)	.95 (.54-1.67)						1.25 (.59-2.66)	
Donor age		.87		.55		.79		.55
Continuous	1.00 (.98-1.02)						.99 (.96-1.02)	
≥50 yr	.81 (.41-1.58)	.60	1.05 (.56-1.97)	.41	1.29 (.87-1.91)	.06	.66 (.23-1.87)	.41
Female donor to male recipient		.54		.43		.54		.43
Yes (vs no)	1.21 (.69-2.13)		1.25 (.59-2.66)		.93 (.56-1.54)		.72 (.28-1.85)	
Type of stem cell product		.49		.30		.77		.49
Cryopreserved (vs fresh)	.70 (.36-1.35)						.63 (.26-1.51)	
Nadir neutropenia >19 d		.29	.98 (.96-1.01) .65 (.23-1.86)	.49	1.01 (.99-1.03) 1.18 (.69-2.02)	.41		.30
Yes (vs no)	1.58 (1.01-2.48)	.60	.67 (.26-1.51) .71 (.28-1.84) 1.38 (.73-2.59)	.31	1.08 (.64-1.82) .79 (.47-1.35) 1.76 (1.18-2.63)	**.005**	1.38 (.74-2.59)	.31
Multivariate regression analysis								
CD34 cell dose					1.01		1.01	
$\leq 5 \times 10^6$ (vs 5-8 $\times 10^6$)	1.15 (.67-1.98)	.59	1.34 (.64-2.82	.43	1.04 (.65-1.68)	.85	1.27 (.60-2.68)	.52
Donor type					1.01		1.01	
Haploidentical (vs others)	.62 (.24-1.62)	.33		.56	1.01	.31	1.37 (.43-4.35)	.59
Donor type and cell dose count			1.40 (.44-4.43)		.67 (.31-1.44) 1.06		1.01	
Haplo receiving $\leq 5 \times 10^6$ (vs others)	6.01 (1.76-2.49)	**.004**	1.91 (.43-8.36)	.39	4.57 (1.59-3.14)	**.004**	1.90 (.42-8.40) 1.01	.40
Age					1.01		1.01	

(*continued*)

progression-free survival, and OS in the low-dose group compared with the intermediate-dose group for the entire series of patients independent of donor type. Although the proportion of nonhaploidentical alloHSCT recipients with a low $CD34^+$ cell dose was clearly higher in our study than in the Elmariah et al. study (58% versus 8%), we found no negative effect on survival outcomes in that group of patients. Other differences between the studies, such as the type and intensity of conditioning regimens or GVHD prophylaxis schedule, might have contributed to these discrepant findings.

The main limitation of our study is its retrospective nature and the limited number of haploidentical alloHSCT recipients in the low-dose group. Nonetheless, we emphasize that although the statistical impact of this small sample size would be a higher likelihood of type II error (false-negative), we were still able to detect significant differences in outcomes between the low-dose and high-dose group. Moreover, our results are in line with those published by Maffini et al. [26] in a large series of haploidentical transplantations from the EBMT database. Finally, the other available study on this subject showed similar results even with a haploidentical population with a lower $CD34^+$ cell dose than ours (n = 5) [17]. Our statistical analysis supports the findings of these 2 previous studies suggesting that the $CD34^+$ dose should not be $<5 \times 10^6$/kg when using haploidentical donors. We also have shown that in MSD, MUD, and MMUD transplantations, the infused $CD34^+$ cell dose had no impact on survival after transplantation. However, considering the benefit of the infusion of a high $CD34^+$ cell dose on engraftment, we also suggest that administration of no less than 5×10^6/kg $CD34^+$ cells could be beneficial in all PBSC PTCy alloHSCT recipients regardless of donor type.

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Authorship statement: C.M., A.P., and M.Q.S. conceived and designed the study; A.P. collected and assembled data; C.M., A.P., and M.Q.S. analyzed and interpreted the data; and A.P. and C.M. wrote the manuscript. All coauthors are physicians at our center, who performed the transplantations, took care of the patients, made significant contributions to the discussion of the results, and participated substantively in the writing of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.12.005.

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Table 4 (*Continued*)

Variables	OS, Cox Regression Model		NRM, Fine-Gray Proportional Hazards Regression Model		RFS, Cox Regression Model		CIR, Fine-Gray Proportional Hazards Regression Model	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
≥60 yr	1 (1.01 - 1.03)	**.03**	1.02 (1.0-1.05)	**.01**	1.01(1.01-.03)	**.041**	1.02(1.01-.01)	**.02**
KPS					1.01			
80-60 (vs 100-90)	1.97 (1.21-3.21)	**.006**		**.001**		**.007**	2.87 (1.51-.47)	**.001**
DRI			2.92 (1.53-5.55)		1.81 (1.17-2.82)			
High-very high (vs low-intermediate)	1.95 (1.20-3.18	**.007**		-		**.014**	1.59 (.80-3.15)	.18
HCT-CI score								
>3 (vs 0-3)	1.72 (1.04-2.85)	**.03**	-	-	1.73 (1.11-2.70)	.100	-	-
Nadir neutropenia >19 d	-	-	-	-	1.46 (.92-2.32)	**.032**	-	-
Yes (vs no)			.		1.58 (1.04-2.40)			

Significant *P* values are in bold type.

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6.3. La puntuación EASIX se correlaciona con los biomarcadores de disfunción endotelial y predice el riesgo de enfermedad injerto contra receptor tras el trasplante alogénico.

"EASIX score correlates with endothelial dysfunction biomarkers and predicts risk of acute graft-versus-host disease after allogeneic transplantation."

Diferentes biomarcadores plasmáticos de disfunción endotelial se han postulado para el diagnóstico y pronóstico de la EICRa. Sin embargo, su uso aún no está validado en la práctica clínica habitual. El llamado índice de estrés y activación endotelial (E*ndothelial Activation and Stress Index*, EASIX), calculado con una fórmula matemática sencilla basada en parámetros de laboratorio de uso rutinario, se considera un marcador indirecto del daño endotelial. Un valor elevado de EASIX se ha relacionado con una peor MRT y SG en los pacientes con EICRa y con un alto riesgo de SOS y MAT-AT. Este estudio investiga los cambios post-trasplante del EASIX y de tres biomarcadores plasmáticos conocidos de daño endotelial y su capacidad de predecir el riesgo de desarrollar EICRa.

Se analizaron los niveles VCAM-1, TNFR1, VWF:Ag y la puntuación de EASIX antes del aloTPH y en los días 0, +3, +7, +14 y +21 después del trasplante en una cohorte experimental de pacientes (n=33). EASIX se transformó a un logaritmo de base 2 para facilitar el análisis. Para los biomarcadores más relevantes, se estimaron los valores de corte óptimos y la capacidad discriminatoria para diferenciar a los pacientes con alto riesgo de EICRa de los de bajo riesgo. Las conclusiones obtenidas en la cohorte experimental respecto a EASIX se validaron en una cohorte más amplia de 321 pacientes en la misma institución.

En la cohorte experimental, los biomarcadores plasmáticos y el EASIX mostraron una dinámica post-trasplante similar y progresivamente ascendente. El análisis multivariado mostró una asociación entre los niveles elevados de TNFR1 y Log2-EASIX medidos el día 7 post-trasplante y un mayor riesgo de desarrollar EICRa (HR=1, p=0,002 y HR=2,31, p=0,013, respectivamente). Los pacientes con TNFR1 ≥1.300 ng/mL (HR =7,19, p=0,006) y Log2-EASIX ≥3 (HR=14,7, p<0,001) en el día +7

tuvieron más probabilidades de desarrollar EICRa con una precisión predictiva elevada (C-*index* del 74% y 81%, respectivamente).

En la cohorte de validación, los pacientes con Log2-EASIX ≥3 el día +7 presentaron una incidencia significativamente mayor de EICRa grado II-IV (HR=1,94, *p*=0,004) independientemente de la profilaxis para la EICR (HR=0,38, *p*=0,004), del régimen de acondicionamiento (HR=0,59, *p*=0,02) y del tipo de donante (HR=2,38, *p*=0,014).

En conclusión, el daño endotelial puede medirse en el post-trasplante temprano utilizando tanto el EASIX como los biomarcadores plasmáticos. Una puntuación elevada de EASIX podría identificar de forma sencilla y temprana a los pacientes con alto riesgo de desarrollar EICRa.

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Full Length Article
Biomarkers

Easix Score Correlates With Endothelial Dysfunction Biomarkers and Predicts Risk of Acute Graft-Versus-Host Disease After Allogeneic Transplantation

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A B S T R A C T

Plasma biomarkers of endothelial dysfunction have been postulated for the diagnosis and prognosis of acute graft-versus-host disease (aGVHD). However, their use is not validated in clinical practice yet. The endothelial activation and stress index (EASIX), a simple score based on routine laboratory parameters, is considered to be an indirect marker of endothelial damage. High value of EASIX was correlated with worse non-relapse mortality (NRM) and overall survival (OS) and a high risk of sinusoidal obstructive syndrome and transplant-associated thrombotic microangiopathy (TA-TMA). This study investigates the predictive value of plasma biomarkers and the EASIX score for the prediction of aGVHD. We assessed vascular cell adhesion molecule-1 (VCAM-1), tumor necrosis factor receptor 1 (TNFR1), and VWF:Ag plasma levels and the EASIX score before allogeneic hematopoietic stem cell transplantation (allo-HSCT) and on days 0, 3, 7, 14, and 21 in an experimental cohort (n = 33). EASIX was transformed to a base-2 logarithm to perform the analysis. For the most relevant biomarkers, we estimate the optimal cutoff values and the discriminatory ability to differentiate patients with high-risk of aGVHD. The conclusions obtained in the experimental cohort were validated in a large cohort of 321 patients at the same institution. Plasma biomarkers and EASIX showed similar post-transplantation dynamics consisting of a progressive increase.

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Multivariate analysis showed an association between high TNFR1 levels and Log-2 EASIX score on day 7 after transplantation with an increased likelihood of developing aGVHD (hazard ratio [HR] = 1, P = .002; HR = 2.31, P = .013, respectively). Patients with TNFR1 ≥1300 ng/mL (HR = 7.19, P = .006) and Log2-EASIX ≥3 (HR = 14.7, P < .001) at day 7 after transplantation were more likely to develop aGVHD with high predictive accuracy (C-index of 74% and 81%, respectively). In the validation cohort, patients with Log2-EASIX ≥3 on day 7 after transplantation presented a significantly higher incidence of grade II-IV aGVHD (HR = 1.94, P = .004) independent of GVHD prophylaxis (HR = 0.38, P = .004), conditioning regimen (HR = 0.59, P = .02) and type of donor (HR = 2.38, P = .014). Differential degree of endothelial damage can be measured using both EASIX score and plasma biomarkers in the early post-transplantation period. Patients at risk of developing aGVHD could be easily identified by a high EASIX score. Both indicators of endothelial activation represent a promising approach to predict aGVHD.

