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Factores clínicos, analíticos y genómicos para refinar el pronóstico de los pacientes con linfoma folicular

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Factores clínicos, analíticos y genómicos para refinar el pronóstico de los pacientes con linfoma folicular

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Yo, Armando López Guillermo, como director, doy el visto bueno a Pablo Javier Mozas Fernández para la presentación de su tesis doctoral.

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Glosario

Abreviatura	Significado
ADN	ácido desoxirribonucleico
ADNtc	ADN tumoral circulante
B2M	β2-microglobulina
BAFF	B-cell activating factor
BCL2	B-cell lymphoma 2
BCL6	B-cell lymphoma 6
BCR	receptor del linfocito B
CAR-T	chimeric antigen receptor T-cell
CCI	*Charlson Comorbidity Index*
CDF	células dendríticas foliculares
CG	centro germinal
CGA	campo de gran aumento
CN-LOH	*copy-neutral loss of heterozygosity*
CPC	célula precursora del cáncer
CR30	mantenimiento de la respuesta completa a los 30 meses de iniciar el tratamiento de primera línea
ECOG	*Eastern Cooperative Oncology Group*
FISH	hibridación fluorescente *in situ*
FLIPI	*Follicular Lymphoma International Prognostic Index*
IAMP	incidencia acumulada de muerte por progresión
IFN	interferón
Ig	inmunoglobulina
IGH	cadena pesada de las inmunoglobulinas
IL	interleucina
IMO	infiltración de médula ósea
IPI	*International Prognostic Index*
IQT	inmunoquimioterapia
LBDCG	linfoma B difuso de células grandes
LDH	lactato deshidrogenasa
LF	linfoma folicular
LFt	linfoma folicular transformado

LMR	ratio linfocito/monocito
LNH	linfoma no Hodgkin
MAT	microambiente tumoral
MO	médula ósea
NFIS	neoplasia folicular in situ
NGS	*next generation sequencing*
NICE	*National Institute for Health and Care Excellence*
PET	tomografía por emisión de positrones
PET-TCft	PET-TC fin de tratamiento
POD24	progresión de la enfermedad en los 24 meses siguientes al inicio del tratamiento de primera línea
R-bendamustina	rituximab-bendamustina
RC	respuesta completa
R-CHOP	rituximab, ciclofosfamida, doxorrubicina, vincristina y prednisona
RSR	razón de supervivencia relativa
SEER	*Surveillance, Epidemiology, and End Results Program*
SEL	supervivencia específica por el linfoma
SG	supervivencia global
SLP	supervivencia libre de progresión
SN	segundas neoplasias
SP	sangre periférica
SR	supervivencia relativa
SUV	*standardized uptake value*
SWOG	*Southwest Oncology Group*
TAPH	trasplante autólogo de progenitores hematopoyéticos
TC	tomografía computarizada
Tfh	*T follicular helper*
Tfr	*T follicular regulatory*
TH	transformación histológica
TNF	factor de necrosis tumoral
Treg	*T regulatory*
VMTT	volumen metabólico tumoral total
VSG	velocidad de sedimentación globular

Introducción

1. Linfoma folicular: aspectos generales

1.1. Definición y epidemiología

El linfoma folicular (LF) es el subtipo más frecuente de linfoma B indolente y representa en torno a un 15% de todos los casos de neoplasias linfoides en nuestro ámbito[1]. Su incidencia se sitúa alrededor de 5 casos por 100.000 personas y año[2], es ligeramente superior en hombres que en mujeres y la mediana de edad al diagnóstico se sitúa en torno a los 60 años.

Los estudios clásicos indicaban que la supervivencia de los pacientes con LF no se había modificado significativamente durante décadas[3]. Sin embargo, análisis más recientes han evidenciado que las mejoras en el tratamiento (especialmente el uso de rituximab) y en las medidas de soporte han contribuido a una mejoría en la supervivencia libre de progresión (SLP) y global (SG)[4–14], haciendo que la mediana de esta última se sitúe actualmente en torno a los 20 años[15] (***Figura 1A***). Sin embargo, la presencia de recaídas constantes durante el seguimiento, con disminución de la duración de la respuesta a cada línea de tratamiento[16] (***Figura 1B***), hacen que la enfermedad sea considerada todavía incurable en la mayoría de casos.

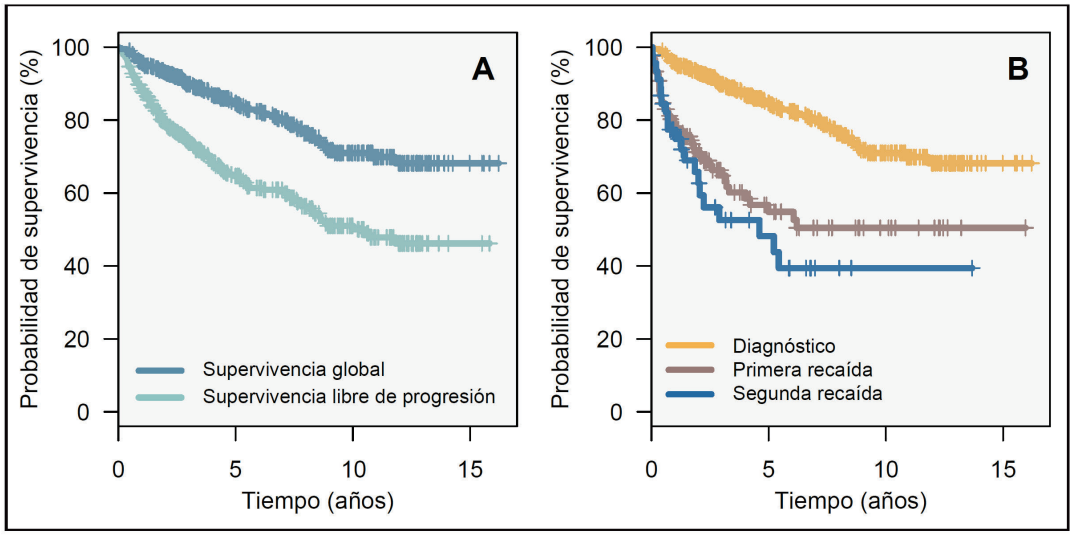

Figura 1. *A: Supervivencia global y libre de progresión de los 414 pacientes con linfoma folicular grado 1-3A de la serie del Hospital Clínic de Barcelona (2002-2018). B: Supervivencia desde el diagnóstico (curva superior), desde la primera recaída (curva central) y desde la segunda recaída (curva inferior). Modificado de* ***Mozas y colaboradores***[17].

1.2. Patogenia

La traslocación t(14;18), que da lugar al reordenamiento *BCL2-IGH*, es característica de la

enfermedad y se encuentra en más de un 85% de los casos. Sin embargo, esta alteración se puede detectar a bajas frecuencias en un 50-70% de adultos sanos[18], por lo que no se considera suficiente para el desarrollo de la enfermedad[19]. El proceso biológico que rige el desarrollo del LF es complejo y se extiende a lo largo de varios años, durante los cuales los síntomas del paciente pasan desapercibidos. El LF se considera el paradigma de neoplasia linfoide B de origen centrogerminal, como lo demuestra la frecuente expresión de marcadores como BCL6 y CD10 y la hipermutación somática del gen de las inmunoglobulinas (Ig), mediada por la *activation-induced deaminase*[20]. La t(14;18) se adquiere de manera temprana en los linfocitos pre-B precoces de la médula ósea, como resultado de un fallo en la reparación durante la recombinación de los genes de las Ig para formar el receptor del linfocito B (BCR)[21]. Como consecuencia, el oncogén *BCL2* queda bajo control transcripcional de las regiones reguladoras del gen de la cadena pesada de las Ig (*IGH*) y se produce una sobreexpresión de la proteína Bcl-2, que favorece la proliferación.

Además de la t(14;18), el desarrollo del LF requiere de la aparición de alteraciones genéticas adicionales (***Figura 2***). Entre ellas, los genes mutados con mayor frecuencia son los modificadores de la cromatina, también llamados modificadores epigenéticos (*KMT2D, CREBBP, EZH2*)[22]. Por otra parte, se ha concedido a los linfocitos T y otras células del microambiente tumoral un papel fundamental en el desarrollo y progresión de esta neoplasia[23], como se comentará con mayor detalle en el apartado referente a los factores pronósticos.

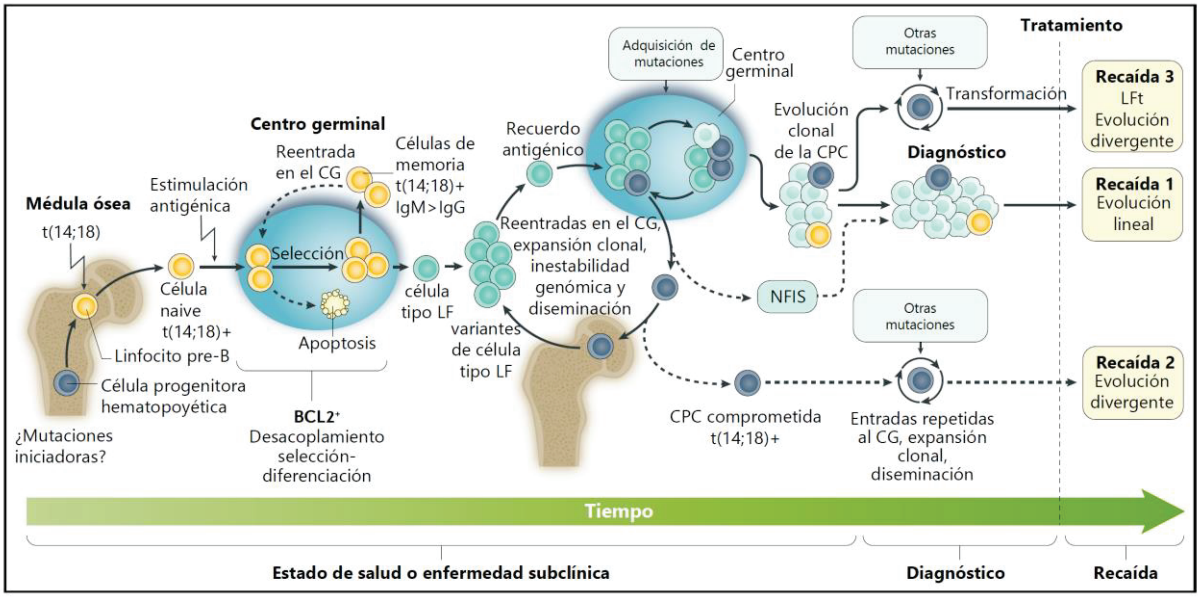

Figura 2. *Modelo de la evolución por pasos del LF. El LF abierto surge a partir de células precursoras del cáncer (CPC) que sufren un proceso dinámico de reentrada en el centro germinal (CG), evolucionando y diseminándose a lo largo de décadas en individuos asintomáticos. Esos clones precoces son probablemente el origen de las recaídas tras el tratamiento. Modificado de Carbone y colaboradores*[24].

1.3. Diagnóstico

Los pacientes que son finalmente diagnosticados de LF tienden a consultar tras la aparición de adenopatías no dolorosas, que pueden haber sufrido variaciones de tamaño durante un tiempo no despreciable[17]. Los ganglios linfáticos aumentados de tamaño son principalmente cervicales o abdominales; estos últimos frecuentemente detectados a raíz de la realización de pruebas de imagen por otro motivo, como el control de neoplasias de órgano sólido. En torno a un 20% de los pacientes presenta alguno de los llamados síntomas "B" (fiebre, sudoración nocturna o pérdida de peso), astenia, infecciones recurrentes o citopenias por infiltración medular.

Para establecer el diagnóstico fehaciente de LF, como en el resto de los linfomas, es primordial disponer de una muestra de ganglio linfático obtenida mediante biopsia excisional o, de manera menos idónea, mediante biopsia con aguja gruesa. En el estudio microscópico, el LF muestra generalmente un patrón de crecimiento folicular o nodular, que remeda la estructura de los folículos linfoides normales del ganglio linfático[25]. Los nódulos tumorales están formados por células pequeñas o medianas (centrocitos) y otras más grandes (centroblastos), entremezcladas con linfocitos T reactivos, células dendríticas, histiocitos y macrófagos. Es importante la determinación del grado histológico de acuerdo al número de centroblastos por campo de gran aumento (CGA): LF de grado 1-2 (hasta 15 centroblastos por CGA, ***Figura 3***), de grado 3A (>15 centroblastos por CGA, con presencia de centrocitos), o de grado 3B (sábanas difusas de centroblastos). Este último se comporta clínica y biológicamente como un linfoma B difuso de células grandes (LBDCG) y se trata como tal.

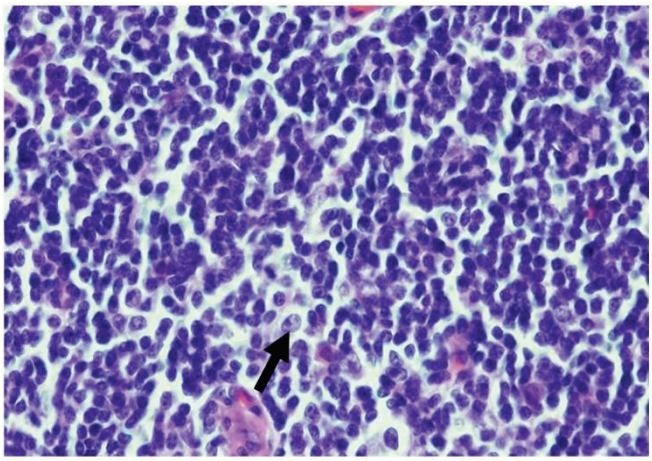

Figura 3. *Histología del linfoma folicular (biopsia de ganglio linfático, hematoxilina/eosina): población predominantemente constituida por linfocitos de tamaño pequeño-mediano (centrocitos), con ocasionales centroblastos (flecha), compatible con un LF de bajo grado (1-2). Cortesía del Servicio de Anatomía Patológica del Hospital Clínic de Barcelona.*

Las células tumorales del LF son generalmente positivas para BCL2, BCL6, CD10 y marcadores B. Si existen dudas diagnósticas, puede ser de utilidad el estudio del reordenamiento del gen de la cadena pesada de las Ig y la demostración por citogenética convencional o por hibridación fluorescente in situ (FISH) de la t(14;18).

En los últimos años se ha desarrollado la detección en plasma de alteraciones genéticas características de tumores sólidos y neoplasias hematológicas (biopsia líquida o ADN tumoral circulante)[26]. Aunque en un futuro cercano puede constituir una herramienta útil para el diagnóstico y pronóstico de los linfomas, aún se encuentra en fases incipientes de su desarrollo.

1.4. Estudio de extensión

Una vez establecido el diagnóstico histológico, se procede al estudio de extensión de la enfermedad, que además de constituir en sí misma un factor pronóstico, es de interés a la hora de evaluar la respuesta al tratamiento. Según las guías internacionales[27], se recomienda la realización de una biopsia de médula ósea antes de iniciar tratamiento, que muestra infiltración por el linfoma en un 50-60% de los casos (típicamente paratrabecular, ***Figura 4A***), y una tomografía por emisión de positrones (PET) asociada a una tomografía computarizada (TC, ***Figura 4B***), además de una analítica sanguínea con bioquímica y hemograma. En base a los hallazgos anteriores, y según la clasificación de Ann-Arbor[28], el estadio de la enfermedad se clasificará de I a IV, algo que posteriormente se simplificó en localizado vs. diseminado en el consenso de Lugano[29].

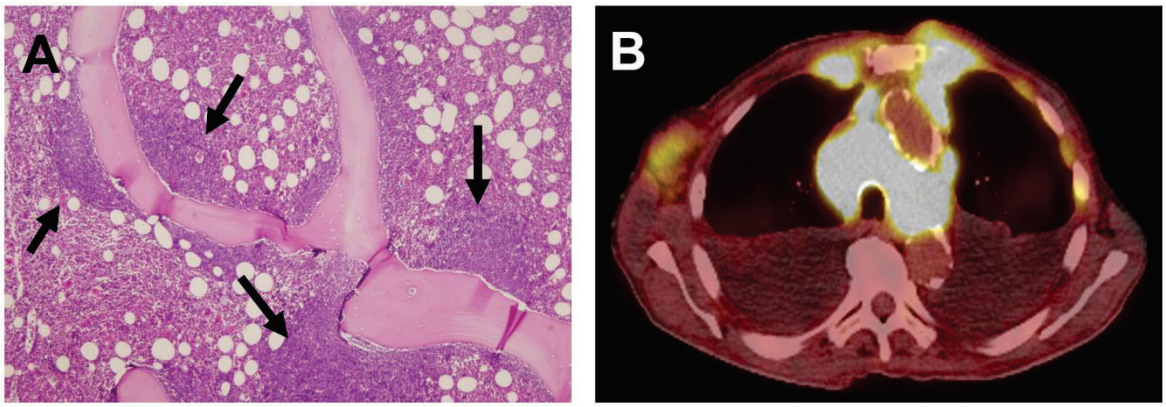

Figura 4. *A: Infiltración de la médula ósea (hematoxilina/eosina) con el característico patrón paratrabecular (flechas). B: Sección axial de PET-TC torácico en el momento del diagnóstico (hipercaptación de radiotrazador en gran conglomerado adenopático mediastínico, cadenas mamarias internas bilaterales y axilares, además de derrame pleural). Cortesía de los Servicios de Anatomía Patológica y Medicina Nuclear del Hospital Clínic de Barcelona.*

1.5. Tratamiento

Las características demográficas de la población a la que afecta con mayor frecuencia el LF, así como la historia natural de la enfermedad, condicionan la elección de los regímenes de tratamiento y su secuencia. Atendiendo a la prolongada supervivencia de la mayoría de los pacientes, es primordial considerar las posibles toxicidades de cada tratamiento, tanto a corto

como a largo plazo.

1.5.1. Tratamiento de primera línea

Actualmente, y desde hace más de dos décadas, la estrategia inicial de tratamiento sigue dependiendo de dos factores principales: el estadio y la presencia de datos de alta carga tumoral[27].

1.5.1.1. Estadios localizados

Un 10-15% de los pacientes con LF se presentan en estadio localizado[30], lo cual constituye una excepción al resto de casos de la enfermedad, puesto que se considera una enfermedad potencialmente curable[31] con radioterapia, asociada o no a rituximab[27].

1.5.1.2. Estadios avanzados

1.5.1.2.1. Baja carga tumoral

La gran mayoría de los pacientes con LF se diagnostica en estadio avanzado. Dentro de este grupo de pacientes cabe distinguir aquellos con baja o alta carga tumoral. Se consideran criterios de alta carga tumoral según el *Groupe d'Étude des Lymphomes Folliculaires*[32] la presencia de síntomas "B", la afectación ganglionar o extraganglionar voluminosa y las alteraciones en el hemograma atribuibles al linfoma. En pacientes con baja carga tumoral (~15%), el tratamiento activo no ha demostrado prolongar la SG ni el tiempo hasta el segundo tratamiento respecto a la abstención terapéutica (conducta expectante o *watchful waiting*), por lo que esta actitud sigue siendo una opción adecuada para pacientes asintomáticos[33].

1.5.1.2.2. Alta carga tumoral

En cambio, los pacientes diagnosticados en estadio avanzado y alta carga tumoral deben recibir tratamiento, generalmente compuesto de una inducción con inmunoterapia (rituximab) o inmunoquimioterapia (R-CHOP, obinutuzumab-CHOP, R-bendamustina o obinutuzumab-bendamustina), seguida de mantenimiento con inmunoterapia (rituximab u obinutuzumab)[27].

1.5.2. Tratamiento de segunda y posteriores líneas

La reaparición de la enfermedad tras un periodo de remisión es la norma en los pacientes con estadio avanzado cuya supervivencia (atendiendo a la edad y a la comorbilidad) es lo suficientemente larga. En la medida de lo posible, es importante biopsiar una de las lesiones sugestivas de recaída para confirmarla y descartar la transformación histológica (TH) a un linfoma de alto grado u otra neoplasia. A pesar de que la evidencia que sustenta la elección del

tratamiento de la recaída es de baja calidad[34], los expertos coinciden en seleccionar regímenes de tratamiento no empleados en la primera línea, con el objetivo de maximizar la eficacia y minimizar la toxicidad solapada.

Es importante analizar el tiempo desde el inicio de la quimioterapia de primera línea hasta la aparición de la primera recaída. Como se verá más adelante, la presencia de una recaída precoz (POD24)[35] es un factor pronóstico adverso que condiciona la elección del esquema de segunda línea. Para pacientes con recaída precoz, la mayoría de expertos recomienda la combinación de obinutuzumab-bendamustina (siempre que no se hayan recibido estos fármacos previamente), seguida de una consolidación con un trasplante autólogo de progenitores hematopoyéticos (TAPH), si la edad y la situación funcional lo permiten, dado que este procedimiento ha demostrado prolongar la SLP y, potencialmente, la SG[36–38]. Para las recaídas tardías, en cambio, son aceptables la abstención terapéutica (siguiendo unas indicaciones de tratamiento similares a la primera línea), radioterapia (para recaídas localizadas), rituximab en monoterapia, combinaciones libres de quimioterapia (rituximab-lenalidomida[39]) e inmunoquimioterapia (en función del esquema utilizado en primera línea)[27].

Los pacientes que presentan ulteriores recaídas, atendiendo a la duración progresivamente más corta de la respuesta en cada línea[16], requieren habitualmente de la utilización de regímenes de tratamiento no empleados previamente, como las pequeñas moléculas de acción específica (idelalisib[40], tazemetostat[41]), la inmunoterapia CAR-T[42] o, en casos muy seleccionados, el trasplante alogénico de progenitores hematopoyéticos.

1.6. Transformación histológica

Una de las características del LF, de manera similar a otros linfomas indolentes, es el potencial de transformación a un linfoma de alto grado relacionado clonalmente, habitualmente un LBDCG[43]. En pacientes tratados durante la era rituximab, la incidencia acumulada de TH se ha establecido alrededor del 5-10% a 10 años, con una aparente reducción en la incidencia para pacientes tratados con rituximab[44]. Actualmente, la aparición de esta situación sigue condicionando un ensombrecimiento marcado del pronóstico (SG del 25% a los 5 años de la TH[45]). A pesar de que la calidad de la evidencia para recomendar la elección del tratamiento en el momento de la TH es baja, se considera aceptado realizar tratamiento con inmunoquimioterapia (IQT) de tipo R-CHOP en aquellos pacientes que no han sido previamente expuestos a antraciclinas, y otros regímenes de IQT seguidos de un TAPH en pacientes previamente tratados con R-CHOP[46], siempre que la edad y la comorbilidad lo permitan.

2. Evaluación pronóstica en el linfoma folicular

La heterogeneidad en el comportamiento clínico de los pacientes con LF hace especialmente atractiva la identificación de factores que permitan predecir, con un grado de precisión suficiente, la probabilidad de que un individuo concreto presente una supervivencia prolongada y fallezca por otras causas o, en cambio, que experimente una recaída precoz o TH, con una marcada disminución de su calidad y cantidad de vida, y una muerte relacionada con el linfoma.

A tal efecto cabe diferenciar los factores predictivos (que proporcionan información sobre la probabilidad de responder o presentar una supervivencia determinada bajo una intervención terapéutica concreta) de los pronósticos (que informan sobre estimaciones de supervivencia, a priori de manera independiente del tratamiento recibido)[47]. Asimismo, los factores pronósticos pueden emplearse de manera aislada, o bien agrupados en forma de índices pronósticos (***Tabla 1***), cuyo objetivo es el de sintetizar en una categoría (generalmente, riesgo bajo, intermedio o alto) la probabilidad de presentar un evento (recaída, muerte, TH, etc.)

Tabla 1. *Factores incluidos en los índices pronósticos desarrollados para el LF. Modificado de* ***Mozas y colaboradores***[48].

Índice pronóstico (ordenados por fecha)	Edad	Sexo	Estadio	ECOG	Síntomas B	N.º áreas ganglionares	Tamaño ganglionar	Afectación extragangl	Infiltración de MO	Anemia	LDH	B2M	Grado histológico	Otros
Gospodarowicz [49], 1984	●			●	●		●						●	
LNH-84 [50], 1991			●				●	●			●			
Romaguera [51], 1991		●					●	●	●					
Leonard [52], 1991	●	●	●	●						●				
Soubeyran [53], 1991	●		●											
Cameron [54], 1993	●		●	●	●									Afectación intestinal
IPI [55,56], 1994	●		●	●				●			●			
Denham [57], 1996	●	●			●	●							●	Esplenomegalia
López-Guillermo [58], 1998												●		EMR tras el tratamiento por PCR en SP o MO
Decaudin [59], 1999	●				●	●								
ILI [60], 2000	●	●			●			●			●			VSG
FLIPI [61], 2004	●		●			●				●	●			
FLIPI2 [62], 2009	●						●		●	●		●		
m7-FLIPI [63], 2015	●		●	●		●				●	●			Estado mutacional de 7 genes en el ganglio
POD24-PI [64], 2016	●		●			●				●	●			Estado mutacional de 3 genes en el ganglio
FCG [65], 2016	●		●			●				●	●		●	Índice de comorbilidad de Charlson
Meignan [66], 2016	●						●		●	●		●		VMTT al diagnóstico
PRIMA-PI [67], 2018									●			●		
Cottereau [68], 2018														VMTT al diagnóstico y *Deauville score* en el PET-TCft
Índice 23-GEP [69], 2018														Expresión génica de 23 genes en el ganglio
FLIPI-L [70], 2019	●		●			●				●	●			Recuento linfocitario en SP
FLEX [71], 2020		●		●			●	●		●	●	●	●	Recuento de células NK en SP

ECOG PS, Eastern Cooperative Oncology Group; MO, médula ósea; LDH, lactato deshidrogenasa; B2M, β_2-microglobulina; EMR, enfermedad mínima residual; SP, sangre periférica; PCR, reacción en cadena de la polimerasa; VSG, velocidad de sedimentación globular; VMTT, volumen metabólico tumoral total; DS, Deauville score; PET-TCft, tomografía por emisión de positrones-tomografía computerizada fin de tratamiento; GEP gene expression profile.

2.1. Factores pronósticos individuales

El estudio de factores pronósticos en el LF ha suscitado un notable interés desde hace décadas, lo cual queda reflejado en la abundancia de publicaciones sobre el tema[72]. Sin embargo, tal como sucede para los índices pronósticos[48], es importante examinarlos a la luz de ciertas consideraciones, a saber: que el tamaño muestral sea lo suficientemente grande como para extraer conclusiones, que las características clínicas, histológicas y terapéuticas de los pacientes del estudio sean comparables a las de la población a las que se quieren aplicar, y que los métodos estadísticos empleados gocen de suficiente robustez. Además, es lógico pensar que diferentes parámetros que miden lo mismo (y, por ende, van paralelos), como aquellos que evalúan la carga tumoral, estén relacionados con el pronóstico. Sin embargo, si no presentan independencia en los análisis multivariados, la contribución al refinamiento del pronóstico será probablemente escasa. Por otra parte, debido a la larga supervivencia de los pacientes con LF, en ocasiones es útil analizar el impacto pronóstico de ciertos factores sobre parámetros refinados, como la supervivencia específica por el linfoma (SEL), la incidencia acumulada de muerte por progresión (IAMP) o la supervivencia relativa (SR).

2.1.1. Previos al inicio del tratamiento

Desde un punto de vista teórico, se pueden identificar factores pronósticos en cualquier momento de la evolución clínica del paciente con LF. En el momento del diagnóstico, tienen la cualidad de no depender de los tratamientos recibidos previamente y, por tanto, de ser extrapolables a cualquier paciente con características clínicas e histológicas similares. Aquellos factores que se identifican durante el seguimiento (p. ej., en el momento de la primera recaída) proporcionan información sobre ese subgrupo concreto de pacientes, con el riesgo de extraer conclusiones a partir de un conjunto de pacientes no del todo homogéneo.

2.1.1.1. Dependientes del paciente

Existe una serie de factores que, en principio, podrían ser considerados independientes de la enfermedad que afecta al paciente, y que este ya presentaba antes de su aparición. Son de especial interés por el impacto que pueden tener en la aparición de complicaciones relacionadas con el tratamiento y en la mortalidad no relacionada con el linfoma.

2.1.1.1.1. Sexo

Varios estudios clásicos habían observado que los varones con LF tenían una supervivencia inferior a la de las mujeres con la misma enfermedad, e incluso se había llegado a incluir esta información

en algunos índices pronósticos[51,52,57,60]. Sin embargo, un estudio más reciente[73] que analizaba la importancia de factores demográficos en la supervivencia de pacientes con LF concluyó que el sexo masculino constituía un factor pronóstico adverso para pacientes diagnosticados entre el año 1992 y 2000, pero no para aquellos diagnosticados entre 2001 y 2009. Estos hallazgos generan la hipótesis de que la inferior supervivencia para los varones en algunas series sea debida a una mayor mortalidad por otras causas (considerando también que la supervivencia de la población general es inferior para los varones). Por este motivo resulta especialmente interesante el análisis de la SEL, la IAMP y la SR en cohortes que evalúan factores pronósticos en LF. El sexo masculino se ha incorporado como factor pronóstico adverso en algunos de los índices pronósticos iniciales[51,52,57,60] y en uno más reciente[71], pero ninguno de ellos ha evaluado las causas de muerte ni la SR.

2.1.1.1.2. Edad

De manera similar a lo que sucede con el sexo, huelga decir que los individuos de edad más avanzada (con o sin linfoma) tienen una supervivencia más reducida. En un estudio realizado en la época pre-rituximab[74] se evidenció una inferior SG para pacientes mayores, y los pacientes de >70 años presentaban una probabilidad mayor de morir por otras causas. En el estudio de registro del SEER citado previamente[75], se evidenció que la SG a 5 años disminuía con cada década que se sumaba a la edad del paciente, pero no se estudió la SR. En esta misma línea fueron los resultados de otro estudio de registro con un gran número de pacientes con edad igual o superior a 66 años diagnosticados de linfoma no Hodgkin (LNH)[76]. Para la mayoría de subtipos de LNH, tanto la mortalidad específica del linfoma como la mortalidad por otras causas se incrementaban con la edad y la comorbilidad. Los autores sugieren que los índices pronósticos actualmente disponibles no informan sobre el riesgo de mortalidad asociada específicamente al linfoma ni de aquel debido a causas diferentes del linfoma.

En un estudio combinado de ensayos clínicos[77] del grupo FLASH, con casi 6 000 pacientes, se observó que los pacientes mayores de 70 años presentaban una mayor frecuencia de lactato deshidrogenasa (LDH) y β2-microglobulina (B2M) elevadas, anemia, un índice funcional ECOG (*Eastern Cooperative Oncology Group*) deteriorado, una menor frecuencia de afectación ganglionar múltiple y un índice FLIPI (*Follicular Lymphoma International Prognostic Index*) similar si no se consideraba el punto atribuido a la edad. Introdujeron además una reflexión novedosa: que la peor SLP de los pacientes mayores (que considera como evento la recaída/progresión o la muerte por cualquier causa) era debida a una mayor incidencia de muertes no relacionadas con el linfoma, sin diferencias en la incidencia acumulada de recaídas, con lo que concluyen que no

existen diferencias en el pronóstico relacionado específicamente con la enfermedad entre los diferentes tramos de edad.

Se han llevado a cabo estudios centrados en subgrupos de edad específicos. En cuanto a los pacientes jóvenes, Conconi y colaboradores[78] analizaron una serie extensa de pacientes con LF, de los cuales 155 tenían 40 años o menos, y evidenciaron que los pacientes jóvenes presentaban ciertas particularidades clínicas (menor frecuencia de LDH elevada y de origen extraganglionar del linfoma, mayor frecuencia de afectación de la médula ósea –MO– y de afectación adenopática extensa). Además, constataron que los pacientes mayores tenían una inferior SLP, SG y SEL y que los jóvenes presentaban una marcada reducción de su esperanza de vida respecto a la población general, que cifraban en un 50%.

En lo referente a los pacientes de edad avanzada, un estudio del SEER sobre 3 705 pacientes de 80 años o más evidenció que recibir algún tratamiento para el LF supuso un 23% de reducción del riesgo de muerte, de manera independiente de la comorbilidad. Con estos datos, los autores animan a valorar activamente las opciones terapéuticas en todo paciente sea cual sea su edad, siempre que tenga criterios de tratamiento.

Desde el punto de vista de la relación entre la edad y la biología del linfoma, un estudio reciente[79] que secuenció muestras de ADN de 258 pacientes con LF evidenció que la edad tenía además un impacto en la genética del LF: el número de mutaciones aumenta con la edad al diagnóstico, especialmente en forma de mutaciones silentes y mutaciones con bajo impacto funcional. Observaron también, de manera similar a lo propuesto por Flowers en 2016[77], que los pacientes de >70 años presentaban una mayor SLP y SG debido a una mayor muerte sin recaída pero una incidencia acumulada de progresión similar.

A pesar de que los estudios que dieron lugar a los índices pronósticos evaluaron la SLP o SG por cualquier causa y no emplearon una evaluación de la SR, la presencia de la edad como factor es casi ubicua en ellos[49,52–54,56,57,59–66,70]. Es de interés la reciente aparición del índice PRIMA-PI[67], un índice pronóstico con solo dos elementos que no contempla la edad, como se comentará más adelante.

2.1.1.1.3. Fragilidad y comorbilidad

En los últimos años ha cobrado una gran importancia la evaluación de la fragilidad y la comorbilidad en pacientes con linfoma. Aunque el desarrollo de este campo ha sido mucho más

prominente en el ámbito de los linfomas agresivos[80–87], se trata de un aspecto particularmente relevante en los linfomas indolentes[88], atendiendo a su larga supervivencia y la intercurrencia de otras posibles causas de muerte.

En un estudio con 224 pacientes diagnosticados de LF y tratados con IQT en los que se evaluó la comorbilidad según el *Charlson Comorbidity Index* (CCI)[65], el 7% de los pacientes presentaba una comorbilidad moderada o grave (CCI ≥2), lo cual fue un predictor de SG independiente del índice FLIPI y del grado histológico. Con estos tres elementos propusieron un nuevo índice pronóstico que aún no ha sido validado en cohortes externas. Por otra parte, en el ya citado estudio de registro[76] se observó que la comorbilidad aumentaba tanto la mortalidad específica como la no relacionada con el linfoma en la mayoría de subtipos de LNH. Hasta donde tenemos constancia, no se ha estudiado el impacto de la comorbilidad en series amplias de pacientes diagnosticados específicamente de LF.

2.1.1.1.4. Hipovitaminosis D

Tras el análisis de muestras de suero de pacientes incluidos en tres ensayos clínicos del grupo SWOG y en el estudio PRIMA[15], se evidenció que el 15% de pacientes con LF con niveles de vitamina D al diagnóstico <20 ng/mL presentaban una SLP y una SG inferiores, aunque estos hallazgos se observaron únicamente en la cohorte SWOG y no se replicaron en la del estudio PRIMA. Los autores reconocen que, aunque este hallazgo podría traducir una inferior actividad macrofágica antitumoral[89], resulta complicado dilucidar si se trata de un factor relacionado con un estilo de vida más saludable y, por tanto, una mejor supervivencia, o un factor modificable a través de la suplementación vitamínica antes de iniciar el tratamiento. El impacto pronóstico adverso de los niveles bajos de vitamina D se confirmó en un estudio prospectivo de 624 pacientes[90] en cuanto a varios parámetros de supervivencia (SLP, SG, SEL) y en pacientes tratados con inmunoterapia, IQT o que no habían recibido tratamiento.