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Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Diagnosis of acute GVHD (aGVHD) is based on clinical features, patient symptoms, laboratory values, and, in most cases, histological confirmation. In recent years, efforts have been made to identify potential noninvasive peripheral blood biomarkers with diagnostic and clinical value in aGVHD, and some promising results have been achieved with markers related to endothelial damage [1–3].

Similar to other early transplant-related complications such as sinusoidal obstruction syndrome, transplant-associated thrombotic microangiopathy (TA-TMA), or engraftment syndrome (ES), endothelial activation and dysfunction have been implicated in the pathophysiology of aGVHD [4–8]. Several soluble biomarkers of endothelial dysfunction have been postulated for the diagnosis and prognosis of aGVHD. These biomarkers include von Willebrand factor (VWF), thrombomodulin (TM), tumor necrosis receptor 1 (TNFR1), plasminogen activator inhibitor type 1, adhesion molecules such as E-selectin, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1), suppression of tumorigenicity-2 (ST2), angiopoietin-2 (ANG2), hyaluronic acid (HA), L-Ficolin, and circulating endothelial cells (CECs), among others [9–14]. In general, the majority of studies have shown an increase in the levels of the different markers compared to baseline, especially in patients with transplant-related complications, including aGVHD [2,3,15-17]. We have previously reported that levels of VWF and TNFR1 above an optimal cutoff at day 7 after allo-HSCT could positively predict that approximately 90% of patients will develop aGVHD [9]. Some biomarkers are now being incorporated into clinical trials design to validate and standardize their value in current clinical practice. In addition to validation, technical issues, such as adequate sample processing, availability of the tests in all centers, and rapidity of results, may be one of the main limitations to the widespread implementation of these plasma biomarkers in clinical practice.

The endothelial activation and stress index (EASIX) is a simple laboratory formula (creatinine [mg/dL] × LDH [U/L]/platelets [$\times 10^9$/L]) considered an indirect measurement of endothelial activation. A high EASIX score before and after allo-HSCT has been correlated with worse nonrelapse mortality (NRM) and overall survival (OS) [1,18-22] and a high risk of post-transplantation vascular endothelial complications such as sinusoidal obstruction syndrome and TA-TMA [23,24]. However, its role in predicting aGVHD has not been extensively investigated.

Based on the ability of EASIX to predict early post-transplantation vascular endothelial complications and the lack of easily applicable biomarkers for predicting aGVHD in the allo-HSCT setting, the present study investigates whether the measurement of endothelial activation biomarkers and EASIX during the early post-transplantation period can predict the onset of aGVHD.

METHODS

Study Design

We hypothesized that the dynamics of the EASIX score in the early post-transplantation period would be similar to the dynamics of endothelial damage plasma biomarkers; therefore it could be used for prediction of clinically relevant aGVHD. The present study was conducted in 2 phases. In the first part, the dynamics plasma

biomarkers of endothelial damage and EASIX score were measured at different time points before and after allo-HSCT in an experimental cohort of 33 allo-HSCT recipients using peripheral blood stem cell grafts and reduced-intensity conditioning (RIC) regimens. Then the potential association between the plasma biomarkers levels and EASIX score with the risk of developing grade II-IV aGVHD was evaluated.

In the second part of the study, we investigated whether EASIX score results in predicting aGVHD were reproducible in a larger validation cohort of 321 patients undergoing allo-HSCT at the same institution. Because endothelial dysfunction after allo-HSCT can be induced by RIC regimens, but more commonly by myeloablative conditioning (MAC) regimens, the validation cohort included patients who received RIC and MAC regimens. This was done to investigate whether the results observed in the analysis were independent of the intensity of the conditioning regimen administered as part of the transplant platform [25]. The local ethics committee approved the study (HCB/2021/0948), which was conducted in accordance with the Declaration of Helsinki.

Patients and Allo-HSCT Procedure

Patients suffering from malignant hematologic diseases who consecutively underwent allo-HSCT at the Hospital Clínic of Barcelona between January 2014 and October 2020 were included in the study. To form the experimental cohort, a group of 33 patients with plasma samples available for biomarker analysis at all predefined timepoints of the study before and after transplantation was selected.

The conditioning regimens used were based on the type of hematological malignancy and patient characteristics. Patients aged >50 years or who had previously undergone autologous HSCT received an RIC regimen; otherwise, MAC regimens were administered. A summary of the different types of conditioning regimens used is shown in Table 1.

Our institutional protocol for GVHD prophylaxis historically consisted of conventional calcineurin inhibitor-based GVHD prophylaxis with cyclosporine (5 mg/kg from days −1 to 3 and then 3 mg/kg to day 270 in the absence of GVHD) plus methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11) or mycophenolate mofetil (10 mg/kg every 8 hours, maximum 3 g daily, from day 0 to day 60). Since 2016, it has been progressively introduced PTCY-based schemes (50 mg/kg/day on days 3 and 4) for the prevention of GVHD, initially in transplants from haploidentical and mismatched unrelated donor and more recently in transplants from matched unrelated donor and matched related donor. Antithymocyte globulin or alemtuzumab were not administered.

Donor selection and supportive care have been described in detail previously [26]. All patients included in the study received T-cell–replete peripheral blood stem cell grafts. None of the patients received letermovir for cytomegalovirus prophylaxis. Acute GVHD was clinically and histologically diagnosed and graded using standard criteria [27,28]. Patients with aGVHD were treated homogeneously throughout the study period.

Plasma Biomarker Assessment

The sample size of the experimental cohort was selected in accordance with the previous studies of our group [9,15,29]. Only patients with plasma samples available at all study time points were chosen for the study.

Based on our previously published work, we chose to analyze plasma levels of VCAM-1, TNFR1, and VWF:Ag as biomarkers of endothelial damage [9,15,29]. Individual blood samples were collected prospectively at different time points during allo-HSCT: before conditioning (pre-transplantation), immediately before transplantation (day 0), and on days 3, 7, 14, and 21 after allo-HSCT. Plasma was obtained by centrifugation of anticoagulated blood samples collected in 3.8% sodium citrate tubes, within 4 hours of collection. The samples were aliquoted in cryovials without additives and stored at −80°C until use. The biomarkers VCAM-1 (R&D Systems, Minneapolis, MN), TNFR1 (BosterBio, Pleasanton, CA), and VWF:Ag (Grifols, Barcelona, Spain) were assessed by enzyme-linked immunosorbent assay.

EASIX Score Assessment

The EASIX score was calculated retrospectively in both cohorts of patients according to the following formula: creatinine (mg/dL) × LDH (U/L) / platelets ($\times 10^9$/L). The analytical parameters used to calculate the score were obtained from routine patient blood samples before conditioning (pre-transplantation), immediately before transplantation (day 0), and on days 3, 7, 14, and 21 after allo-HSCT.

Statistical Methods

First, using the experimental cohort data, our study examined the dynamics of VCAM-1, TNFR1, VWF:Ag plasma levels and EASIX score during the

Table 1
Patient and Transplantation Characteristics of Experimental and Validation Cohorts

	Experimental Cohort (N = 33) (%)	Validation Cohort (N = 321) (%)
Patient information		
Age (yr), median (range)	60 (41-69)	52 (18 -70)
Male sex	16 (48)	170 (53)
Baseline diagnosis		
AML	11 (33)	128 (40)
ALL	2 (6)	44 (14)
MDS/CMML	10 (30)	55 (17)
MPN	3 (9)	30 (9)
NHL/CLL	4 (13)	46 (15)
Others	3 (9)	18 (5)
High-very high Disease Risk Index	16 (48)	54 (17)
60%-80% Karnofsky Performance Status	12 (36)	80 (25)
HCT-CI score >3	10 (30)	74 (23)
Transplant information		
Age donor median (y), (range)	41 (19-68)	38 (15-73)
Donor sex		
Male	20 (61)	208 (65)
Female to Male (Donor/recipient)	13 (39)	50 (16)
Donor selection		
HLA MSD	10 (30)	87 (27)
10/10 HLA MUD	13 (40)	125 (39)
7/8 HLA MMUD	10 (30)	68 (21)
Haploidentical	–	41 (13)
Conditioning regimen		
Myeloablative (MAC)		
Cy/Bu 4 days	–	19 (6)
Cy/TBI 12Gy	–	13 (4)
FLUBU 4 days	–	58 (18)
FLU/TBI 12Gy	–	38 (12)
RIC		
FLUBU 2–3 days	26 (79)	116 (36)
FLUTBI 2-8Gy	4 (12)	38 (12)
FLUMEL	–	13 (4)
IDA/FLAG/MEL (sequential)	2 (6)	13 (4)
FLU/CFM/TBI2Gy (Baltimore)	1 (3)	13 (4)
GVHD Prophylaxis		
CNI/MTX	-	48 (15)
CNI/MMF	11 (33)	68 (21)
CNI/RAPA	1 (3)	2 (1)
PTCY/TK	18 (54)	148 (46)
PTCY/TK/MMF	3 (10)	55 (17)
Main post-transplantation outcomes		
Cumulative Incidence of aGVHD (95% CI)		
Grade II-IV acute GVHD (+100)	18.8 (7.5-34)	26.2 (21.5-31.1)
Grade III-IV acute GVHD (+100)	9.1 (2.3-21.9)	7.5 (4.9-10.7)
2-year non-relapse mortality [% (95% CI)]	25.2 (11.6-41.4)	14.9 (11.2-19.1)
2-year Cumulative incidence of relapse, % (95% CI)	31.3 (16.1-47.8)	27.8 (23.0-32.9)
2-year Overall survival, % (95% CI)	49.5 (31.2-65.4)	67.8 (62.3-72.8)
2-year Relapse free survival, % (95% CI)	43.5 (26.0-59.7)	57.3 (51.5-62.6)
Follow-up (mo), median (IQR)	21 (4.1-45.1)	28.8 (9.3-53.8)

HCT-CI indicates hematopoietic cell transplantation comorbidity index; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor.

early post-transplant period and the ability of EASIX and the selected endothelial damage biomarkers to predict grade II-IV aGVHD. Plasma biomarkers and EASIX score results are presented as medians and ranges. To graphically represent the statistical trend in which plasma biomarkers and EASIX score move over time after transplantation with respect to the pretransplantation time point, we used the Stacked Line Chart provided by the Microsoft Excel program. Second, the ability of EASIX to predict grade II-IV aGVHD was evaluated in the 321 adults included in the validation cohort.