2.1.1.1.5. Condición socioeconómica

A pesar de que el impacto de la situación socioeconómica en la supervivencia de pacientes con linfoma es posiblemente menor en países con cobertura sanitaria universal como el nuestro, es otro de los factores iniciales que pueden modificar el cumplimiento terapéutico, el manejo de las complicaciones y, por ende, la supervivencia. En un estudio estadounidense de registro[91] con más de 40 000 pacientes con FL diagnosticados en la época del rituximab, el hecho de tener cobertura sanitaria privada (por contraposición a pertenecer al programa *Medicare, Medicaid* o a no tener cobertura de ningún tipo) se identificó como un predictor favorable de la SG.

2.1.1.2. Dependientes de la enfermedad

2.1.1.2.1. Situación funcional (performance status)

La evaluación de la situación funcional (*performance status*) mediante el empleo de escalas como la de Karnofsky o ECOG es una herramienta esencial en la valoración del paciente oncológico en general, y del paciente con LF en particular. Aunque el deterioro de la situación funcional al diagnóstico es menos frecuente que en los pacientes con linfoma agresivo, por lo larvado de la enfermedad, en torno a un 10% presenta un ECOG ≥2[61], y es un parámetro incluido en varios índices pronósticos[49,52,54,56,63,71]. El índice FLIPI[61] no lo incluyó pese a ser altamente significativo, debido a la pequeña proporción de pacientes en la cual estaba alterado y a diferencias inexplicables entre pacientes procedentes de centros europeos y americanos.

La ventaja teórica de este parámetro es que se trata de una situación clínica atribuible al linfoma y, por tanto, reversible. Es por este motivo que no debería ser un factor tan limitante en la elección de la modalidad ni intensidad del tratamiento. Sin embargo, en la práctica diaria, edad, comorbilidad, fragilidad y situación funcional se entremezclan y complican la toma de decisiones terapéuticas.

2.1.1.2.2. Estadio

El grado de diseminación del linfoma fue uno de los primeros factores en ser considerado relevante para evaluar el pronóstico de la enfermedad. Tanto es así, que el estadio de Ann-Arbor (***Figura 5***) forma parte de un gran número de índices pronósticos, diseñados desde los años 1980 hasta la actualidad[50,52–54,56,61,63–65,70]. Hoy en día, conserva relevancia por su claro valor pronóstico y por ser uno de los factores empleados en la elección del esquema terapéutico (ver arriba).

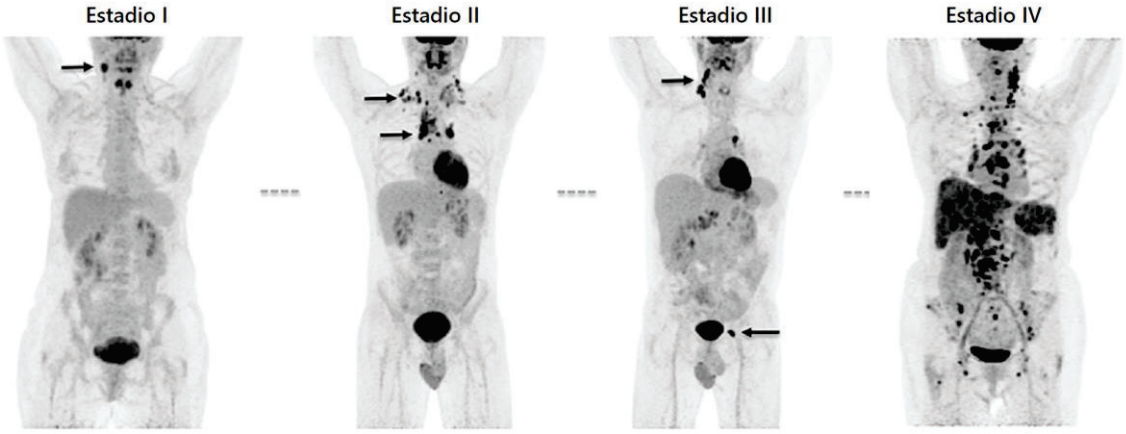

Figura 5. *PET-TC de cuatro pacientes con linfoma en el momento del diagnóstico. Las flechas indican la afectación ganglionar. Modificado de El-Galaly y colaboradores*[92].

2.1.1.2.3. Extensión de la afectación ganglionar y enfermedad voluminosa

De manera similar al estadio de Ann-Arbor, se ha establecido que el número de áreas ganglionares, agrupadas de diversas maneras en función del estudio, y el tamaño de la región ganglionar más afectada (también llamado "masa voluminosa" o "*bulky*") tienen impacto pronóstico. Ciertos índices han incluido información sobre el número de territorios ganglionares[57,59,61,63–65,70] y otros sobre la presencia de una masa voluminosa[49–51,62,66,71].

2.1.1.2.4. Presencia de síntomas B

La liberación de sustancias proinflamatorias por parte de las células tumorales y del microambiente está asociada a un estadio más avanzado y otros factores pronósticos adversos, por lo que la presencia de alguno de los llamados síntomas "B" es un marcador de actividad de la enfermedad y, según las guías internacionales, una indicación para iniciar tratamiento[27,32]. Es un factor incluido en algunos de los índices clásicos[49,54,57,59,60].

2.1.1.2.5. Marcadores de proliferación

De manera similar a lo que ocurre con los síntomas "B", existe una gran cantidad de marcadores séricos, plasmáticos o del hemograma que reflejan un mayor recambio celular, son indicativos de una enfermedad más proliferativa y, en consecuencia, se han identificado como factores pronósticos adversos.

2.1.1.2.5.1. Niveles séricos de LDH

La LDH es una enzima que, además de encontrarse en altas concentraciones en múltiples tejidos[93], juega un papel importante en el efecto Warburg[94], por el cual las células tumorales experimentan un cambio de un metabolismo aeróbico a otro predominantemente anaeróbico y la glucosa es convertida en lactato. Este aumento de la glucolisis aun en presencia de concentraciones suficientes de oxígeno ("glucolisis aerobia") es una característica común a muchas neoplasias y participa en los mecanismos de progresión tumoral. En el caso concreto del LF, la LDH se encuentra elevada en alrededor de un 20% de los casos al diagnóstico[61] y se ha relacionado con mayor actividad proliferativa y peor pronóstico. Este parámetro ha sido incorporado a varios índices pronósticos[50,56,60,61,63–65,70,71].

2.1.1.2.5.2. Niveles séricos de β_2-microglobulina

La B2M es una proteína sintetizada por todas las células nucleadas que forma la cadena ligera del complejo mayor de histocompatibilidad tipo I[95]. La B2M soluble se detecta en varios tipos de líquidos orgánicos, tras la liberación de la superficie celular o del citoplasma. Tras la identificación

de los niveles elevados de B2M en suero como un factor pronóstico adverso, se ha incorporado a diversos índices[58,62,66,71]. No fue incluido en el índice FLIPI[61] debido a que existía una elevada proporción de pacientes en los que no se disponía de información para este parámetro. Es oportuno recordar que el reciente índice PRIMA-PI[67] incorpora únicamente la infiltración de médula ósea y los niveles de B2M (en este caso, superiores a 3 mg/L, independientemente del rango de normalidad del laboratorio).

2.1.1.2.5.3. Anemia, niveles séricos de albúmina y velocidad de sedimentación globular

La anemia puede sobrevenir en pacientes con LF a través de distintos mecanismos: el bloqueo del metabolismo férrico (similar al que ocurre en la anemia de trastorno crónico), la infiltración medular y el hiperesplenismo en casos con esplenomegalia, entre otros. La presencia de anemia se encuentra incluida en el FLIPI y otros índices[52,62–66,70,71]. La velocidad de sedimentación globular (VSG) es un parámetro relacionado con el estado inflamatorio y el aumento de las proteínas circulantes, que se incluyó en el índice ILI[60]. La hipoalbuminemia, por su parte, es un factor presente en varias situaciones clínicas de claro impacto pronóstico adverso: desnutrición y fragilidad, ciertas comorbilidades o una alteración metabólica en el contexto de una enfermedad agresiva. De manera similar a lo sucedido con la B2M, a pesar de ser altamente significativos, en el índice FLIPI no se incluyeron la albúmina ni la VSG por existir un número inaceptablemente alto de pacientes de la cohorte en los que no se habían medido.

2.1.1.2.5.4. Niveles plasmáticos de ADN tumoral

La caracterización del ADN circulante derivado del tumor (ADNtc) ha surgido como un método para identificar alteraciones genéticas específicas de la neoplasia en muestras de sangre periférica[26]. En varios subtipos de linfoma, especialmente linfoma de Hodgkin y LBDCG, se ha establecido como factor pronóstico al diagnóstico y en la evaluación de la respuesta, aunque su llegada a la práctica clínica aún no se ha producido. Se está explorando su utilidad en otro tipo de linfomas, incluido el LF[96,97].

2.1.1.2.6. Afectación extranglionar y de la médula ósea

La afectación extraganglionar (infiltración de órganos diferentes a los ganglios linfáticos) se ha establecido como un pronóstico adverso y se ha incluido en algunos índices[50,51,56,60,71]. Una situación particular es la infiltración de médula ósea (IMO), frecuente en el LF (la presenta un 48% en la cohorte del FLIPI), e incluida en ciertos índices pronósticos[51,62,66,67].

Este parámetro es único de los dos (junto con la B2M) evaluados por el índice PRIMA-PI[67]. En este índice, los niveles de B2M >3 mg/L adscriben al paciente directamente a la categoría de alto riesgo, mientras que, entre los pacientes con B2M ≤3 mg/L era la infiltración de MO la que diferenciaba entre riesgo bajo e intermedio. Es importante destacar, sin embargo, que los pacientes de riesgo bajo e intermedio presentaban una SG similar, por lo que las dos categorías se fusionaron a este efecto. En un análisis posterior del Grupo Nórdico de Linfoma[98] sobre pacientes tratados sin quimioterapia, a pesar de que el PRIMA-PI de manera global predecía la SLP y la SG, no se observaron diferencias significativas cuando se analizaron únicamente en base a la infiltración de MO, lo que sugiere que el factor pronóstico realmente importante es la B2M. En efecto, la controversia sobre el impacto pronóstico de la infiltración de MO existía ya antes de la creación de este índice. Algunos estudios iniciales habían establecido una asociación entre la IMO y una supervivencia inferior[99], otros no habían observado impacto pronóstico[100–102], mientras que ciertos estudios habían determinado que era el patrón[103] o el grado[51] de infiltración lo que definía el impacto pronóstico.

Más allá del estudio histológico, se ha explorado el impacto de la IMO evaluada por otros métodos, como la citometría de flujo (CMF) o la biología molecular. Aunque se ha evidenciado una fuerte asociación entre la CMF y la histología en la evaluación de la IMO[104–106], el impacto de la IMO por CMF no se ha establecido hasta hace poco[107]. Se consideran dos los posibles riesgos de la CMF en la evaluación de la infiltración medular: la sobreestimación (detectar una infiltración de pequeña cuantía sin impacto pronóstico) y la infraestimación (no detectar una infiltración parcheada)[72]. Por su parte, la infiltración medular evaluada por biología molecular (PCR del reordenamiento *IGH-BCL2*) no parece estar estrechamente correlacionada con la infiltración evaluada por histología[108,109] y, además, su impacto pronóstico no está claro[108,110–114].

2.1.1.2.7. PET inicial

La incorporación del PET a la práctica asistencial ha permitido una optimización en el estadiaje de los pacientes con linfoma: contribuye a detectar lesiones hipermetabólicas morfológicamente normales (con el consiguiente *upstaging*) y la categorización como residuales de lesiones aumentadas de tamaño pero con una captación normal de radiotrazador, especialmente en el contexto de la evaluación de la respuesta al tratamiento (la llamada "masa residual"). Esto hace que el estudio combinado PET-TC sea el recomendado por las guías más recientes[27]. Varias son las situaciones clínicas en las que la PET-TC tiene claras implicaciones terapéuticas, como la exclusión de enfermedad a distancia en casos supuestamente localizados y la identificación de la lesión más hipermetabólica para dirigir la biopsia en situaciones en las que se sospecha una TH.

Más recientemente, se ha establecido con firmeza el impacto pronóstico de los parámetros del PET en LF. En un análisis combinado de 185 pacientes[66] con LF grado 1-3A en estadio avanzado y tratados con IQT, provenientes de tres ensayos prospectivos, se analizaron datos clínicos y de PET-TC, y se estableció que el punto de corte óptimo del volumen metabólico tumoral total (VMTT) para predecir la SLP y la SG era 510 cm^3. Además, se estableció que el VMTT se asociaba con características iniciales de alto riesgo. En el análisis multivariado se estableció que el VMTT y el FLIPI2 eran predictores independientes de la SLP, por lo que se combinaron en un índice pronóstico, que selecciona un pequeño grupo de pacientes (14%, VMTT elevado y FLIPI2 intermedio-alto) con una baja SLP (20% a 5 años). Las limitaciones de este estudio son un seguimiento demasiado corto como para identificar diferencias significativas en SG y la ausencia de validación en una cohorte externa.

Por otra parte, se ha evidenciado que la extensión de la enfermedad medida por PET-TC tiene también impacto pronóstico. Un estudio unicéntrico retrospectivo que revisó los datos de PET-TC de 613 pacientes observó que la afectación de >2 áreas ganglionares y la esplénica, ósea o de partes blandas estaban asociados de manera independiente a un mayor riesgo de recaída precoz[115]. Aunque el uso de los parámetros de imagen es muy prometedor en LF, la falta de estandarización de las medidas del PET-TC, la existencia de múltiples métodos para calcular el VMTT y la variabilidad de los puntos de corte para considerarlo de alto riesgo complican su aplicabilidad clínica. Como se verá más adelante, el empleo del PET tiene más importancia, si cabe, en la evaluación de la respuesta.

2.1.1.3. Dependientes de la biología del tumor

Finalmente, existe una serie de factores pronósticos en el momento del diagnóstico o cualquier recaída que son relativos al comportamiento de las células tumorales, sus características histológicas, determinantes genéticos y relaciones con el microambiente tumoral (MAT).

2.1.1.3.1. Grado histológico

El reconocimiento del grado histológico fue una de las primeras aproximaciones a la caracterización del comportamiento de la enfermedad. Como se ha definido en las secciones precedentes, establecer el grado histológico es de crucial importancia, en especial en el caso del LF grado 3B, en el que las antraciclinas se consideran un pilar del tratamiento. Existen, sin embargo, ciertas limitaciones en reconocer el grado histológico como un factor pronóstico potente, como la baja frecuencia de casos de LF grado 3, la limitada concordancia interobservador

al establecer el grado y la mayor intensidad del tratamiento recibido por los pacientes con LF grado 3B como posible factor confusor. Se suma a estas dificultades la heterogeneidad espacial del LF (sesgo de muestreo), por la cual biopsias de diferentes localizaciones anatómicas tomadas en el mismo momento pueden mostrar características histológicas dispares. Por todo lo anterior, se requieren más estudios para establecer de manera definitiva el impacto pronóstico del grado histológico[116–126].

2.1.1.3.2. Biología de la célula tumoral

El factor posiblemente más determinante, y menos conocido, en el pronóstico del LF son las alteraciones genéticas y epigenéticas de la población de células tumorales.

2.1.1.3.2.1. Alteraciones citogenéticas

Como se ha comentado previamente, la t(14;18) es una alteración frecuente (85%) en las muestras histológicas de LF, pero no parece ser ni suficiente (algunos individuos sanos presentan el reordenamiento *BCL2*/*IGH* en sangre periférica) ni necesaria para el desarrollo de la enfermedad (existe un 15% de pacientes con LF que no presentan la traslocación). Un estudio reciente que ha analizado este subgrupo de LF sin la t(14;18)[127] evidenció que se daba más frecuentemente en mujeres, se presentaba en estadios menos avanzados y tenía un pronóstico excelente. El perfil de alteraciones en el número de copias (CNA) y mutaciones era similar al de los LF con t(14;18).

De manera adicional a la t(14;18), el LF presenta habitualmente otras alteraciones cromosómicas recurrentes. En los estudios anteriores a la introducción de la inmunoterapia se asoció la deleción de 6q y la deleción de 17p con un peor pronóstico[116,128,129]. En un análisis más reciente de alteraciones cromosómicas evaluadas por cariotipo con bandas G[130], se estudiaron 201 muestras de pacientes con LF, un 87% de los cuales había recibido rituximab como parte del tratamiento. En el análisis inicial se evidenció que la deleción de 17p, anomalías en 3q27, la trisomía 7, trisomía 21 o un cariotipo complejo (≥3 alteraciones cromosómicas) tenían impacto adverso en la SG, aunque solo la trisomía 21 conservó significación en el análisis multivariado. Sin embargo, la trisomía 21 es una alteración citogenética poco frecuente (6%).

En un estudio de la cohorte S0016 del SWOG en el que se emplearon *arrays*[131], se relacionó la recaída precoz con ganancias en 2p y con la pérdida de heterocigosidad (CN-LOH) en 2p. La deleción de 9p, que alberga *CDKN2A/B,* se relacionó con una SLP inferior. La deleción de 16p, que contiene *CREBBP*, y la de 17p se asociaron con una inferior SG. Estos datos animaron a los autores a recomendar el empleo de *arrays* en el estudio inicial de pacientes con LF para optimizar

su estratificación pronóstica, pues la identificación de alteraciones citogenéticas es más eficaz que mediante FISH. Sin embargo, esta técnica no está implantada en la rutina asistencial.

2.1.1.3.2.2. Mutaciones

En la última década, se ha avanzado de manera significativa en el conocimiento del perfil genético del LF a través de los estudios de secuenciación masiva (*next generation sequencing, NGS,* ***Tabla 2***). Se ha establecido que los genes con papel de regulación epigenética (también llamados genes modificadores de la cromatina o de histonas) están frecuentemente mutados en LF. Por ejemplo, *KMT2D* se encuentra mutado en un 75% de casos, *CREBBP* en un 65% y *EZH2* en un 25%[22,132,133]. Sin embargo, no se debe equiparar la importancia patogénica de estas mutaciones, que es indiscutible, con un supuesto impacto pronóstico, que está aún por establecer.

Las mutaciones en reguladores epigenéticos se centran fundamentalmente en la modificación postraduccional de histonas. Este tipo de mutaciones son eventos generalmente precoces, clonales y temporalmente estables, lo que les atribuye un papel central en el inicio y mantenimiento de la enfermedad[134,135]. La mayoría de los casos presenta varias alteraciones de este tipo, y la suma de estas mutaciones con pérdida de función genera de manera global (con la excepción de *EZH2*) una represión de la transcripción.

Otras vías frecuentemente alteradas en LF son la del reconocimiento inmune (*TNFRSF14*, *CTSS*), la de BCR/NF-κB (*CARD11, TNFAIP3, MYD88*), vía de mTOR (*RRAGC, ATP6AP1, ATP6V1B2, SESTRIN1*), y señalización de JAK-STAT (*STAT6*)[132–134,136–140].

Existen también mutaciones en dos genes supresores de tumores que han demostrado tener implicaciones pronósticas independientes: *TP53* y *CDKN2A*. A pesar de que la mutación de *TP53* se encuentra solo en un 6% de los LF que no han recibido tratamiento, su presencia se relacionó con una peor SLP y SG, de manera independiente del *International Prognostic Index* (IPI)[141]. Además, la adquisición de la mutación de *TP53* se ha encontrado en un 28% de pacientes que presentan una TH a un LBDCG[142]. Por su parte, la deleción o metilación de *CDKN2A* se halla en un 27% de muestras de LF al diagnóstico y se ha asociado con una inferior SG, especialmente en pacientes tratados con rituximab[143]. Además de su papel en el reordenamiento con *IGH*, se han estudiado las mutaciones de *BCL2* como factor pronóstico, y se ha observado que tienen impacto en el riesgo de TH y en la SG[144].

Tabla 2. *Alteraciones genéticas presentes en al menos el 10% de los casos de LF.*

Vía	Gen	Tipo de alteración y efecto	Frecuencia mutacional en LF (%)	Efecto o función
Proliferación	***KMT2D***	Mutación (↓)	80–90	Modificación de histonas, supresor de tumores
	IgHV, IgLV	Mutación (↑)	75–90	Glicosilación del BCR
	RB1	Deleción (↓)	12	Interferencia con el control del ciclo celular
	CDK4	Ganancia en el número de copias (↑)	29	Interferencia con el control del ciclo celular
	BCL6	Traslocación o mutación (↑)	6–15	Factor de transcripción, progresión tumoral
	H1-2, H1-4	Mutación (↓)	47	Remodelado de la cromatina
	MEF2B	Mutación (↓)	44	Factor de transcripción, activador transcripcional
	EP300	Mutación (↓)	13–15	Modificación de histonas
	SESN1	Silenciamiento epigenético (↓)	10–20	Promoción de la actividad de mTOR
	RRAGC$^{ATP6V1B2, ATP6AP1}$	Mutación (↑)	~20	Señal de supervivencia de mTORC1
	EZH2	Mutación (↑)	17	Modificación de histonas
	ARID1A	Mutación (↓)	7–30	Remodelado de la cromatina
	GNA13	Mutación (↓)	15	Proliferación y diseminación del linfocito B
	SGK1	Mutación (↓)	~10	Desregulación de factores de transcripción FOXO y NF-κB
	FOXO1	Mutación (↑)	~10	Factor de transcripción, supervivencia y proliferación
	CARD11	Mutación (↑)	~10	Aumento de señalización BCR
	STAT6	Mutación (↑)	10	Activación de señalización JAK-STAT
Supervivencia	***BCL2***	Traslocación o mutación (↑)	10	Supresión de la apoptosis
	TNFAIP3	Mutación (↓)	80–90	Pérdida de supresión de tumores
Evasión inmune	***EPHA7***	Deleción o silenciamiento epigenético (↓)	50	Supresor de tumores
	TNFRSF14	Mutación (↓)	2–26	Supresor de tumores, aumento de señalización BCR
	CREBBP	Mutación (↓)	70	Modificación de histonas, supresor de tumores

↑, *ganancia de función;* ↓, *pérdida de función; BCR, receptor de la célula B. Modificado de Carbone y colaboradores*[24].

Kridel y colaboradores[138] estudiaron las poblaciones clonales en muestras de LF tomadas en el momento de progresión de la enfermedad o TH. Sus hallazgos ponen de manifiesto diferencias en las mutaciones asociadas con la recaída precoz (*KMT2C, BTG1, TP53, MKI67, XBP1, SOCS1, IKZF3, B2M, FAS* y *MYD88*) en comparación con las de la TH (*TP53, B2M, EZH2, MYC, CCND3, EBF1, PIM1, GNA13, ITPKB, CHD8, P2RY8* y *S1PR2*).

El grupo alemán de linfoma de bajo grado (GLSG), en colaboración con la *British Columbia Cancer Agency* (BCCA) ha desarrollado recientemente un índice pronóstico con información clínica (FLIPI

y ECOG) y genética, denominado m7-FLIPI[63]. Aplicaron un panel de secuenciación de 74 genes a 151 muestras de ganglio de pacientes con LF en estadio avanzado y alta carga tumoral tratados con R-CHOP +/- mantenimiento con interferón. Los autores encontraron que las mutaciones en *EP300, FOXO1, CREBBP* y *CARD11* (de mal pronóstico), y las de *MEF2B*, *ARID1A* y *EZH2* (de buen pronóstico) eran suficientemente frecuentes y con peso pronóstico como para mejorar la predicción conseguida por los parámetros clínicos. De los genes evaluados en el índice, todos son modificadores de la cromatina, a excepción de *FOXO1* (un factor de transcripción esencial en la supervivencia celular y la proliferación) y *CARD11* (implicado en la activación del linfocito B). Dicho estudio presenta varias limitaciones, a saber: 1) las mutaciones individuales no tenían impacto pronóstico por sí mismas en los análisis multivariados, 2) solo se consideraron las mutaciones con una frecuencia alélica ≥10%, 3) solo se incluyeron pacientes con alta carga tumoral y 4) las biopsias se obtuvieron como máximo un año antes de iniciar el tratamiento, independientemente del momento del diagnóstico y el intervalo durante el cual se siguió una actitud expectante. Con todo, el citado estudio supone un avance en el entendimiento de la biología y pronóstico del linfoma folicular. Un estudio posterior intentó simplificar el m7-FLIPI mediante el análisis de únicamente tres genes (*EP300, FOXO1* y *EZH2*), y consiguió identificar de manera adecuada aquellos pacientes con recaída precoz[64].

A pesar de las múltiples técnicas genómicas disponibles, la NGS sigue siendo la que ha conseguido una mayor difusión experimental, pero aún se encuentra lejos de establecerse en la práctica clínica. El hecho de que la mayoría de pacientes con LF tenga una supervivencia prolongada hace que la inclusión de subgrupos particulares de pacientes en lo que respecta a la necesidad de tratamiento o la duración de la respuesta sea un reto para los estudios genómicos de cohortes de pacientes no seleccionados (que a lo sumo cuentan con uno o dos centenares de pacientes). Es por este motivo que es de especial interés diseñar estudios genómicos sobre poblaciones específicas de pacientes con LF, como aquellos que no requieren tratamiento durante largos periodos de tiempo, presentan una recaída precoz o recaen de manera tardía.

2.1.1.3.3. Microambiente tumoral

Tanto o más importante que el comportamiento de las células tumorales en sí mismas lo es el de las células no tumorales que las acompañan y constituyen una gran parte de la histología del LF (linfocitos, células estromales y componentes extracelulares). Un MAT permisivo puede favorecer el desarrollo, persistencia y reaparición de cualquier tumor, y los linfomas B no son una excepción.

Existe amplia evidencia del papel fundamental del MAT en el LF[145]. En primer lugar, los linfocitos del LF residen en nichos específicos y adoptan una arquitectura espacial con las células dendríticas foliculares (CDF) vecinas y con los linfocitos T *follicular helper* (Tfh) que recuerda a los centros germinales reactivos. En segundo lugar, los linfocitos B del LF no son capaces de crecer *in vitro* en ausencia de señales del microambiente ni de implantarse en ratones inmunosuprimidos.

Históricamente, la identificación de los componentes del MAT se ha basado principalmente en la morfología, inmunohistoquímica y citometría de flujo, lo que les ha otorgado un papel pronóstico incierto[146], debido en parte a su estudio en cohortes de pacientes pequeñas, tratadas de manera heterogénea, a la variabilidad en la interpretación de la IHQ y a una subestimación de la complejidad del equilibro y localización espacial de las células inmunes en el MAT. El empleo de técnicas de mayor resolución, como análisis inmunes espaciales y de citometría de masas tipo c*ytometry by time of flight* (CyTOF) han contribuido y seguirán contribuyendo al entendimiento del MAT.

Cabe tener en cuenta, además, que la relación entre la genómica y el comportamiento del microambiente es estrecha. Ciertas lesiones genéticas alteran el microambiente normal del centro germinal y con esto favorecen la linfomagénesis y la progresión. Un ejemplo destacado es la proteína HVEM, que se expresa de manera normal en linfocitos B y T y regula las respuesta inmunes estimuladoras e inhibidoras a través de vías de señalización bidireccionales[147]. Un 40% de los casos de LF presentan una pérdida de HVEM, codificada por el gen *TNFRSF14,* mediante mutación, deleción o CN-LOH[133,137,148]. Su papel en la linfomagénesis parece depender de su interacción con la molécula inhibidora BTLA.

Los componentes del MAT se han clasificado en tres grandes grupos de agentes: linfocitos T infiltrantes del tumor, macrófagos asociados al tumor y células del estroma *(**Figura 6***).

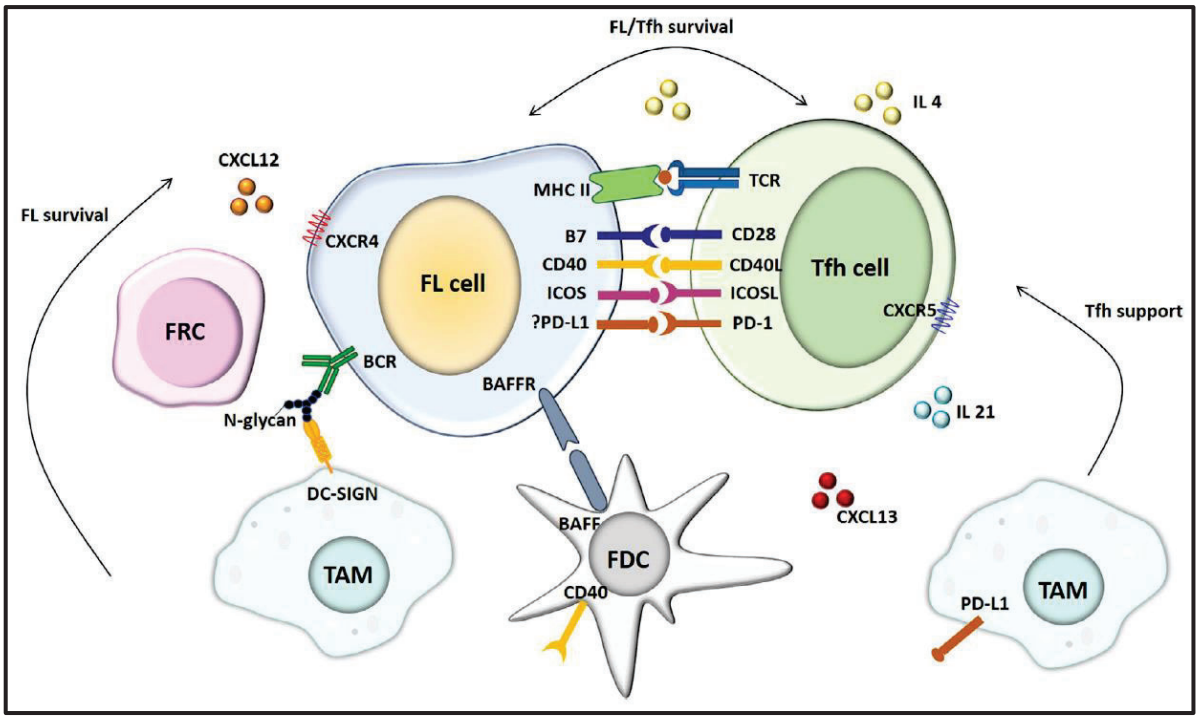

Figura 6. *Interacciones entre la célula tumoral y el microambiente del LF. Las células del LF expresan CD40, mientras que las células* T follicular helper *(Tfh) expresan CD40L. Estas células también secretan interleucinas que favorecen el crecimiento de las células tumorales. Las quimiocinas (CXCL12 y CXCL13) secretadas por las células estromales se unen al receptor CXCR5 de las células Tfh y las células del LF. La N-glicosilación del BCR induce su activación independiente de antígeno y activa señales de supervivencia mediante su interacción con macrófagos asociados al tumor (TAM) que expresan la molécula DC-SIGN. FDC, célula dendrítica folicular; PD-L1,* programmed cell death ligand 1*; MHC,* major histocompatibility complex*; TCR,* receptor de la célula T; *ICOS,* inducible T-cell co-stimulatory*; FRC, célula fibroblástica reticular. Tomado de Kumar y colaboradores*[146].

2.1.1.3.3.1. Linfocitos infiltrantes del tumor

Los ganglios linfáticos afectos de LF tienen un claro aumento de varios tipos de linfocitos T CD4$^+$, como linfocitos Tfh (*T follicular helper*), Treg (*T regulatory*) y linfocitos Tfr (*T follicular regulatory*) respecto a ganglios linfáticos normales[149,150].

Los linfocitos Tfh son un grupo especializado de linfocitos T CD4$^+$ con un papel fundamental en el desarrollo y función de los CG normales. En el LF los linfocitos Tfh presentan unos perfiles de expresión génica y de citocinas específicos, con sobreexpresión de IL-2 e IL-4, que median la señalización a través de STAT6, contribuyen a la proliferación y evitan la apoptosis. Se han descrito también alteraciones en vías de señalización inmunosupresoras y en la secreción de IL-4 y TNF-α. que facilitan la evasión del sistema inmune por parte del tumor[151]. De hecho, los niveles séricos de citocinas, potencialmente secretadas por células del microambiente, se han correlacionado con el pronóstico de los pacientes con LF[152].

Los linfocitos Treg, identificados por la expresión de CD25 y FOXP3 son linfocitos T $CD4^+$ de perfil inmunosupresor que, en condiciones de homeostasis, son cruciales en el mantenimiento de la tolerancia inmune periférica. La elevada presencia de Tregs en los ganglios linfáticos del LF apunta a otro mecanismo de evasión inmune por parte del tumor.

Por último, recientemente se ha descrito un conjunto especializado de linfocitos Treg $CXCR5^+$, los llamados Tfr, que residen principalmente en los ganglios linfáticos y tienen características fenotípicas intermedias entre linfocitos Tfh y Tregs clásicos. El delicado equilibrio entre Tfh y Tfr parece importante en la configuración biológica del LF.

Por otra parte, los linfocitos T $CD8^+$ (citotóxicos) son un componente esencial de la inmunidad antitumoral. En el LF, un número aumentado de este tipo de células se ha asociado con mejor supervivencia, de manera independiente de otros factores pronósticos[153,154].

Se han estudiado otros marcadores con potencial utilidad para identificar subpoblaciones de linfocitos relevantes en el MAT del LF. La presencia en el MAT de células que expresan PD-1 se ha relacionado con una mejor supervivencia, y además se ha evidenciado un menor número de estas células en muestras de LF transformado[155].

En un estudio reciente[156], Tobin y colaboradores demostraron que la recaída precoz de los pacientes con LF se asociaba con una menor infiltración inmune del tumor. Mediante perfiles de expresión génica digital (Nanostring®) de 12 genes aplicados a 132 pacientes en estadio avanzado y con alta carga tumoral, evidenciaron que la expresión de PD-L2 fue el marcador más sensible y específico para diferenciar a pacientes según el riesgo de recaída precoz. Los pacientes con un perfil de infiltración inmune alta (niveles altos de PD-L2) tenían abundantes macrófagos y poblaciones T expandidas. En cambio, los pacientes con baja infiltración inmune (PD-L2 bajo) tenían mayor probabilidad de recaer precozmente.

Mondello y colaboradores[157] caracterizaron mediante CyTOF y CODEX (*co-detection by antibody indexing*) los inmunofenotipos intratumorales de 496 casos de LF grado 1-3A. La falta de expresión de CD4 intrafolicular fue el único predictor de recaída precoz. Posteriormente desarrollaron un modelo clínico de riesgo combinando esta información con la del índice FLIPI.

Un ámbito de especial interés es la correlación de los parámetros macroscópicos (como el VMTT medido por PET-TC) con los del MAT. En ese sentido, Nath y colaboradores[158] estudiaron una

cohorte de 83 pacientes y observaron que la presencia de un bajo número de linfocitos T intratumorales se asociaba con un VMTT unas 6 veces superior, y los ganglios linfáticos de pacientes con VMTT elevado tenían una mayor infiltración por linfocitos B tumorales y menor por linfocitos T $CD8^+$ y $CD4^+$ Tfh. Evidenciaron también que la captación de radiotrazador medida mediante el SUV (*standardized uptake value*) de la lesión estaba asociada con la proporción de linfocitos T, pero no de linfocitos B. Con estos datos, concluyeron que el VMTT está más asociado con la carga de linfocitos B tumorales, mientras que los linfocitos T intratumorales determinan el SUV.

2.1.1.3.3.2. Macrófagos asociados al tumor

Los macrófagos son una parte esencial del sistema inmune innato que actúa mediante la fagocitosis de patógenos y células apoptóticas, pero también son células presentadoras de antígeno de alta eficiencia. La importancia de este conjunto de células, de función variada y compleja, ha quedado reconocida en el LF y en otras enfermedades. La clasificación tradicional defendía una polarización de los macrófagos hacia M1 (dependientes de lipopolisacárido e IFN-γ) o M2 (dependientes de IL-4 e IL-13, más relacionados con la inmunidad antitumoral). Sin embargo, hoy en día se considera que esta clasificación solo captura de manera parcial la plasticidad, heterogeneidad y dinamicidad de los macrófagos *in vivo*[159].

Los macrófagos asociados al tumor contribuyen directamente al crecimiento de las células tumorales a través de las vías del BCR, de BAFF y de IL-15[160]. En cambio, la fagocitosis de células dependiente de anticuerpos está también mediada por macrófagos, y es esencial en la eliminación de células tumorales inducida por el rituximab[161].

Dave y colaboradores[162] demostraron que los casos de LF enriquecidos en genes principalmente expresados en macrófagos y CDF (lo que denominaron "perfil de respuesta inmune 2") tenían una supervivencia inferior, mientras que en aquellos que expresaban genes propios de linfocitos T ("perfil de respuesta inmune 1") era superior. Actualmente, queda por definir la importancia pronóstica exacta de los macrófagos asociados al tumor en el LF, especialmente cuando se evalúan mediante IHQ. Estas células se identifican típicamente por expresar los marcadores CD68 y CD163. Aunque un mayor número de células $CD68^+$ se relacionó con un peor pronóstico en pacientes con LF tratados con QT, este efecto se perdió en pacientes tratados con rituximab[163,164]. Estos hallazgos aparentemente contradictorios sugieren que el tratamiento con QT o IQT puede modular la composición del MAT.

2.1.1.3.3.3. Células del estroma

Aunque la atención de las últimas décadas se ha centrado en los componentes inmunológicos del MAT, el estudio de los elementos no inmunes (células endoteliales, fibroblastos y células mesenquimales del estroma) está ganando importancia. Por ejemplo, la infiltración de la médula ósea, presente en un 70% de pacientes con LF, se caracteriza por una diferenciación ectópica de células estromales pseudolinfoides y enriquecimiento local de linfocitos T CD4^{+}[165]. Algunos estudios en desarrollo tienen como objetivo caracterizar las diferencias en la composición y organización de las células de los ecosistemas que componen el ganglio linfático y la médula ósea.

En base a la idea teórica de que los componentes del MAT podrían tener un reflejo en compartimentos celulares circulantes (linfocitos y monocitos en sangre periférica), algunos autores han evaluado la importancia de la linfopenia[70,166,167], la monocitosis[167–169] o una ratio linfocito/monocito (LMR) disminuida[170–172] en el pronóstico de los pacientes con LF. Sin embargo, se trata de series con un número reducido de pacientes, muchos de ellos tratados antes de la llegada de la inmunoterapia, y con resultados contradictorios respecto a su impacto en la supervivencia.

Finalmente, con el objetivo de combinar información proveniente de las células tumorales y del MAT, a partir de una cohorte de pacientes del estudio PRIMA[15] se ha propuesto un índice pronóstico basado en la expresión de 23 genes[69]. Se identificaron dos firmas de expresión, de bajo y alto riesgo de progresión. La firma de alto riesgo se asoció con una SLP inferior y mayor riesgo de recaída precoz, y los resultados se validaron en 3 cohortes independientes. Un hallazgo interesante fue que las firmas de respuesta inmune identificadas por Dave[162] no tuvieron impacto pronóstico en el estudio de Huet y colaboradores, probablemente debido a que en la cohorte del estudio de Dave los pacientes no habían recibido tratamiento con rituximab. A pesar de que dicho estudio se considera un avance en el conocimiento sobre la biología del LF, será necesario validar el impacto pronóstico del perfil de expresión génica en cohortes de pacientes con baja carga tumoral o estadios localizados antes de aplicarlo en la práctica clínica para seleccionar estrategias terapéuticas.

2.1.2. Relacionados con la profundidad y duración de la respuesta

Existen algunos factores pronósticos que dependen de la respuesta obtenida al tratamiento de primera línea y que, por tanto, no son conocidos en el momento del diagnóstico. Aunque por definición no constituyen una buena herramienta para decidir sobre la necesidad de iniciar

tratamiento o el esquema más adecuado, dan información sobre el comportamiento de la enfermedad para cada paciente de manera individual y permitirían, una vez validada su importancia, adaptar el tratamiento tras la inducción (estrategias de mantenimiento, trasplante autólogo, tratamiento con CAR-T, entre otras).

2.1.2.1. Enfermedad mínima residual en SP y MO y niveles plasmáticos de ADN tumoral

Desde un punto de vista conceptual, es fácilmente entendible que la cuantificación de la alteración genética característica de las células del LF en sangre periférica (SP) y MO después del tratamiento tenga correlación pronóstica. Se han explorado varias técnicas para monitorizar le enfermedad mínima residual (EMR). Sin embargo, la FISH no tiene suficiente sensibilidad y la citometría de flujo no puede identificar adecuadamente las células del LF por la inexistencia de un patrón inmunofenotípico específico. Por lo tanto, ha sido la biología molecular la que ha aportado más al seguimiento de la EMR en esta enfermedad.

Varios estudios han empleado la detección del tránscrito *IGH-BCL2* después del tratamiento. Como era de esperar, los pacientes con EMR negativa tienen una SLP más larga, tanto en los estudios realizados en la época pre-rituximab como en la era de la inmunoterapia, y evaluada ya sea en SP o en MO[58,113,173]. Sin embargo, se prefiere la reevaluación en MO por su mayor sensibilidad (la cantidad de células con el reordenamiento es inferior en SP, entre otras cosas, por su rápida eliminación por los regímenes de IQT)[110,114,173]. Otros estudios, en cambio, no han encontrado asociación entre la EMR y la supervivencia, aunque algunos autores han explicado estos resultados negativos por la ausencia de aleatorización y el pequeño tamaño muestral de los estudios[112].

Existen varios factores que limitan la aplicabilidad práctica del análisis de la EMR[72]. En primer lugar, el punto de ruptura de la t(14;18) es variable. Aunque alrededor de la mitad de los casos ocurre en la *major breakpoint region*[108,174], existen otros puntos que no tienen una detección estandarizada. El diseño de una PCR específica para esos reordenamientos sería teóricamente posible, pero de un coste y una complejidad inaceptables. En segundo lugar, como se ha comentado previamente, un 10-15% de pacientes con LF no presenta la t(14;18), por lo que no podrían ser sometidos a este método de seguimiento de la EMR y, además, el gen de fusión *BCL2-IGH* puede detectarse en individuos sanos, sobre todo a medida que avanza la edad[175]. En tercer lugar, no está bien definido en qué momento clínico se debería medir la EMR, en parte debido a que los estudios sobre el tema utilizaban estrategias terapéuticas diferentes (inducción, inducción y mantenimiento, consolidación con inmunorradioterapia). Con todo, lo que sí parece claro es

que, a mayor persistencia de la negatividad de la EMR, mayor diferencia en SLP[58,108,112], por lo que probablemente no exista ningún punto óptimo de medición, sino que se trate de un parámetro continuo. Por último, a diferencia de algunas leucemias, el LF es una enfermedad principalmente ganglionar, y la ausencia del reordenamiento característico en MO no informa necesariamente de la evolución de la afectación a ese nivel.

Se prevé que parte de estas limitaciones sean superadas en un futuro próximo por la implantación de la tecnología del ADNtc[26]. Se trata del análisis mediante NGS de ADN liberado por células tumorales necróticas y apoptóticas. Una de las ventajas de esta técnica es que no evalúa exclusivamente los compartimentos sanguíneo o medular y que no está limitada a la detección de un reordenamiento concreto, ya que puede utilizar la secuenciación de todos los reordenamientos V(D)J de *IGH*, o bien el estudio de un panel de genes, potencialmente diseñado en base a las alteraciones genéticas halladas en la biopsia ganglionar. Sin embargo, esta tecnología se encuentra aún en desarrollo preclínico y sus aplicaciones en LF han sido de momento limitadas.

2.1.2.2. Respuesta evaluada por imagen

De manera similar a lo recomendado para el diagnóstico, los pacientes deben ser evaluados con PET-TC tras el tratamiento de inducción[27], pues el resultado de esta prueba proporciona información pronóstica y ayuda a elegir el tratamiento posterior. En un estudio combinado de tres cohortes de ensayos clínicos con IQT, Trotman y colaboradores evaluaron el PET-TC realizado en los 3 meses posteriores al tratamiento de inducción[176]. La presencia de un PET-TC fin de tratamiento (PET-TCft) positivo (algo que ocurrió en el 17% de los pacientes) se asoció con una SLP (23 vs 63% a 4 años) y SG inferiores (87 vs 97% a 4 años).

Cottereau y colaboradores[68] analizaron 159 pacientes incluidos en estudios prospectivos y establecieron un índice pronóstico combinado basado exclusivamente en parámetros de imagen. Se atribuía un punto si el VMTT al diagnóstico era >510 cm^3 y otro si el PET-TCft presentaba un *Deauville score* >3. La SLP a 5 años fue claramente diferente en función del número de puntos (67 vs 33 vs 23% para 0, 1 y 2 puntos, respectivamente). Sin embargo, el hecho de no contar con el resultado del PET-TCft en el momento del diagnóstico limita la estratificación pronóstica y el tratamiento adaptado, aunque se podría emplear para la elección de estrategias posinducción. Por otra parte, solo 10 pacientes del estudio recibieron rituximab de mantenimiento, por lo que su validez en pacientes tratados con estrategia queda aún por definir.

Por último, el impacto pronóstico del resultado del PET-TCft se analizó también en la cohorte del ensayo clínico GALLIUM[177,178]. Entre los 595 pacientes que tenían información disponible, se evidenció que la obtención de una respuesta completa (RC) evaluada tanto por TC como por PET-TC se asociaba con la SLP a 30 meses. Sin embargo, solo la respuesta evaluada por PET-TC se asociaba con la SG, lo que sugería que su valoración mediante PET-TC tiene un mayor significado clínico.

2.1.3. Factores pronósticos en el momento de la progresión o recaída

2.1.3.1. Duración de la respuesta

Como se ha expuesto previamente, las recaídas en el LF son la norma, y la duración de la respuesta al tratamiento en cada línea es progresivamente menor[16]. Esto hace que la duración de la respuesta, también llamada tiempo hasta la recaída o SLP tras el tratamiento de primera línea, tenga impacto pronóstico en sí misma.

Un análisis del *National Lymphocare Study*[35], que ha gozado de gran difusión, identificó 588 pacientes con LF tratados con R-CHOP en primera línea. De ellos, un 19% presentó una progresión en los primeros 24 meses desde el diagnóstico (que en dicho estudio coincidía con el momento de iniciar tratamiento, porque los pacientes incluidos no realizaron abstención terapéutica), lo que fue denominado POD24. El hecho de ser POD24 se asociaba con un FLIPI de más alto riesgo y se observó una marcada reducción de la SG para los pacientes POD24 (50 vs 90% a 5 años). El impacto pronóstico de la recaída precoz se ha validado en series posteriores, incluida la del estudio PRIMA[67].

Aunque el impacto pronóstico de la recaída precoz está claro para pacientes tratados con IQT, está por definir si el concepto de POD24 es igualmente válido en pacientes que reciben rituximab en monoterapia o combinaciones como rituximab-lenalidomida[179,180].

Peor todavía es el pronóstico de los pacientes primariamente refractarios al tratamiento de primera línea. El concepto de refractariedad a rituximab se ha definido como una ausencia de respuesta o la recaída dentro de los 6 meses posteriores a haber recibido rituximab. En este sentido, el ensayo GADOLIN[181] evaluó el beneficio aportado por la adición de obinutuzumab a la bendamustina en pacientes con linfoma indolente refractario a rituximab, lo que dio lugar a la aprobación de esta combinación por la *Food and Drug Administration*. Por otra parte, los pacientes refractarios presentan un mayor riesgo de TH y de refractariedad a los tratamientos de rescate[182,183].

La otra cara de la moneda la constituyen los pacientes que permanecen libres de recaída a los 24 meses ("no POD24") o aquellos que conservan una RC a los 30 meses (CR30) desde el inicio del tratamiento (que coincide con el periodo de tratamiento de inducción y dos años de mantenimiento para los que se someten a este tipo de esquema). El concepto de CR30 se acuñó por Shi y colaboradores[8] con datos de casi 4 000 pacientes y estableció que era un parámetro subrogado de la SLP y que, por lo tanto, podría ser un nuevo objetivo de los ensayos clínicos para acelerar la aprobación de tratamientos en desarrollo. Posteriormente, el grupo español demostró que los pacientes que permanecían en CR30 tenían una esperanza de vida similar a la de individuos de la población general de la misma edad y sexo[184], lo cual podría ser considerado un factor de buen pronóstico tras el tratamiento, y uno de los objetivos del esquema terapéutico de primera línea.

2.1.3.2. Otros factores pronósticos en la recaída

La mayoría de los factores pronósticos señalados al diagnóstico tienen importancia también en el momento de la recaída, pues traducen una mayor carga tumoral o una biología del linfoma más agresiva. Un ejemplo es la importancia del índice FLIPI, que se ha demostrado útil en este contexto[185].

2.2. Índices pronósticos

Durante las últimas décadas se han realizado esfuerzos para combinar los factores pronósticos individuales en forma de índices que sinteticen el riesgo individual aportado por cada elemento en una puntuación que permita comparar y agrupar pacientes con probabilidades similares de presentar un determinado evento puntual (respuesta completa, recaída precoz) o dependiente del tiempo (SLP, SG, TH, ***Figura 7***). Después de los índices iniciales como el IPI[55,56] o el ILI[60], se desarrolló el índice FLIPI[61], que tiene en cuenta la edad, el estadio de Ann-Arbor, el número de áreas ganglionares afectas, la elevación de la LDH y la presencia de anemia. A pesar de haber sido desarrollado en la época previa a la introducción del rituximab, se ha validado en pacientes tratados con IQT[186]. En un estudio reciente[187] se ha observado que el aumento de la puntuación del índice FLIPI entre el diagnóstico y el inicio del tratamiento en los pacientes inicialmente considerados candidatos a abstención es un factor pronóstico adverso para la SG. Posteriormente al FLIPI se desarrolló de manera prospectiva el índice FLIPI2[62] (edad, anemia, niveles de B2M, diámetro del mayor ganglio linfático y afectación de MO) y el PRIMA-PI[67] (niveles séricos de B2M y afectación de médula ósea).

En un **estudio recientemente publicado**, aplicamos cinco índices clínicos (IPI, ILI, FLIPI, FLIPI2 y PRIMA-PI) a una serie de 414 pacientes diagnosticados de LF grado 1-3A en la época del rituximab y comparamos su capacidad pronóstica[188]. La concordancia global (proporción de pacientes clasificados en la misma categoría de riesgo por los cinco índices) fue del 24%. Los índices FLIPI y FLIPI2 predijeron el tiempo hasta el primer tratamiento. Los cinco índices predijeron la respuesta, la recaída precoz (POD24), la SLP y la SG. Sin embargo, solo el FLIPI predijo el riesgo de TH. El índice IPI identificó un pequeño grupo de pacientes (7%) de muy alto riesgo (SG a 10: 16%). En los análisis por subgrupos, el índice ILI tuvo utilidad para establecer el pronóstico de los pacientes en estado localizado, mientras que el PRIMA-PI es una herramienta independiente de la edad que puede identificar un grupo de pacientes mayores de alto riesgo. La evaluación estadística de los parámetros de capacidad pronóstica no mostró grandes diferencias entre índices. Con los resultados mencionados, concluimos que el FLIPI era la herramienta pronóstica más potente, ya que predecía el mayor número de *endpoints*.

Las iniciativas más recientes de índices pronósticos han incorporado información molecular a los datos clínicos, como el mencionado m7-FLIPI[63] o el índice de 23 genes medidos por perfiles de expresión génica[69]. Por su parte, se ha incorporado el VMTT, sumado al FLIPI2, en un nuevo índice que predice la SLP[66]. A día de hoy, aunque todos los intentos de refinar los índices pronósticos clásicos mediante nuevos elementos suponen avances en el entendimiento en la biología y comportamiento clínico del LF, ninguno de ellos ha conseguido influir suficientemente la práctica diaria como para ayudar en la toma de decisiones respecto al momento de iniciar tratamiento, el esquema idóneo de primera línea o las estrategias posinducción (mantenimiento o consolidación con terapias celulares). Cabe citar, como excepción, la aprobación de la combinación de obinutuzumab y quimioterapia en primera línea para pacientes con un índice FLIPI de riesgo intermedio o alto por parte del *National Institute for Health and Care Excellence* (NICE) británico[189].

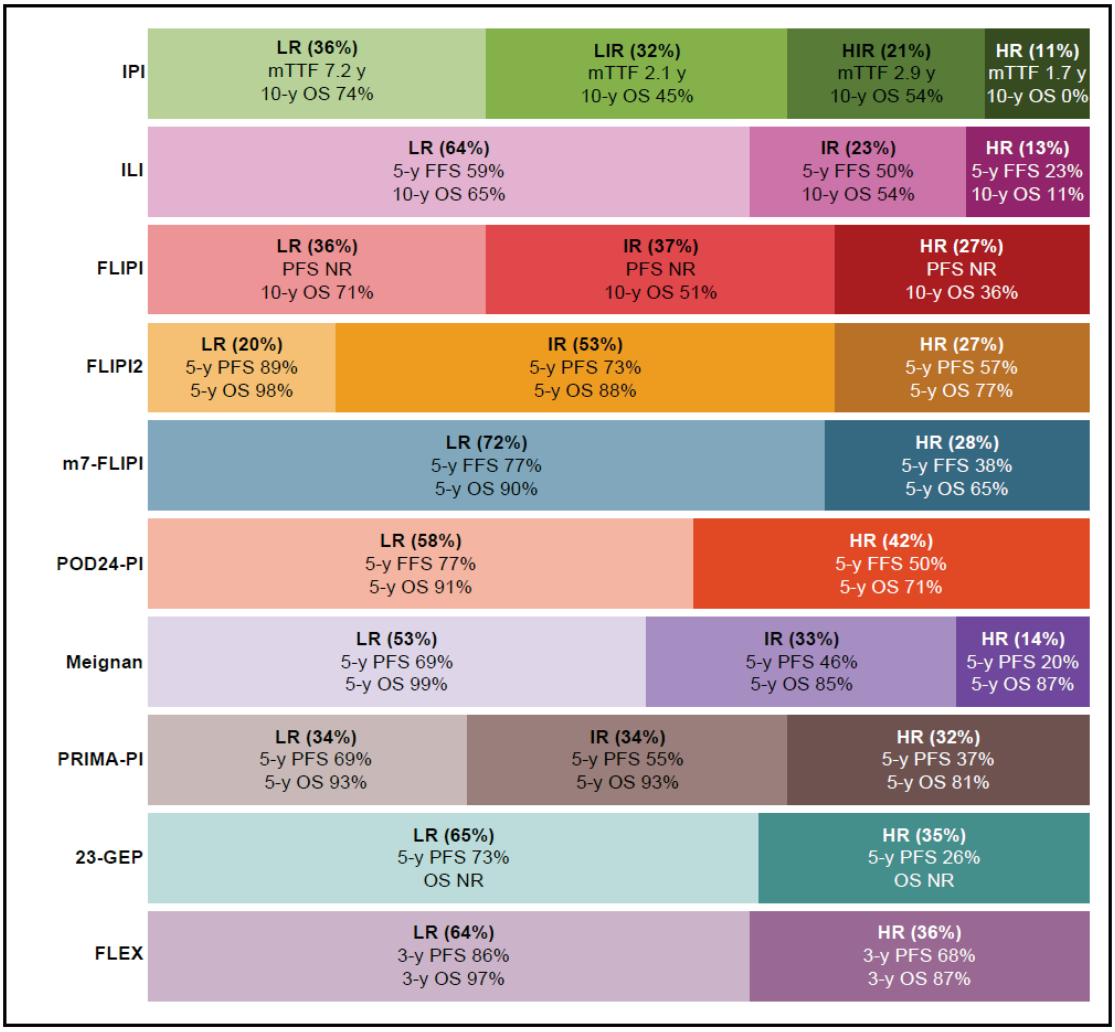

Figura 7. *Distribución de los pacientes en categorías de riesgo y estimaciones de supervivencia según los índices pronósticos más utilizados en LF, ordenados por fecha de publicación (más antiguo arriba). Cuando en el estudio existe una cohorte de* training *y una de validación, los datos corresponden a la primera. LR, riesgo bajo; LIR, riesgo intermedio-bajo; HIR, riesgo intermedio-alto; HR, alto riesgo; mTTF, mediana de tiempo hasta el fallo del tratamiento; y, años; OS, supervivencia global; FFS, supervivencia libre de fallo del tratamiento; PFS, supervivencia libre de progresión; NR, no alcanzada. Referencias: IPI*[55]*, ILI*[60]*, FLIPI*[61]*, FLIPI2*[62]*, m7-FLIPI*[63]*, POD24-PI*[64]*, Meignan*[66]*, PRIMA-PI*[67]*, 23-GEP*[69]*, FLEX*[71]*. Modificado de* ***Mozas y colaboradores***[48].

Hipótesis

El LF es una enfermedad con un comportamiento clínico heterogéneo, en la que la mayoría de los pacientes goza de una supervivencia prolongada y un pequeño subgrupo presenta una recaída precoz o transformación histológica, con alta mortalidad.

Las hipótesis de trabajo de los proyectos que convergen en la presente tesis doctoral pueden sintetizarse de la siguiente manera:

1. Los cambios en el tratamiento de soporte y los esquemas terapéuticos a lo largo de las últimas décadas podrían haber supuesto una mejoría en la tasa de respuestas, supervivencia global y libre de progresión e incidencia de transformación histológica y segundas neoplasias de los pacientes con LF.

2. La edad más avanzada y la presencia de enfermedades concomitantes podrían tener impacto en la tasa de respuestas, supervivencia global y libre de progresión e incidencia de transformación histológica y segundas neoplasias de los pacientes con LF.

3. La ratio linfocito/monocito en sangre periférica podría ser un marcador del microambiente tumoral fácilmente accesible a través de la sangre periférica de los pacientes con LF que permitieran predecir su supervivencia y riesgo de otros eventos (riesgo de transformación y de segundas neoplasias).

4. La presencia de una inmunofijación sérica positiva al diagnóstico podría tener impacto en la supervivencia de los pacientes con LF, ya que posiblemente traduce una desregulación inmunológica, una biología alterada del linfocito B tumoral y sus relaciones con el microambiente.

5. Ciertos grupos de pacientes de LF con un comportamiento clínico concreto (ausencia prolongada de necesidad de tratamiento, ausencia de recaída, recaída precoz, recaída tardía, o refractariedad primaria) podrían estar enriquecidos en ciertas alteraciones genéticas, tanto mutaciones como alteraciones en el número de copias.

Objetivos

1. Objetivo general

Identificar factores históricos, demográficos, analíticos y genómicos que permitan profundizar en el conocimiento sobre la patogenia del linfoma folicular y refinar la estratificación pronóstica de los pacientes.

2. Objetivos específicos

- Evaluar los cambios en el tratamiento, la respuesta, la supervivencia libre de progresión, global y relativa y el riesgo de transformación histológica y segundas neoplasias a lo largo de las últimas décadas de los pacientes con LF diagnosticados en nuestra institución ***(Primer trabajo).***

- Analizar el impacto de la edad, la comorbilidad y la interacción entre ellas en la respuesta al tratamiento, la supervivencia libre de progresión, global y relativa, las causas de muerte y el riesgo de transformación y segundas neoplasias en los pacientes con LF *(**Segundo trabajo**)*.

- Establecer las asociaciones de la ratio linfocito/monocito con las características iniciales de los pacientes con LF y explorar su potencial impacto pronóstico *(**Tercer trabajo**)*.

- Explorar la prevalencia y la correlación clínica y analítica de la presencia de un componente monoclonal sérico al diagnóstico de los pacientes con LF, y evaluar una posible relevancia pronóstica *(**Cuarto trabajo**)*.

- Determinar si existen alteraciones genéticas (mutaciones o alteraciones en el número de copias) en las que estén enriquecidos los pacientes de LF con un comportamiento clínico concreto, en lo referente a la necesidad de tratamiento o a la duración de la respuesta *(**Quinto trabajo**)*.

Material, métodos y resultados

1. Métodos comunes a los trabajos 1 a 4

1.1. Pacientes y parámetros clínicos

Los pacientes incluidos en los estudios fueron diagnosticados de manera consecutiva de LF grado 1-3A en el Hospital Clínic de Barcelona. No se incluyeron los pacientes con LF grado 3B, LF primario cutáneo o gastrointestinal o LF compuesto (con un componente de LBDCG). Se analizaron retrospectivamente las características iniciales, tipo de tratamiento de primera línea, SLP, SG y riesgo de TH y segundas neoplasias (SN) (***Tabla 3***). Los tratamientos empleados siguieron los criterios del Departamento de Hematología y las guías internacionales. Los estudios se desarrollaron de acuerdo al Comité de Ética de Investigación del Hospital Clínic y en línea con la Declaración de Helsinki.

El estadiaje se realizó utilizando TC o PET-TC, además de una biopsia de médula ósea unilateral. La decisión de iniciar tratamiento (pero no la modalidad de tratamiento) respondió a los criterios GELF[32], independientemente de la edad o la comorbilidad. La evaluación de la respuesta se realizó utilizando TC o PET-TC tras el tratamiento de inducción. Las definiciones de RC o RC no confirmada (RCnc), respuesta parcial (RP) o fracaso terapéutico fueron las estándares[29,190].

La SLP se calculó desde la fecha de la primera dosis de tratamiento de primera línea hasta el momento de la progresión (si se alcanzó una RP tras el tratamiento de primera línea), recaída (si se alcanzó una RC/RCnc) o muerte por cualquier causa. Por lo tanto, los pacientes recaídos precoces (POD24) se definieron como aquellos que experimentaron una recaída/progresión en los 24 meses siguientes al inicio del tratamiento de primera línea, de manera similar a trabajos previos sobre el tema[35]. Este análisis solo incluyó pacientes que recibieron tratamiento, consiguieron al menos una RP y tuvieron un seguimiento superior a dos años.

Para el análisis de la SG se consideraron todos los pacientes, y este parámetro se calculó desde la fecha del diagnóstico a la de último seguimiento o muerte por cualquier causa. Para los análisis multivariados de SLP, SG o riesgo de TH se construyeron modelos de regresión de riesgos proporcionales (Cox).

La TH se definió en base a criterios histológicos o citológicos. Se definieron las SN como todos los tumores malignos (incluido el cáncer de piel no melanoma) que aparecieron una vez

establecido el diagnóstico de LF. Se registró el tiempo desde el diagnóstico de LF hasta el de la SN. En caso de desarrollo de más de una SN en el mismo paciente, para el análisis solo se tuvo en cuenta la primera.

Tabla 3. *Características clínicas iniciales y evolutivas de los pacientes incluidos en los cinco estudios que componen la tesis.*

	Primer trabajo	**Segundo trabajo**	**Tercer trabajo**	**Cuarto trabajo**	**Quinto trabajo**
	Patrones de evolución por décadas	***Edad y comorbilidad***	***Ratio linfocito / monocito***	***Componente monoclonal***	***Genómica***
Número de pacientes	727	414	384	311	55
Edad, mediana (rango)	57 (23–93)	60 (26–91)	61 (26–91)	60 (26–88)	57 (26–79)
Sexo femenino (%)	389 (53)	230 (56)	212 (55)	169 (54)	30 (55)
Grado histológico 1 o 2 (%)	419 (84)	331 (82)	309 (82)	231 (74)	43 (78)
ECOG ≥2 (%)	64 (9)	27 (7)	25 (7)	19 (6)	4 (7)
Estadio de Ann-Arbor III-IV (%)	546 (76)	310 (75)	288 (75)	236 (76)	41 (75)
LDH elevada (%)	126 (19)	77 (20)	71 (19)	53 (17)	13 (25)
β_2-microglobulina elevada (%)	241 (43)	163 (43)	178 (49)	145 (47)	24 (47)
Índice FLIPI de alto riesgo (%)	163 (25)	100 (25)	96 (25)	73 (24)	13 (25)
Tratamiento con R-CHOP (%)	217 (30)	204 (56)	185 (55)	129 (47)	45 (82)
RC/RCnc (%)	437 (64)	268 (73)	247 (73)	197 (71)	40 (83)
Mediana de seguimiento (años)	7.6	5.1	5.1	4.6	12.9
SLP a 10 años, % (IC a 95%)	34 (30–38)	50 (44–57)	51 (44–58)	49 (40–57)	40 (28–56)
Recaída precoz (POD24), %	204 (30)	71 (20)	60 (19)	51 (19)	11 (20)
SG a 10 años, % (IC a 95%)	65 (66–73)	71 (65–77)	71 (65–77)	72 (65–80)	79 (69–91)
Riesgo de TH a 10 años, % (IC a 95%)	10 (7–11)	8 (5–11)	7 (4–10)	8 (4–13)	–
Riesgos de SN a 10 años, % (IC a 95%)	10 (8–13)	13 (9–17)	16 (11-22)	16 (11–23)	–

ECOG, Eastern Cooperative Oncology Group; LDH, lactato deshidrogenasa; FLIPI, Follicular Lymphoma International Prognostic Index; R-CHOP, rituximab, ciclofosfamida, doxorrubicina, vincristina y prednisona; RC, respuesta completa; RC, respuesta completa no confirmada; SLP, supervivencia libre de progresión; IC, intervalo de confianza, POD24, progresión de la enfermedad en los 24 meses siguientes al inicio del tratamiento de primera línea; SG, supervivencia global; TH, transformación histológica; SN, segundas neoplasias.

1.2. Métodos estadísticos

Se trazaron curvas de supervivencia de Kaplan-Meier de acuerdo al parámetro de interés y se compararon mediante la prueba de log-rank. Para el estudio de la supervivencia relativa, se comparó la SG observada en nuestra cohorte con la de la población general, ajustada por edad y sexo, de individuos españoles de la *Human Mortality Database*, que tiene en cuenta todas las causas de muerte y proporciona una estimación de la supervivencia específica por causa mediante el paquete *relsurv* del programa R. Posteriormente, se calculó la razón de supervivencia relativa (RSR), mediante la división de la supervivencia observada en nuestra cohorte por la supervivencia esperada de la población general. Del RSR se derivó el parámetro denominado "exceso de mortalidad" o "reducción de la supervivencia", expresado en porcentaje, calculado según la fórmula [1–(supervivencia de la cohorte/supervivencia de la población general)] x 100. Este parámetro pretendía reflejar la reducción de la esperanza de vida respecto a la población general.

En cuanto a las causas de muerte, en la línea de estudios previos[191], se consideraron las siguientes cuatro categorías: progresión del LF, toxicidad del tratamiento, neoplasias distintas del LF y otras causas/causa desconocida. Las dos primeras causas se agruparon posteriormente en "muerte relacionada con el linfoma" y, las dos últimas, en "muerte no relacionada con el linfoma". Para estimar el riesgo de TH y SN, situaciones en las cuales son posibles eventos competitivos (es decir, muerte sin TH o muerte sin SN), se calculó la incidencia acumulada (paquete *cmprsk* del programa R) y se comparó de acuerdo al parámetro de interés mediante el test de Gray[192]. Se emplearon también riesgos competitivos para examinar las causas de muerte mediante la incidencia acumulada de muerte específica por causas.

Las variables categóricas se compararon mediante la prueba de $\chi 2$ o la prueba exacta de Fisher. Se emplearon pruebas no paramétricas cuando correspondía. Se consideraron estadísticamente significativos los valores con una $P<0.05$.

2. Trabajos que componen la tesis

2.1. Primer trabajo

Patterns of change in treatment, response, and outcome in patients with follicular lymphoma over the last four decades: a single-center experience

Pablo Mozas, Ferran Nadeu, Alfredo Rivas-Delgado, Andrea Rivero, Marta Garrote, Olga Balagué, Blanca González-Farré, Luis Veloza, Tycho Baumann, Eva Giné, Julio Delgado, Neus Villamor, Elías Campo, Laura Magnano, Armando López-Guillermo

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Patrones de cambio en el tratamiento, respuesta y resultados de los pacientes con linfoma folicular a lo largo de las últimas cuatro décadas: experiencia de un solo centro

Resumen

Aunque la introducción de la inmunoterapia ha mejorado la supervivencia de los pacientes con linfoma folicular (LF), la transformación histológica (TH) y la recaída precoz aún confieren un mal pronóstico. Nuestro objetivo fue describir los cambios en el tratamiento, respuesta y supervivencia de los pacientes con LF en nuestro centro a lo largo de las últimas cuatro décadas. La población del estudio estaba constituida por 727 pacientes (389 M/338 H, edad mediana: 57 años), diagnosticados de manera consecutiva de LF grado 1-3A entre 1980 y 2017 y categorizados en cuatro décadas según el momento del diagnóstico. Se evaluaron las características iniciales, tratamiento, respuesta, supervivencia absoluta y relativa, TH, incidencia de segundas neoplasias (SN) y causas de muerte. La mediana de SG para el conjunto de pacientes fue de 17.6 años. Se evidenció un aumento en la tasa de respuestas completas de la década 1 a la 4 (48 al 70%), así como una mejoría de la SLP a 5 años (40 a 56%), de la SG a 5 años (77 a 86%) y de la razón de supervivencia relativa a 5 años (0.83 a 0.94). No se observaron diferencias significativas en el riesgo de TH ni de SN. El LF fue la causa de muerte más frecuente en las cuatro décadas. Estos hallazgos ilustran la mejoría general de la supervivencia de los pacientes con LF, pero subrayan la necesidad de continuar investigando en el campo de la estratificación del riesgo y del tratamiento.

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Patterns of change in treatment, response, and outcome in patients with follicular lymphoma over the last four decades: a single-center experience

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Abstract

Although the introduction of immunotherapy has improved outcomes for follicular lymphoma (FL) patients, histological transformation (HT) and early relapse still confer a poor prognosis. We sought to describe the patterns of change in treatment, response, and outcome of FL patients at our institution over the last four decades. Seven hundred and twenty-seven patients (389 F/338 M; median age, 57 years) consecutively diagnosed with grade 1-3a FL between 1980 and 2017, categorized into four decades according to the time of diagnosis, constituted the study population. Clinical characteristics, treatment, response, absolute and relative survival, HT, second malignancies (SM), and causes of death were assessed. Median OS for the entire cohort was 17.6 years. From decade 1 to 4, there was an increase in the complete response rate (48 to 70%), progression-free survival (40 to 56% at 5 years), OS (77 to 86% at 5 years), and relative survival ratio (0.83 to 0.94 at 5 years), with no significant differences in the risk of HT or SM. Lymphoma remained the most common cause of death in all four decades. These findings illustrate the overall improvement in outcome for FL patients, but support the need for further research into risk stratification and management.

Introduction

Follicular lymphoma (FL), the most common type of indolent B-cell lymphoma[1], is characterized by a long survival, despite a pattern of continuous relapses during follow-up[2], and shorter duration of responses in each relapse[3]. Although data initially indicated that survival of FL patients had remained unchanged during the last three decades of the 20th century[4], more recent analyses have acknowledged that improvements in therapy, most likely related to the use of rituximab, as well as supportive measures, may have contributed to a better progression-free (PFS) and overall survival (OS) in recent years[5–15]. Despite a median OS now approaching 20 years, early progression (progression of disease within 24 months, or POD24), defined as a relapse within the first 2 years of frontline treatment, confers a much poorer prognosis[16]. Likewise, histologic transformation (HT) to a high-grade lymphoma significantly reduces survival[17,18], although recent studies have suggested that its incidence might be lower in the rituximab era[19]. Some single-center and registry studies have described the changes in survival of FL patients over the last decades and trends in relative survival according to the general population[7,9]. However, to our knowledge, no single-center series has concurrently examined the trends in observed and relative survival, the risk of HT and second malignancies (SM), and the causes of death over a long period of time.