EASIX was transformed into a base-2 logarithm (Log2-EASIX) for statistical analysis. Descriptive information was calculated using counts and percentages and compared using the chi-square test, Fisher's exact test, and Mann-Whitney U test. The cumulative incidence function of grade II-IV aGVHD was estimated using cumulative incidence regression analyses and accounting for death and relapse as competing events. The variations of Log^2-EASIX, VCAM-1, TNFR1 and VWF:Ag values during the early post-transplantation period, and their correlation with grade II-IV aGVHD was explored using landmark and regression analyses. Those patients who died or those who were diagnosed with the study time-dependent adverse event before each landmark point were excluded from the next time point.

The association between higher endothelial activation and damage (measured using endothelial damage biomarkers and EASIX score) and grade II-IV aGVHD risk occurring during the first 100 days after allo-HSCT was analyzed using Fine-Gray proportional hazard regression for competing events. This association was investigated treating the explanatory variables as continuous and discrete after estimating the optimal cutoff values for discriminating high-risk patients. Because of the sample size, in the experimental cohort, the variables included in the univariate analysis as predictive of aGVHD were the research variables (plasma biomarkers of endothelial damage and log2-EASIX). A multivariate analysis was performed to investigate whether the predictive ability of a particular biomarker was superior to that of the others. In the validation cohort, in addition to the experimental variable log2-EASIX, all clinical variables classically associated with the risk of developing aGVHD were included in the univariate and multivariate analysis. Last, the accuracy of Log2-EASIX, VCAM-1, TNFR1 and VWF:Ag for predicting grade II-IV aGVHD was explored using the concordance index (C-index). This index confirms the predictive power of the evaluated score for values superior to 0.5 with perfect discrimination of 1. All P values were 2-sided, and $P < .05$ indicated a statistically significant result. SPSS and R software were used to perform statistical analysis.

RESULTS

Patient, Transplant Characteristics, and Outcomes

The baseline characteristics of the patients and the transplant procedure are summarized in Table 1. The experimental and validation cohorts included 33 and 321 patients, respectively. Acute leukemia and myelodysplastic syndrome were the most common pretransplantation diagnoses in both cohorts. All patients in the experimental cohort and 60% of the validation cohort received RIC regimens. Most patients in both groups received allo-HSCT from HLA-identical related and unrelated donors. PCTy-based prophylaxis was used in 64% of the patients included in the experimental cohort and in 63% of the adults included in the validation cohort.

Overall, 32% of patients developed grade aGVHD at a median of 40 days (interquartile range [IQR] = 26-64) after transplantation [40 days for grade II-IV (IQR = 26-59) and 42 days for grade III-IV (IQR = 27-67)]. The cumulative incidences of grade II-IV and grade III-IV aGVHD at day 100 were 18.8% and 9.1% for the experimental cohort, and 26.2% and 7.5% for the validation cohort. The median follow-up of survivors was 21 (range 4.1-45.1) and 28.8 (range 9.3-53.8) months, respectively. For all patients, the 2-year overall survival (OS) and progression-free survival rates were 67.8% (95% confidence interval [CI], 62.3-72.8) and 57.3 (95% CI, 51.5-62.6), respectively.

Association Between Plasma Biomarkers, the EASIX Score, and the Development of Acute GVHD in the Experimental Cohort

Plasma levels of VCAM-1, TNFR1 and VWF:Ag showed an increasing dynamic in the post-transplantation period in all patients from before transplantation until day 21. A graphical representation of the statistical trend in which plasma biomarkers and Log2-EASIX data move over time after transplantation with respect to pretransplantation values is shown in Figure 1. Log^2-EASIX score increased rapidly after transplantation and peaked at day 7 (median Log^2-EASIX 1.64, IQR = 0.53-3.16), followed by a slight decline at days 14 (median Log2-EASIX 1.45, IQR 0.22-

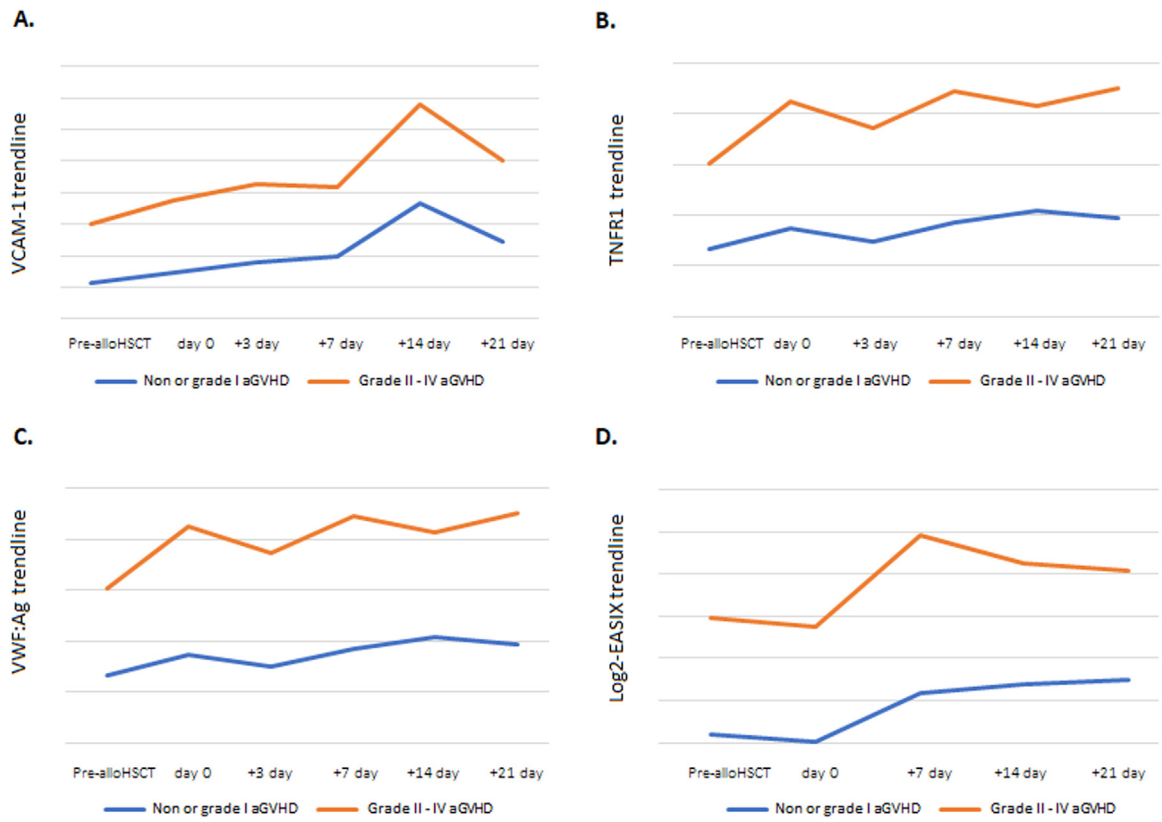

Figure 1. Graphical representation of the statistical trend in which plasma biomarkers and Log2-EASIX data move over time after transplant with respect to pre-transplant values at the experimental cohort: (A) VCAM-1, (B) TNFR1, (C) VWF:Ag, and (D) Log2-EASIX.

2.58) and +21 (median Log^2-EASIX 1.07, IQR = 0.57-1.47), remaining above the pretransplantation baseline (Table 2, Figure 1D).

Patients who developed grade II-IV aGVHD had higher levels of plasma biomarkers and the Log^2-EASIX score than those without aGVHD or grade I aGVHD (Table 2). In the univariate analysis, VCAM-1 levels measured on day 7 and TNFR1 levels measured at all-time points after transplantation were significantly associated with an increased risk of grade II-IV aGVHD (Table 3). Multivariate analysis confirmed the association between high TNFR1 levels on day 7 after transplantation with an increased likelihood of developing grade II-IV aGVHD (hazard ratio [HR] = 1, P = .002). Similarly, the Log^2-EASIX score measured on days 0, 7, 14, and 21 was predictive of grade II-IV aGVHD, with the highest hazard ratio (HR) for day + (HR - 2.31, P = .013) (Table 3).

Considering these results, optimal cutoff values for TNFR1 and Log2-EASIX score on day 7 were estimated to discriminate patients at high risk of developing aGVHD. We found that patients with TNFR1 $\geq$ 1300 ng/mL (HR = 7.19, P = .006) and Log2-EASIX $\geq$ 3 (HR = 14.7, $P < .001$) at day 7 after transplantation were more likely to develop grade II-IV aGVHD with high predictive accuracy (C-index of 74% and 81%, respectively) (Figure 2).

EASIX Dynamics and Log2-EASIX Cutoff Applicability in the Validation Cohort

Similar to the experimental cohort, the Log2-EASIX score increased from pretransplantation levels to a peak on day 7, followed by a slight decrease on days 14 and 21 after transplantation, which remained above baseline (Figure 3A). Patients who developed aGVHD had higher Log^2-EASIX scores throughout the analysis period, with a peak on day 7 after transplantation (Figure 3A). Patients with Log2-EASIX values $\geq$3 on day 7 after transplantation had a significantly higher cumulative incidence of grade II-IV aGVHD than those with low values (HR = 1.82, P = .004) (Table 4, Figure 3B). Moreover, the univariate analysis show that the use of post-transplant high-dose cyclophosphamide–based GVHD prophylaxis (HR = 0.58, P = .009) and RIC regimen (HR = 0.64, P = .037) were associated with a low risk of aGVHD (Table 4). Multivariate analysis confirmed that Log2-EASIX $\geq$3 had a predictive value for aGVHD (HR = 1.98, P = .003) independently of the age (HR = 0.99, P = .54), the type of GVHD

Table 2
VCAM-1, TNFR1, VWF:Ag, and Log2-EASIX Median Values According to Presence of Grade II–IV aGVHD

	Pre-transplantation	Day 0	Day 3	Day 7	Day 14	Day 21
Experimental cohort						
VCAM-1 (ng/mL)						
None or grade I aGVHD (IQR)	58.1 (35.2-120.7)	73.5 (36.3-114)	93 (75-152)	97.9 (45-225.3)	181(50.2-277)	147.5 (55-247.3)
Grade II-IV aGVHD (IQR)	84 (54.8-241.4)	92.9 (61.8-175.3)	117.1 (68.7-149)	104 (40.7-237.7)	169 (51.5-238.5)	123.9 (51.5-198.8)
TNFR1 (ng/mL)						
None or grade I aGVHD (IQR)	658.7 (521.9-745.3)	868.2 (567.4-1019.8)	745.1 (685-862.2)	791.3 (628.4-1212.6)	981 (748-1278.2)	942.7 (794.4-1429.5)
Grade II-IV aGVHD	849.6 (578.2-980.4)	1115.7 (759.6-1488.8)	965.8 (646.3-1254.9)	932.3 (716.2-1481.4)	1140.6 (993.8-1700.5)	1108.3 (937.3-1421.6)
VWF:Ag (ng/mL)						
None or grade I aGVHD (IQR)	114.3 (98.7-167.3)	166.4 (154.7-208.6)	185 (136.2-242.9)	173.1 (112.7-322.5)	237.8 (119.5-402.6)	284.7 (216.1-404.1)
Grade II-IV aGVHD (IQR)	137.1 (111.4-167.1	182.9 (136.1-253.8)	198 (159.4-353.6)	187.4 (146.6-344.2)	194.3 (155.4-350.5)	250.5 (169.9-373)
Log2-EASIX						
None or grade I aGVHD (IQR)	0.33 (−0.48 to 0.56)	0.72 (−0.69 to 0.96)	—	1.49 (0.78-2.58)	1.12 (0.68-2.11)	0.54 (−1.07 to 1.23)
Grade II-IV aGVHD (IQR)	0.37 (-1.09-3.02)	1.18 (0.68-2.72)	—	2.72 (0.23-4.26)	2.41 (0.55-3.33)	1.34 (0.32-2.05)
Validation cohort						
Log2-EASIX						
None or grade I aGVHD (IQR)	0.4 (−0.37 to 1.38)	0.54 (−0.16 to 1.36)	—	2.4 (1.49-3.30)	1.95 (0.81-3.12)	1.95 (0.51-3.22)
Grade II-IV aGVHD (IQR)	0.88 (−0.02 to 1.97)	0.95 (0.14-2.03)	—	2.91 (1.45-3.63)	2.26 (1.02-3.36)	2.01 (0.97-3.52)

Table 3
Predictive Value of VCAM-1, TNFR1, VWF:Ag, and Log2-EASIX for Grade II-IV Acute GVHD in the Experimental Cohort

	Pretransplantation		Day 0		Day 3		Day 7		Day 14		Day 21	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Analysis of the predictive value of VCAM-1, TNFR1, VWF:Ag for grade II-IV aGVHD												
	Univariate Analysis											
VCAM-1 ng/mL	1 (0.99-1)	.90	1 (0.99-1)	.92	1 (0.99-1)	.54	1 (1-1)	<**.001**	0.99 (0.99-1)	.27	0.99 (0.99-1)	.44
TNFR1 ng/mL	0.99 (0.99-1)	.49	1 (0.99-1)	.42	1 (1-1)	**.001**	1 (1-1)	<**.001**	1 (1-1)	<**.001**	1 (1-1)	**.002**
VWF:Ag ng/mL	1 (0.99-1.01)	.62	1 (0.99-1)	.89	1 (0.99-1)	.31	1 (0.99-1.01)	.15	0.99 (0.99-1)	.75	1 (0.99-1)	.99
Multivariate regression analysis of the three plasma biomarkers at day 7												
VCAM-1 ng/mL	—		—		—		0.7 (0.99-1.01)	.97	—		—	
TNFR1 ng/mL	—		—		—		1.01(1.00-1.01)	**.002**	—		—	
VWF:Ag ng/mL	—		—		—		1.01 (0.99-1.01)	.99	—		—	
Analysis of the predictive value of log2-EASIX for grade II-IV aGVHD												
	Univariate Analysis											
Log2-EASIX	1.68 (0.96-2.98)	.06	2.03 (1.14-3.61)	**.01**	—		2.31 (1.19-4.48)	**.013**	1.89 (1.16-3.07)	**.009**	1.64 (1.14-2.37)	**.007**

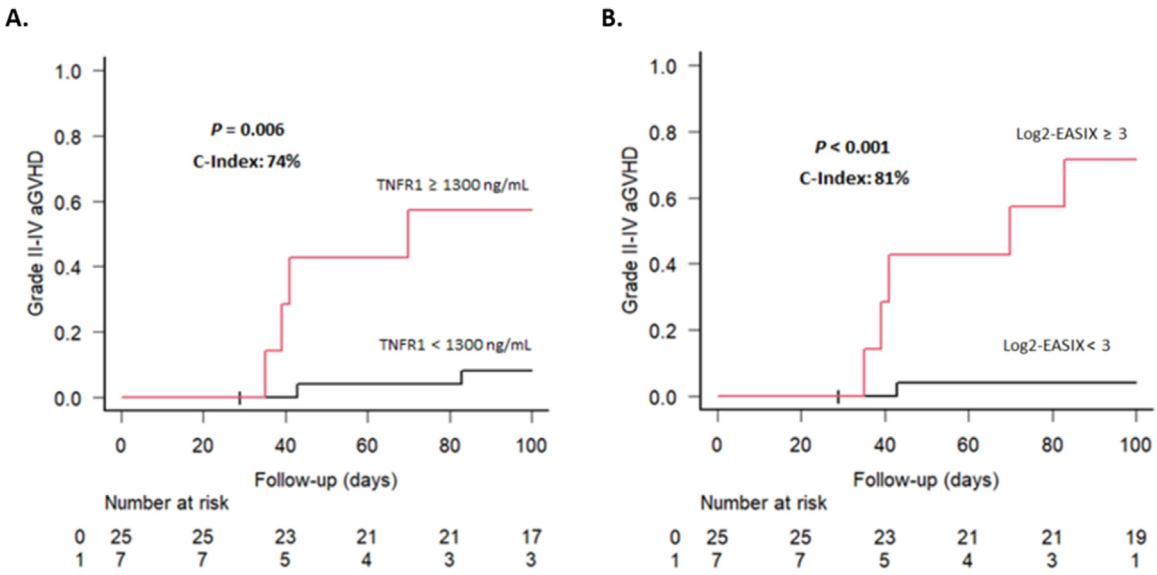

Figure 2. Cutoff value at day 7 to evaluate II-IV aGVHD in the experimental cohort for (A) TNFR1 and (B) Log2-EASIX.

prophylaxis (HR = 0.38, P = .005), intensity of conditioning regimen (HR = 0.65, P = .10), and the type of donor (HR = 2.29, P = .021) (Table 4). Log^2-EASIX values ≥3 on day 7 after transplantation were also significantly associated with higher NRM (HR = 1.18; 95% CI, 1.0-1.3; P = .03), and lower OS (HR = 1.15; 95% CI, 1.0-1.2; P = .01).

DISCUSSION

In the present study we demonstrated that plasma biomarkers of endothelial dysfunction, mainly TNFR1, and the EASIX score showed a parallel post-transplantation dynamic, consisting of a progressive increase through the period of observation after transplantation. Both parameters, TNFR1 levels and EASIX score, were significantly higher in patients who developed grade II-IV aGVHD. We were able to identify a cutoff value of these parameters in an experimental cohort of patients that was significantly associated with an increased risk of developing aGVHD. The predictive value of the EASIX score as a surrogated of endothelial dysfunction in predicting aGVHD was confirmed in a large cohort. Our results suggest that measuring endothelial damage using EASIX score or plasma biomarkers is a promising

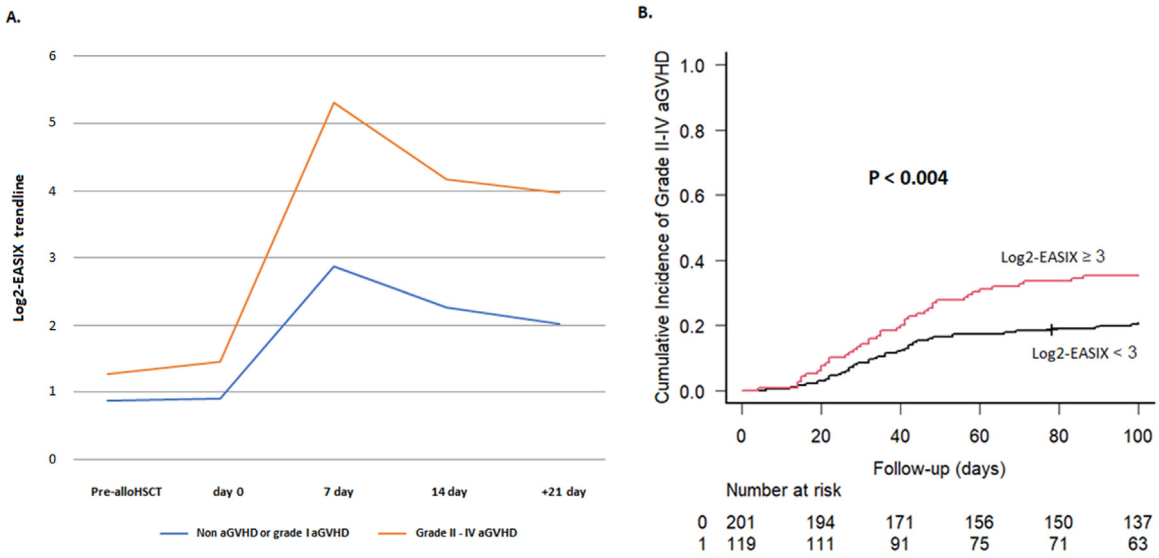

Figure 3. Graphical representation of the statistical trend in which (A) Log2-EASIX data move over time after transplant with respect to pretransplantation values at the validation cohort, and (B) Log2-EASIX cutoff value at day 7 to evaluate II-IV aGVHD.

Table 4
Predictive value of Log2-EASIX for grade II-IV aGVHD in the validation cohort.

	HR (95% CI)	*P* value
Univariate Analysis		
Log2-EASIX ≥3	1.82 (1.21-2.74)	.004
Age (continuous variable)	0.98 (0.97-1.01)	.12
PTCy-based GVHD prophylaxis (versus other)	0.58 (0.38-0.87)	.009
Mismatched and Haplo donor (MSD and 10/10 MUD)	1.12 (0.73-1.71)	.60
RIC (versus MAC)	0.64 (0.43-0.97)	.037
Donor cells from female to male (versus others)	0.89 (0.49-1.61)	.72
Multivariate Regression Analysis: Predictors for grade II-IV aGVHD		
Log2-EASIX ≥3 at day 7	1.98 (1.26-3.13)	.003
Age (continuous variable)	0.99 (0.97-1.01)	.540
PTCy-based GVHD prophylaxis (vs. other)	0.38 (0.19-0.75)	.005
Mismatched and Haplo donor (MSD and 10/10 MUD)	2.29 (1.13-4.64)	.021
RIC (versus MAC)	0.65 (0.39-1.09)	.100

PTCy indicates post-transplantation high-dose cyclophosphamide; MSD, HLA-matched sibling donors.

approach to predict aGVHD development. In comparison to plasma biomarkers, the main advantage of the EASIX score could be its simplicity, low cost, and rapid results.