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In this retrospective analysis of a single-center cohort, we sought to describe the patterns of change in baseline characteristics, treatment, response, PFS, absolute and relative OS, risk of HT and SM, and causes of death of FL patients over the last four decades at our institution.

Methods

Patients

Seven hundred and twenty-seven patients (389 females, 338 males; median age, 57 years) consecutively diagnosed with grade 1-3a FL at Hospital Clínic of Barcelona between January 1980 and December 2017 were included in the present study. All the available diagnostic specimens (most of them dating from 1990 onwards) underwent pathological review. Patients with grade 3b FL, primary gastrointestinal or cutaneous FL, or composite lymphoma were excluded. Baseline patient and disease characteristics, type of frontline treatment, PFS and OS, and risk of HT and SM were assessed retrospectively. Considering the time necessary for a complete diagnosis and staging, frontline treatment was defined as "immediate" if it was started within the first 2 months after diagnosis, or as a "watchful waiting" policy if it was deferred for a longer period of time. Treatment choices in each of the decades were in accordance with the policies of our Department. During decades 1 and 2, a "watchful waiting" strategy was undertaken only in patients 65 years old or older, while in decades 3 and 4, the initiation of treatment responded to the GELF criteria[20].

Since our study cohort spans a 38-year period, we categorized patients into four groups according to the decade of diagnosis: decade 1, 1980–1989; decade 2, 1990–1999; decade 3, 2000–2009; and decade 4, 2010–2017. We decided to include the last period in the analyses, although, due to short follow-up, data concerning survival and the risk of HT and SM should be interpreted with caution. The study was conducted according to the Hospital Clínic Institutional Review Board and in accordance with the Declaration of Helsinki.

Response assessment and outcome

In most cases diagnosed during decades 1 and 2, response to treatment was evaluated using only computed tomography (CT). With the advent of positron emission tomography (PET), PET-CT was increasingly used as a staging and response assessment tool during decades 3 and 4. Therefore, we use the term complete response unconfirmed (CRu) when there was a residual mass on the CT scan without fluorodeoxyglucose uptake on the PET study. For response assessment purposes, CR and CRu were considered equivalent. Definitions of complete response (CR), partial response (PR), and treatment failure were the standard[21].

HT was defined based on histologic or cytological criteria. It is challenging to affirm that second malignancies (SM) are causally related to FL or its treatment (especially for younger patients followed for a long period of time). We define SM as all types of malignant tumors appearing after the diagnosis of FL (including non-melanoma skin cancer). Time from the diagnosis of FL to that of the second neoplasm was registered. In the event of the development of more than one additional neoplasm in the same patient, only the first one was considered for the analysis.

Since the definition of disease-related or unrelated death might be a matter of discussion, we considered the following causes of death: progressive FL, treatment-related toxicities, neoplasms different from FL, and other/unknown causes.

Statistical analysis

PFS was calculated from the date of the first dose of frontline treatment to the date of progression (if a PR was achieved after frontline treatment) or relapse (if a CR/CRu was achieved after frontline treatment). Hence, early progressors (POD24) were defined as patients experiencing progressive disease in the first 24 months after the initiation of frontline treatment. This analysis only included patients who received treatment and had a follow-up of more than 2 years. All patients were included for OS analysis, which was calculated from the date of diagnosis to the date of last follow-up or death from any cause. Kaplan-Meier survival curves were plotted for the four decades and compared for statistical differences by use of the log-rank test. To compare the OS observed in our cohort with that of the general population, patients were matched by age and sex with Spanish individuals from the Human Mortality Database, which accounts for other causes of death and provides an estimate of the cause-specific survival through relative survival analysis (relsurv R package). The relative survival ratio (RSR), calculated by dividing the observed survival of our cohort by the expected survival of the general population, was intended to reflect the reduction in life expectancy due to lymphoma. To estimate the risk of HT and of SM, where competitive events are possible, cumulative incidence was calculated (cmprsk R package) and compared by use of Gray's test[22]. Likewise, when causes of death were evaluated through cause-specific cumulative incidence of mortality, competing risk of death was used. The χ^2 test or p-exact test were used for comparison of categorical variables among the four decades. Nonparametric tests were used when necessary. P values <0.05 were considered to indicate statistical significance.

Results

Patient characteristics

Baseline characteristics of the 727 patients are shown in Table 1. Patients were distributed according to the year of

Table 1 Baseline features and response to frontline treatment of the 727 patients of the series.

Characteristics	Entire cohort ($n = 727$)	Decade 1 (1980–1989) ($n = 79$)	Decade 2 (1990–1999) ($n = 163$)	Decade 3 (2000–2009) ($n = 254$)	Decade 4 (2010–2017) ($n = 231$)	*P* value
Age, median (range)	57 (23–93)	54 (24–85)	54 (24–88)	57 (23–93)	61 (26–91)	<0.001
Female sex (%)	389 (53)	33 (42)	92 (56)	138 (54)	126 (54)	NS
Histologic grade 1 or 2 (%)	419 (84)	NA	59 (89)	184 (83)	172 (83)	NS
ECOG ≥ 2 (%)	64 (9)	16 (21)	18 (11)	15 (6)	15 (7)	<0.001
Ann Arbor stage IV (%)	433 (60)	45 (57)	97 (62)	149 (59)	142 (62)	NS
Elevated LDH (%)	126 (19)	17 (25)	30 (22)	42 (19)	37 (17)	NS
Elevated β2-microglobulin (%)	241 (43)	4 (57)	45 (36)	80 (37)	112 (52)	0.005
High-risk FLIPI (%)	163 (25)	20 (30)	38 (27)	39 (17)	66 (29)	0.009
CR/CRu (%)	437 (64)	37 (48)	85 (53)	177 (73)	138 (70)	<0.001
PR (%)	186 (28)	33 (42)	57 (36)	45 (19)	51 (26)	
Refractory disease (%)	54 (8)	8 (10)	18 (11)	19 (8)	9 (4)	

Response data are calculated only on the 677 patients of the series who received any treatment. Data concerning β2-microglobulin levels should be interpreted with caution for decade 1, since only seven patients had available data.
ECOG Eastern Cooperative Oncology Group, *LDH* lactate dehydrogenase, *FLIPI* Follicular Lymphoma International Prognostic Index, *NA* not available, *NS* not statistically significant, *CRu* complete response unconfirmed, *PR* partial response.

diagnosis: decade 1 (1980–1989), $n = 79$ (11%); decade 2 (1990–1999), $n = 163$ (22%); decade 3 (2000–2009), $n = 254$ (35%); and decade 4 (2010–2017), $n = 231$ (32%). Median follow-up for the entire series (calculated only for surviving patients) was 7.6 years, and 31.8, 20.7, 12, and 3.2 years for decades 1–4, respectively. Most features did not follow a significant trend over decades, except for the more advanced age at diagnosis and better performance status in decade 4 ($P < 0.001$).

Treatment and response to therapy

Initial treatment strategies given to patients in the entire cohort and according to the decade of diagnosis are depicted in Supplementary Table 1. Second- and third-line treatments are detailed in Supplementary Tables 2 and 3. The proportion of patients who underwent an initial "watchful waiting" strategy was 3%, 3%, 13%, and 30% for decades 1 to 4, respectively ($P < 0.001$), with a median time of observation of 1.8 years (range, 3 months–9.3 years). Rituximab was part of the initial treatment in none of the patients in decades 1 and 2, and in 66 and 85% of patients in decades 3 and 4, respectively ($P < 0.001$). Of the patients treated with chemoimmunotherapy during decades 3 and 4, 15 and 60% received rituximab maintenance therapy, respectively. Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) were the most frequent frontline chemotherapy regimen used in decades 1 and 2 (64 and 75% of patients, respectively), while this regimen was only used in combination with immunotherapy in decade 4. The advent of the anti-CD20 antibody rituximab made its combination with CHOP (R-CHOP) the preferred regimen in decades 3 and 4 (39 and 47%, respectively). Response to treatment is detailed in Table 1. For the entire cohort, the percentage of patients achieving a CR/CRu and a PR after frontline treatment was 64 and 28%, respectively, while 8% of patients had refractory disease. A steady increase in the rate of CR/CRu was seen, being of 48%, 53%, 73%, and 70% for decades 1–4, respectively, with the corresponding decrease in the rate of PR and refractory cases. Interestingly, cases not responding to frontline therapy decreased from 10% in decade 1–4% in decade 4 ($P < 0.001$).

Risk of relapse/progression,

Data concerning PFS, POD24, OS, risk of HT, and risk of SM are depicted in Table 2. Three hundred and sixty-four patients (54%) were refractory or experienced relapse/progression during follow-up, with a median PFS for the entire cohort of 4.3 years, and a 10-year PFS of 34%. A significant increase in PFS was observed over the decades, with a median PFS of 2.3, 2.1, 7.5 years, and unreached for decades 1 to 4, respectively ($P < 0.001$, Fig. 1a). In addition, the proportion of patients progressing within the first 2 years after the initiation of frontline therapy (early progressors or POD24) significantly decreased over the decades (44%, 46%, 25%, and 19% for decades 1–4,

Table 2 PFS, POD24, OS, risk of HT, and risk of SM of the 727 patients with follicular lymphoma.

	Entire cohort (*n* = 727)	Decade 1 (1980–1989) (*n* = 79)	Decade 2 (1990–1999) (*n* = 163)	Decade 3 (2000–2009) (*n* = 254)	Decade 4 (2010–2017) (*n* = 231)	*P* value
PFS						
Median PFS, years	4.3	2.3	2.1	7.5	NR	<0.001
5-year PFS, %	46	40	26	55	56	
10-year PFS, %	34	29	14	43	NR	
Early progression (POD24) (%)	204 (30)	34 (44)	73 (46)	60 (25)	37 (19)	<0.001
OS						
Median OS, years	17.6	12.3	11.8	NR	NR	<0.001
5-year OS, %	81	77	74	83	86	
10-year OS, %	65	52	56	72	–	
Histological transformation						
5-year risk, %	7	7	9	5	8	NS
10-year risk, %	10	8	15	7	–	
Second malignancy						
5-year risk, %	5	1	5	7	4	NS
10-year risk, %	10	5	9	12	–	

Due to short follow-up for Decade 4, data concerning survival and risk of histological transformation and second malignancies should be interpreted with caution. *PFS* progression-free survival, *POD24* progression of disease within the first 24 months of frontline treatment initiation, *OS* overall survival, *NR* not reached, *NS* not statistically significant.

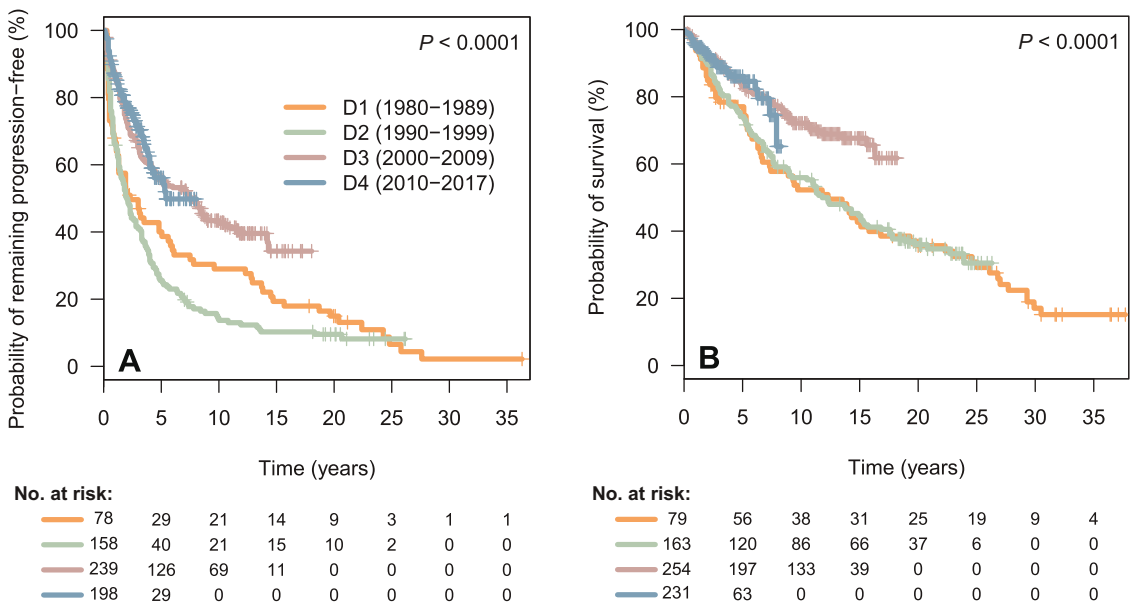

Fig. 1 a progression-free survival of the 677 FL patients who received any treatment, according to the decade of diagnosis. **b** overall survival of the 727 FL patients of the series, according to the decade of diagnosis. D decade, FL follicular lymphoma.

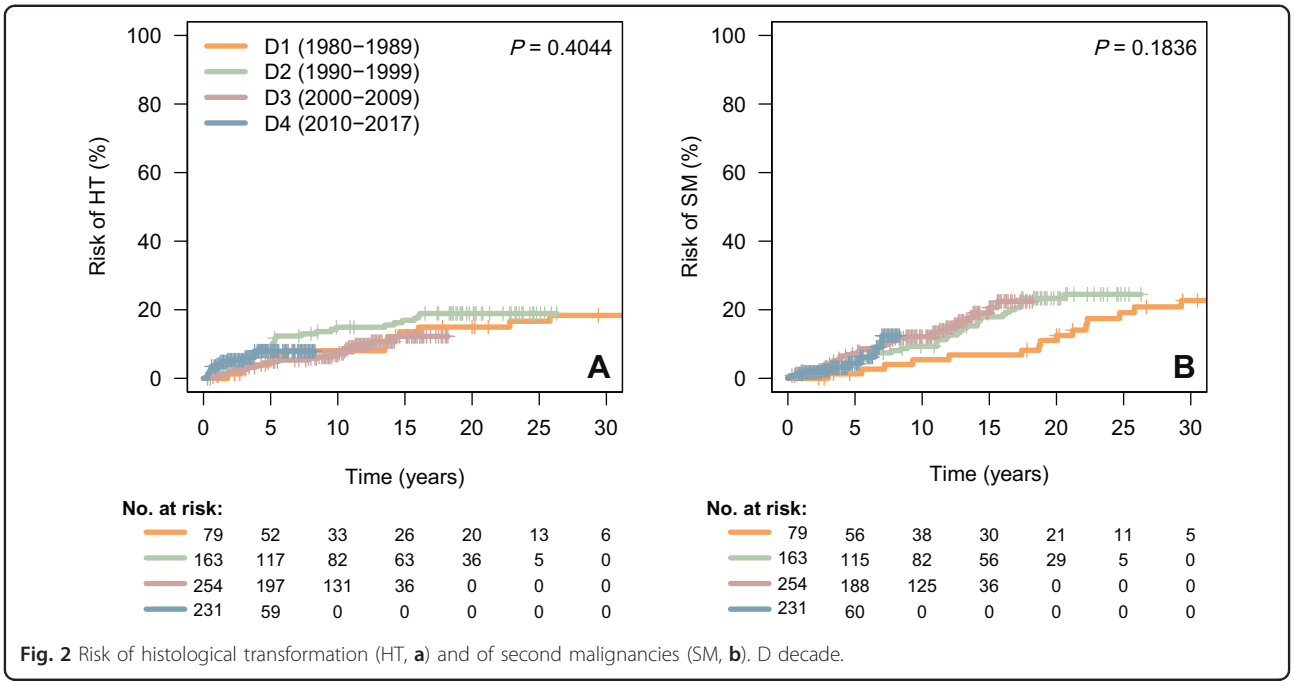

Fig. 2 Risk of histological transformation (HT, **a**) and of second malignancies (SM, **b**). D decade.

respectively, $P < 0.001$). When evaluating survival from an event-defining timepoint (the time of progression for POD24 patients, or 2 years of follow-up for non-POD24 patients), no significant differences were noted among decades, although early progression retained its negative impact on survival for patients diagnosed in all four decades ($P < 0.0001$; Supplementary Fig. 1).

Histological transformation and second malignancies

Risk of HT and SM are plotted in Fig. 2 and Supplementary Fig. 2. HT to diffuse large B-cell lymphoma (DLBCL) was seen in 13 (17%), 30 (18%), 23 (9%), and 15 (7%) patients diagnosed during decades 1–4, respectively. For the entire series, the median time to HT was 4.1 years (range, 3 months–25.8 years). The 5- and 10-year risks of HT for the entire cohort were 7 and 10%, respectively, with no significant differences across decades. Likewise, we did not find significant differences in the rate of HT according to the frontline exposure of rituximab, with or without maintenance.

The diagnosis of a second malignancy after the diagnosis of FL was made in 16 (20%), 37 (23%), 39 (15%), and 10 (4%) patients for those diagnosed during decades 1–4, respectively. The median time to the development of a SM was 7.7 years (range, 1 month–33 years), with a 10-year risk of SM of 10% for all patients, and 5%, 9%, and 12% for decades 1–3, respectively, with no significant differences across decades. Hematological malignancies constituted the most frequent type of SM, which accounted for 20% of cases, including five cases of acute myeloid leukemia. Other frequent sites for the development of SM were the genitourinary tract (17%), the lung and ear, nose, and throat area (15%), and the skin (non-melanoma skin cancer, NMSC, 11%). When the cumulative incidence of SM was evaluated according to the site (hematological malignancies, solid tumors excluding NMSC, and NMSC alone), no significant differences were observed across decades. Likewise, we did not find significant differences in the risk of SM according to the treatment with fludarabine or stem cell transplantation (autologous or allogeneic).

Overall and relative survival

Two hundred and sixty-eight patients (37%) eventually died during the time of follow-up. Median OS for the entire cohort was 17.6 years, and the 10-year OS rate was 65%. As seen for PFS, there was a significant increase in OS, the median OS being 12.3 and 11.8 years for decades 1 and 2, respectively, and unreached for decades 3 and 4 ($P < 0.001$, Fig. 1b). The 10-year OS rate for decades 1 to 4 was 52%, 56%, 72%, and not calculable, respectively.

The outcome of patients along the decades was studied in terms of relative survival (RS) with respect to the general Spanish population (Fig. 3, Supplementary Table 4, and Supplementary Fig. 3). As mentioned above, PFS and OS greatly improved across the decades. This improvement was substantially more evident for FL patients than the gains in life expectancy for the sex- and age-matched general population. Importantly, however, the diagnosis of FL remained a burden for OS, which was significantly inferior to the expected survival of the general population. The 5-year relative survival ratios (RSR) for decades 1 to 4 were 0.83, 0.79, 0.89, and 0.94

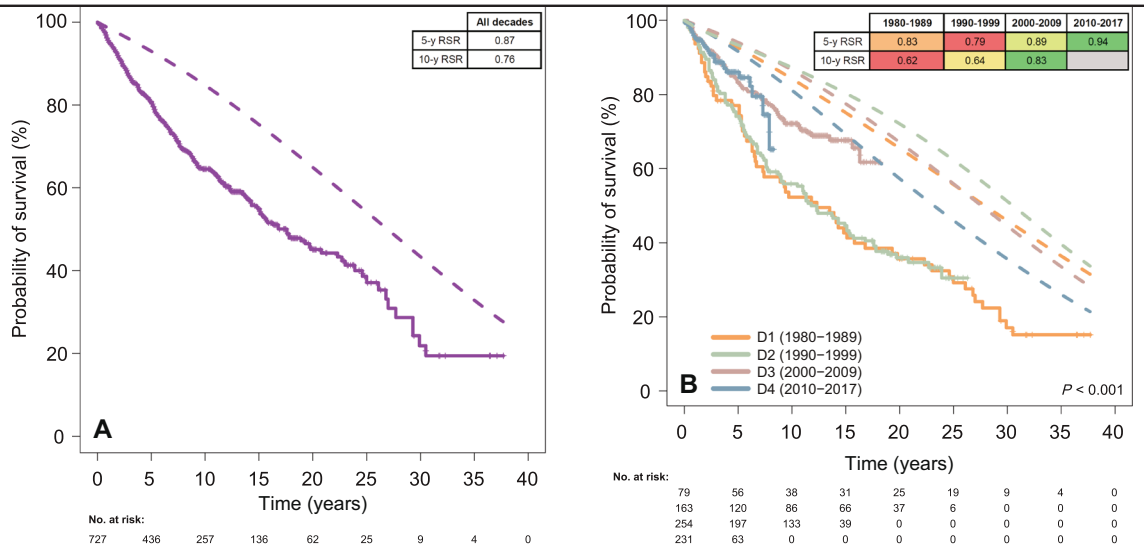

Fig. 3 Relative survival ratios (RSR, calculated by dividing observed by expected survival) are detailed in the built-in tables and color-coded within each row, red and green representing poorer and better RSR values, respectively. a Comparison of observed (solid line) versus expected OS (dashed line) for the global series. **b** Comparison of observed (solid line) versus expected OS (dashed line) according to the decade of diagnosis. D decade, y year.

Table 3 Causes of death in the 204 patients who died within the first 10 years after diagnosis.

	Entire cohort ($n = 204$)	Decade 1 (1980–1989) ($n = 36$)	Decade 2 (1990–1999) ($n = 71$)	Decade 3 (2000–2009) ($n = 66$)	Decade 4 (2010–2017) ($n = 31$)
Progression of FL (%)	111 (54)	22 (61)	47 (66)	28 (42)	14 (45)
Complications of therapy (%)	18 (9)	1 (3)	5 (7)	9 (14)	3 (10)
Other neoplasms (%)	31 (15)	3 (8)	9 (13)	16 (24)	3 (10)
Others/unknown (%)	44 (22)	10 (28)	10 (14)	13 (20)	11 (35)

The Others/unknown group is composed of 23 patients (11%) dying of other causes (mainly geriatric complications and cardiovascular disease), and 21 patients (10%) for whom information concerning the exact cause of death was not available.
FL follicular lymphoma.

respectively. The 10-year RSR were 0.62, 0.64, 0.83, and not calculable, for decades 1–4, respectively. When assessing the evolution of RS according to age (less than 50 years, 50–69 years, and 70 years or more) and sex, the trend of improvement was seen across decades for both sexes and all three age groups.

Causes of death

The exact cause of death was known for 233 (95.2%) of the 268 deceased patients. For patients dying within the 10 years of the diagnosis of FL, 61% of those diagnosed in decade 1 died due to progression of FL, while this percentage progressively decreased during the last two decades (42 and 45% for decades 3 and 4, respectively, $P = 0.020$, Table 3). Consequently, the percentage of patients dying from other/unknown causes increased (28 and 35% for decades 1 and 4, respectively).

Using cumulative incidence of mortality with competing risk of death, 5-year mortality due to progressive FL was estimated at 14 and 20% for decades 1 and 2, respectively, in contrast with 7 and 7% for decades 3 and 4, respectively ($P < 0.0001$). Cumulative incidence of death from complications of therapy or that due to other neoplasms was not significantly different across decades (Fig. 4 and Supplementary Table 5).

Discussion

In the analysis of our cohort of 727 FL patients, spanning almost four decades, we sought to describe variations in care and outcome. Patients diagnosed during decade 4 were older but had a better performance status. These trends presumably reflect changes in the reference population of our institution, and the performance of a greater number of diagnostic tests in otherwise

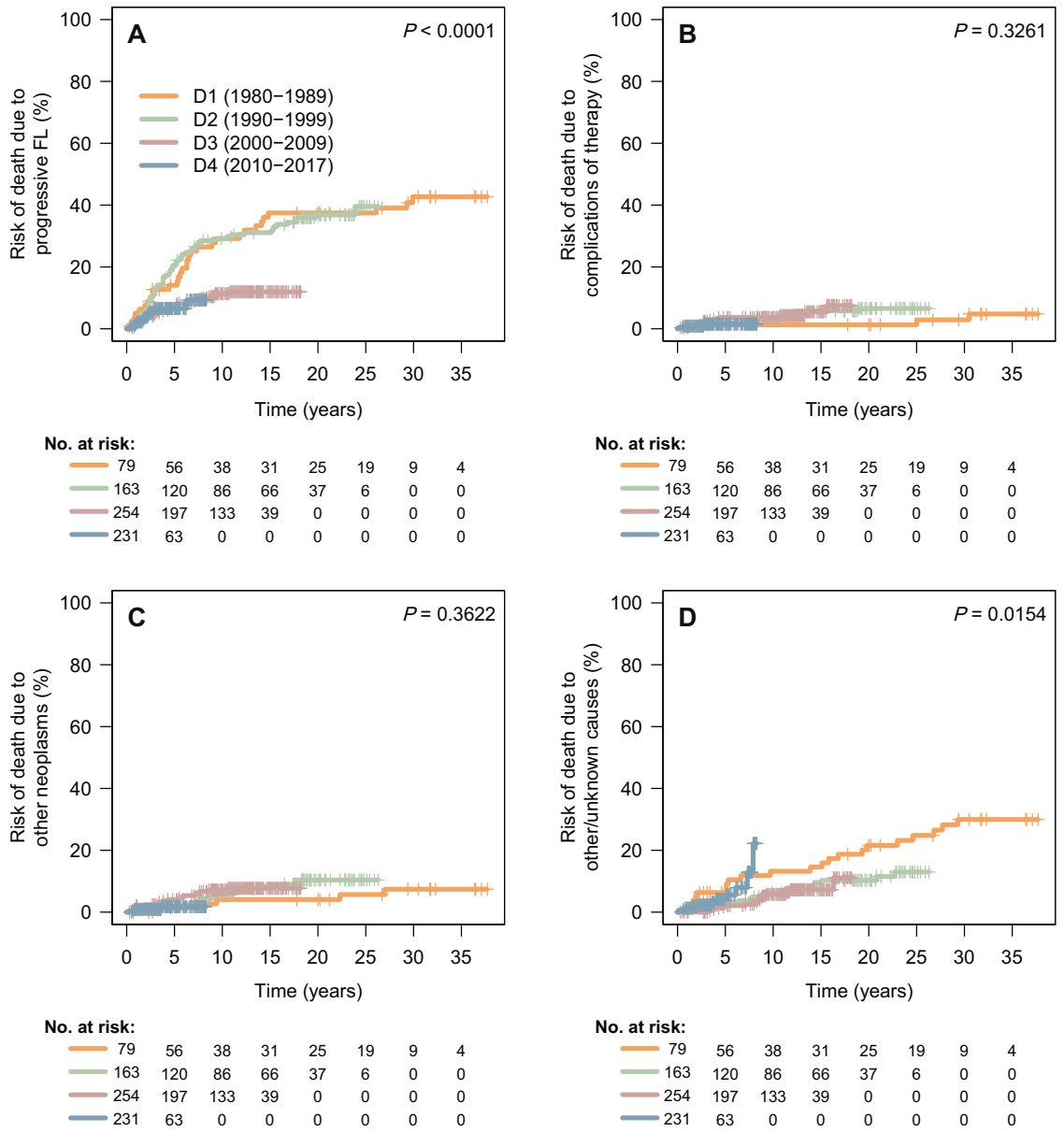

Fig. 4 Cause-specific cumulative incidence of mortality, using competing risk of death. a death due to progressive FL; **b** death due to complications of therapy; **c** death due to other neoplasms; **d** death due to other/unknown causes.

asymptomatic elderly individuals, which might recognize FL at an older age. No patient received immunotherapy during decades 1 and 2, being CHOP-like regimens the most common therapeutic options. With the advent of rituximab, R-CHOP was the most used first line treatment (around 40% of patients in decades 3 and 4).

The fraction of patients achieving a CR after frontline treatment markedly increased over time, likely on account of the adoption of immunotherapy. However, taking into account that PET-CT can help consider as a CR a study where there is a residual mass without metabolic activity, the advent of this technique cannot be discarded as a contributing factor to an improvement in observed responses. This deepening of responses translated into a better PFS (40–56% at 5 years, for decades 1 and 4, respectively) and OS (77–86% at 5 years, for decades 1 and 4, respectively), as previously shown by multicenter studies in the rituximab era[23].

Evidences of improvement in PFS and OS in patients with FL over the last decades have been published in recent years. Some single-center and registry studies have shown an improved survival of FL patients with respect to a sex- and age-matched population[7,9]. Our series shows the same trend, with a 5-year RSR increasing from 0.83 in

decade 1 to 0.94 in decade 4 and a 10-year RSR increasing from 0.62 in decade 1 to 0.83 in decade 3. To evaluate the causes of death in the present series, we first considered only patients dying within the first decade after diagnosis, with the intention of equalizing follow-up among decades. Although the proportion of patients dying due to progressive FL has decreased across decades (61–45% from decades 1–4), most patients still die from lymphoma-related causes (55% in decade 4, considering progressive disease and therapy-related complications), in line with recent results arising from large cohorts[24]. This fact underscores the need for new therapies with improved toxicity profiles and a lower potential to induce SM. When evaluating causes of death of the entire series using a cumulative mortality risk analysis with competing causes of death, we were able to identify a marked reduction in the cumulative incidence of death due to progressive disease.

In contrast with recent reports describing a reduced risk of HT in the rituximab era[19], we were not able to identify significant differences in its incidence among decades, presumably to the relatively small number of this type of event in each of the decades. However, HT rates in the last two decades of the study are superimposable to that reported in the Aristotle study[19].

The appearance of a SM represents one of the concerns of the use of cytotoxic agents, which has led to a growing interest in the development of chemotherapy-free regimens. In the rituximab era, its cumulative incidence has been estimated to be of 2.9% at 10 years for SM arising at any site[24], and of 2.7% at 10 years for second hematological malignancies specifically[25]. In our series, the 10-year incidence of SM of any site was 10%, with no significant differences among decades, and hematological malignancies were the most common subtype. Although follow-up is short, the incidence of SM observed in decade 4 strongly compares with that reported by Sarkozy and colleagues[24].

We have to acknowledge some limitations of our study. First, due to short follow-up of patients in decade 4, data concerning survival and the risk of HT and SM should be interpreted with caution. Second, baseline characteristics were not totally uniform among decades. However, the fact that patients in decade 4 were older might further underscore the improvement in outcomes of FL patients, since older individuals would be expected to fare more poorly. Third, due to historical reasons, the number of patients in decade 1 was lower, as was the availability of a diagnostic specimen for histological review, and data regarding the cause of death, which might explain the higher percentage of patients dying from other/unknown causes in this period of time. Fourth, the low number of patients developing a HT in each decade might hamper the ability to observe a decreased HT rate in the rituximab era. Finally, considering the nature of this disease, an analysis of second and subsequent lines of therapy might help to better characterize progression/relapse patterns and outcomes. Among the strengths of the current study, however, are the large number of patients from a single institution, diagnosed and treated with comparable standards within each decade, the high availability of clinical characteristics and follow-up data, and the use of robust statistical methods to analyze relative survival and competing risks (HT, SM, and causes of death).

In conclusion, in our series, we observed that FL patients have experienced an increase in the CR rate after frontline treatment, with a positive impact on PFS, OS, RS, and cumulative incidence of death due to progressive FL. Conversely, the risk of HT and SM, as well as the percentage of lymphoma-related deaths have remained essentially unchanged, highlighting the need for further advances in prognostic and therapeutic tools for this disease.

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Conflict of interest
The authors declare that they have no conflict of interest.

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References

1. Armitage, J. O. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. *Blood* **89**, 3909–3918. (1997).
2. Horning, S. J. & Rosenberg, S. A. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. *N. Engl. J. Med.* **311**, 1471–1475 (1984).
3. Rivas-Delgado, A. et al. Response duration and survival shorten after each relapse in patients with follicular lymphoma treated in the rituximab era. *Br. J. Haematol.* **184**, 753–759 (2019).
4. Horning, S. J. Natural history of and therapy for the indolent non-Hodgkin's lymphomas. *Semin. Oncol.* **20**, 75–88 (1993).
5. Fisher, R. L. et al. New treatment options have changed the survival of patients with follicular lymphoma. *J. Clin. Oncol.* **23**, 8447–8452 (2005).

6. Swenson, W. T. et al. Improved survival of follicular lymphoma patients in the United States. *J. Clin. Oncol.* **23**, 5019–5026 (2005).
7. Tan, D. et al. Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. *Blood* **122**, 981–987 (2013).
8. Shi, Q. et al. Thirty-month complete response as a surrogate end point in first-line follicular lymphoma therapy: An individual patient-level analysis of multiple randomized trials. *J. Clin. Oncol.* **35**, 552–560 (2017).
9. Junlén, H. R. et al. Follicular lymphoma in Sweden: nationwide improved survival in the rituximab era, particularly in elderly women: a Swedish Lymphoma Registry Study. *Leukemia* **29**, 668–676 (2015).
10. Herold, M. et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German study group hematology and oncology study. *J. Clin. Oncol.* **25**, 1986–1992 (2007).
11. Hiddemann, W. et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: Results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* **106**, 3725–3732 (2005).
12. Marcus, R. et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood* **105**, 1417–1423 (2005).
13. Marcus, R. et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. *J. Clin. Oncol.* **26**, 4579–4586 (2008).
14. Salles, G. et al. Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: Results of the GELA-GOELAMS FL2000 study. *Blood* **112**, 4824–4831 (2008).
15. Liu, Q. et al. Improvement of overall and failure-free survival in stage IV follicular lymphoma: 25 years of treatment experience at the University of Texas M.D. Anderson Cancer Center. *J. Clin. Oncol.* **24**, 1582–1589 (2006).
16. Casulo, C. et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: an analysis from the National LymphoCare Study. *J. Clin. Oncol.* **33**, 2516–2522 (2015).
17. Alonso-Álvarez, S. et al. Risk of, and survival following, histological transformation in follicular lymphoma in the rituximab era. A retrospective multicentre study by the Spanish GELTAMO group. *Br. J, Haematol*, **178**, 699–708 (2017).
18. Montoto, S. et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J. Clin. Oncol.* **25**, 2426–2433 (2007).
19. Federico, M. et al. Rituximab and the risk of transformation of follicular lymphoma: a retrospective pooled analysis. *Lancet Haematol.* **5**, e359–e367 (2018).
20. Brice, P. et al. Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: a randomized study from the Groupe d'Etude des Lymphomes Folliculaires. *J. Clin. Oncol.* **15**, 1110–1117 (1997).
21. Cheson, B. D. et al. Recommendations for initial evaluation, staging, and response assessment of hodgkin and non-hodgkin lymphoma: the lugano classification. *J. Clin. Oncol.* **32**, 3059–3067 (2014).
22. Gray, R. J. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann. Stat.* **16**, 1141–1154 (1988).
23. Bachy, E. et al. Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: long-term results of the PRIMA study. *J. Clin. Oncol.* **37**, 2815–2824 (2019).
24. Sarkozy, C. et al. Cause of death in follicular lymphoma in the first decade of the rituximab era: a pooled analysis of French and US cohorts. *J. Clin. Oncol.* **37**, 144–152 (2019).
25. Prusila, R. E. I. et al. Risk of secondary haematological malignancies in patients with follicular lymphoma: an analysis of 1028 patients treated in the rituximab era. *Br. J. Haematol.* **187**, 364–371 (2019).

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Initial treatment strategies.