The early allogeneic post-transplantation period is characterized by endothelial activation and dysfunction, which can be detected by quantification of various biomarkers of endothelial injury. The origin of this endothelial damage is multifactorial and includes the conditioning regimen, cytokines produced by the injured tissues, bacterial endotoxins translocated through the damaged gastrointestinal tract, use of the granulocyte colony-stimulating factor and calcineurin inhibitors, the process of engraftment, and allogeneic reactions with donor-derived immune cells, among others [29,30]. There is increasing evidence that endothelial damage is the pathophysiological substrate for the development of aGVHD and a key factor in the development of steroid refractory forms [8].

Several studies have shown that inflammatory angiogenesis and neovascularization are central events in endothelial damage during the development of aGVHD and refractoriness to steroid treatment [31–33]. Other findings indicative of endothelial dysfunction include increased numbers of circulating endothelial cells and elevated levels of the endothelial stress biomarkers such as ST2, VWF, angiopoietin 2, and thrombomodulin [34–36]. Some of these factors have also been related to an increase in mortality in patients with steroid-refractory aGVHD [10,37]. However, there is less information on their value as predictors of aGVHD risk.

We have previously shown that exposure of endothelial cells in culture to sera from patients who have received allo-HSCT causes endothelial activation and damage, with a more pronounced proinflammatory and prothrombotic phenotype associated with aGVHD [9]. In this study, plasma levels of VWF:Ag and TNFR1 above an optimal cutoff at day 7 after transplantation were able to positively predict that approximately 90% of patients would develop aGVHD. Consistent with these findings, in the present study we observed a progressive increase in the plasma levels of VCAM-1, TNFR1 and VWF:Ag after conditioning, as early as day 0, and before infusion of hematopoietic progenitors. Patients who developed aGVHD had higher levels of all biomarkers than those without aGVHD. Among all the plasma biomarkers, multivariate analysis identified TNFR1 measured on days 7 as having the greatest impact on developing aGVHD. All of these data support the concept that aGVHD is associated with severe endothelial injury, and that this endothelial injury occurs several days before the clinical diagnosis of aGVHD.

The quantification of plasma biomarkers of endothelial dysfunction as predictors of aGVHD risk has, however, technical limitations in clinical practice. In that sense, the EASIX, a simple score based on three laboratory parameters used worldwide, was developed as a surrogate value of endothelial dysfunction. Luft et al. [1] showed that EASIX measured at the time of aGVHD diagnosis was a powerful predictor of NRM and OS. In a second study from the same authors, EASIX score measured before conditioning regimen (EASIX-pre) correlated with biomarkers of endothelial

homeostasis such as CXCL8, interleukin-18, and insulin-like growth-factor-1 serum levels [22]. They also found that higher values of EASIX-pre predicted higher NRM, lower OS, and tended to be associated with a higher risk of grade III-IV aGVHD after allo-HSCT. In agreement with these results, other studies have shown that higher EASIX scores measured at different time points before and after transplantation are associated with poorer overall outcomes [18–20]. In the experimental cohort of our study, endothelial dysfunction biomarkers and the EASIX score were quantified from the pretransplantation period to day 21 after transplantation. We observed a parallel dynamic consisting of a progressive increase in both parameters that was significantly higher in patients who developed aGVHD. Patients with TNFR1 $\geq$ 1300 ng/mL (HR = 7.19, P = .006) and Log2-EASIX $\geq$ 3 (HR = 14.7, $P < .001$) at day 7 after transplantation were more likely to develop grade II-IV aGVHD with high predictive accuracy (C-index of 74% and 81%, respectively). We confirmed the predictive value of EASIX at day 7 in a large validation cohort that was independent of the type of conditioning regimen, GVHD prophylaxis protocol, and type of donor. In agreement with previous published studies, we also found an association between EASIX score and post-transplantation outcomes [18-20,38]. Thus patients with Log^2-EASIX $\geq$ 3 at day 7 had a significant higher risk of NRM and lower OS.

In the experimental cohort, only patients who received RIC allo-HSCT were included, whereas the validation cohort was composed of patients who received both RIC and MAC transplants. This could be considered a limitation of the study. Previous studies from our group have shown that endothelial dysfunction after allo-HSCT can be induced by any type of conditioning regimen [39]. MAC regimens seem to be associated with a higher risk of endothelial damage-related clinical manifestations than RIC [25]. In our study, the analysis conducted in the experimental cohort showed that plasma biomarkers and log^2-EASIX had similar post-transplant dynamics. Patients with TNFR1 $\geq$1300 ng/mL and Log^2-EASIX $\geq$3 at day 7 after transplantation were more likely to develop aGVHD with high predictive accuracy. Therefore patients at risk of developing aGVHD could be easily identified by a high EASIX score. Based on these results, we aimed to confirm the predictive value of EASIX in a large and heterogeneous cohort including both types of conditioning regimens, RIC and MAC. Findings in the validation cohort regarding log^2-EASIX were similar to those observed in the experimental cohort. As expected, RIC was associated with a lower risk of aGVHD. Multivariate analysis confirmed that Log^2-EASIX $\geq$3 had a predictive value for aGVHD independently of the type of GVHD prophylaxis, intensity of conditioning regimen and the type of donor. In contrast to plasma biomarkers, EASIX can be easily used in clinical practice worldwide to predict individual risk of developing aGVHD.

In conclusion, we showed, using 2 different approaches, a differential degree of endothelial damage in the early post-transplantation period between patients who developed aGVHD and those who did not. This endothelial damage appears some days before the clinical diagnosis of aGVHD onset. Patients at risk of developing aGVHD could be easily identified by a high EASIX score. Our results, together with those published previously, may open a new window for preventive strategies in patients at high risk of aGVHD aimed at reducing endothelial dysfunction and damage, such as defibrotide, statins or sildenafil.

DISCLOSURE OF CONFLICTS OF INTEREST

All authors declare no competing financial interests.

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AUTHORSHIP CONTRIBUTIONS

M.Q.S, A.P, C.M and M.P conceived and designed the study; A.P and S.E.S collected and assembled data; M.Q.S, A.P and C.M, analyzed and interpreted the data; and A.P and C.M wrote the manuscript. All coauthors are physicians at our center, who performed the transplants, took care of the patients, made significant contributions to the discussion of the results, and participated substantively in the writing of the manuscript.

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7. DISCUSIÓN

La EICR continúa siendo hoy en día una de las principales complicaciones del aloTPH por su impacto sobre la supervivencia y la calidad de vida de los pacientes. Probablemente, uno de los mayores avances en este campo ha sido la introducción de la PTCy en la última década como parte de los esquemas de profilaxis. Aplicada en sus inicios al trasplante haploidéntico, PTCy se ha ido incorporando progresivamente al aloTPH de DnE y DE, reduciendo de forma significativa la incidencia de las formas graves de EICR. Sin embargo, a pesar de la popularidad de la PTCy, aún existen cuestiones pendientes sobre su uso.

Al igual que muchos otros centros alrededor del mundo, nuestro grupo incorporó inicialmente la PTCy junto con Tac y MMF al programa de trasplante haploidéntico y en una segunda fase, al aloTPH de DnE y DE. Estos cambios se han realizado de una forma gradual y basada en el análisis de los resultados propios y de la bibliografía. En la presente Tesis Doctoral se recogen algunos de los trabajos realizados en nuestro centro que nos han permitido incorporar la PTCy más Tac (PTCy/Tac) como el estándar de profilaxis de EICR independientemente del tipo de donante.

PTCy en el trasplante de donante no emparentado con y sin identidad HLA

Para aquellos pacientes con indicación de aloTPH pero sin DE o DnE HLA idéntico, las opciones disponibles son un donante familiar haploidéntico o un donante, habitualmente DnE, con una disparidad antigénica en el HLA. En un primer análisis de nuestro grupo pudimos demostrar que en el aloTPH a partir de DnE con identidad HLA 7/8, el uso de PTCy con Tac +/- MMF obtenía unos resultados similares al DnE HLA 8/8 con profilaxis estándar en términos de incidencia de EICRa y crónica, MRT, SLP y SG [183]. Observamos además que existía una tendencia hacia una menor incidencia de EICRc moderada-grave y hacia una mejor SLP y SG en los DnE HLA 7/8 con PTCy vs. DnE HLA 8/8 con profilaxis convencional. En este estudio, el 69% de los pacientes recibieron únicamente un ICN junto con la PTCy, sin MMF, sin que ello afectara la incidencia de EICR. Estos hallazgos nos animaron a continuar con el esquema PTCy/Tac en el aloTPH de DnE HLA 7/8 y a testarlo en el trasplante de DnE HLA 8/8.

En el **primer estudio** presentado en esta Tesis Doctoral quisimos confirmar los resultados anteriores en una cohorte más amplia de DnE HLA 7/8 así como evaluar los resultados de la profilaxis con PTCy/Tac en el aloTPH de DnE HLA 8/8. Históricamente, el uso de DnE con una disparidad antigénica HLA se ha asociado a mayor MRT, mayor incidencia de EICR y de fallo del injerto. La mejor opción de profilaxis de la EICR para estos pacientes no está establecida. Hasta la fecha de la publicación de nuestro estudio existía escasa información sobre los resultados de PTCy en el contexto del aloTPH con DnE HLA 7/8. Además, la mayoría de los estudios utilizaban el modelo de inmunosupresión triple del trasplante haploidéntico que combina PTCy con ICN y MMF o MTX.

Nuestro estudio retrospectivo incluyó 109 receptores consecutivos de aloTPH de DnE HLA 7/8 (n=55) y DnE HLA 8/8 (n=54) usando PTCy/Tac y progenitores hematopoyéticos de SP, procedentes de un único centro. No se observaron diferencias entre los grupos con respecto a la incidencia de EICRa grados II-IV y III-IV y EICRc moderada-grave, siendo estas inferiores a lo reportado en la literatura con profilaxis de EICR convencional. Hubo sólo un caso de fallo de injerto primario. Ambos grupos mostraron una incidencia acumulada similar de MRT y de recaída, resultando en SLP y SG similares entre ambos grupos. Con todo ello pudimos concluir que la profilaxis de la EICR con PTCy/Tac hace de los DnE HLA 7/8 una excelente alternativa para los pacientes que no dispongan de un DE o DnE HLA 8/8.