Treatment	Entire cohort (N=727)	Decade 1 (1980-1989) (N=79)	Decade 2 (1990-1999) (N=163)	Decade 3 (2000-2009) (N=254)	Decade 4 (2010-2017) (N=231)
Watchful waiting (%)	110 (15)	2 (3)	5 (3)	34 (13)	69 (30)
R-CHOP (%)	174 (24)	0	0	**92 (36)**	**82 (35)**
R-CVP (%)	43 (6)	0	0	25 (10)	18 (8)
Other R-containing regimens (%)	45 (6)	0	0	25 (10)	20 (9)
Single-agent rituximab (%)	48 (7)	0	0	8 (3)	40 (17)
CHOP-like (%)	199 (27)	**50 (63)**	**122 (75)**	27 (11)	0
Fludarabine-based (%)	34 (5)	0	3 (2)	31 (12)	0
Other treatments/unknown (%)	74 (10)	27 (34)	33 (20)	12 (5)	2 (1)

Most used treatment modality in each decade is highlighted in bold.

CVP, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; R, rituximab.

Supplementary Table 2. Second-line treatment strategies

Second-line treatment		1980-1989	1990-1999	2000-2009	2010-2017	Total
No treatment	n	2	2	6	5	15
	%	4%	2%	6%	10%	4%
R-CHOP/R-CVP	n	1	4	15	5	25
	%	2%	3%	14%	10%	7%
Single-agent rituximab	n	3	8	25	2	38
	%	5%	6%	23%	4%	11%
Benda/Fluda-based (+/- R)	n	1	34	19	15	69
	%	2%	26%	17%	29%	20%
Other chemo regimens	n	25	40	17	16	98
	%	45%	31%	16%	31%	28%
Chlorambucil and other agents	n	23	15	1	0	39
	%	41%	12%	1%	0%	11%
ASCT	n	1	23	22	7	53
	%	2%	18%	20%	14%	15%
Allo-SCT	n	0	3	4	1	8
	%	0%	2%	4%	2%	2%

CVP, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; R, rituximab; Benda, bendamustine; Fluda, fludarabine; chemo, chemotherapy; ASCT, autologous stem cell transplantation; Allo-SCT, allogeneic stem cell transplantation.

Supplementary Table 3. Third-line treatment strategies

Third-line treatment		1980-1989	1990-1999	2000-2009	2010-2017	Total
No treatment	n	0	1	4	0	5
	%	0%	1%	6%	0%	3%
R-CHOP/R-CVP	n	1	7	6	0	14
	%	3%	9%	10%	0%	7%
Single-agent rituximab	n	2	6	8	1	17
	%	6%	7%	13%	7%	9%
Benda/Fluda-based (+/- R)	n	1	15	9	2	27
	%	3%	18%	15%	13%	14%
Other chemo regimens	n	18	30	16	12	76
	%	53%	37%	26%	80%	39%
Chlorambucil and other agents	n	8	1	0	0	9
	%	24%	1%	0%	0%	5%
ASCT	n	4	18	11	0	33
	%	12%	22%	18%	0%	17%
Allo-SCT	n	0	4	8	0	12
	%	0%	5%	13%	0%	6%

CVP, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; R, rituximab; Benda, bendamustine; Fluda, fludarabine; chemo, chemotherapy; ASCT, autologous stem cell transplantation; Allo-SCT, allogeneic stem cell transplantation.

Supplementary Table 4. 5- and 10-year survival (observed, expected, and relative survival ratio, RSR) for all patients, and according to the decade of diagnosis, sex, and age at diagnosis. RSR are color-coded within each row, red and green representing poorer and better RSR values, respectively.

			5-y OS				
			All decades	1980-1989	1990-1999	2000-2009	2010-2017
All patients		Series	80.7	77.0	74.1	83.3	86.0
		Pop	93.0	92.7	93.3	94.1	91.6
		RSR	0.87	0.83	0.79	0.89	0.94
Sex	Male	Series	77.0	69.4	71.8	81.1	81.9
		Pop	92.5	93.3	93.4	93.8	90.3
		RSR	0.83	0.74	0.77	0.86	0.91
	Female	Series	84.0	87.8	75.9	85.1	89.4
		Pop	93.4	91.8	93.3	94.3	92.8
		RSR	0.90	0.96	0.81	0.90	0.96
Age	<50 y	Series	91.0	83.9	93.1	87.6	94.1
		Pop	99.1	98.8	99.0	99.0	99.3
		RSR	0.92	0.85	0.94	0.88	0.95
	50-69 y	Series	82.7	74.5	69.4	90.6	90.0
		Pop	95.6	94.5	93.6	96.3	96.7
		RSR	0.87	0.79	0.74	0.94	0.93
	70+ y	Series	61.3	66.7	36.8	61.9	70.9
		Pop	79.9	71.4	74.8	82.4	80.7
		RSR	0.77	0.93	0.49	0.75	0.88

			10-y OS				
			All decades	1980-1989	1990-1999	2000-2009	2010-2017
All patients		Series	64.5	52.3	55.9	72.1	
		Pop	84.8	84.3	87.3	86.6	81.2
		RSR	0.76	0.62	0.64	0.83	
Sex	Male	Series	63.0	45.4	59.1	70.3	
		Pop	84.2	85.6	87.4	86.3	79.0
		RSR	0.75	0.53	0.68	0.81	
	Female	Series	65.8	61.8	53.4	73.7	
		Pop	85.3	82.4	87.2	86.8	82.9
		RSR	0.77	0.75	0.61	0.85	
Age	<50 y	Series	80.2	72.9	72.3	86.1	
		Pop	97.7	97.0	97.6	97.8	98.3
		RSR	0.82	0.75	0.74	0.88	
	50-69 y	Series	67.2	47.7	52.7	79.7	
		Pop	90.2	86.8	88.0	91.2	92.0
		RSR	0.75	0.55	0.60	0.87	
	70+ y	Series	31.5	16.7	19.7	37.4	
		Pop	57.1	43.6	52.6	61.1	57.4
		RSR	0.55	0.38	0.37	0.61	

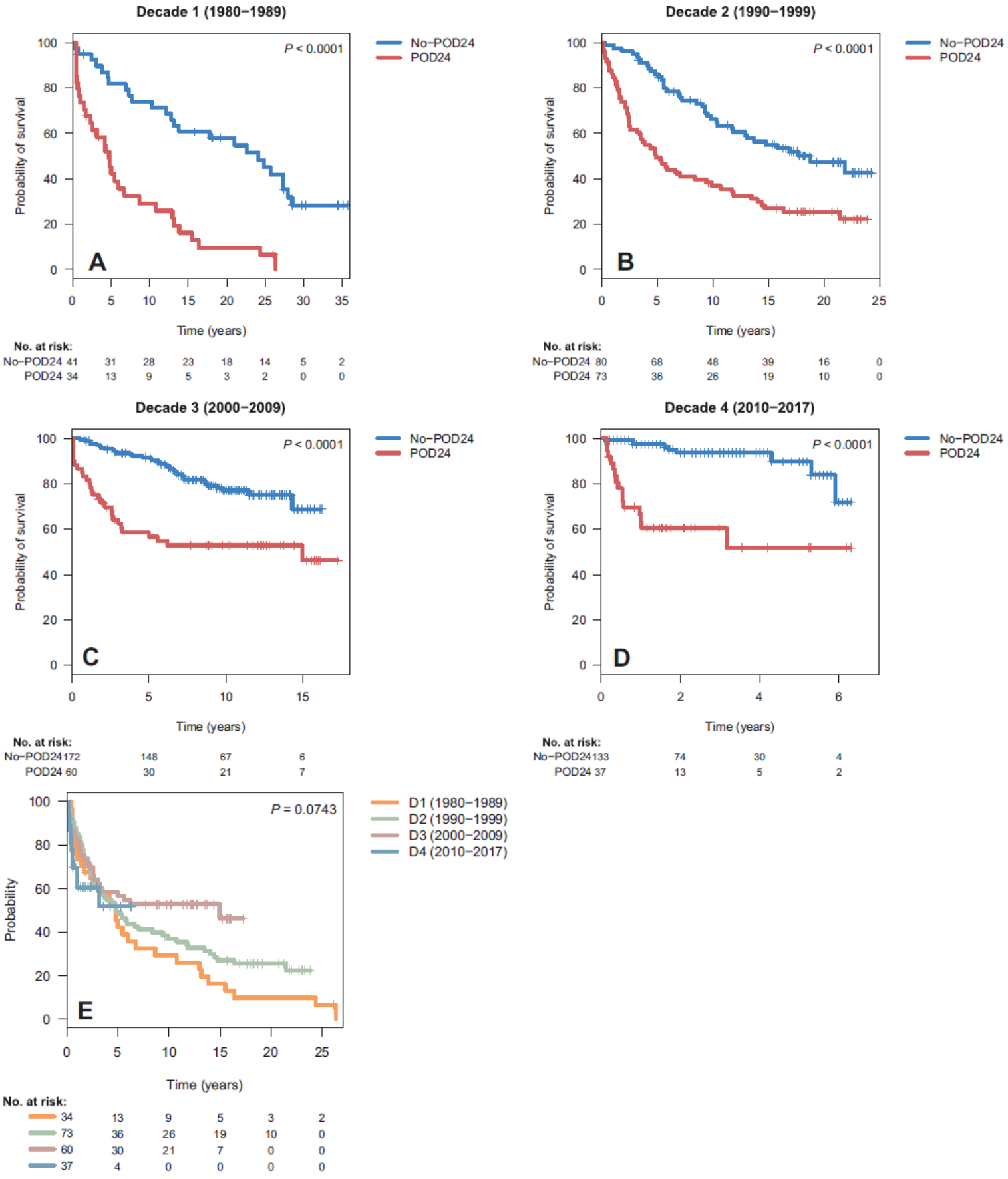
y, year; OS, overall survival, Pop, population; RSR, relative survival risk. OS from the Series and the Population are expressed in %.

Supplementary Table 5. Cause-specific cumulative incidence of mortality, using competing risk of death, for all deceased patients.

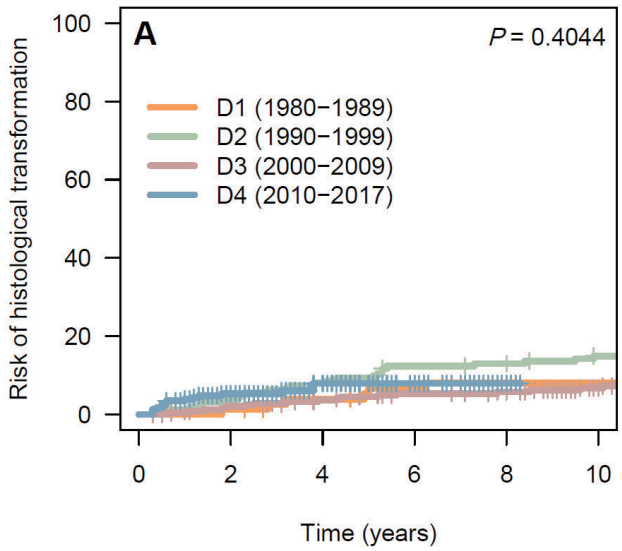
	Entire cohort (N=204)	**Decade 1 (1980-1989) (N=36)**	**Decade 2 (1990-1999) (N=71)**	**Decade 3 (2000-2009) (N=66)**	**Decade 4 (2010-2017) (N=31)**	***P* value**
Death due to progressive FL						
5-year risk, %	11	14	20	7	7	<0.0001
10-year risk, %	19	29	29	12	-	
Death due to complications of therapy						
5-year risk, %	2	1	1	4	2	NS
10-year risk, %	3	1	3	4	-	
Death due to other neoplasms						
5-year risk, %	3	1	2	4	2	NS
10-year risk, %	6	4	6	7	-	

FL, follicular lymphoma.

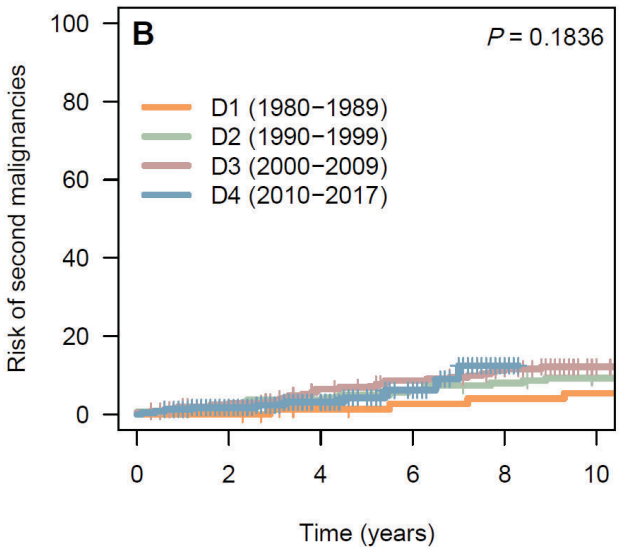
Supplementary Figure 1. Survival from an event-defining timepoint (the time of progression for POD24 patients, or 2 years of follow-up for non-POD24 patients), according to the decade of diagnosis (A through D), and only for POD24 patients (E).



Supplementary Figure 2. Risk of histological transformation (A) and of second malignancies (B) with a follow-up limited to 10 years, according to the decade of diagnosis.

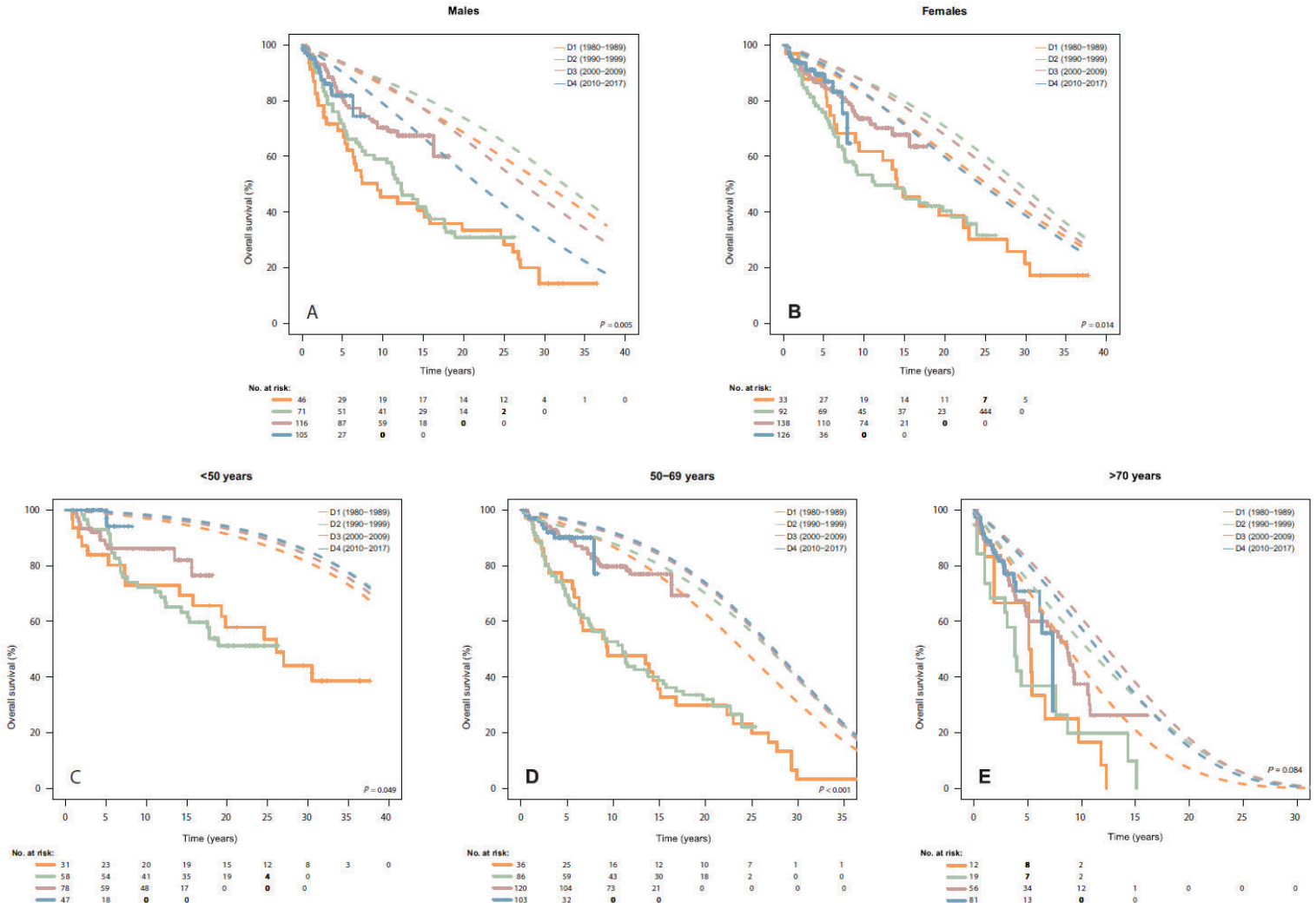


No. at risk:						
D1 (1980−1989)	79	67	55	44	37	33
D2 (1990−1999)	163	143	124	106	92	82
D3 (2000−2009)	254	227	205	185	173	131
D4 (2010−2017)	231	175	85	35	5	0



No. at risk:						
D1 (1980−1989)	79	68	58	48	42	38
D2 (1990−1999)	163	144	123	104	89	82
D3 (2000−2009)	254	229	200	178	165	125
D4 (2010−2017)	231	180	87	34	5	0

Supplementary Figure 3. Comparison of observed (solid line) versus expected (dashed line) OS, according to the decade of diagnosis. A: male patients only; B: female patients only; C: patients younger than 50 years; D: patients between 50 and 69 years; and E: patients of 70 years or older.



2.2. Segundo trabajo

Age and comorbidity are determining factors in the overall and relative survival of patients with follicular lymphoma

Pablo Mozas, Andrea Rivero, Alfredo Rivas-Delgado, Ferran Nadeu, Juan Gonzalo Correa, Carlos Castillo, Alex Bataller, Tycho Baumann, Eva Giné, Julio Delgado, Neus Villamor, Elías Campo, Laura Magnano, Armando López-Guillermo

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La edad y la comorbilidad son factores determinantes en la supervivencia global y relativa de los pacientes con linfoma folicular

Resumen

La fragilidad y la comorbilidad son factores cruciales en el manejo de los pacientes con linfoma folicular (LF). En nuestro estudio evaluamos el impacto pronóstico de la edad y la comorbilidad en la supervivencia, causas de muerte, transformación histológica (TH) y segundas neoplasias (SN) en una serie unicéntrica amplia de pacientes con LF grado 1-3A. Se estudiaron 414 pacientes diagnosticados en la época del rituximab, categorizados en tres grupos de edad (≤60, 61–70, >70 años) y dos grupos de comorbilidad (*Charlson Comorbidity Index*, CCI, 0-1 y ≥2). A pesar de una incidencia acumulada de recaída similar, los pacientes más mayores y más comórbidos tenían una SG a 10 años inferior (88, 65 y 41% para pacientes ≤60, de 61–70 y >70 años, respectivamente, *P* <0.0001; 76 vs 51% para pacientes con CCI 0-1 y ≥2, respectivamente, *P* <0.0001). En el análisis multivariado para la SG, la comorbilidad retuvo su impacto pronóstico independiente (HR=2.5, *P*=0.0003). La proporción de pacientes que fallecieron debido al LF fue más alta entre pacientes ≤60 años (74%) y aquellos con un CCI 0-1 (67%). Además, el exceso de mortalidad (reducción de la supervivencia) a 10 años fue más marcado en pacientes >70 años (30%) y aquellos con un CCI ≥2 (32%). Los pacientes con un CCI ≥2 también tuvieron una mayor incidencia de SN. Estos hallazgos son un estímulo para una evaluación integral previa al tratamiento y una estrategia terapéutica adaptada para todos los pacientes con LF.

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ORIGINAL ARTICLE

Age and comorbidity are determining factors in the overall and relative survival of patients with follicular lymphoma

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Abstract

Frailty and concurrent medical conditions are crucial factors in the management of follicular lymphoma (FL). We evaluated the impact of age and comorbidity on survival, causes of death, histological transformation (HT), and second malignancies (SM) in a large single-center series of grade 1–3A FL. We studied 414 patients diagnosed in the rituximab era, categorized into three age groups (≤60, 61–70, >70 years) and two comorbidity groups (Charlson Comorbidity Index, CCI, 0–1 and ≥2). Despite a similar cumulative incidence of relapse, older and comorbid patients had a lower 10-year overall survival (OS, 88, 65, and 41% for patients ≤60 years, 61–70 years, and >70 years, $P<0.0001$; and 76 vs. 51% for CCI 0–1 and ≥2, $P<0.0001$). In a multivariate analysis for OS, comorbidity retained its prognostic impact (HR=2.5, $P=0.0003$). The proportion of patients dying due to FL was higher among those ≤60 years (74%) and those with a CCI 0–1 (67%). Furthermore, 10-year excess mortality (survival reduction) was more prominent for patients >70 years (30%) and those with a CCI ≥2 (32%). Patients with a CCI ≥2 also had a higher incidence of SM. These data encourage a comprehensive pre-treatment evaluation and a tailored therapeutic approach for all FL patients.

Keywords Follicular lymphoma · Age · Comorbidity · Prognosis · Survival

Introduction

Follicular lymphoma (FL), the most common indolent B-cell lymphoma [1], has seen great improvements in response to treatment, progression-free (PFS), and overall survival (OS) in recent times [2]. Despite its high risk of relapse, survival is generally prolonged, with a median OS currently approaching 20 years [3].

Large registry studies have established the median age at diagnosis for this disease at 65 years [4], which makes frailty and comorbidity challenges in the management of these patients. In an effort to consider the increased mortality inherent to older patients, widely used prognostic scores have included age as an item [5–7], while others do not take this parameter into account [8], thus only examining lymphoma-related variables. Indeed, the impact of age on the course of the disease has been explored in several studies. Patients above 70 years have been shown to have a poorer OS [9, 10], and a higher likelihood of dying of causes different from the lymphoma [9]. Age has been associated with higher-risk baseline features

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and a lower PFS, but not a higher cumulative incidence of relapse (CIR), concluding that older patients should also be considered for standard treatments or clinical trials [11]. Moreover, the benefit of treating very old patients with FL (≥80 years) has also been demonstrated, irrespective of their comorbidity level [12].

The appearance of very prevalent conditions, such as arterial hypertension, diabetes mellitus, or chronic respiratory diseases increases with age, and cancer patients are not an exception. In a retrospective study of 224 FL patients, 7% had a Charlson Comorbidity Index (CCI) ≥2, and this parameter was an independent predictor of OS [13]. However, histological grade 3 was not subdivided into 3A or 3B, and relative survival (RS) and the risks of histological transformation (HT) or second malignancies (SM) were not assessed. Furthermore, survival was not stratified by age at diagnosis, a parameter that can show significant interaction with comorbidity. Likewise, considering the prolonged survival of FL patients, the description of causes of death (COD) is of particular interest. Lymphoma- or treatment-related death has been shown to be the most common COD in all age groups, including those >70 years [14].

In our retrospective analysis of a large single-center cohort of FL patients, we aim to describe the impact of age and comorbidity on the response to treatment, PFS, OS, COD, RS, risk of HT, and of SM.

Methods

Patients

Four hundred and fourteen patients (230 females, 184 males; median age, 60 years; range, 26 to 91) consecutively diagnosed with grade 1–3A FL at Hospital Clínic of Barcelona between February 2002 and February 2018 were included in the present study. All available diagnostic specimens underwent pathological review. Patients with grade 3B FL, primary gastrointestinal or cutaneous FL, or composite lymphoma were not included. Baseline patient and disease characteristics, type of frontline treatment, PFS and OS, and risk of HT and SM were assessed retrospectively. The decision of initiating therapy (but not the treatment modality) responded to the Groupe d'Étude des Lymphomes Folliculaires (GELF) criteria [15], irrespective of age or comorbidity. The study was conducted according to the Hospital Clínic Institutional Review Board and in accordance with the Declaration of Helsinki.

Age and comorbidity

In line with previous studies [9, 11], we stratified patients into three groups, according to their age at diagnosis (≤60, 61–70, and >70 years). To assess comorbidity, data concerning patient- and medical history-reported conditions, mental status, concomitant medications, and laboratory tests at the time of diagnosis were retrospectively evaluated, and the CCI was calculated [16] (Supplementary Table 1), without considering the points allocated to the lymphoma. We then categorized patients into those having no/minor comorbidity (CCI 0–1) or moderate/severe comorbidity (CCI ≥2). The prevalence of other relevant conditions not considered by the CCI was also documented (arterial hypertension and hyperlipidemia).

Response assessment, endpoints, and statistical analysis

Computed tomography (CT) and unilateral bone marrow biopsy were used as staging and response assessment tools, response was evaluated after induction, and definitions of complete response (CR), partial response (PR), and treatment failure were the standard [17]. Positron emission tomography-CT (PET-CT) was systematically performed since 2012 and was available in 195 cases.

PFS was calculated from the date of the first dose of frontline treatment to the date of progression, relapse, or death of any cause. Hence, early progressors (POD24) were defined as patients experiencing relapse or progressive disease in the first 24 months after the initiation of frontline treatment. This analysis only included patients who received treatment, achieved at least a PR, and had a follow-up of more than 2 years.

All patients were included for OS analysis, which was calculated from the date of diagnosis to the date of last follow-up or death from any cause. Kaplan-Meier survival curves were plotted according to age and comorbidity and compared for statistical differences by use of the log-rank test. For the construction of a multivariate model including comorbidity and the rest of variables with statistical significance in the univariate analysis for OS, Cox proportional hazards regression was used. Age was not independently incorporated in the multivariate model, since it is part of the FLIPI score.

To compare the OS observed in our cohort with that of the general population, patients were matched by age and sex with Spanish individuals from the Human Mortality Database [18], which provides an estimate of the cause-specific survival through relative survival analysis (relsurv package, R software, Vienna, Austria). Excess mortality (survival reduction), expressed as a percentage, was calculated with the formula [1 – (cohort survival/population survival)] × 100 and was intended to reflect the reduction in life expectancy compared with the general population. We considered the following causes of death: progressive FL, treatment-related toxicities, neoplasms different from FL, and other/unknown causes. We then grouped the first two into an endpoint called lymphoma-related death (LRD) and the last two into one called lymphoma-unrelated death (LuRD), in line with previous studies [14].

HT was defined based on histological and/or cytological criteria. Data concerning all types of malignant tumors appearing after the diagnosis of FL were collected (including non-melanoma skin cancer), and the time from the diagnosis of FL to that of the second neoplasm was registered. To estimate the risk of HT and of SM, where competitive events are possible, cumulative incidence was calculated (cmprsk R package) and compared by use of Gray's test [19]. This method was also used for the calculation of cause-specific cumulative incidence of mortality, and for the CIR vs. non-relapse mortality. The χ^2 test or Fisher's exact test was used for comparison of categorical variables. *P* values <0.05 were considered to indicate statistical significance.

Results

Patient characteristics

Baseline characteristics of the 414 patients of the series are shown in Table 1. Patient distribution according to the age at diagnosis was as follows: ≤60 years, *n*=211 (51%); 61–70 years, *n*=90 (22%); and >70 years, *n*=113 (27%). Median follow-up for surviving patients was 5.1 years. The proportion of female patients increased with age (51% in ≤60 years to 66% in >70 years). Information on comorbidity was available for 406 patients (98%) and, while most patients (60%) had no comorbidity, the presence of moderate/severe comorbidity (CCI ≥2) increased with age (12% in ≤60 years to 41% in >70 years). Patients >70 years had a worse Eastern Cooperative Oncology Group performance status (ECOG PS), a higher frequency of elevated β_2-microglobulin (B2M) and/or lactate dehydrogenase (LDH) levels, and higher-risk disease, per the established prognostic scores (FLIPI, FLIPI2, and PRIMA-PI). When FLIPI was re-calculated without considering the point attributed to age, older patients still had higher-risk disease.

Frontline treatment and response to therapy

Initial treatment strategies and the obtained responses, according to age and comorbidity, are detailed in Table 2. The proportion of patients who received any treatment during follow-up was similar among age groups, as was that of patients undergoing an initial wait and watch approach longer than 6 months. Younger patients were more likely to receive R-CHOP as frontline treatment (67 and 61% for patients ≤60 and 61–70 years, respectively), while older patients were more frequently treated with R-CVP (35%). No significant differences were found in the proportion of patients treated with single-agent rituximab or other rituximab-based combinations, according to age. Comorbidity was not associated with

Table 1 Main baseline features of the 414 patients of the series, according to the age at diagnosis

Characteristic [available data]	All patients	Age			
		≤60 y	61–70 y	>70 y	*P* value
Number of patients (%)	414 (100)	211 (51)	90 (22)	113 (27)	–
Female sex (%)	230 (56)	107 (51)	49 (54)	74 (66)	0.038
Charlson comorbidity index [*n*=406 (98%)]					
0	242 (60)	154 (75)	47 (54)	41 (37)	<0.001
1	69 (17)	28 (13)	16 (18)	25 (22)	
≥2	95 (23)	24 (12)	25 (28)	46 (41)	
Arterial hypertension (%) [*n*=407 (98%)]	113 (28)	24 (12)	35 (40)	54 (48)	<0.001
Hyperlipidemia (%) [*n*=407 (98%)]	82 (20)	29 (14)	27 (31)	26 (23)	0.003
Performance status, ECOG ≥2 (%) [*n*=413 (99.8%)]	27 (7)	8 (4)	4 (4)	15 (13)	0.003
B symptoms (%) [*n*=414 (100%)]	50 (12)	26 (12)	8 (9)	16 (14)	NS
Ann Arbor stage III–IV (%) [*n*=414 (100%)]	310 (75)	158 (75)	66 (73)	86 (76)	NS
LDH above normal (%) [*n*=391 (94%)]	77 (20)	34 (17)	16 (19)	27 (25)	NS
β2-microglobulin above normal (%) [*n*=376 (91%)]	163 (43)	60 (31)	31 (38)	72 (71)	<0.001
Hemoglobin <120 g/L (%) [*n*=388 (94%)]	73 (19)	29 (15)	9 (11)	35 (32)	<0.001
High-risk FLIPI (%) [*n*=397 (96%)]	100 (25)	29 (14)	20 (24)	51 (46)	<0.001
High-risk FLIPI, not considering age (%) [*n*=397 (96%)]	60 (15)	29 (14)	7 (8)	24 (21)	0.002
High-risk FLIPI2 (%) [*n*=386 (93%)]	111 (29)	26 (13)	29 (36)	56 (51)	<0.001
High-risk PRIMA-PI (%) [*n*=392 (95%)]	98 (25)	31 (15)	17 (20)	50 (49)	<0.001

ECOG Eastern Cooperative Oncology Group, *LDH* lactate dehydrogenase, *FLIPI* Follicular Lymphoma International Prognostic Index, *NA* not available, *PRIMA-PI*, PRIMA Prognostic Index, *NS* not statistically significant, *y* years

Table 2 Treatment and response data for the 414 patients of the series, according to the age and comorbidity

Characteristic	All patients	Age				Comorbidity		
		≤60 y	61–70 y	>70 y	*P* value	CCI 0–1	CCI ≥2	*P* value
Never treated (%)	49 (12)	19 (9)	16 (18)	14 (12)	NS	33 (11)	16 (17)	NS
Treated during follow-up (%)	365 (88)	192 (91)	74 (82)	99 (88)		278 (89)	79 (83)	
Watch and wait period >6 months (%)	53 (15)	31 (16)	10 (14)	12 (12)	NS	35 (13)	18 (23)	0.025
Frontline treatment regimens								
R-CHOP (%)	204 (56)	128 (67)	45 (61)	31 (31)	<0.001	168 (61)	29 (37)	0.003
R-CVP (%)	46 (13)	4 (2)	7 (10)	35 (35)		29 (10)	17 (22)	
Single-agent rituximab (%)	60 (16)	30 (16)	14 (19)	16 (17)		45 (16)	15 (19)	
Others (%)	55 (15)	30 (15)	8 (10)	17 (17)		36 (13)	18 (22)	
CR rate (%)	268 (73)	150 (78)	54 (73)	64 (65)	0.048	202 (73)	58 (73)	NS

R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; *R-CVP* rituximab, cyclophosphamide, vincristine, and prednisone; *CR* complete response; *CCI* Charlson Comorbidity Index; *NS* not statistically significant; *y* years

the probability of receiving treatment during follow-up. However, patients with a CCI ≥2 were more likely to undergo an initial wait and watch approach (23 vs. 13%, *P*=0.025). In patients with a CCI 0–1, R-CHOP was the most widely used treatment (61%), compared with 37% for those with a CCI ≥2, in favor of other less intensive regimens. There was a slight but statistically significant reduction in the CR rate with increasing age (78, 73, and 65% for patients ≤60 years, 61–70 years, and >70 years, respectively), while comorbidity did not impact on response.

Risk of relapse/progression

Outcomes according to age and comorbidity are described in Table 3. One hundred ant twenty-one patients (29%) experienced relapse/progression during follow-up, with a median PFS for the entire cohort of 8.9 years and a 10-year PFS of 50%. Increasing age was associated with a significantly lower PFS (61, 43, and 32% at 10 years, for patients ≤60 years, 61–70 years, and >70 years, respectively, *P*=0.0015, Fig. 1a), but comorbidity was not (Fig. 1b). However, when competing risk analysis was performed (relapse vs. non-relapse mortality), neither age nor comorbidity impacted on the CIR (Fig. 1 c and d). There were no significant differences in the proportion of POD24 patients according to age, while this percentage was slightly higher in the low comorbidity group.

Histological transformation and second malignancies

HT to diffuse large B-cell lymphoma (DLBCL) was seen in 27 patients (6.5%), the median time to HT was 1.8 years (range, 3 months to 10.7 years), and the risk of HT was not significantly different according to age or comorbidity (Supplementary Figure 1).

The diagnosis of a second malignancy after the diagnosis of FL was made in 39 patients (9.4%, Supplementary Table 2). Lung and ear/nose/throat malignancies were the most common type (23%), followed by breast cancer (15%) (Fig. 2). Patients of 61–70 years had a higher rate of SM (28% at 10 years, Fig. 3a), compared with younger and older patients. The presence of a CCI ≥2 was also associated with a higher risk of SM (23 vs. 10%, *P*=0.004, Fig. 3b), although this increased risk was not independent from age. No significant differences were seen in the site of the SM according to age or comorbidity. Eighteen patients who developed a SM died during the time of follow-up, and the SM was the cause of death in most of them (16/18, 89%).

Overall survival

Eighty-one patients (20%) died during follow-up. Median OS for the entire cohort was not reached, and the 10-year OS rate was 71%. Older patients had a lower 10-year OS (88, 65, and 41% for patients ≤60 years, 61–70 years, and >70 years, respectively, *P*<0.0001, Fig. 2a, continuous lines), as did patients with a CCI ≥2 (51 vs. 76% at 10 years, *P*<0.0001, Fig. 2b, continuous lines). When the concurrent impact of age and comorbidity on OS was explored, it remained statistically significant (*P*<0.0001, Fig. 2c). To avoid attributing the prognostic impact of comorbidity solely to the older age, we also performed a bivariate for OS including the CCI and age (as a quantitative and categorical variable), and both variables retained statistical significance.