Durante los últimos años, los esquemas de profilaxis de EICR basados en ATG se han considerado el estándar para el aloTPH de DnE con disparidad HLA [228, 229]. En la Tabla 2 se muestran los resultados de tres estudios retrospectivos recientes que han comparado ATG vs. PTCy en este contexto junto con los resultados de nuestro estudio con PTCy [179-181]. En todos ellos, ATG o PTCy se combinó con otros dos inmunosupresores a diferencia de nuestro estudio en el que sólo usamos Tac. En el análisis de registro del EBMT publicado por Battipaglia y cols [179], PTCy se asoció a tasas más bajas de EICRa grado III-IV y tasas similares de EICRa grado II-IV y EICRc en comparación con la ATG. Además, la PTCy se asoció con mejor SLP y supervivencia sin recaída y libre de EICR clínicamente significativa. Las incidencias de EICRa y EICRc, SLP y SG observadas en nuestro estudio con la estrategia PTCy/Tac fueron similares a las

notificadas en la cohorte con PTCy del estudio del EBMT. En el estudio de Soltermann y cols, se exploró la eficacia de un protocolo con PTCy utilizando una dosis reducida (40 mg/kg/día) en combinación con 2 inmunosupresores, comparando los resultados de la cohorte que recibió PTCy con la que recibió ATG [180]. La incidencia de EICRa grado II-IV fue significativamente menor en el grupo de PTCy, y se observó una tendencia a una menor incidencia de EICRc y MRT. Los pacientes tratados con PTCy tuvieron un mejor pronóstico en términos de SG a 1 año, con una SLP similar, a la de los pacientes tratados con ATG. Finalmente, en un estudio prospectivo de Mehta y cols [181], utilizando principalmente injertos de MO, no se observaron diferencias con respecto a la incidencia de EICRa, EICRc, MRT, recaídas, SLP y SG entre PTCy y ATG, equiparando ambas estrategias de profilaxis. En este caso, el uso de MO en lugar de SP podría haber contribuido a la ausencia de diferencias entre PTCy y ATG.

Nuestros resultados coinciden con los reportados por los estudios que utilizan PTCy en combinación con ICN más MMF o MTX, lo que sugeriría que una profilaxis menos intensiva (PTCy/Tac) es suficiente para prevenir de forma eficaz los casos graves de EICR (Tabla 2 y 3) [179-182, 230]. En este sentido, la combinación óptima de inmunosupresores asociados a la PTCy sigue siendo un tema de debate. Aunque la administración de PTCy en monoterapia ha demostrado ser factible y eficaz en el trasplante mieloablativo de MO de DnE HLA idéntico, se ha descrito una mayor incidencia de EICRa y MRT con el uso de injertos de SP, lo que sugiere que en este contexto es necesario añadir un inmunosupresor más a los esquemas de profilaxis [171, 174, 231, 232]. En el caso del aloTPH de DnE, la mayoría de los estudios se han realizado utilizando esquemas de profilaxis para la EICR basados en la estrategia original del trasplante haploidéntico, con tres fármacos: PTCy más ICN y MMF, MTX o sirólimus (Tabla 2 y 3) [179-182, 230, 233]. En un estudio retrospectivo del EBMT, Ruggeri y cols. [234] analizaron 423 pacientes que recibieron un aloTPH de DE y DnE HLA idénticos usando PTCy en monoterapia o en combinación con otros IS. En comparación con la administración de PTCy en monoterapia, el análisis multivariado reveló que añadir 2 inmunosupresores al esquema de profilaxis se asocia con una reducción significativa de la incidencia de la EICRc extensa y del riesgo de MRT, así como con una mayor SG. Cabe señalar que, una proporción importante de pacientes en

Tabla 2. Estudios recientes aloTPH de DnE con disparidad HLA en un locus y esquemas basados PTCy vs ATG y nuestros resultados.

Estudio	Cohorte	Profilaxis	Régimen de acondicionamiento	EICRa	EICRc	MRT	Recaída	SLP	SG
Pedraza y cols. Retrospectivo	DnE HLA 7/8 n=55 DnE HLA 8/8 n=54	PTCy +Tac	MAC: 48% RIC: 52%	II-IV: 31% vs. 32% (p=0.9) III-IV: 9% vs. 7% (p=0.7)	A 1 año Global: 33% vs. 19% (p=0.1) Mod-sev: 18% vs. 14% (p=0.6)	A 1 año 13% vs. 9% (p=0.4)	A 3 año 24% vs.25% (p=0.7)	A 3 año 56% vs. 57% (p=0.9)	A 3 año 64% vs. 65% (p=0.6)
Battipaglia y cols. Retrospectivo EBMT [179]	DnE HLA 9/10 n=272	PTCy, n=93 vs. ATG, n=179 Asociado a CsA/Tac + MMF/MTX	MAC: 50% RIC: 50%	II-IV: 30% vs. 32% (p=0.39) III-IV: 9% vs. 19% (p<0.04)	A 2 años Extensiva 17% vs. 20% (p=0.31)	A 2 años 16% vs. 29% (p=0.06)	A 2 años 29% vs. 37% (p=0.31)	A 2 años 55% vs. 34% (p<0.05)	A 2 años 56% vs. 38% (p=0.07)
Soltermann y cols. Retrospectivo [180]	DnE HLA 9/10 n=80	PTCy (40mg/Kg) n=22 vs. ATG, n=58 Asociado a ICN+MTX/MMF	MAC: 56% RIC:44%	II-IV: 15% vs. 50% (p=0.006)	26% vs. 35% (p=0.14)	A 1 año 5% vs. 28% (p=0.07)	A 1 años 18% vs. 15% (p=0.74)	A 1 años 77% vs. 57% (p=0.11)	A 1 años 91% vs. 64% (p=0.008)
Mehta y cols. Prospectivo [181]	DnE HLA 9/10 n=113 DnE HLA 7/8 n=84	PTCy, n=41 vs. ATG, n 72 Asociado a ICN+MTX/MMF	MAC: 48% RIC: 52%	II-IV: 37% vs. 36% (p=0.8) III-IV: 17% vs.12% (p=0.5)	A 2años 30% vs. 42% (p=0.6)	A 2 años 35% vs. 25% (p>0.5)	A 2 años 20% vs. 31% (p>0.5)	A 2 años 42% vs. 38% (p>0.5)	A 2 años 52% vs. 40% (p>0.5)
Jiménez y cols. Prospectivo CIBMTA [235]	DnE HLA 7/8 n=102 DnE HLA < 7/8 n=26	PTCy, n=82 vs. ATG, n =46 Asociado a Tac/Sirólimus +MMF		III-IV: 15% vs. 31% (p=0.03)	A 1 año Global: 9% vs. 22% (p=0.03)	A 1 año 16% vs. 38% (p<0.001)	A 1 años 16% vs. 24% (p=0.3)	A 1 años 69% vs. 38% (p<0.001)	A 1 años 75% vs. 45% (p<0.001)

Tabla 3. Estudios recientes con aloTPH de DnE con disparidad HLA en un locus usando esquemas basados en PTCy.

Estudio	Cohorte	Profilaxis	Régimen de acondicionamiento	EICRa	EICRc	MRT	Recaída	SLP	SG
Pedraza y cols. Retrospectivo	DnE HLA 7/8 n=55 DnE HLA 8/8 n=54	PTCy +Tac	MAC: 48% RIC: 52%	II-IV: 31% vs. 32% (p=0.9) III-IV: 9% vs. 7% (p=0.7)	A 1 año Global: 33% vs. 19% (p=0.1) Mod-sev: 18% vs. 14% (p=0.6)	A 1 año 13% vs. 9% (p=0.4)	A 3 año 24% vs.25% (p=0.7)	A 3 año 56% vs. 57% (p=0.9)	A 3 año 64% vs. 65% (p=0.6)
Gaballa y cols. Prospectivo [182]	DnE HLA 9/10 n=46	PTCy +Tac +MMF	RIC: 100%	II-IV: 33% III-IV: 13%	A 2 años 19%	A 1 años 31%	A 1 años 19%	A 1 años 47%	A 1 años 60%
Kasamon y cols. Prospectivo [233]	DnE HLA 9/10 n=11 DnE HLA 8/10 n=4 DnE HLA <7/10 n=5	PTCy +MMF+ Sirólimus	RIC:100%	II-IV: 20% III-IV: 0%	A 1 año 16%	A 1 año 0% A 2 años 6%	A 1 años 35%	A 3 años 52%	A 3 años 62%
Lorentino y cols. Retrospectivo [230]	DnE HLA 10/10 n=305 DnE HLA 9/10 n=159	PTCy en monoterapia o en combinación con otros IS	MAC: 54% RIC: 46%	II-IV: 28% vs. 28% (p=0.84) III-IV: 10 vs.8% (p=0.51)	A 2años 35% vs. 44% (p=0.21)	A 2 año 20% vs. 16% (p=0.15)	A 2 año 24% vs. 28% (p=0.41)	A 2 años 56% vs. 56% (p=0.64)	A 2 años 62% vs. 59% (p=0.86)

los grupos que recibieron uno (36%) o dos (49%) fármacos también realizaron una depleción de linfocitos T con ATG, a diferencia del grupo que solo recibió PTCy. Por el contrario, en un estudio publicado recientemente que comparó pacientes que recibieron un trasplante de DnE HLA idéntico y DnE con disparidad HLA usando profilaxis con PTCy en monoterapia o en combinación con uno o dos IS, no se observaron diferencias en los resultados entre los grupos que se estratificaron según el número total de IS utilizados [230]. Los autores reportaron incidencias de EICRa y EICRc muy similares a las observadas en nuestro estudio con PTCy/Tac.

Trabajos posteriores a nuestra publicación, usando esquemas con triple inmunosupresión, coinciden con nuestros hallazgos. Jiménez y cols. [235] analizaron los registros de 128 pacientes receptores de un aloTPH de DnE HLA 7/8 o inferior a 7/8, administrando como profilaxis para la EICR PTCy (n=82) o ATG (n=46) en combinación con sirólimus o Tac más MMF. De nuevo, el grupo PTCy presentó menor MRT e incidencia de EICRa y crónica, y mayor SLP, SG y supervivencia sin recaída libre de EICR en comparación con ATG, con valores muy similares a los de nuestra investigación. En un estudio prospectivo de Shaw y cols. el análisis de los datos de una cohorte de aloTPH de MO de DnE HLA igual o menor a 7/8 (n=80), utilizando como profilaxis PTCy más Tac y MMF, mostro resultados semejantes a los nuestros en referencia a la MRT, incidencia de EICR y SG [236]. En el último congreso anual de la Sociedad Americana de Hematología, Auletta y cols. [237] presentaron los resultados de un estudio de registro del CIBMTR en pacientes que habían recibido un aloTPH con PTCy de DnE HLA 7/8 (n=613) y HLA 8/8 (n=1681). Al igual que lo observado por nosotros, no encontraron diferencias entre ambos grupos con respecto a la SG y supervivencia sin recaída y libre de EICR.