We designed a multivariate model for OS, with 365 cases and 67 events (Supplementary Figure 2). The variables included in the analysis were the presence of B symptoms, ECOG PS, presence of a bulky mass, FLIPI score, elevated B2M levels, and a CCI ≥2. All the aforementioned variables retained an independent impact on OS, except for B2M and

Table 3 Outcomes of the patients of the series, according to age and comorbidity

Characteristic	All patients	Age				Comorbidity		
		≤60 y	61–70 y	>70 y	*P* value	CCI 0–1	CCI ≥2	*P* value
CIR, % at 10 y (95% CI) [*n*=365]	39 (33–45)	36 (28–44)	44 (30–57)	41 (29–52)	NS	42 (35–49)	32 (20–43)	NS
PFS, % at 10 y (95% CI) [*n*=365]	50 (44–57)	61 (54–70)	43 (31–60)	32 (21–48)	0.015	52 (45–60)	39 (28–56)	NS
POD24 (%) [*n*=365]	71 (20)	42 (22)	11 (13)	19 (20)	NS	61 (22)	10 (12)	0.044
OS, % at 10 y (95% CI) [n=414]	71 (65–77)	88 (83–93)	65 (52–81)	41 (29–58)	<0.0001	76 (70–83)	51 (38–67)	<0.0001
Cause of death [*n*=81]								
Lymphoma-related (%)	43 (53)	14 (74)	8 (40)	21 (50)	0.047*	32 (67)	11 (33)	0.01*
Progressive FL (%)	31 (38)	7 (37)	7 (35)	17 (41)		21 (44)	10 (30)	
Treatment complications† (%)	13 (16)	7 (37)	1 (5)	5 (12)		11 (23)	2 (6)	
Lymphoma-unrelated (%)	38 (47)	5 (26)	12 (60)	21 (50)		16 (33)	22 (67)	
Other malignancies (%)	15 (19)	2 (10)	7 (35)	6 (14)		9 (19)	6 (18)	
Other causes/unknown (%)	22 (27)	3 (16)	5 (25)	14 (33)		7 (15)	15 (46)	
Excess mortality, % at 10 y [*n*=414]	15	8	25	30	–	12	32	–
Risk of HT, % at 10 y (95% CI) [*n*=414]	8 (5–11)	7 (4–12)	10 (4–20)	6 (2–12)	NS	7 (4–11)	9 (4–17)	NS
Risk of SM, % at 10 y (95% CI) [*n*=414]	13 (9–17)	5 (2–10)	28 (16–41)	14 (7–24)	<0.0001	10 (6–15)	23 (12–36)	0.0039

CIR cumulative incidence of relapse, *PFS* progression-free survival, *CI* confidence interval, *POD24* progression of disease within 24 months of treatment initiation, *OS* overall survival, *FL* follicular lymphoma, *RS* relative survival, *HT* histological transformation, *SM* second malignancies, *NR* not reached, *CCI* Charlson Comorbidity Index, *NS* not statistically significant, *y* years

*Chi-square test has been performed comparing the four categories of the COD variable, not the LRD/LuRD variable. Percentages are calculated within the LRD/LuRD variable, and within the four COD variable

†Treatment complications include those associated with any line of treatment, including rescue regimens and autologous/allogeneic stem cell transplantation

B symptoms. Of note, a CCI ≥2 was independently associated with a shorter OS (HR=2.5; *P*=0.0003).

Causes of death

The exact cause of death was known for 75 (95%) of the 79 deceased patients. In the entire series, most patients died of lymphoma-related causes (53%), this percentage being significantly higher in those ≤60 years (74%) than in those >70 years (50%). Most patients (67%) with a CCI 0–1 died due to lymphoma-related causes, while most patients (67%) with a CCI ≥2 died due to lymphoma-unrelated causes. Age impacted on a higher cumulative incidence of LRD, while comorbidity did not affect it (Supplementary Figure 3).

Relative survival

The outcome of patients according to age and comorbidity was studied with respect to the general Spanish population. Patients from our series experienced a 15% survival reduction (also called excess mortality) when compared with a sex- and age-matched population. The 10-year excess mortality was 8, 25, and 30% for patients ≤60 years, 61–70 years, and >70 years, respectively (Fig. 2a, dashed lines). Patients with a CCI ≥2 had a higher excess mortality than those with a CCI 0–1 (32 vs. 12% at 10 years, Fig. 2b, dashed lines).

Discussion

In the analysis of our cohort of 414 FL patients diagnosed at a single tertiary institution, we sought to describe the influence of age and comorbidity on outcome. The median age at diagnosis in our series was somewhat lower than that reported in population studies [4], likely due to the referral of patients with more unfavorable clinical behavior who are candidates for intensive approaches. Since FLIPI and FLIPI2 consider age as a risk factor, older patients had higher-risk scores, but this distribution was maintained when age was omitted from the calculation, likely reflecting a more aggressive tumor biology in older patients, as it has been previously described [20]. Age influenced treatment choices, as shown by the omission of anthracyclines in the 35% of patients >70 years treated with R-CVP. Despite a shorter PFS, older patients did not have a higher CIR, which brings to light the limitations of this combined endpoint.

As expected and previously reported [11], older patients had a poorer OS. In the entire series, 38% of patients died due to progressive FL, a percentage that is lower than that

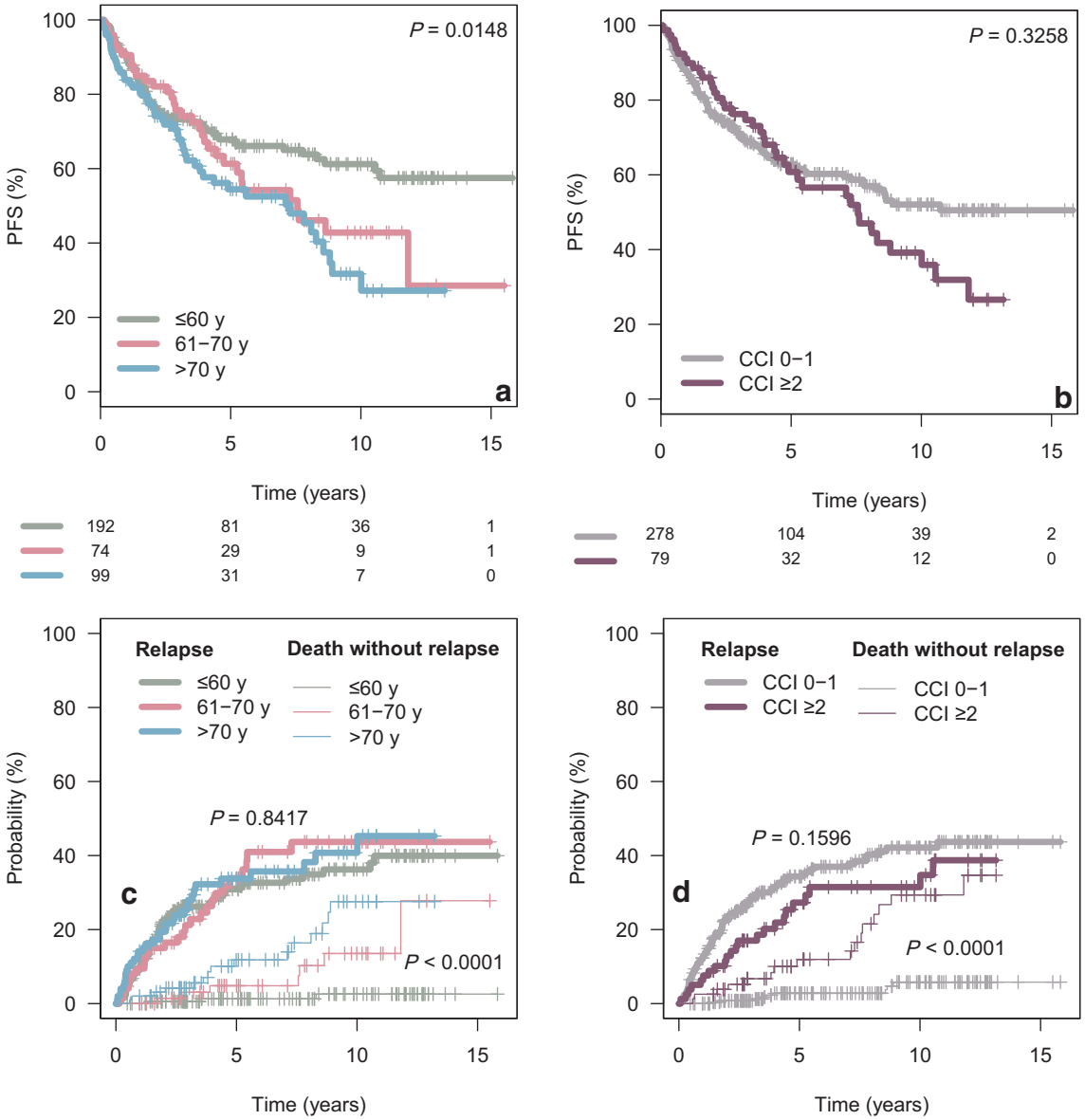

Fig. 1 Progression-free survival according to age (a) and the Charlson Comorbidity Index (CCI) (**b**). Cumulative incidence of relapse (thick lines) and non-relapse mortality (thin lines) according to age (**c**) and CCI (**d**). PFS, progression-free survival; CCI, Charlson Comorbidity Index; y, years

reported for a large cohort of patients in which causes of death were evaluated [14]. When combining progressive FL and treatment-related complications, the percentage of patients dying due to the so-called lymphoma-related death (LRD) was significantly higher in the younger than the older age group. Additionally, patients in our series had a 15% excess mortality when compared to the general population, and this burden was more prominent in elderly patients (30%), underscoring the need for a risk- and life expectancy-adapted treatment of FL in all age groups, as stated by Albarmawi and colleagues [12].

Considering that FL is mostly diagnosed in elderly patients, frailty and comorbidity are crucial issues in the management of this disease. In line with the only published study assessing accompanying medical conditions in FL, with 224 cases [13], the majority of the patients of our series (60%) did not have any comorbidity, and age and comorbidity were tightly related. Comorbidity determined the choice of less intensive therapies, as demonstrated by the fact that only 37% of patients with a CCI ≥2 was treated with R-CHOP. Despite a similar CIR, comorbid patients had a shorter OS, especially in the older subgroup, and comorbidity retained its adverse prognostic impact in the multivariate model, as suggested by Mihaljevic and colleagues [13]. However, we believe that, in order to propose a new prognostic model

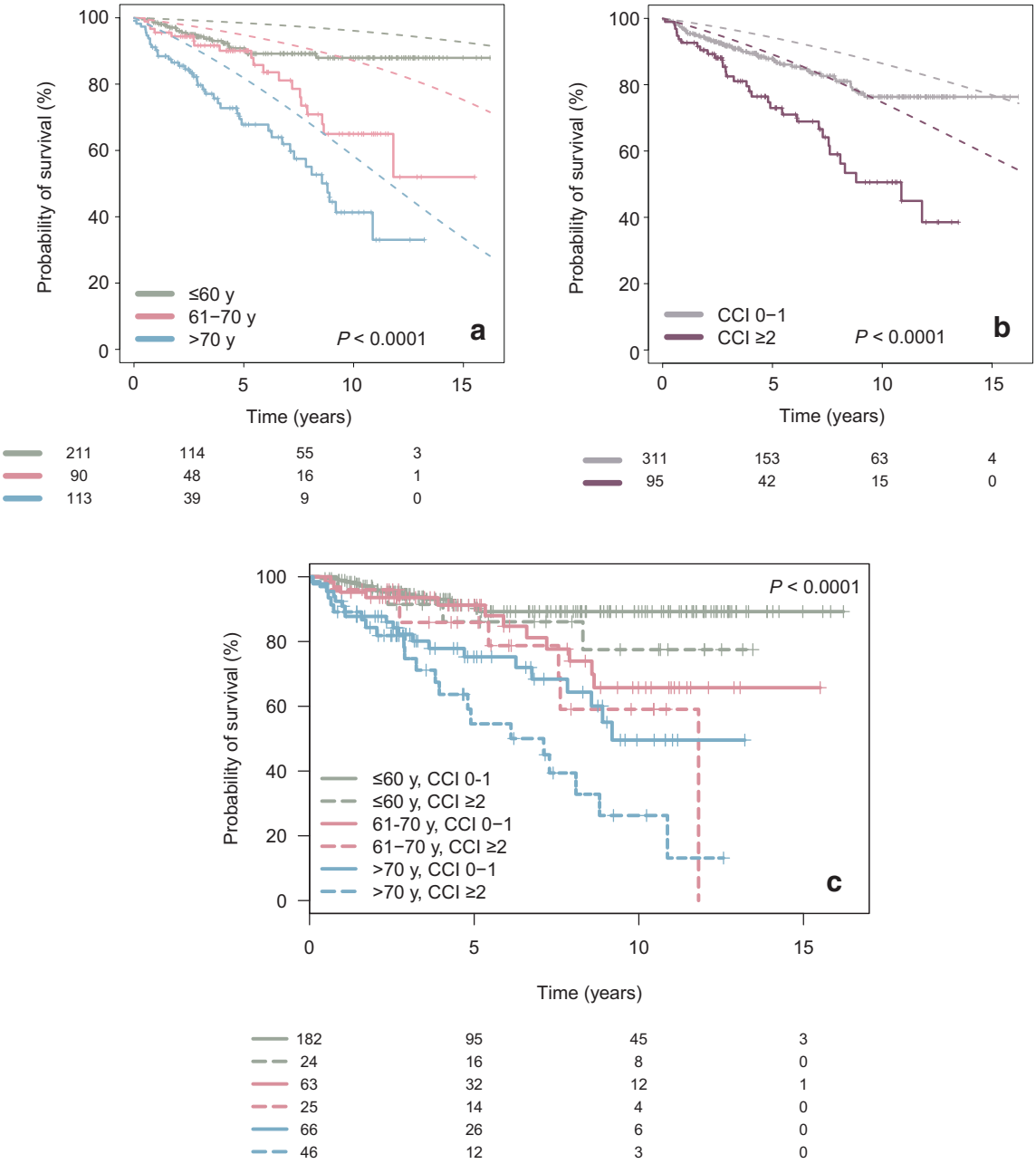

Fig. 2 **a** Overall survival (OS) according to age for all the patients of the series (continuous lines) and for the sex- and age-matched general population (dashed lines). **b** OS according to the Charlson Comorbidity Index (CCI) for all the patients of the series (continuous lines) and for the sex- and age-matched general population (dashed lines). **c** OS considering age and comorbidity. y, years

including comorbidity, these findings should be validated in larger series of patients.

In our series, patients aged 61–70 years had a higher risk of SM compared with younger and older patients, a finding that could be explained by the lower intrinsic risk of second neoplasms in the younger age group, and the performance of fewer screening or invasive diagnostic tests in the older one. Concerning causes of death, comorbid patients were more likely to die due to causes unrelated to the lymphoma (67%), while the same fraction of patients with no or mild comorbidity experienced lymphoma-related death. When excess mortality was calculated, comorbidity was an additional burden for relative survival. Although comorbidity did not impact the risk of HT, it did increase the risk of SM, likely reflecting an older age and the existence of shared risk factors between other cancers (lung, ear, nose, and throat area, genitourinary tract) and accompanying medical conditions (chronic obstructive respiratory disease, ischemic heart disease).

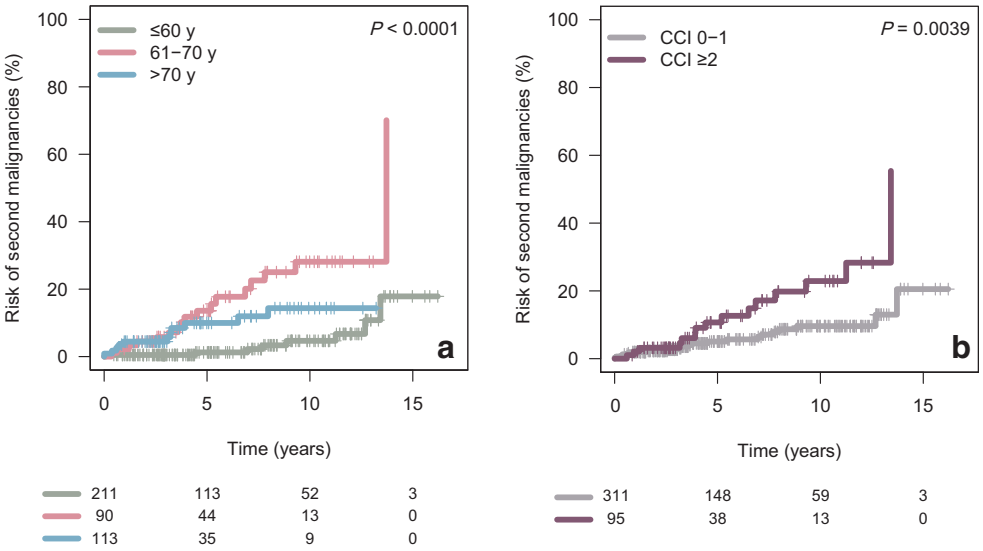

Fig. 3 Risk of second malignancies according to age (**a**) and the Charlson Comorbidity Index (CCI) (**b**)

Conclusion

In our series, we observed that age and comorbidity are independent predictors of OS, but not of CIR. Younger patients and those with no or mild comorbidity are more likely to die from lymphoma-related causes. Relative survival analysis demonstrated that the mortality burden of FL is higher in older patients and those with moderate-severe comorbidity, warranting a tailored treatment approach for all patients diagnosed with this disease.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00277-021-04470-7.

Author contribution PM designed the study, collected the data, performed the analysis, and wrote the paper. AR, ARD, FN, JGC, CC, AB, TB, EG, JD, NV, EC, LM, and ALG contributed to data collection and approved the final version of the manuscript.

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Availability of data and material Available upon request.

Code availability Not applicable.

Declarations

Ethics approval The study was conducted according to the Hospital Clínic Institutional Review Board and in accordance with the Declaration of Helsinki.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Swerdlow SH, Campo E, Harris NL et al (2017) WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France
2. Mozas P, Nadeu F, Rivas-Delgado A, Rivero A, Garrote M, Balagué O, González-Farré B, Veloza L, Baumann T, Giné E, Delgado J, Villamor N, Campo E, Magnano L, López-Guillermo A (2020) Patterns of change in treatment, response, and outcome in patients with follicular lymphoma over the last four decades: a single-center experience. Blood Cancer J 10:1–9. https://doi.org/10.1038/s41408-020-0299-0
3. Bachy E, Seymour JF, Feugier P, Offner F, López-Guillermo A, Belada D, Xerri L, Catalano JV, Brice P, Lemonnier F, Martin A, Casasnovas O, Pedersen LM, Dorvaux V, Simpson D, Leppa S, Gabarre J, da Silva MG, Glaisner S, Ysebaert L, Vekhoff A, Intragumtornchai T, le Gouill S, Lister A, Estell JA, Milone G, Sonet A, Farhi J, Zeuner H, Tilly H, Salles G (2019) Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: long-term results of the PRIMA study. J Clin Oncol 37:2815–2824. https://doi.org/10.1200/JCO.19.01073
4. Junlén HR, Peterson S, Kimby E, Lockmer S, Lindén O, Nilsson-Ehle H, Erlanson M, Hagberg H, Rådlund A, Hagberg O, Wahlin BE (2015) Follicular lymphoma in Sweden: nationwide improved survival in the rituximab era, particularly in elderly women: a Swedish Lymphoma Registry Study. Leukemia 29:668–676. https://doi.org/10.1038/leu.2014.251
5. Solal-Céligny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D, Coiffier B, Conde-Garcia E, Doyen C, Federico M, Fisher RI, Garcia-Conde JF, Guglielmi C, Hagenbeek A, Haïoun C, LeBlanc M, Lister AT, Lopez-Guillermo A, McLaughlin P, Milpied N, Morel P, Mounier N, Proctor SJ, Rohatiner A, Smith P, Soubeyran P, Tilly H, Vitolo U, Zinzani PL, Zucca E, Montserrat E (2004) Follicular lymphoma international prognostic index. Blood 104:1258–1265. https://doi.org/10.1182/blood-2003-12-4434
6. Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, Pro B, Pileri S, Pulsoni A, Soubeyran P, Cortelazzo S,

Martinelli G, Martelli M, Rigacci L, Arcaini L, di Raimondo F, Merli F, Sabattini E, McLaughlin P, Solal-Céligny P (2009) Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol 27: 4555–4562. https://doi.org/10.1200/JCO.2008.21.3991

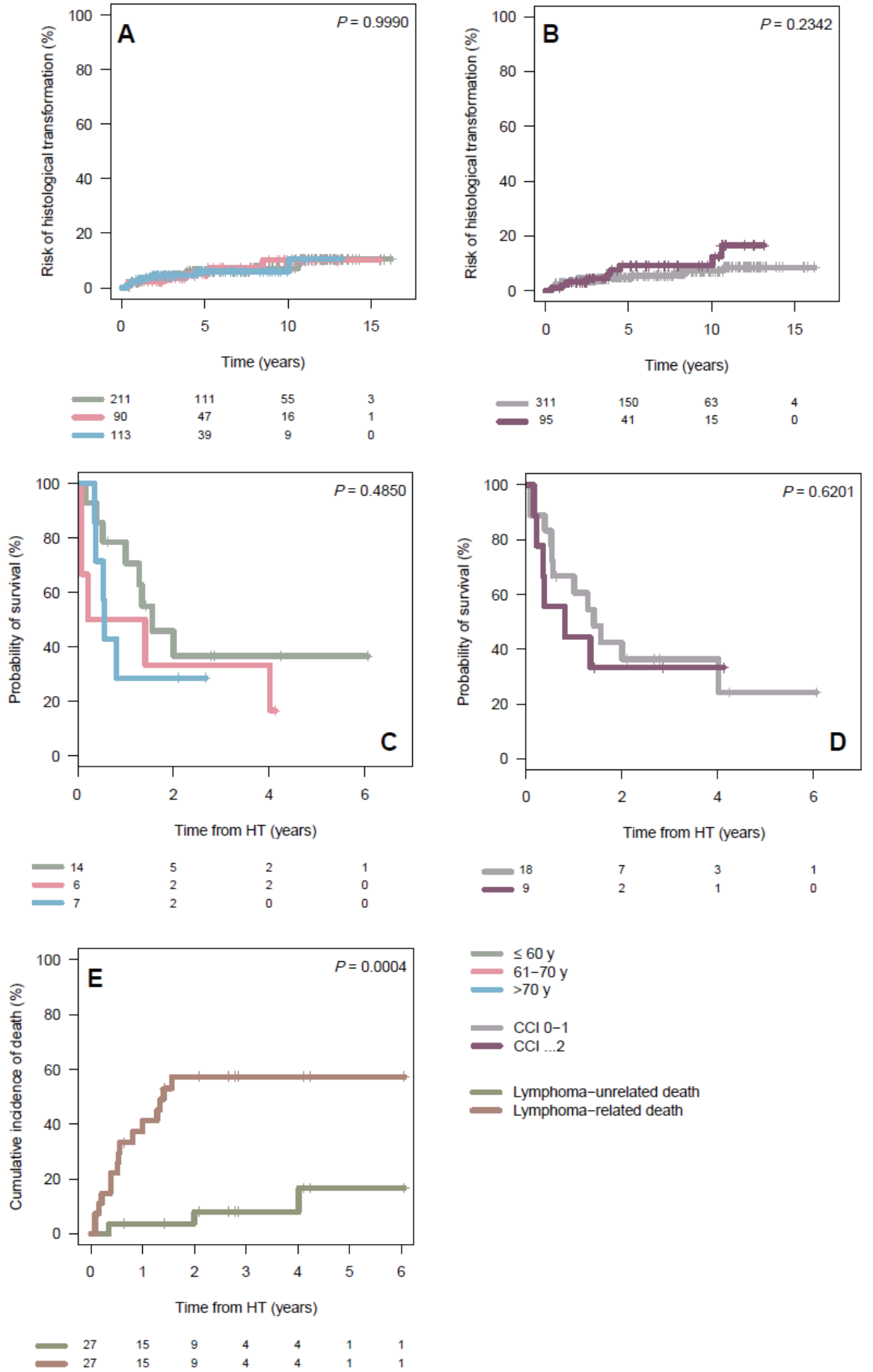
7. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, Pott C, Kopp N, Murakami M, Horn H, Leich E, Moccia AA, Mottok A, Sunkavalli A, van Hummelen P, Ducar M, Ennishi D, Shulha HP, Hother C, Connors JM, Sehn LH, Dreyling M, Neuberg D, Möller P, Feller AC, Hansmann ML, Stein H, Rosenwald A, Ott G, Klapper W, Unterhalt M, Hiddemann W, Gascoyne RD, Weinstock DM, Weigert O (2015) Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol 16:1111–1122. https://doi.org/10.1016/S1470-2045(15)00169-2
8. Bachy E, Maurer MJ, Habermann TM, Gelas-Dore B, Maucort-Boulch D, Estell JA, van den Neste E, Bouabdallah R, Gyan E, Feldman AL, Bargay J, Delmer A, Slager SL, Gomes da Silva M, Fitoussi O, Belada D, Maisonneuve H, Intragumtornchai T, Ansell SM, Lamy T, Dartigues P, Link BK, Seymour JF, Cerhan JR, Salles G (2018) A simplified scoring system in de novo follicular lymphoma treated initially with immunochemotherapy. Blood 132:49–58. https://doi.org/10.1182/blood-2017-11-816405
9. Maartense E, Le Cessie S, Kluin-Nelemans HC et al (2002) Age-related differences among patients with follicular lymphoma and the importance of prognostic scoring systems: analysis from a population-based non-Hodgkin's lynphoma registry. Ann Oncol 13:1275–1284. https://doi.org/10.1093/annonc/mdf198
10. Nabhan C, Aschebrook-Kilfoy B, Chiu BCH, Kruczek K, Smith SM, Evens AM (2014) The impact of race, age, and sex in follicular lymphoma: a comprehensive SEER analysis across consecutive treatment eras. Am. J. Hematol. 89:633–638
11. Flowers C, Ou F-S, Shi Q, Hoster E, Peterson BA, Hochster HS, Brice P, Ladetto M, Hiddemann W, Marcus RE, Kimby E, Herold M, de Bedout S, Nielsen T, Morschhauser F, Rummel M, Hagenbeek A, Vitolo U, Salles GA, Sargent D (2016) Outcomes for elderly patients (pts) with follicular lymphoma (FL) using individual patient data (IPD) from 5922 pts in 18 randomized controlled trials (RCTs): a Follicular Lymphoma Analysis of Surrogate Hypothesis (FLASH) group study. Blood 128:1102–1102. https://doi.org/10.1182/blood.v128.22.1102.1102
12. Albarmawi H, Onukwugha E, Keating KN, Appukkuttan S, Yared J (2020) Survival benefit associated with treating follicular lymphoma in patients 80 years or older. J Geriatr Oncol 11:55–61. https://doi.org/10.1016/j.jgo.2019.10.003
13. Mihaljevic B, Jelicic J, Andjelic B, Antic D, Markovic O, Petkovic I, Jovanovic MP, Trajkovic G, Bila J, Djurasinovic V, Sretenovic A, Vukovic V, Smiljanic M, Balint MT (2016) FCG (FLIPI, Charlson comorbidity index, and histological grade) score is superior to FLIPI in advanced follicular lymphoma. Int J Hematol 104: 692–699. https://doi.org/10.1007/s12185-016-2099-7
14. Sarkozy C, Maurer MJ, Link BK, Ghesquieres H, Nicolas E, Thompson CA, Traverse-Glehen A, Feldman AL, Allmer C, Slager SL, Ansell SM, Habermann TM, Bachy E, Cerhan JR, Salles G (2019) Cause of death in follicular lymphoma in the first decade of the rituximab era: a pooled analysis of French and US cohorts. J Clin Oncol 37:144–152. https://doi.org/10.1200/JCO.18.00400
15. Brice P, Bastion Y, Lepage E, Brousse N, Haïoun C, Moreau P, Straetmans N, Tilly H, Tabah I, Solal-Céligny P (1997) Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: a randomized study from the Groupe d'Etude des Lymphomes Folliculaires. J Clin Oncol 15:1110–1117. https://doi.org/10.1200/JCO.1997.15.3.1110
16. Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40:373–383. https://doi.org/10.1016/0021-9681(87)90171-8
17. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, Coiffier B, Fisher RI, Hagenbeek A, Zucca E, Rosen ST, Stroobants S, Lister TA, Hoppe RT, Dreyling M, Tobinai K, Vose JM, Connors JM, Federico M, Diehl V, International Harmonization Project on Lymphoma (2007) Revised response criteria for malignant lymphoma. J. Clin. Oncol. 25:579–586
18. University of California, Berkeley (USA) and MPI for DR (Germany) (2019) Human Mortality Database. Available at www.mortality.org
19. Gray RJ (1988) A class of *K*-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 16:1141–1154. https://doi.org/10.1214/aos/1176350951
20. Alig S, Jurinovic V, Pastore A, Bararia D, Häbe S, Hellmuth JC, Kridel R, Gascoyne R, Schmidt C, Zöllner AK, Buske C, Dreyling M, Unterhalt M, Hiddemann W, Hoster E, Weigert O (2018) Impact of age on genetics and treatment efficacy in follicular lymphoma. Haematologica 103:e364–e367

Supplementary Table 1. Charlson Comorbidity Index (adapted from Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *J Chronic Dis* 1987; **40**: 373–383.)

Assigned weight	Condition
1	Ischemic heart disease
	Congestive heart failure
	Peripheral vascular disease
	Cerebrovascular disease
	Cognitive impairment
	Chronic pulmonary disease
	Connective tissue disease
	Peptic ulcer disease
	Mild liver disease
	Diabetes mellitus without end-organ damage
2	Hemiplegia
	Moderate or severe chronic kidney disease
	Diabetes with end-organ damage
	Any solid tumor (excluding NMSC)
	Leukemia
	Lymphoma (excluded in our series)
3	Moderate or severe liver disease
6	Metastatic solid tumor
	AIDS

Assigned weights for each condition that a patient has. The total equals the score. Example: chronic pulmonary disease (1) and lung cancer (2) = total score (3). NMSC, non-melanoma skin cancer; AIDS, acquired immunodeficiency syndrome.

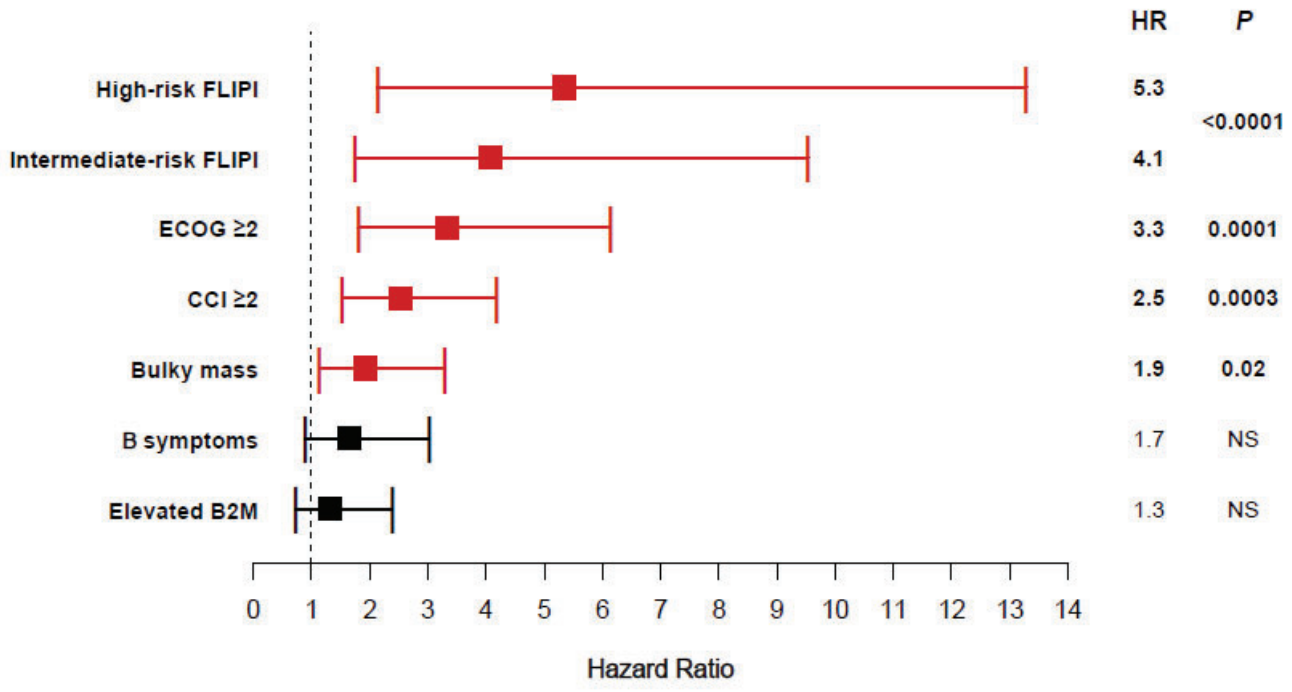
Supplementary Figure 1. A: risk of histological transformation (HT) according to age. B: risk of HT according to Charlson Comorbidity Index (CCI). C: survival from HT according to age. D: survival from HT according to CCI. E: cumulative incidence of lymphoma-related and unrelated death for patients in whom a HT was documented. Survival from HT was similar, irrespective of age or comorbidity (C and D). Among patients in which a HT was documented, the cumulative incidence of LRD was much higher to that of LuRD (E).



Supplementary Figure 2. Upper part: Univariate and multivariate analyses for OS, using Cox proportional hazards regression. **Lower part:** Forest plot of the multivariate analysis for OS.

Parameter	Risk category	OS (365 cases, 67 events)			
		Univariate analysis		Multivariate analysis	
		HR	*P*	HR	*P*
Sex	Female	0.9	NS	NI[a]	
Age	>60 years	4	<0.0001	NI[b]	
Ann–Arbor stage	III–IV	3.2	0.002	NI[b]	
B symptoms	Present	2.5	0.001	1.7	NS
ECOG	≥2	6	<0.0001	3.3	0.0001
Bulky mass	Present	1.9	0.01	1.9	0.02
No. of nodal sites	>4	1.6	NS	NI[a,b]	
FLIPI score	Intermediate risk	3.4	<0.0001	4.1	<0.0001
	High risk	7.2		5.3	
Hemoglobin	<120 g/L	1.6	NS	NI[a,b]	
LDH	Above ULN	2.1	0.003	NI[b]	
B2M	Above ULN	3.1	<0.0001	1.3	NS
Charlson Comorbidity Index	≥2	2.5	0.0001	2.5	0.0003

ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index; LDH, lactate dehydrogenase; B2M, β2-microglobulin; NI, not included in the multivariate model; HR, hazard ratio; NS, not statistically significant; OS, overall survival; ULN, upper limit of normal. [a] Due to absence of statistical significance in the univariate analysis. [b] To avoid redundancy with the FLIPI score.

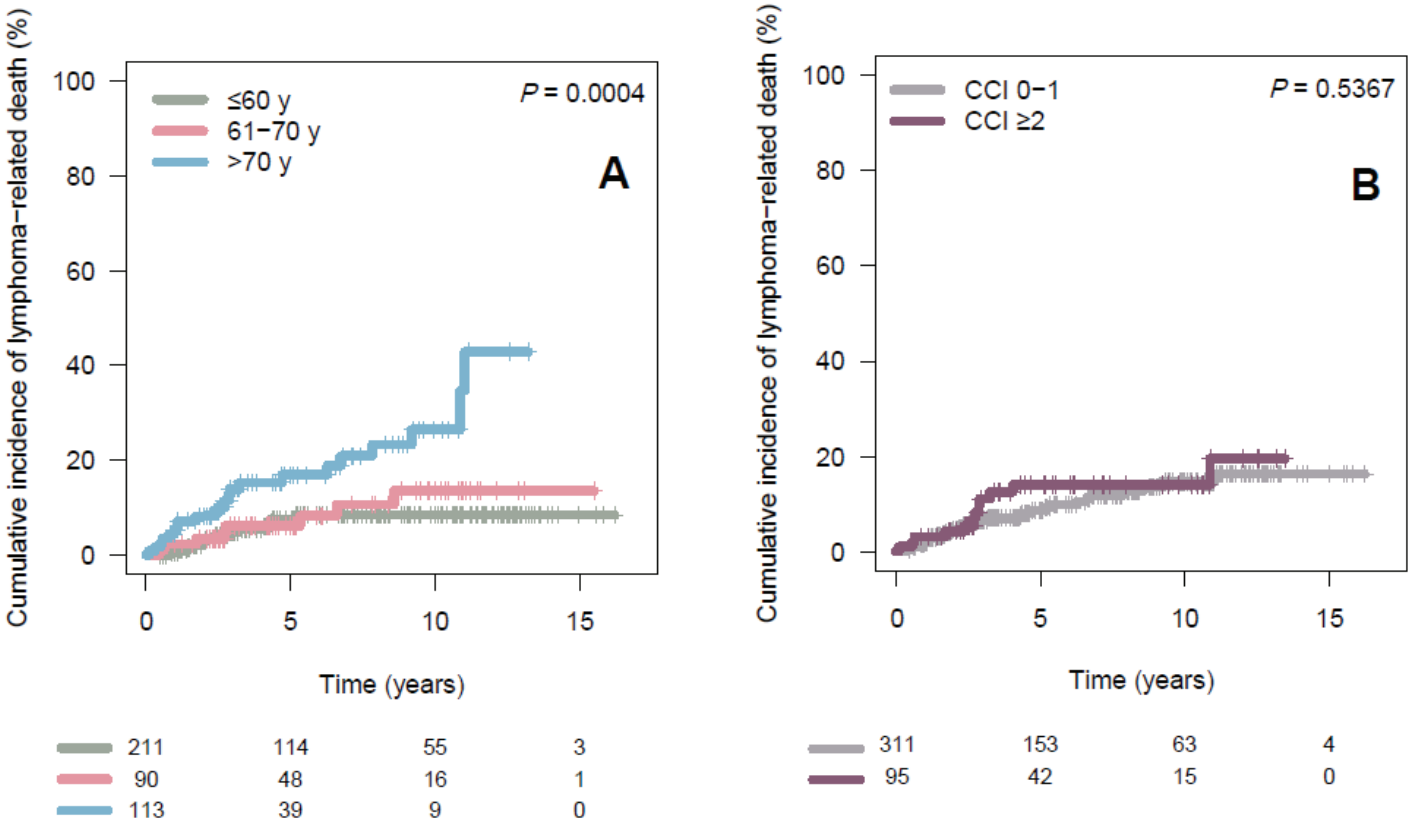


FLIPI, Follicular Lymphoma International Prognostic Index; ECOG, Eastern Cooperative Oncology Group; CCI, Charlson Comorbidity Index; B2M, beta2-microglobulin; HR, hazard ratio; NS, not statistically significant.

Supplementary Figure 3. **Upper part:** Cumulative incidence of lymphoma-related and lymphoma-unrelated death, using competing risks, according to age and comorbidity. **Lower part:** Cumulative incidence of lymphoma related death, using competing risks, according to age (A), and Charlson Comorbidity Index (CCI, B).

Cumulative incidence of death, % at 10 y (95% CI)	All patients	Age				Charlson Comorbidity Index		
		≤60 y	61-70 y	>70 y	*P* value	0-1	≥2	*P* value
Lymphoma-related	14 (10-19)	8 (5-13)	14 (6-25)	26 (16-38)	0.0004	15 (10-20)	13 (NC)	NS
Lymphoma-unrelated	15 (11-21)	4 (1-8)	23 (12-36)	32 (20-46)		9 (10-20)	33 (NC)	

y, year; NS, not statistically significant; CI, confidence interval; NC, not calculable.



2.3. Tercer trabajo

A low lymphocyte-to-monocyte ratio is an independent predictor of poorer survival and higher risk of histological transformation in follicular lymphoma

Pablo Mozas, Andrea Rivero, Alfredo Rivas-Delgado, Ferran Nadeu, Guillem Clot, Juan Gonzalo Correa, Carlos Castillo, Alex Bataller, Tycho Baumann, Eva Giné, Julio Delgado, Neus Villamor, Elías Campo, Patricia Pérez-Galán, Laura Magnano, Armando López-Guillermo

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Una ratio linfocito-monocito baja es un predictor independiente de peor supervivencia y mayor riesgo de transformación histológica en el linfoma folicular

Resumen

La ratio linfocito/monocito (LMR) en sangre periférica es un factor pronóstico en diferentes neoplasias, pero su importancia en el linfoma folicular (LF) no está bien definida. Analizamos 384 pacientes que tenían datos sobre el LMR al diagnóstico. Se compararon las características iniciales y supervivencia entre pacientes con un LMR ≤2.5 y >2.5. Los 76 pacientes (20%) que tenían un LMR ≤2.5 tenían una edad más avanzada y tenían parámetros de más alta carga tumoral. Un LMR bajo fue predictor de una SLP a 10 años inferior (32 vs 55%, *P*=0.001), así como una SG a 10 años inferior ((35 vs 78%, *P*<0.0001; HR=2.3, *P*=0.003 en un modelo multivariado de 6 elementos). Un LMR bajo también fue un factor de riesgo independiente para la transformación histológica (11 vs 6% a 10 años, *P*=0.01). Además, los pacientes con LMR bajo tuvieron una mayor incidencia de segundas neoplasias. La potencial utilidad de este parámetro ampliamente disponible y su contribución a índices pronósticos bien establecidos deberían ser exploradas en series independientes y prospectivas de pacientes.

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ORIGINAL ARTICLE

A low lymphocyte-to-monocyte ratio is an independent predictor of poorer survival and higher risk of histological transformation in follicular lymphoma

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ABSTRACT

The lymphocyte-to-monocyte ratio (LMR) is a prognostic factor in different neoplasms, but its potential importance in follicular lymphoma (FL) is not well defined. We studied 384 FL patients for which the LMR was available at diagnosis. Baseline features and outcomes were compared between patients with an LMR $\leq/>2.5$. The 76 patients (20%) who had an LMR ≤ 2.5 were older and had a higher tumor burden. A low LMR was predictive of a lower 10-y progression-free survival (32 vs. 55%, $p = .001$) and overall survival (35 vs. 78%, $p < .0001$; HR = 2.3, $p = .003$ in a 6-element multivariable model). A low LMR was also an independent risk factor for histological transformation (11 vs. 6% at 10 years, $p = .01$). Likewise, patients with a low LMR had a higher rate of second malignancies. The potential utility of this widely available parameter and its contribution to well-established prognostic scores need to be explored in independent, prospective series.

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Introduction

Although outcomes for most patients with follicular lymphoma (FL), the most common indolent B-cell neoplasm, have improved since the advent of immunotherapy, some specific clinical scenarios remain a challenge, namely early relapse (POD24) [1] and histological transformation (HT) [2–4]. In an attempt to predict the clinical behavior and to design risk-adapted therapeutic strategies, various prognostic indexes considering clinical and biological variables have been proposed [5–7].

Lymphomas are a heterogeneous group of neoplasms in which the tumor microenvironment (TME) plays a major role in the development and progression of the disease. Non-malignant lymphocytes and macrophages constitute a large proportion of the TME, and the absolute lymphocyte count (ALC) and absolute monocyte count (AMC) could be considered a quantitative measure of their counterparts in the blood [8]. Among other complete blood count (CBC) values, these two parameters and a combined measure thereof, the lymphocyte-to-monocyte ratio (LMR), have been analyzed in several lymphoid disorders, with lower values being predictive of poorer outcomes [9–15].

It has been postulated that the secretion of cytokines by the TME could induce an increase in the proliferation of monocytes, which would in turn reduce the ability of the host to control tumor progression [16]. As for lymphocytes, the ALC could be a simple reflection of the host's immunity in general, and against cancer in particular, with a lower ALC implying a greater degree of immune suppression [17,18]. Furthermore, rituximab, an essential component of the current treatment of B-cell malignancies, acts through antibody-dependent cellular cytotoxicity [19], among other mechanisms. Thus, response to anti-CD20

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Supplemental data for this article can be accessed here.

antibodies could be impaired if the number of lymphocytes is decreased [16].

Despite the plethora of articles examining the role of AMC, ALC, and LMR in other neoplasms, only three studies have analyzed the importance of the LMR in FL specifically, where the TME is crucial to pathogenesis [20,21]. Kumagai et al. reported a decreased progression-free survival (PFS) for patients with an LMR <4.7, but the study was not powered to evaluate overall survival (OS) [22]. Belotti et al. also found a negative impact of an LMR <2 on PFS, without differences in OS [23]. Finally, in a small cohort of patients treated in the pre- and rituximab eras, an LMR ≥ 3.2 was associated with a longer PFS, but OS was not reported [24]. Therefore, the aim of our study was to further explore the baseline correlations of the LMR in a large single-center series of FL patients diagnosed in the rituximab era, and analyze its impact on outcomes.

Methods

Patients

We studied 384 grade 1–3A FL patients (212 females, 172 males; median age, 61 years) consecutively diagnosed at a single institution between 2000 and 2018, who had available data on the ALC and AMC at diagnosis. Pathological review was performed when there was sample availability. Patients with the following entities were not included: grade 3B FL, primary gastrointestinal or cutaneous FL, and composite lymphoma. Baseline characteristics, treatment, and outcomes were evaluated and compared according to the LMR. The study was approved by our Institutional Review Board and followed the Declaration of Helsinki.

CBC parameters

Information about CBC data was collected from the initial blood analysis performed at diagnosis. Cell counts were determined by means of an automated cell counter. In cases with prominent CBC abnormalities, such as marked lymphocytosis, the peripheral blood smear was manually reviewed and the resulting counts were used for calculations. The LMR was calculated by dividing the ALC by the AMC. Peripheral blood involvement (PBI) by FL was defined as the presence of immunophenotypically-characterized FL cells in the blood, a study that was generally prompted by the presence of an elevated ALC.

Response assessment and survival endpoints

Computed tomography, with or without positron emission tomography, and unilateral bone marrow biopsy were used for staging and response assessment purposes. Definitions of response to frontline treatment were the standard [25]. An initial observation approach was defined as >6 months between diagnosis and initiation of frontline therapy. PFS was calculated from frontline treatment to relapse or death of any cause. Thus, early progressors (POD24) were patients who relapsed within the first 24 months of initial treatment. OS was calculated from diagnosis to last follow-up or death from any cause.

Statistical analysis

The method of maximally selected rank statistics (maxstat package, R software, Vienna, Austria), was used to calculate the best LMR cut-off to predict OS, and a value of 2.5 was obtained (Supplementary Figure 1(A)). We performed a 100,000-sample bootstrap validation of the maxstat-obtained cut-off (mean: 2.508529; 95% CI: $1.9-3.0$, Supplementary Figure 1(B)). The maxstat test was then applied to PFS and yielded the exact same cut-off (Supplementary Figure 1(C)). CBC values were compared using the Mann–Whitney's U test. The χ^2 or Fisher's exact test were used to compare categorical variables. We plotted Kaplan–Meier survival curves and used the log-rank test to explore differences based on the LMR. For outcomes where a possible competing event exists (HT and second malignancies, SM), the primary event was HT or SM, and the competing event was death without HT or SM. Cumulative incidence was then calculated (cmprsk R package) and Gray's test [26] was used for comparisons between both groups. For the analysis of causes of death, we used cause-specific cumulative incidence of mortality, with competing risk of death. For the estimation of hazard ratios in the uni- and multivariable analyses, Cox proportional hazards regression was used. Statistical significance was defined as a p value $<.05$.

Results

Initial characteristics of the patients and LMR

Table 1 details baseline characteristics of all the 384 patients, and according to the LMR. The median LMR for the entire series was 5.8 (range, $0.7-52.7$). Seventy-six patients (20%) had an LMR below the established cut-off of 2.5. Other values of the CBC can

Table 1. Clinical features of the 384 patients with FL, globally and according to the LMR.

		LMR		
	All patients	≤2.5	>2.5	*p*-value
Number of patients, *n* (%)	384 (100)	76 (20)	308 (80)	–
Age (median, range)	61 (26-91)	71 (32-91)	59 (26-86)	**<.0001**
Female sex, *n* (%)	212 (55)	38 (50)	174 (57)	.3
Histological grade 1-2, *n* (%)	309 (82)	53 (70)	256 (85)	**.002**
Ki67 index >30%, *n* (%)	117 (41)	20 (37)	97 (41)	.3
ECOG PS ≥2, *n* (%)	25 (7)	10 (13)	15 (5)	**.02**
B symptoms, *n* (%)	44 (12)	16 (21)	28 (9)	**.006**
Ann-Arbor stage III/IV, *n* (%)	288 (75)	62 (82)	226 (73)	.1
Secondary extranodal involvement, *n* (%)	244 (64)	48 (63)	196 (64)	.9
Bone marrow involvement, *n* (%)	199 (54)	28 (42)	171 (57)	**.02**
Peripheral blood involvement, *n* (%)	43 (11)	2 (3)	41 (13)	**.02**
5 or more lymph node areas, *n* (%)	119 (31)	27 (37)	92 (30)	.3
Bulky mass, *n* (%)	82 (22)	28 (37)	54 (18)	**<.001**
Elevated serum LDH, *n* (%)	71 (19)	19 (26)	52 (17)	.09
Elevated β2-microglobulin, *n* (%)	178 (49)	47 (68)	131 (44)	**<.001**
Hemoglobin <120 g/L, *n* (%)	71 (19)	24 (32)	47 (15)	**.002**
High-risk FLIPI score, *n* (%)	96 (25)	31 (41)	65 (21)	**<.001**

ECOG: Eastern Cooperative Oncology Group Performance Status; LDH: lactate dehydrogenase; LMR: lymphocyte-to-monocyte ratio; FLIPI: Follicular Lymphoma International Prognostic Index.
p-values in bold indicate statistical significance.

be found in Supplementary Table 1. The age distribution according to the LMR was significantly different (median age, 71 vs. 59 years, for LMR ≤/>2.5, respectively). Grade 3a FL was more frequent among patients with an LMR ≤2.5 (30 vs. 15%, $p = .002$). Patients with an LMR ≤2.5 had a poorer performance status, a higher frequency of B symptoms, a bulky mass, and elevated β2-microglobulin (B2M) levels. No differences were seen with regard to the stage of the disease, the frequency of extranodal involvement, or the proportion of patients with elevated LDH. The proportion of patients with a high-risk FLIPI score was higher among patients with a low LMR (41 vs. 21%, $p < .001$).

Frontline treatment and responses

First-line treatment and the obtained responses are summarized in Supplementary Table 2. Among all patients, 97 (25%) underwent initial observation, without significant differences according to the LMR. Likewise, the proportion of patients who received initial treatment with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) was similar in both groups. For the entire cohort, the proportion of patients achieving a complete response (CR) after frontline treatment was 73%, a rate that was significantly lower for patients with a low LMR (58 vs. 77%, $p = .002$). However, when this analysis was performed for patients diagnosed after 2012, year in which PET-CT was systematically available, no significant differences were found.

Progression-free and overall survival

Outcomes are summarized in Table 2. The median follow-up of the cohort was 5.1 years (range, 0.6–16.6 years). Among treated patients, 103 patients (31%) relapsed or progressed during follow-up, with a median PFS of 10 years. Patients with an LMR ≤2.5 had a significantly shorter PFS, compared with those with an LMR >2.5 (median PFS, 5.7 years vs. not reached; 10-year PFS, 32% vs. 55%, $p = .001$, Figure 1(A)). The POD24 rate was not significantly different according to the LMR.

Seventy-four patients (19%) died during follow-up. Median OS for all patients was not reached. As seen for PFS, the LMR had a major impact on OS (median OS, 7.1 years vs. not reached; 10-year OS, 35% vs. 78%, $p < .0001$, Figure 1(B)). Likewise, when analyzing competing causes of death, patients with an LMR ≤2.5 had a significantly higher 10-year mortality due to progressive FL (28 vs. 7% at 10 years) (Figure 2(A)).

Considering the statistically significant factors from the univariable analyses, a multivariable model was built for PFS and OS, excluding the variables that are comprised in the FLIPI score (Table 3). Although the LMR did not retain statistical significance in the multivariable analysis for PFS (Supplementary Figure 2(A)), in the model for OS, with 364 cases and 68 events, including the presence of B symptoms, ECOG performance status, the presence of a bulky mass, the FLIPI score, and the levels of B2M, the LMR retained its adverse impact [HR = 2.3 (95% CI: 1.3 – 4), $p = .003$, Supplementary Figure 2(B)].

As expected, 41 of the 43 patients who had PBI (95%) had an LMR >2.5. Among patients with PBI, the

Table 2. Outcomes of the patients, globally and according to the LMR.

	All patients	LMR		
		≤2.5	>2.5	*p*-value
PFS				
% at 5 y (95% CI)	66 (61 – 72)	55 (48 – 63)	70 (64 – 76)	**.001**
% at 10 y (95% CI)	51 (44 – 58)	32 (14 – 49)	55 (47 – 62)	
POD24, *n* (%)	60 (19)	16 (27)	44 (17)	.07
OS				
% at 5 y (95% CI)	85 (82 – 90)	64 (53 – 78)	90 (87 – 94)	**<.0001**
% at 10 y (95% CI)	71 (65 – 77)	35 (20 – 60)	78 (72 – 85)	
Cumulative incidence of death due to progressive FL, % at 10-y (95% CI)	11 (7 – 16)	28 (15 – 42)	7 (4 – 12)	**<.0001**
Risk of HT, % at 10 y (95% CI)	7 (4 – 10)	11 (5 – 19)	6 (3 – 10)	**.01**
Risk of SM, % at 10 y (95% CI)	16 (11 – 22)	22 (9 – 38)	14 (9 – 20)	**.006**

CI: confidence interval; HT: histological transformation; FL: follicular lymphoma; LMR: lymphocyte-to-monocyte ratio; NS: not statistically significant; PFS: progression-free survival; POD24: progression of disease within 24 months of treatment initiation; OS: overall survival; SM: second malignancies.
p-values in bold indicate statistical significance.

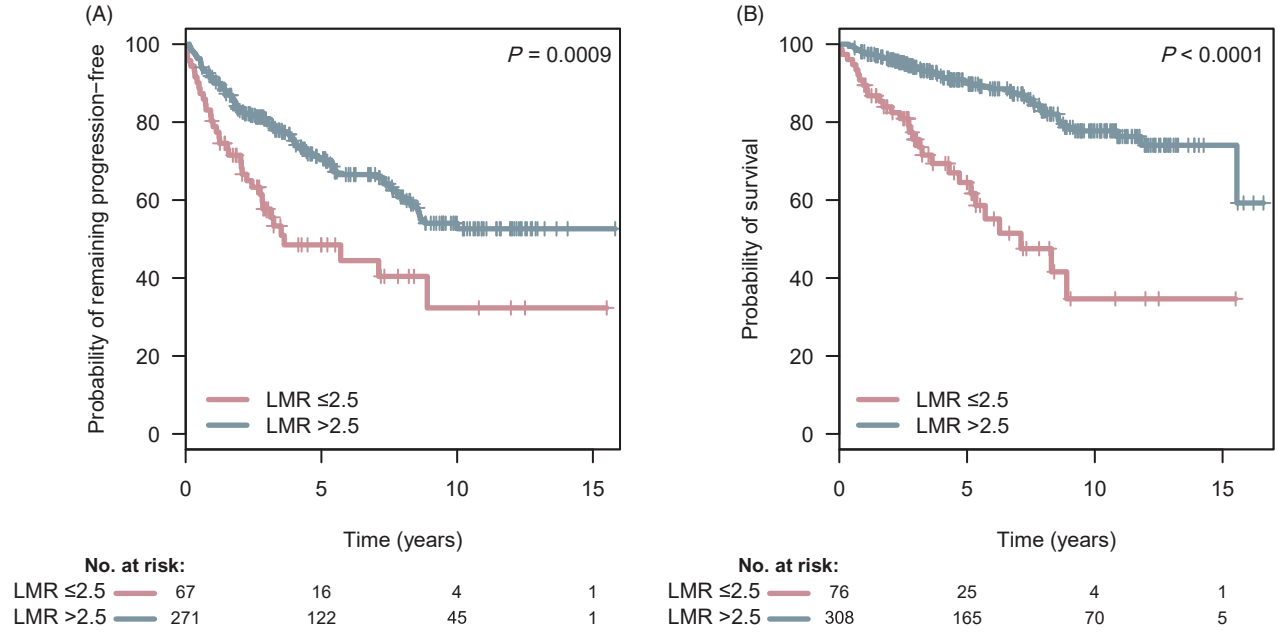

Figure 1. Progression-free (A) and overall survival (B) of the series according to the lymphocyte-to-monocyte ratio (LMR).

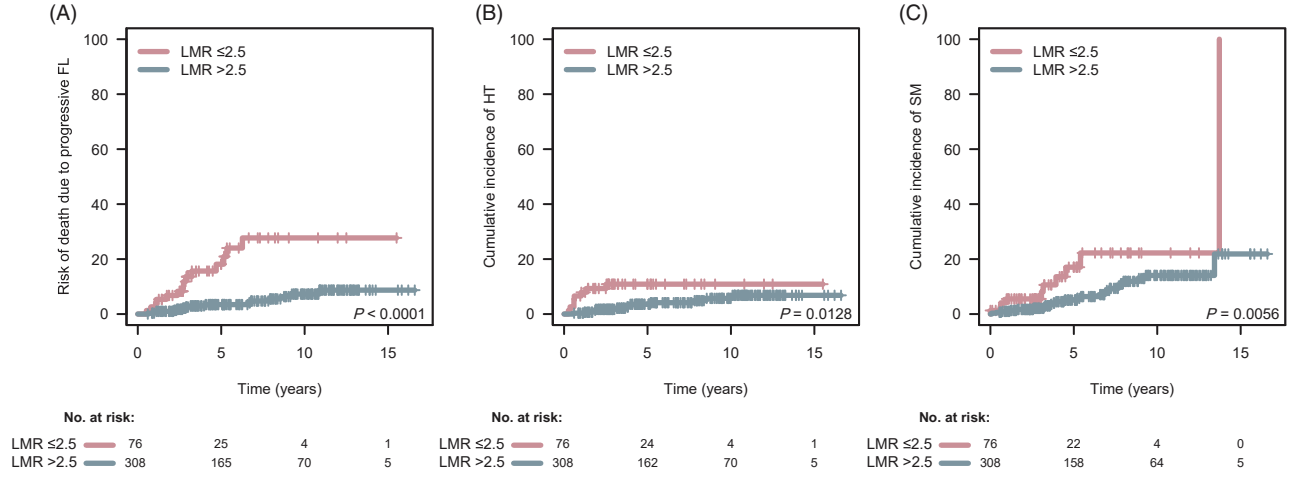

Figure 2. Risk of death due to progressive FL (A), risk of HT (B) and of SM (C) according to the LMR. FL: follicular lymphoma; HT: histological transformation; SM: second malignancies; LMR: lymphocyte-to-monocyte ratio.

Table 3. Univariable and multivariable analyses for PFS and OS using Cox proportional hazards regression.

		PFS (323 cases, 122 events)				OS (364 cases, 68 events)			
		Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis	
Parameter	Risk category	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Sex	Male	1.3 (0.9 – 1.9)	.1	NI[a]		1.3 (0.8 – 2.1)	.2	NI[a]	
Age	>60 years	1.7 (1.2 – 2.5)	**.003**	NI[b]		4.3 (2.4 – 7.4)	**<.0001**	NI[b]	
Histological grade	3A	1.1 (0.7 – 1.7)	.7	NI[a]		1.6 (0.9 – 2.7)	.1	NI[a]	
Ann – Arbor stage	III – IV	2.5 (1.5 – 4.2)	**.0004**	NI[b]		2.8 (1.4 – 5.9)	**.006**	NI[b]	
B symptoms	Present	2.4 (1.6 – 3.8)	**.0004**	1.8 (1.1–2.9)	**.02**	2.8 (1.6 – 4.8)	**.0003**	1.7 (0.9 – 3.2)	.08
ECOG PS	≥2	3.4 (2 – 5.8)	**<.0001**	2.4 (1.3–4.2)	**.003**	7.5 (4.4 – 13)	**<.0001**	4.6 (2.4 – 8.7)	**<.0001**
Bulky mass	Present	1.6 (1.1 – 2.4)	**.01**	1.5 (1–2.2)	**.048**	1.8 (1.1 – 3)	**.02**	1.6 (0.9 – 2.6)	.1
No. of nodal sites	>4	1.6 (1.1 – 2.4)	**.008**	NI[b]		1.6 (1 – 2.6)	.053	NI[a,b]	
FLIPI score	Intermediate risk	2.2 (1.4 – 3.5)	**.0006**	2 (1.2 – 3.2)	**.02**	3.3 (1.7 – 6.6)	**.0007**	3.8 (1.7 – 8.5)	**.001**
	High risk	3.3 (2.1 – 5.2)	**<.0001**	1.9 (1.1 – 3.3)		6.5 (3.3 – 12.9)	**<.0001**	3.6 (1.5 – 8.7)	
Hemoglobin	<120 g/L	1.2 (0.8 – 1.9)	0.3	NI[a,b]		1.8 (1 – 2.9)	**.03**	NI[b]	
LDH	Above ULN	2 (1.4 – 3)	**.0003**	NI[b]		1.9 (1.2 – 3.2)	**.01**	NI[b]	
B2M	Above ULN	2.5 (1.7 – 3.7)	**<.0001**	1.7 (1.1 – 2.6)	**.02**	3.2 (1.9 – 5.4)	**<.0001**	1.6 (0.9 – 2.6)	.1
LMR	≤2.5	2 (1.3 – 2.9)	**.001**	1.2 (0.8 – 1.9)	.4	4.3 (2.7 – 6.9)	**<.0001**	2.3 (1.3 – 4)	**.003**

ECOG PS: Eastern Cooperative Oncology Group Performance Status; FLIPI: Follicular Lymphoma International Prognostic Index; LDH: lactate dehydrogenase; B2M: β2-microglobulin; CI: confidence interval; HR: hazard ratio; LMR: lymphocyte-to-monocyte ratio; PFS: progression-free survival; OS: overall survival; ULN: upper limit of normal; NI: not included in the multivariable model.
[a]Due to absence of statistical significance in the univariable analysis.
[b]To avoid redundancy with the FLIPI score.
p-values in bold indicate statistical significance.

median ALC was 4.1×10^9/L (range, 0.9 – 93.8). In the global series, OS was not significantly different for patients according to the presence of PBI, although the Kaplan–Meier estimate for OS involvement was visibly inferior for patients with PBI (Supplementary Figure 3(A)). When performing an OS analysis excluding patients with PBI, the LMR retained its prognostic impact ($p < .0001$, Supplementary Figure 3(B)).

Concerning age, patients older than 60 years were more likely to have an LMR ≤2.5 (28 vs. 10% compared with patients ≤60 years, $p < .0001$). Considering the marked differences in age distribution between patients with an LMR ≤ and >2.5, and to avoid attributing the adverse prognostic impact of a low LMR to the older age, we plotted stratified Kaplan–Meier PFS and OS curves for patients younger and older than 60 years. PFS was not significantly different according to the LMR in younger patients (Supplementary Figure 4(A)). However, LMR retained its prognostic impact for PFS in older patients (Supplementary Figure 4(C)) and for OS in both age groups (Supplementary Figures 4(B) and 4(D)).

The LMR could improve the FLIPI score

We aimed at improving the predictive value of the FLIPI score by adding LMR information. Six categories were defined, considering the FLIPI risk category (low-, intermediate-, and high-risk) and the LMR (≤/>2.5), and six curves were plotted for OS (Figure 3), and the corresponding HR on univariable Cox regression was analyzed. As expected from the multivariable analysis, the LMR was of importance in all three FLIPI risk categories.

Risk of histological transformation

Twenty-one patients (6%) developed HT to DLBCL. For all patients, the median time to HT was 1.8 years (range, 0.3–10 years). A low LMR was predictive of a higher cumulative incidence of HT (11 vs. 6% at 10 years, $p = .01$, Figure 2(B)). To elucidate a potential independent impact of the LMR on the risk of HT, we first identified variables that were risk factors for HT. In a final multivariable model of 383 cases and 21 events, including the presence of extranodal involvement, the FLIPI score, and the LMR, the latter was the only independent predictor of a higher risk of HT [HR = 2.6 (95% CI: 1.1 – 6.3), $p = .03$, Supplementary Table 3 and Supplementary Figure 5].

Risk of second malignancies

Thirty-four patients (9%) developed a second tumor after the diagnosis of FL, and the 10-year risk of developing a SM was of 16% for all patients. The LMR also predicted for a higher risk of SM (22 vs. 14% at 10 years, $p = .006$, Figure 2(C)).

Discussion

Although the median OS for newly diagnosed FL patients now approaches 20 years [27,28], some clinical scenarios remain a challenge, namely early progression and HT. As patients who will fare poorly

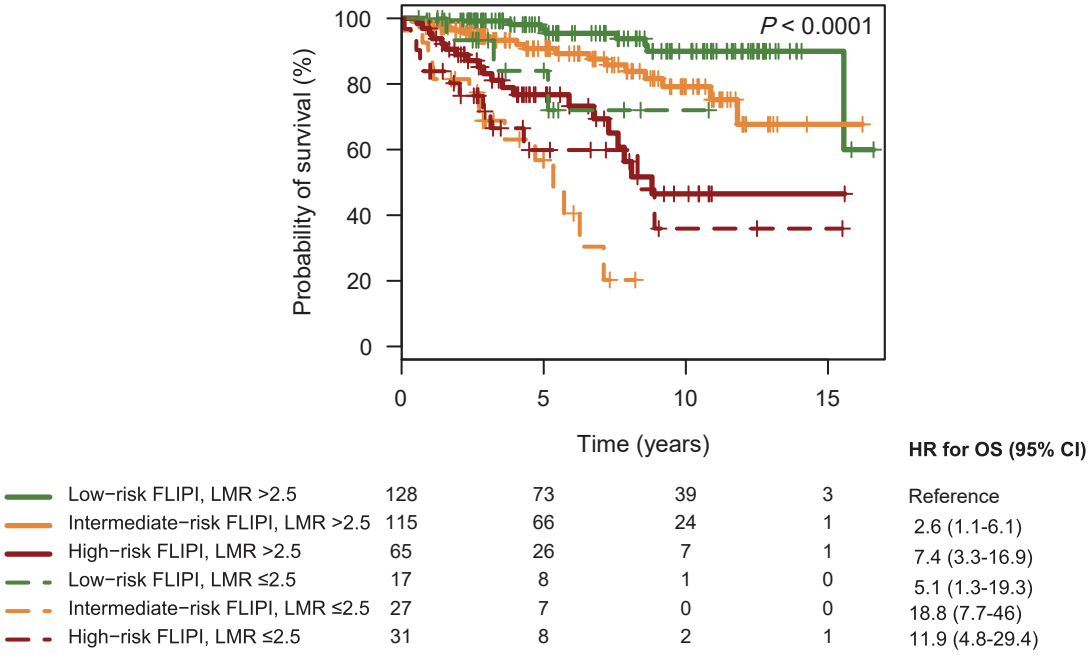

Figure 3. Exploratory Kaplan–Meier plots of OS according to the combination of the FLIPI score and the LMR. OS: overall survival; FLIPI: Follicular Lymphoma International Prognostic Index; HR, hazard ratio; CI, confidence interval; LMR: lymphocyte to monocyte ratio. The colors of the curves represent FLIPI risk categories , and the stroke of the line represents the LMR (continuous for LMR >2.5, and dashed for LMR ≤ 2.5).

cannot always be identified upon diagnosis, new variables are being sought to further refine established prognostic indexes and tailor therapeutic strategies.

The TME has been shown to be of paramount importance in the biology of FL, with the cell composition of the tumor determining the dynamics of disease progression and response to therapy [20]. In this regard, lymphocytes and monocytes have been considered the circulating counterparts of tumor infiltrating lymphocytes and macrophages [8], the most relevant TME cells, and the LMR has been identified as a combined measure of both parameters, with lower values identifying patients with a poorer prognosis in many cancers [11,13,29].

We analyzed CBC values in a cohort of 384 FL patients and, although observational studies like the present one can suffer from selection bias, we identified a group of patients (20%) with an LMR ≤ 2.5, who were significantly older, had a poorer performance status, higher tumor burden, and a higher-risk FLIPI score. Without significant differences in initial treatment strategies, patients with a low LMR were less likely to achieve a CR after frontline therapy, although this finding could be explained by the higher persistence of a bulky mass in patients in which a PET study was not available.

In line with previous reports [22–24], in our series, patients with an LMR ≤ 2.5 had a shorter PFS (32 vs. 55% at 10 years, $p = .001$). Concerning OS, patients with an LMR ≤ 2.5 had a 10-year OS less than half than that of patients with an LMR >2.5 (35 vs. 78%, $p < .0001$). To confirm that the adverse prognostic impact of a low LMR was not merely associated with an older age, we performed a stratified analysis, and observed that the impact on OS remained both in patients younger and older than 60 years. Moreover, the adverse prognostic impact of the LMR on OS remained significant in a multivariable model including other five clinically relevant characteristics. When evaluating causes of death, we identified that patients with a low LMR were more likely to die due to progressive FL.

A recent report showed that a low ALC was an independent risk factor for transformation to an aggressive lymphoma [30]. In our series, we found that patients with a low LMR were more likely to experience HT (11 vs. 6% at 10 years, $p = .01$), and in the multivariable model to predict HT, the LMR was the only parameter retaining independent prognostic impact. Furthermore, patients with a low LMR were more likely to develop a SM.

Two studies have made an attempt to refine established FL prognostic scores using CBC values: Wilcox et al. proposed the AMC-FLIPI, considering an AMC $\geq 5.7 \times 10^9$/L as an additional risk factor [31], and Yang et al. gave an additional point to patients with an ALC $< 1 \times 10^9$/L and proposed the FLIPI-L score [30]. In our cohort, we combined the three FLIPI risk categories

and the LMR to define six exploratory categories, and analyzed their HR for OS. The LMR added relevant information to all FLIPI categories. However, we believe that, in order to generalize the use of the LMR to inform prognosis and modify well-established prognostic indexes, validation in large, independent series is required.

The biological interactions that lie behind this clinical impact remain elusive. Some groups have suggested that the secretion of cytokines by the TME could induce an increase in the number of monocytes, which would in turn compromise anti-tumor immunity and facilitate disease progression [16]. In this regard, the correlation between circulating monocytes and tumor-infiltrating macrophages needs to be established in FL specifically. Considering the fact that FL is an immune-rich tumor, there has been great interest in the lymphocyte compartment to elucidate the mechanisms of disease progression and response to therapy. A reduced ALC, and therefore, a low LMR, could be an approximate measure of a decreased tumor-specific T-cell repertoire and ineffective antitumor immunity. Moreover, since rituximab depends on lymphocytes to exert antibody-dependent cellular cytotoxicity, a low ALC could also compromise the efficacy of anti-CD20 immunotherapy.

In conclusion, our study represents the largest series studying the LMR in FL. We observed that, in newly diagnosed FL patients, an LMR $\leq$2.5 is associated with higher tumor burden features, a lower CR rate, shorter survival, and higher risk of SM and HT. Upon validation in other series, information provided by the LMR could be an additional tool to improve the prognostic classification of FL patients, in order to avoid toxicities of overtreating low-risk patients, and to intensify therapy in patients at a higher risk of early progression or HT.