Un dato importante para destacar de nuestro estudio es que, aún llevando un único IS adicional a la PTCy, la mayoría de los pacientes vivos y sin recaída estaban libres de inmunosupresión a los 12 y 24 meses después del trasplante tanto para los que recibieron injertos de DnE HLA 8/8 (88.5% y 96.6%, respectivamente) como de DnE HLA 7/8 (88.5% y 96.8%, respectivamente). Ello refleja la eficacia del esquema de prevención de la EICR y podría tener efectos positivos sobre la reconstitución inmune post-trasplante reduciendo el riesgo de infecciones tardías.

La principal limitación de nuestro estudio es su carácter retrospectivo. Sin embargo, como punto fuerte destacamos que todos los pacientes fueron tratados consecutivamente en una sola institución utilizando los mismos criterios de selección de donante, protocolos de acondicionamiento y cuidados básicos. Nuestros resultados nos han permitido establecer en nuestro centro el esquema PTCy/Tac en el aloTPH de DnE como estándar de profilaxis de EICR, independientemente de la histocompatibilidad HLA. Los análisis posteriores realizados como control de calidad de nuestro programa de aloTPH corroboran los resultados publicados en esta Tesis Doctoral.

Dosis de progenitores hematopoyéticos en el ámbito de la PTCy

En el **segundo estudio** nos planteamos analizar el efecto de la dosis de células CD34+ infundidas sobre los resultados del aloTPH en pacientes que recibieron PTCy como profilaxis de la EICR. Al igual que en los aloTPH con profilaxis convencional de EICR, la fuente de progenitores más utilizada en el aloTPH con PTCy es la SP.

Analizamos retrospectivamente en nuestro centro, los datos de 221 pacientes que recibieron un aloTPH con PTCy a partir de diferentes tipos de donante: DE, DnE HLA 8/8, DnE HLA 7/8 y haploidéntico. El punto de cohorte de las células CD34+ administradas se estableció en 5 x 10^6/kg, lo que permitió dividir la cohorte en dos grupos, teniendo en cuenta que 8 x 10^6/kg células CD34+ es la dosis máxima recomendada en nuestro protocolo institucional: dosis alta (de 5 a 8 x 10^6/kg células CD34+) y dosis baja (≤ 5 x 10^6/kg células CD34+).

Nuestra investigación mostró que el impacto de la dosis de células CD34+ en los resultados de supervivencia del aloTPH de SP con PTCy depende del tipo de donante. Así, la administración de una dosis $\underline{<}$ 5 x 10^6/kg de células CD34+ se asoció con una peor SG (HR 6,01, *p*=0,004) y SLP (HR 4,57, *p*=0,004) en los trasplantes haploidénticos pero no cuando se utilizaron DE, DnE HLA 8/8 o DnE HLA 7/8. Por otro lado, en consonancia con estudios previos, la dosis alta de células CD34+ se asoció con una recuperación más rápida de neutrófilos y plaquetas en todos los pacientes, independientemente del tipo

de donante elegido para el trasplante [238]. La dosis de células CD34+ administrada no influyó en la incidencia de la EICRa o crónica.

Varios estudios han analizado el efecto que tiene la dosis de progenitores hematopoyéticos sobre los resultados del aloTPH en el contexto de la profilaxis convencional de EICR, no PTCy, obteniendo resultados dispares. [90-94, 239]. Aunque los umbrales de la dosis de células CD34+ usados en estos análisis son variados, algunos estudios mostraron que las dosis más altas se asocian con mayor riesgo de EICRc, MRT y recaída [96, 98, 240].

Recientemente, algunos grupos de investigación han evaluado el efecto de la dosis de células CD34+ en los resultados del aloTPH con PTCy [241, 242]. El EBMT publicó un estudio retrospectivo basado en los registros de 414 trasplantes haploidénticos, incluyendo como profilaxis para la EICR esquemas basados en PTCy o ATG [241]. Los pacientes fueron divididos en dos grupos según la dosis de CD34+ administrada, teniendo en cuenta como punto de corte 4,96 x 10^6/kg, muy similar al de nuestro estudio. Los pacientes del grupo que recibió dosis altas tuvieron mejores tasas de injerto de neutrófilos y plaquetas, y lograron una recuperación hematopoyética en menos tiempo que los que recibieron dosis bajas. De forma similar a lo observado en nuestro estudio, la dosis de células CD34+ no afectó al desarrollo de EICRa o crónica. La supervivencia de los pacientes que recibieron > 4,96 x 10^6/kg células CD34+ fue superior, gracias principalmente a que presentaron menos MRT. El tipo de profilaxis de la EICH administrada (PTCy o ATG) no influyó en la supervivencia de los pacientes. Nuestros resultados en los trasplantes haploidenticos, usando un punto de corte prácticamente idéntico, fueron similares a los del estudio del EBMT.

En un reciente estudio, Salas y cols. evaluaron el impacto de la dosis de células CD34+ en 68 pacientes que recibieron un trasplante haploidéntico de SP utilizando acondicionamientos no mieloablativos y profilaxis para la EICR con PTCy más ATG [242]. Los pacientes se dividieron en 2 grupos utilizando como punto de corte una dosis de células CD34+ de 9 x 10^6/kg. Los autores reportaron que los trasplantes con injertos de mayor celularidad (≥ 9 x 10^6/kg) tenían una SG significativamente inferior y una tendencia a mayor MRT y menor SLP. No se observaron diferencias significativas en los resultados del trasplante cuando se utilizaron otros valores de corte sobre la dosis de

progenitores (7 x 10^6/kg, 8 x 10^6/kg, y 10 x 10^6/kg). El análisis no incluyo los resultados sobre el uso de injertos con dosis inferiores a 5 x 10^6/kg de células CD34+. En nuestro estudio, la administración de progenitores se limitó a una dosis máxima de 8 x 10^6/kg de células CD34+, de acuerdo con las directrices de nuestro protocolo institucional basado en publicaciones previas que asociaron las dosis altas de células CD34+ con peores resultados en el post-trasplante cuando se utiliza una profilaxis convencional de la EICR [95, 96, 98, 240]. Por lo tanto, en nuestro estudio no pudimos analizar el impacto de dosis > 8 x 10^6/kg en los resultados del aloTPH. Sin embargo, ambos estudios ponen de manifiesto la relevancia del tipo de donante en cuanto al potencial impacto de la dosis de células CD34+ sobre la supervivencia post-trasplante y podrían sugerir que en el contexto haploidéntico la dosis óptima estaría situada entre 5 y 9 x 10^6/kg células CD34+.

Con respecto a los resultados en los trasplantes de otro tipo de donantes, Elmariah y cols. analizaron retrospectivamente los datos de pacientes que recibieron un aloTPH de SP de DE, DnE HLA 10/10, DnE HLA 7/8 y haploidéntico con esquemas de profilaxis para la EICR basados en PTCy [238]. La dosis de células CD34+ infundidas se estratificó en un grupo de dosis baja (5 < 10^6/kg), otro de dosis intermedia (5 a 10 x 10^6/kg) y un último grupo de dosis alta (> 10 x 10^6/kg). Al igual que en nuestro estudio, la dosis de células CD34+ no tuvo un impacto significativo en la incidencia de EICRa y crónica, ni en la tasa de recaída. Sin embargo, a diferencia de nuestros hallazgos, el análisis multivariado mostró peores tasas de MRT, SLP y SG en el grupo de dosis baja en comparación con el grupo de dosis intermedia, independientemente del tipo de donante. Es importante mencionar que el número de aloTPH de DE y DnE fue muy inferior al analizado en nuestro estudio (8% vs 59%) y que además los tipos de acondicionamiento y los esquema de profilaxis de la EICR utilizados fueron diferente entre ambos estudios, todo ello podría haber contribuido con estas discrepancias.

La principal limitación de nuestra investigación es su carácter retrospectivo y el número relativamente pequeño de pacientes que recibieron un aloTPH haploidéntico en el grupo de dosis bajas de células CD34+. Aunque el tamaño muestral de este grupo puede afectar el impacto estadístico, destacamos que nuestro análisis fue capaz de

detectar diferencias en los resultados entre los grupos de dosis baja y alta, hallazgos acordes con los de otros estudios en el mismo contexto [238, 241].

Biomarcadores de daño endotelial como predictores de EICRa

Otro aspecto clave en la investigación dirigida a mejorar los resultados clínicos del aloTPH es la identificación precoz de los pacientes con alto riesgo de padecer EICR. En el **tercer estudio** presentado en esta Tesis Doctoral nos propusimos evaluar la capacidad potencialmente predictiva de EICRa de tres biomarcadores plasmáticos de lesión endotelial y del EASIX.

Tras analizar los niveles VCAM-1, TNFR1, VWF:Ag e EASIX antes del aloTPH y en los días 0, +3, +7, +14 y +21 después del trasplante en una cohorte experimental de 33 pacientes, nuestros resultados revelaron que estos biomarcadores plasmáticos de disfunción endotelial, principalmente el TNFR1, e EASIX presentaban una dinámica post-trasplante paralela, progresivamente ascendente. Ello nos sugirió que EASIX es equiparable a los biomarcadores plasmáticos, con la ventaja de ser fácilmente calculable y con resultados inmediatos. Pudimos además identificar un punto de corte para ambos parámetros relacionado con el riesgo de EICRa. Así, los pacientes con TNFR1 ≥1.300 ng/mL (HR =7,19, p=0,006) y el logaritmo en base 2 de EASIX (Log2-EASIX) ≥3 (HR=14,7, p<0,001) en el día +7 tuvieron más probabilidades de desarrollar EICRa con una precisión predictiva alta (C-*index* del 74% y 81%, respectivamente).

El valor predictivo de EASIX como marcador indirecto de daño endotelial en la predicción de la EICR se confirmó en una cohorte más amplia de validación (n=321), donde los pacientes con Log2-EASIX ≥3 el día +7 presentaron una incidencia significativamente mayor de EICRa grado II-IV (HR=1,94, p=0,004) independientemente de la profilaxis para la EICR, del régimen de acondicionamiento y del tipo de donante. Además, los pacientes con Log2-EASIX $\underline{\geq}$3 en el día +7 presentaron un riesgo significativamente mayor de MRT y menor SG. Nuestros resultados sugieren que la evaluación del daño endotelial mediante el EASIX o los biomarcadores plasmáticos constituye un enfoque prometedor para predecir el desarrollo de EICRa y que, en

comparación con los biomarcadores plasmáticos, la principal ventaja del EASIX es su sencillez, bajo coste y resultados rápidamente accesibles.

En un subanálisis posterior a la publicación de este estudio pudimos comprobar que los resultados mostrados para EASIX tanto en la cohorte experimental como en la cohorte de validación eran similares en el grupo formado únicamente por pacientes que recibieron profilaxis de la EICR con PTCy. En estos pacientes los valores de Log2-EASIX ≥3 el día +7 eran significativamente superiores en aquellos que presentaron EICRa grado II-IV (HR=1,9, *p*=0,04) independientemente del tipo de donante y régimen de acondicionamiento utilizado. Estos resultados se han confirmado en una reciente publicación de nuestro grupo, mostrando que tanto con PTCy como con profilaxis convencional de EICR, EASIX puede predecir el riesgo de MRT, la SG, y el riesgo de complicaciones endoteliales como el SOS hepático, la MAT y la EICRa [243].

Cada vez existen más pruebas de que el daño endotelial es el sustrato fisiopatológico para el desarrollo de EICRa y un factor clave en el desarrollo de las formas clínica refractarias a corticoides [203]. En el periodo post-trasplante temprano, el endotelio experimenta una activación e incluso una disfunción que puede ser identificada mediante la cuantificación de diferentes biomarcadores plasmáticos de lesión endotelial [244]. El origen de este daño endotelial es multifactorial e incluye causas farmacológicas (acondicionamiento e ICN), la producción de citoquinas proinflamatorias por parte de los tejidos lesionados, traslocación de endotoxinas bacterianas desde el tracto gastrointestinal, el proceso derivado del injerto hematopoyético o la alorreactividad del injerto, entre muchos otros [192, 245]. Varios estudios han demostrado que la angiogénesis inflamatoria y la neovascularización son eventos centrales en la lesión endotelial durante el desarrollo de la EICRa y la refractariedad al tratamiento esteroideo [26, 246, 247]. Otros hallazgos indicativos de disfunción endotelial incluyen un mayor número de células endoteliales circulantes y niveles elevados de biomarcadores de estrés endotelial como ST2, VWF, angiopoyetina 2 y trombomodulina [248, 249, 250]. Algunos de estos factores también se han relacionado con un aumento de la mortalidad en pacientes con EICRa refractaria a esteroides [251,252].

El Grupo del Endotelio de Barcelona (BET, por sus siglas en inglés) demostró previamente a esta Tesis Doctoral que la exposición de cultivos de células endoteliales in vitro a sueros de pacientes que han recibido un aloTPH produce activación y daño endotelial, con un fenotipo proinflamatorio y protrombótico más destacado en los pacientes con EICRa [214]. En este estudio, los niveles plasmáticos de VWF y TNFR1 por encima de un límite óptimo medidos el día +7 post-trasplante pudieron predecir el 90% de los pacientes con riesgo de desarrollar EICRa. Estos datos fueron la base para el diseño de nuestro trabajo. De forma similar, nosotros observamos un aumento progresivo en los niveles plasmáticos de VCAM-1, TNFR1 y VWF:Ag después del acondicionamiento hasta el día +21 tras la infusión de progenitores hematopoyéticos. Los niveles de todos los biomarcadores analizados fueron más altos en los pacientes que desarrollaron EICRa en comparación con aquellos que no presentaron esta complicación. Entre todos los biomarcadores plasmáticos evaluados, el análisis multivariado identificó al TNFR1 medido en el día +7 como el que tenía mayor capacidad para discriminar los pacientes que desarrollarían EICRa. Todos estos datos respaldan el concepto de que la EICRa se asocia con una lesión endotelial grave y que esta lesión endotelial ocurre varios días antes del diagnóstico clínico de la EICRa.

Sin embargo, la cuantificación de los biomarcadores plasmáticos de disfunción endotelial como factores predictivos del riesgo de EICRa tiene limitaciones técnicas de implementación y reproductibilidad que dificultan su uso en la práctica clínica habitual. En este sentido, el uso de EASIX, un cálculo simple basado en tres parámetros de laboratorio de rutina utilizados de manera universal por los servicios de trasplante, como evaluador indirecto de la disfunción endotelial, podría facilitar la identificación de los pacientes con mayor riesgo de presentar EICRa. Luft y cols. demostraron que EASIX medido en el momento del diagnóstico de EICRa era un potente predictor de MRT y SG [223]. En un segundo estudio de los mismos autores, la puntuación de EASIX medida antes del régimen de acondicionamiento (EASIX-pre) se correlacionó con biomarcadores de la homeostasis endotelial como CXCL8, interleucina-18 y niveles séricos del factor de crecimiento similar a la insulina-1 [224]. Estos autores también demostraron que los valores más altos de EASIX-pre eran predictivos de una mayor MRT, una menor SG, y tendían a asociarse con un mayor riesgo de EICRa grado III-IV. De

acuerdo con estos resultados, otros trabajos han demostrado que las puntuaciones de EASIX más altas medidas en diferentes momentos antes y después del trasplante se asocian con peores resultados a nivel general [253-255]. De forma similar, nuestros hallazgos muestran una asociación entre EASIX elevado y mayor mortalidad y menor SG.

Una limitación de este estudio podría ser el hecho de que la cohorte experimental estaba formada solo por pacientes que recibieron aloTPH con acondicionamientos no mieloablativos, mientras que la cohorte de validación estuvo compuesta por pacientes que recibieron trasplantes con acondicionamientos mieloablativos y RIC. Sin embargo, estudios anteriores del BET demostraron que, aunque los regímenes mieloablativos parecen estar asociados con un mayor riesgo de manifestaciones clínicas relacionadas con el daño endotelial que los RIC [256], la disfunción endotelial después del aloTPH se puede desarrollar a partir de la administración de cualquier tipo de acondicionamiento [189].

PERSPECTIVAS DE INVESTIGACIÓN FUTURAS

Los trabajos presentados en esta Tesis Doctoral han ayudado a consolidar la profilaxis de EICR con PTCy/Tac como el estándar en nuestro centro, a establecer una recomendación sobre la dosis de células CD34+ administrada a los pacientes y a identificar el EASIX como un marcador de riesgo de EICRa y mayor mortalidad en los receptores de un aloTPH.

Esta línea de investigación en torno a la PTCy se encuentra activa y ha dado lugar a la publicación de numerosos estudios de nuestro grupo donde se han analizado aspectos como los resultados de su aplicación en aloTPH en pacientes añosos [257], el riesgo de infecciones [258], el riesgo cardiológico [259], análisis farmacocinéticos y farmacogenómicos del esquema PTCy/Tac [260, 261] , la capacidad predictiva de MRT del EASIX [262] y la relación entre EASIX y los eventos cardíacos post-trasplante [263], entre otros. Otros análisis en este contexto se encuentran en marcha, como por ejemplo la modificación del protocolo asistencial PTCy/Tac con reducción de la dosis de PTCy en el aloTPH de DE y DnE HLA 8/8 con la finalidad de optimizar los resultados del aloTPH reduciendo la toxicidad y preservando su eficacia en la prevención de la EICR, cuyos resultados preliminares están siendo evaluados a fecha de hoy para su publicación.

8. CONCLUSIONES

1. La profilaxis de la enfermedad injerto contra receptor (EICR) con altas dosis de ciclofosfamida post-trasplante (PTCy) y tacrólimus en el trasplante alogénico de progenitores hematopoyéticos de donantes no emparentados HLA 7/8 y HLA 8/8 consigue tasas EICR aguda grave y EICR crónica moderada-grave inferiores a 10% y 20%, respectivamente, sin diferencias entre ambos grupos.

2. La supervivencia global, libre de progresión y la supervivencia sin recaída libre de EICR son similares entre los pacientes que reciben un trasplante alogénico de donante no emparentado HLA 7/8 y de donante no emparentado HLA 8/8.

3. La mayoría de los receptores de un trasplante a partir de un donante no emparentado con PTCy y tacrólimus están libres de tratamiento inmunosupresor a los 12 meses post-trasplante (80% si HLA 7/8 y 88% si HLA 8/8).

4. Esta estrategia profiláctica es capaz de superar el impacto negativo que supone la disparidad del HLA en un solo locus y hace de los donantes no emparentados HLA 7/8 una alternativa factible y segura para los pacientes que carecen de un donante emparentado o no emparentado HLA idéntico.

5. En el contexto de los esquemas profilácticos de EICR basados en PTCy, la dosis de células CD34+ no influye en los resultados de supervivencia tras el trasplante de donante emparentado, donante no emparentado HLA idéntico y donante no emparentado con disparidad en un locus. Sin embargo, en los trasplantes haploidénticos de sangre periférica, la administración de una dosis menor o igual a 5×10^6/kg células CD34+ reduce significativamente la supervivencia global y supervivencia libre de progresión de los pacientes.

6. La dosis de progenitores hematopoyéticos influye significativamente sobre la velocidad del injerto hematopoyético en los receptores de un trasplante alogénico de sangre periférica cuando se usan esquemas de profilaxis para la EICR basados en

PTCy. Así, la administración de una dosis de células CD34+ entre 5 – 8 x10^6/Kg logra un injerto de neutrófilos y plaquetas más rápido, independientemente del tipo de donante elegido.

7. De acuerdo con lo anterior, es recomendable el uso de una dosis de células CD34+ superior a 5 x 10^6/kg en todos los trasplantes alogénico de sangre periférica que usan como profilaxis esquemas que incluyen PTCy, independientemente del tipo de donante.

8. La dinámica post-trasplante de los biomarcadores plasmáticos de daño endotelial: antígeno del factor Von Willebrand (VWF:Ag), molécula de adhesión de células vasculares (VCAM-1) y receptor del factor de necrosis tumoral α (TNFR1), y del Índice de Activación y Estrés Endotelial (EASIX) es similar y progresivamente ascendente en el post-trasplante inmediato de todos los receptores de un trasplante alogénico de progenitores hematopoyéticos. Por ello, se podría considerar que el EASIX es equiparable a los biomarcadores plasmáticos de daño endotelial.

9. Los valores elevados de TNFR1 e EASIX en el día +7 post-trasplante predicen el desarrollo de la EICR aguda grado II-IV con una precisión alta.

10. Una puntuación elevada de EASIX puede identificar de forma sencilla, rápida y económica a los pacientes con alto riesgo de desarrollar EICR aguda, independientemente del régimen de acondicionamiento, la profilaxis de la EICR y el tipo de donante utilizados en el trasplante. Este hallazgo podría permitir una intervención preventiva dirigida a proteger el endotelio vascular en los pacientes con alto riego de EICR aguda.

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