Author contributions

P.M. designed the study, collected the data, performed the analysis, and wrote the article. A.R., A.R.D., F.N., A.B., T.B., E.G., J.D., N.V., E.C., P.P.G., L.M., and A.L.G. contributed to data collection and reviewed the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Casulo C, Byrtek M, Dawson KL, et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: an analysis from the National LymphoCare Study. J Clin Oncol. 2015;33(23):2516–2522.
[2] Montoto S, Fitzgibbon J. Transformation of indolent B-cell lymphomas. J Clin Oncol. 2011;29(14):1827–1834.
[3] Alonso-Álvarez S, Magnano L, Alcoceba M, et al. Risk of, and survival following, histological transformation in follicular lymphoma in the rituximab era. A retrospective multicentre study by the Spanish GELTAMO group. Br J Haematol. 2017;178(5):699–708.
[4] Federico M, Caballero Barrigón MD, Marcheselli L, et al. Rituximab and the risk of transformation of follicular lymphoma: a retrospective pooled analysis. Lancet Haematol. 2018;5(8):e359–e367.
[5] Solal-Céligny P, Roy P, Colombat P, et al. Follicular lymphoma international prognostic index. Blood. 2004;104:1258–1265.
[6] Federico M, Bellei M, Marcheselli L, et al. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Cin Oncol. 2009;27(27):4555–4562.
[7] Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16(9):1111–1122.

[8] Geissmann F, Manz MG, Jung S, et al. Development of monocytes, macrophages, and dendritic cells. Science (80. 2010;327(5966):656–661.
[9] Li ZM, Huang JJ, Xia Y, et al. Blood lymphocyte-to-monocyte ratio identifies high-risk patients in diffuse large B-cell lymphoma treated with R-CHOP. PLoS One. 2012;7(7):e41658
[10] Rambaldi A, Boschini C, Gritti G, et al. The lymphocyte to monocyte ratio improves the IPI-risk definition of diffuse large B-cell lymphoma when rituximab is added to chemotherapy. Am J Hematol. 2013;88(12): 1062–1067.
[11] Bento L, Díaz-López A, Barranco G; the Grupo Español de Linfomas y Trasplante Autólogo (GELTAMO), et al. New prognosis score including absolute lymphocyte/monocyte ratio, red blood cell distribution width and beta-2 microglobulin in patients with diffuse large B-cell lymphoma treated with R-CHOP: Spanish Lymphoma Group Experience (GELTAMO). Br J Haematol. 2020;188(6):888–897.
[12] Watanabe R, Tomita N, Itabashi M, et al. Peripheral blood absolute lymphocyte/monocyte ratio as a useful prognostic factor in diffuse large B-cell lymphoma in the rituximab era. Eur J Haematol. 2014;92(3): 204–210.
[13] Porrata LF, Ristow K, Habermann TM, et al. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in nodular lymphocyte-predominant Hodgkin lymphoma. Br J Haematol. 2012;157(3):321–330.
[14] Porrata LF, Ristow K, Colgan JP, et al. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma. Haematologica. 2012;97(2):262–269.
[15] Reddy JP, Hernandez M, Gunther JR, et al. Pre-treatment neutrophil/lymphocyte ratio and platelet/lymphocyte ratio are prognostic of progression in early stage classical Hodgkin lymphoma. Br J Haematol. 2018;180(4): 545–549.
[16] Marcheselli L, Bari A, Anastasia A, et al. Prognostic roles of absolute monocyte and absolute lymphocyte counts in patients with advanced-stage follicular lymphoma in the rituximab era: an analysis from the FOLL05 trial of the Fondazione Italiana Linfomi. Br J Haematol. 2015;169(4):544–551.
[17] Cox MC, Nofroni I, Ruco L, et al. Low absolute lymphocyte count is a poor prognostic factor in diffuse-large-B-cell-lymphoma. Leuk Lymphoma. 2008; 49(9):1745–1751.
[18] Lenz G, Wright G, Dave SS; Lymphoma/Leukemia Molecular Profiling Project, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008; 359(22):2313–2323.
[19] Weiner GJ. Rituximab: Mechanism of action. Semin Hematol. 2010;47(2):115–123.
[20] De Jong D. Molecular pathogenesis of follicular lymphoma: a cross talk of genetic and immunologic factors. J Clin Oncol. 2005;23(26):6358–6363.
[21] Küppers R, Stevenson FK. Critical influences on the pathogenesis of follicular lymphoma. Blood. 2018; 131(21):2297–2306.
[22] Kumagai S, Tashima M, Fujikawa J, et al. Ratio of peripheral blood absolute lymphocyte count to absolute monocyte count at diagnosis is associated with progression-free survival in follicular lymphoma. Int J Hematol. 2014;99(6):737–742.
[23] Belotti A, Doni E, Bolis S, et al. Peripheral blood lymphocyte/monocyte ratio predicts outcome in follicular lymphoma and in diffuse large b-cell lymphoma patients in the rituximab era. Clin Lymphoma, Myeloma Leuk. 2015;15(4):208–213.
[24] Lee SF, Luque-Fernandez MA. Prognostic value of lymphocyte-to-monocyte ratio and neutrophil-to-lymphocyte ratio in follicular lymphoma: a retrospective cohort study. BMJ Open. 2017;7(11):e017904.
[25] Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Cin Oncol. 2007;25(5):579–586.
[26] Gray RJ. A class of *K*-sample tests for comparing the cumulative incidence of a competing risk. Ann Statist. 1988;16(3):1141–1154.
[27] Bachy E, Seymour JF, Feugier P, et al. Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: long-term results of the PRIMA study. J Cin Oncol. 2019;37(31): 2815–2824.
[28] Mozas P, Nadeu F, Rivas-Delgado A, et al. Patterns of change in treatment, response, and outcome in patients with follicular lymphoma over the last four decades: a single-center experience. Blood Cancer J. 2020;10(3):1–9.
[29] Wang L, Wang H, Xia ZJ, et al. Peripheral blood lymphocyte to monocyte ratio identifies high-risk adult patients with sporadic Burkitt lymphoma. Ann Hematol. 2015;94(10):1645–1654.
[30] Yang G, Mills M, Kim Y, et al. Enhancement of the follicular lymphoma international prognostic index (FLIPI) with lymphopenia (FLIPI-L): a predictor for overall survival and histologic transformation. Blood Cancer J. 2020;9(12):104
[31] Wilcox RA, Ristow K, Habermann TM, et al. The absolute monocyte count is associated with overall survival in patients newly diagnosed with follicular lymphoma. Leuk Lymphoma. 2012;53(4):575–580.

SUPPLEMENTARY TABLES

Supplementary Table 1. Descriptive statistics of the CBC parameters of the entire series, and according to the LMR.

	Normal range	All patients	LMR		
			≤2.5	>2.5	*P**
Hb, median in g/L (range)	120–170	135 (72-167)	123 (72-162)	136 (72-167)	**<0.001**
Leukocytes, median in x10^9/L (range)	4–11	8.1 (2.3-121.4)	6.9 (2.3-26.1)	6.5 (2.6-121.4)	0.7
Neutrophils, median in x10^9/L (range)	2.5–7	4.6 (1.2-22.6)	5 (1.5-22.6)	4 (1.2-16.4)	**<0.001**
Lymphocytes, median in x10^9/L (range)	0.9–4.5	2.6 (0.3-93.8)	0.9 (0.3-2.8)	1.6 (0.3-93.8)	**<0.001**
Monocytes, median in x10^9/L (range)	0.1–1	0.4 (0.09-5)	0.5 (0.2-1.3)	0.38 (0.09-5)	**<0.001**
Platelets, median in x10^9/L (range)	130–400	241 (9-1250)	250 (84-1250)	224 (9-819)	**0.03**
Lymphocyte-to-monocyte ratio, median (range)	–	5.8 (0.7-52.7)	1.9 (0.7-2.5)	4.9 (2.6-52.7)	**<0.001**

CBC, complete blood count; LMR, lymphocyte-to-monocyte ratio, Hb, haemoglobin; NS, not statistically significant.
**Mann-Whitney's U test. P-values in bold indicate statistical significance.*

Supplementary Table 2. Treatment and response data of the series, globally and according to the LMR.

		All patients	LMR		
			≤2.5	>2.5	*P*
Initial watch and wait approach (>6 months), n (%)		97 (25)	15 (20)	82 (27)	0.2
Treated during follow-up, n (%)		338 (88)	67 (8)	271 (88)	0.9
	Rituximab-containing frontline regimen, n (%)	335 (99)	65 (97)	270 (99)	**0.04**
	Frontline treatment with R-CHOP, n (%)	185 (55)	38 (57)	147 (54)	0.7
CR rate, n (%)		247 (73)	39 (58)	208 (77)	**0.002**

R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; LMR, lymphocyte-to-monocyte ratio. P-values in bold indicate statistical significance.

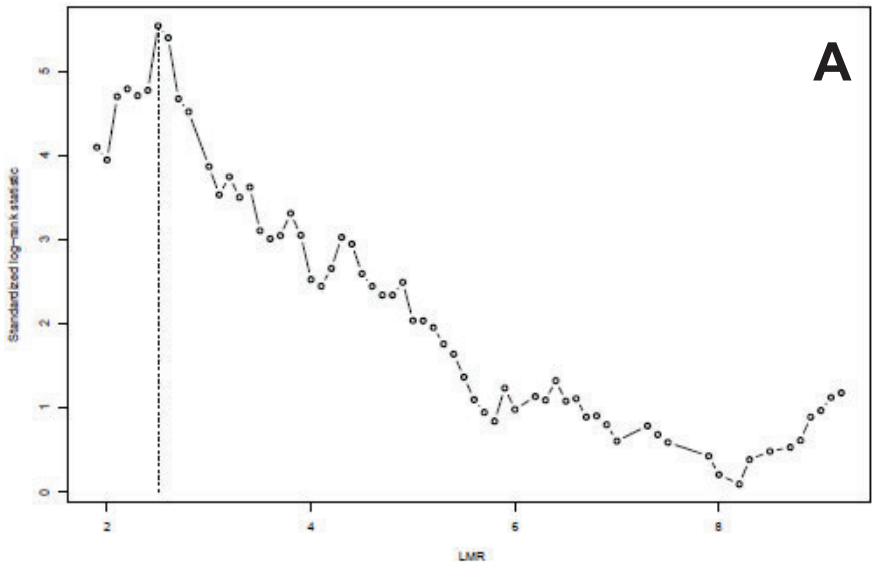
Supplementary Table 3. Univariable and multivariable analyses for the cumulative incidence of histological transformation using Fine-Gray competing risk regression.

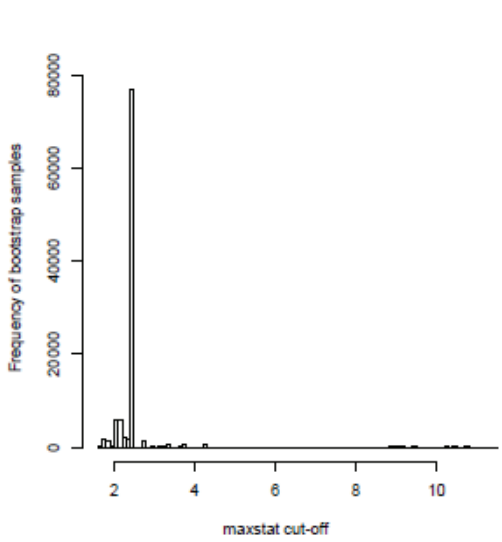
Parameter	Risk category	Risk of HT			
		Univariable analysis		Multivariable analysis (383 cases, 21 events)	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Age	>60 years	1.3 (0.6–3.1)	0.5	NI[a,b]	
Sex	Male	0.9 (0.4–2.2)	0.9	NI[a]	
Histological grade	3A	1.8 (0.7–4.7)	0.2	NI[a]	
B symptoms	Present	0.8 (0.2–3.5)	0.8	NI[a]	
ECOG PS	≥2	1.5 (0.3–6.6)	0.6	NI[a]	
Bulky mass	Present	1.8 (0.7–4.5)	0.2	NI[a]	
Extranodal involvement	Present	3.4 (1–11.5)	**0.047**	2.4 (0.6–10.3)	0.2
FLIPI score	Intermediate risk	3.5 (1–12.6)	0.054	2.3 (0.6–9.4)	0.2
	High risk	4.5 (1.2–16.6)	**0.03**	2.2 (0.5–10.6)	0.3
Hemoglobin	<120 g/L	0.7 (0.2–2.5)	0.6	NI[a,b]	
LDH	Above ULN	2.3 (0.9–5.9)	0.09	NI[a,b]	
B2M	Above ULN	0.7 (0.3–1.7)	0.4	NI[a]	
LMR	≤2.5	2.9 (1.2–6.8)	**0.02**	2.6 (1.1–6.3)	**0.03**

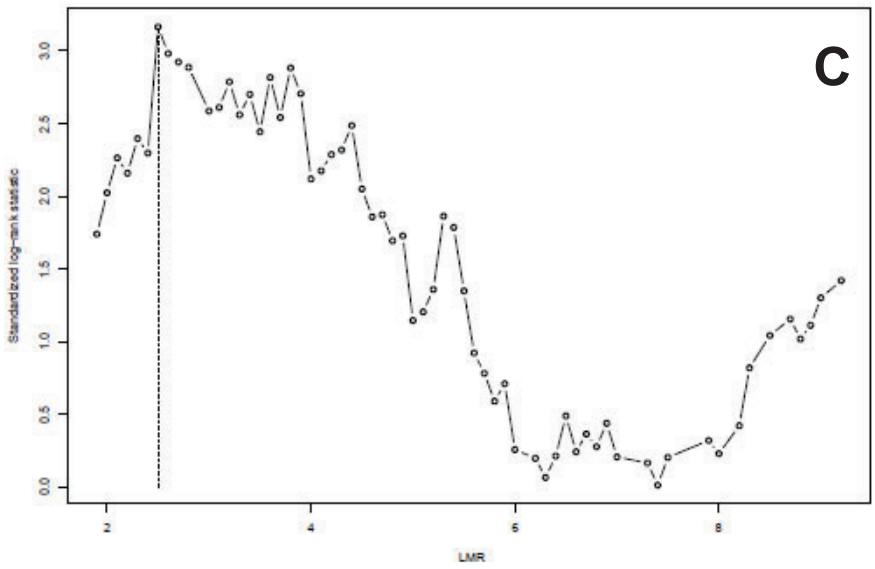
ECOG, Eastern Cooperative Oncology Group Performance Status; FLIPI, Follicular Lymphoma International Prognostic Index; LDH, lactate dehydrogenase; B2M, β2-microglobulin; CI, confidence interval; HR, hazard ratio; HT, histological transformation; LMR, lymphocyte-to-monocyte ratio; ULN, upper limit of normal; NI, not included in the multivariable model; [a] Due to absence of statistical significance in the univariable analysis; [b] To avoid redundancy with the FLIPI score. P-values in bold indicate statistical significance.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Standardized logrank statistics plots for the identification of the best cut-off value of the LMR to predict OS (A). Histogram of bootstrap samples of maxstat-obtained cut-offs to predict OS (B). Standardized logrank statistics plots for the identification of the best cut-off value of the LMR to predict PFS (C).

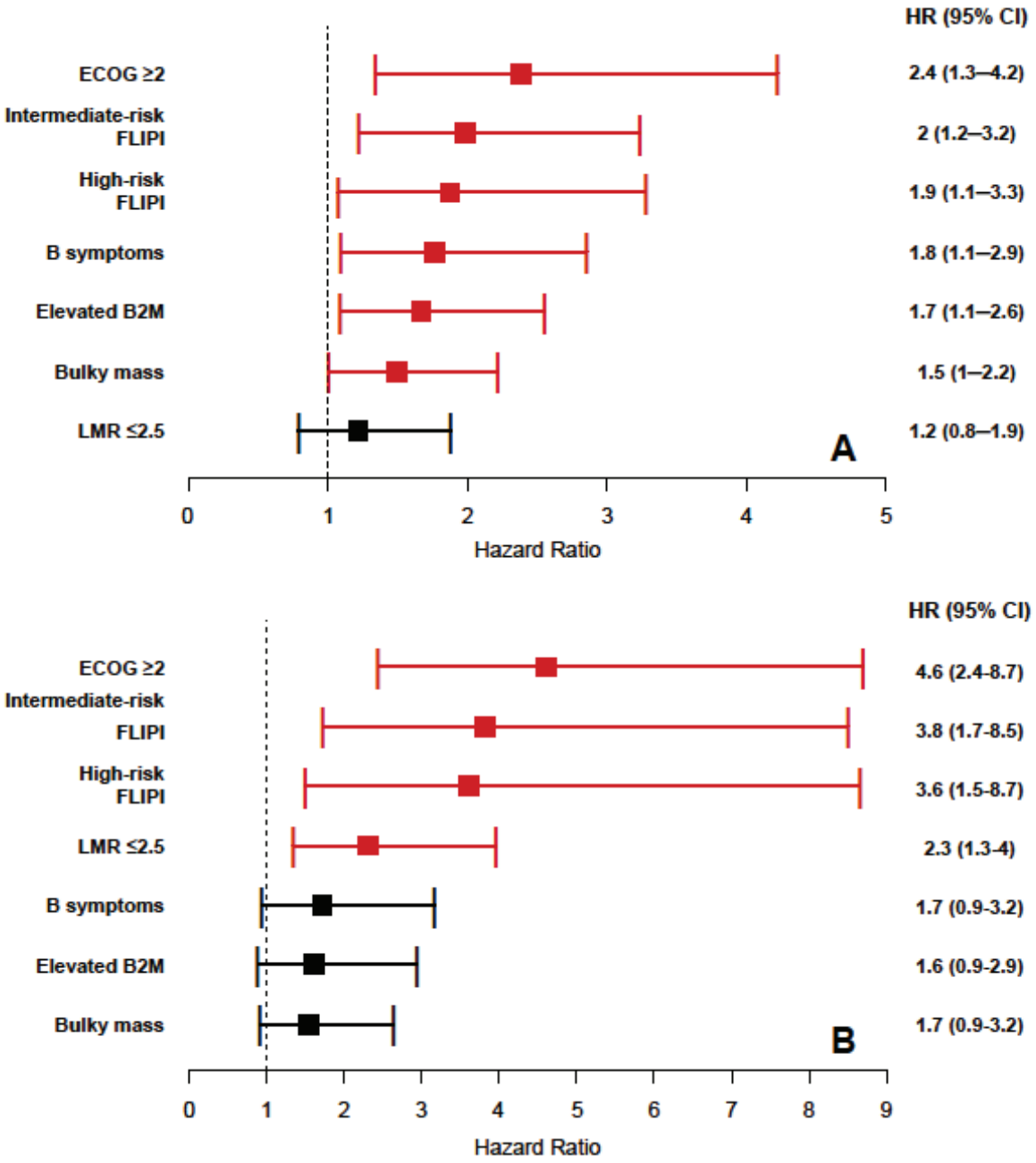






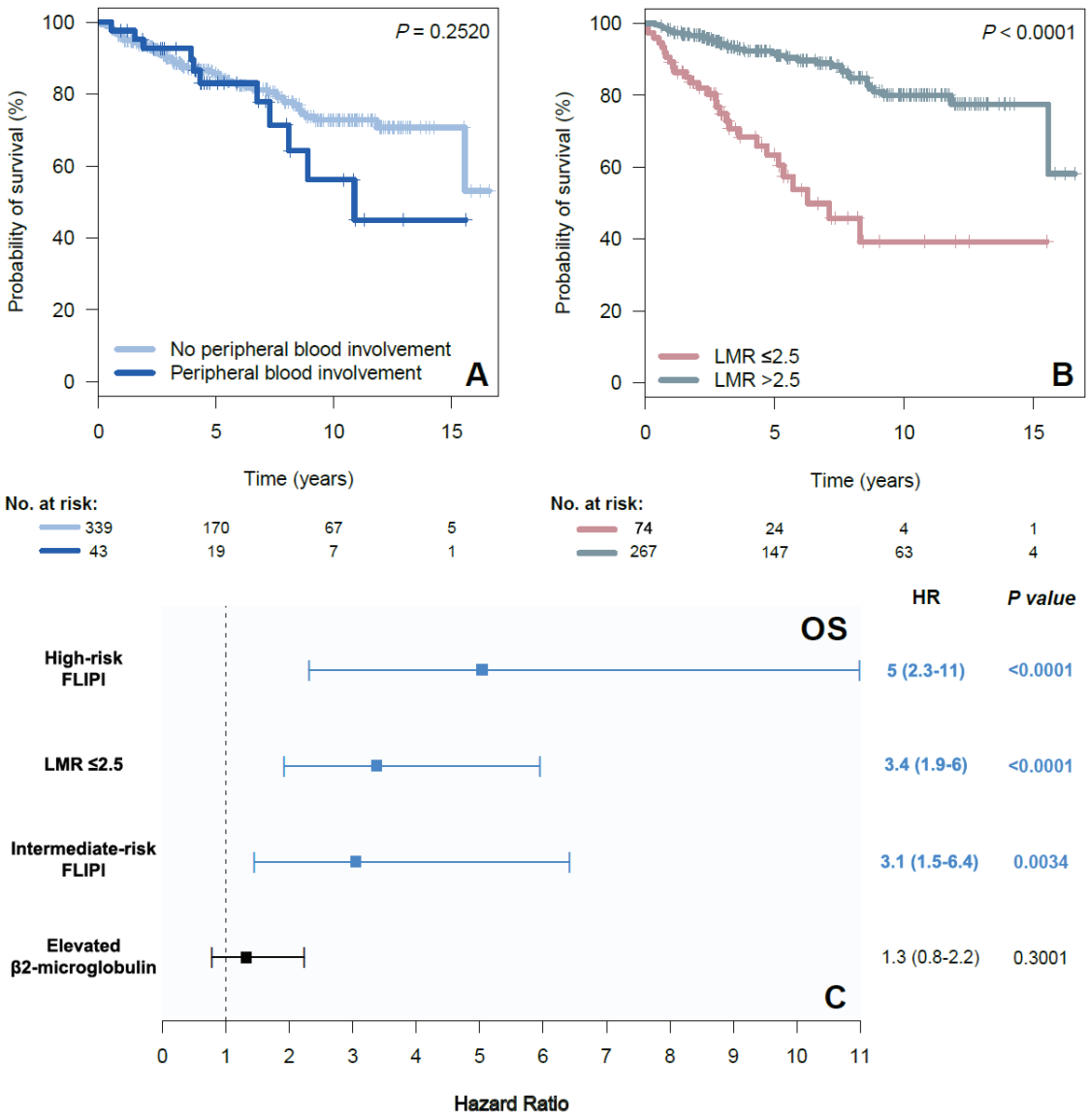
PFS, progression-free survival; OS, overall survival; LMR, lymphocyte to monocyte ratio.

Supplementary Figure 2. Forest plots of the multivariable analyses for PFS (A) and OS (B).



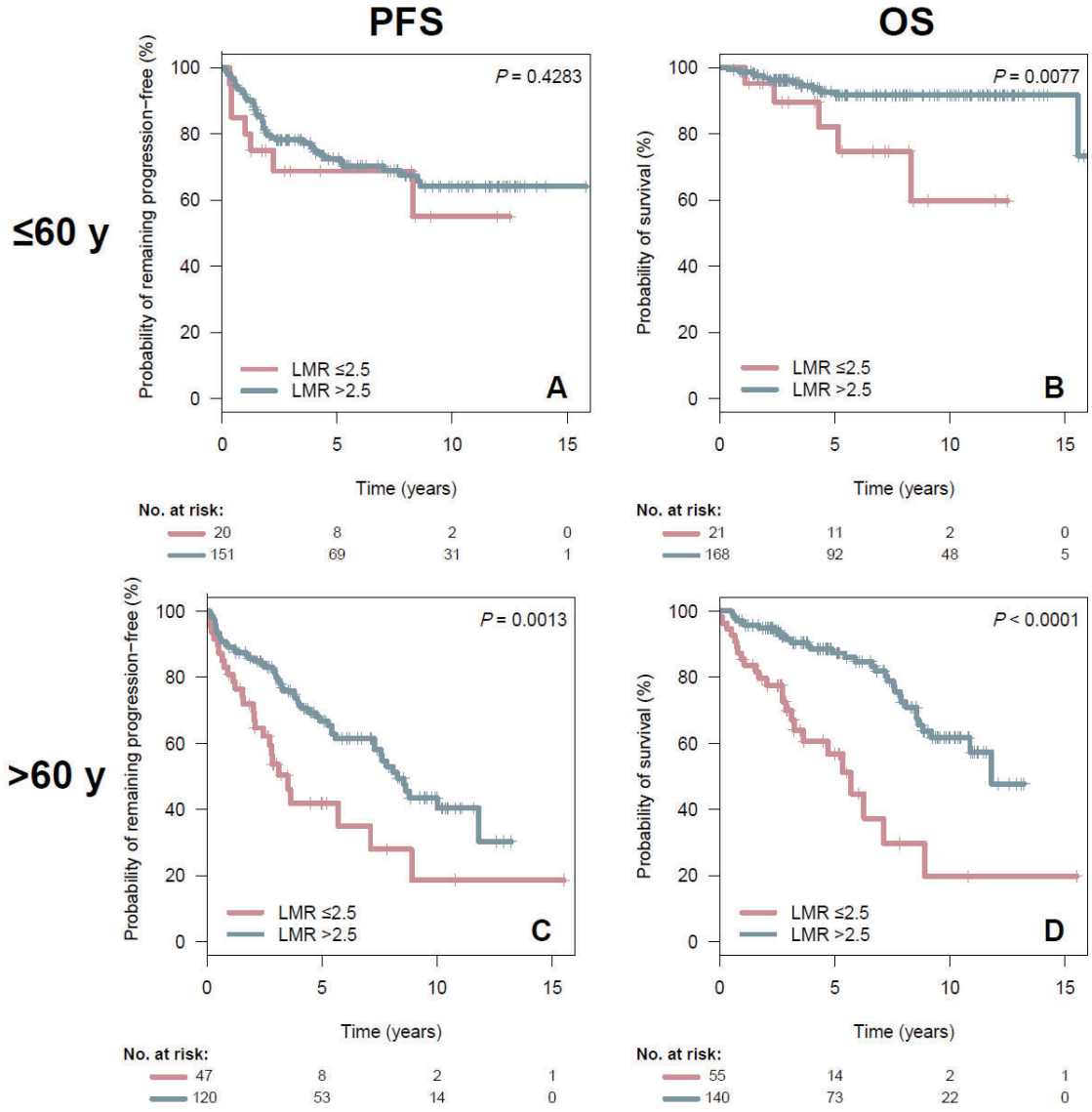
PFS, progression-free survival; OS, overall survival; ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index; B2M, β2-microglobulin; LMR, lymphocyte to monocyte ratio.

Supplementary Figure 3. OS of the entire series according to PBI (A). OS according to the LMR, only for patients without PBI (B).



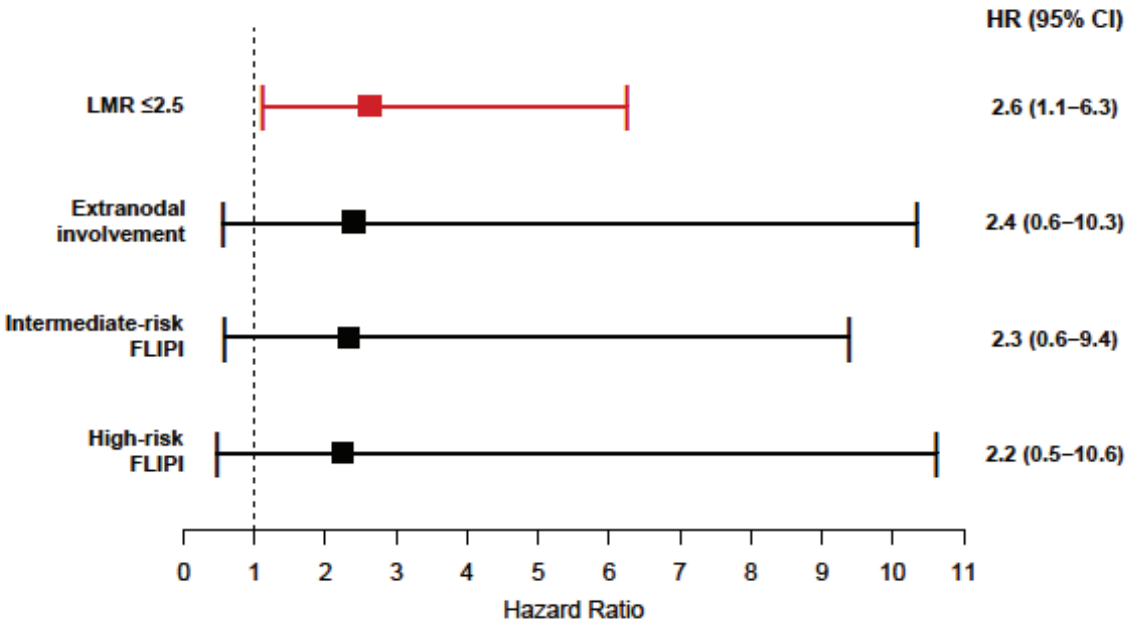
OS, overall survival; PBI, peripheral blood involvement; LMR, lymphocyte to monocyte ratio.

Supplementary Figure 4. PFS and OS for patients ≤60 years (A and B), and >60 years (C and D).



PFS, progression-free survival; OS, overall survival; LMR, lymphocyte to monocyte ratio.

Supplementary Figure 5. Forest plot of the multivariable analysis for the risk of HT.



HT, histological transformation; LMR, lymphocyte to monocyte ratio; FLIPI, Follicular Lymphoma International Prognostic Index; HR, hazard ratio; CI, confidence interval.

2.4. Cuarto trabajo

Baseline correlations and prognostic impact of serum monoclonal proteins in follicular lymphoma

Pablo Mozas, Andrea Rivero, Alfredo Rivas-Delgado, Aleix Fabregat, Juan A. Piñeyroa, Juan G. Correa, Ferran Nadeu, Aina Oliver, Alex Bataller, Eva Giné, Julio Delgado, Neus Villamor, Maria T. Cibeira, Carlos Fernández de Larrea, Laura Rosiñol, Elías Campo, Juan I. Aróstegui, Joan Bladé, Laura Magnano, Armando López-Guillermo

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Correlación con factores iniciales e impacto pronóstico de las proteínas monoclonales séricas en el linfoma folicular

Resumen

La presencia de un componente monoclonal sérico se ha relacionado con peor pronóstico en algunos linfomas. Sin embargo, los datos en linfoma folicular (LF) son escasos. Estudiamos 311 pacientes con LF diagnosticados en un solo centro, con información disponible sobre la inmunofijación sérica (IFs). Se compararon las características iniciales y la evolución entre los pacientes con una IFs positiva y negativa. La IFs fue positiva en 82 pacientes (26%). Las características iniciales fueron similares entre ambos grupos, salvo una mayor edad y niveles más elevados de β_2-microglobulina en el grupo con IFs positiva. Con una mediana de seguimiento de 4.6 años, la IFs positiva se asoció con un mayor riesgo de recaída precoz (POD24, 27 vs 15%, *P*=0.02), una menor supervivencia libre de progresión (42 vs 52% a 5 años, *P*=0.008) y menor supervivencia global (SG, 59 vs 77% a 10 años, *P*=0.046). En pacientes mayores de 60 años, la IFs positiva fue un factor predictivo independiente de la SG (*hazard ratio* = 2.4, intervalo de confianza al 95%: 1.2-5.0, *P*=0.02). Aproximadamente un cuarto de los pacientes con LF presenta una IFs positiva al diagnóstico, lo cual es un factor pronóstico adverso. Estos hallazgos animan a continuar investigando su relación con la biología del linfocito B y del microambiente tumoral.

bjh research paper

Baseline correlations and prognostic impact of serum monoclonal proteins in follicular lymphoma

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Summary

The presence of a serum monoclonal component has been associated with poor outcomes in some lymphomas. However, data in follicular lymphoma (FL) are scarce. We studied 311 FL patients diagnosed at a single institution, for whom information on serum immunofixation electrophoresis (sIFE) at diagnosis was available. Baseline characteristics and outcomes were compared between patients with a positive (+sIFE) and a negative sIFE (−sIFE). sIFE was positive in 82 patients (26%). Baseline features were comparable between both groups, except for an older age and higher proportion of elevated β_2-microglobulin levels in the +sIFE group. With a median follow-up of 4.6 years, a +sIFE was associated with a higher risk of early relapse (POD24, 27% vs. 15%, $P = 0{\cdot}02$), shorter progression-free survival (PFS; 42% vs. 52% at 5 years, $P = 0{\cdot}008$), and shorter overall survival (OS; 59% vs. 77% at 10 years, $P = 0{\cdot}046$). In patients >60 years, a +sIFE was an independent predictor of OS [hazard ratio (HR) = 2·4, 95% confidence interval (CI): 1·2–5·0; $P = 0{\cdot}02$]. Approximately one quarter of patients with FL has a +sIFE at diagnosis, which is a predictor of poor outcome. These findings encourage further investigation of its relationship with B-cell biology and the tumour microenvironment.

Keywords: follicular lymphoma, serum immunofixation electrophoresis, serum monoclonal component, prognosis, survival.

The overall survival (OS) of follicular lymphoma (FL), the most common indolent B-cell lymphoma,[1] now approaches 20 years.[2] However, two specific clinical scenarios remain challenging: early relapse, also called POD24,[3] and histological transformation (HT) to a diffuse large B-cell lymphoma (DLBCL).[4–6] In an effort to anticipate outcomes for each individual patient and eventually tailor treatment strategies accordingly, the impact of clinical and biological variables has been examined and prognostic indexes have been designed.[7–9]

In 1957, Azar and colleagues studied 13 patients with malignant lymphoma and lymphatic leukaemia who had a serum monoclonal component (sMC),[10] which represents the first report on the presence of a monoclonal immunoglobulin (Ig) in lymphoproliferative disorders. The presence of a serum monoclonal IgM is one of the hallmarks and diagnostic criteria of Waldenström's macroglobulinaemia,[11] and a sMC has been described in several other B-cell malignancies.

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In a cohort of 1 150 cases of lymphoma and chronic lymphocytic leukaemia (CLL), Alexanian and colleagues reported a 4·5% prevalence of IgG spikes in the protein pattern of patients with diffuse lymphomas, although their impact on outcome was not evaluated.[12] Economopoulos *et al.*[13] studied a series of 255 non-Hodgkin lymphoma (NHL) cases, 89 of which were indolent NHL (iNHL). The prevalence of a sMC was 17·2% in the entire series, and it was associated with advanced disease. There were also differences according to the lymphoma subtype, IgM being the most frequent isotype in iNHL cases. In this study, no significant differences were found regarding survival.

In DLBCL, several recent studies have reported an impact of a sMC on progression-free survival (PFS) and OS.[14–17] Likewise, in a small cohort of extranodal marginal zone lymphoma (MZL), the prevalence of a sMC was found to be 27%, and all positive cases had stage IV disease.[18] To our knowledge, no study has examined the prevalence and potential prognostic impact of the presence of a sMC in FL patients in the rituximab era. Therefore, our aim was to determine the prevalence of a positive serum immunofixation electrophoresis (sIFE) in our cohort of FL patients, describe their main clinical, biological and immunochemical features at diagnosis, and correlate the sIFE with survival, the risk of HT, and second malignancies (SM).

Patients and methods

Patients

Three hundred and eleven patients (169 females, 142 males; median age, 60 years; range, 26–88 years) consecutively diagnosed with grade 1–3A FL at a single institution between 1991 and 2018, with available data on sIFE at diagnosis, constituted the study population. All available diagnostic specimens underwent pathological review. Patients diagnosed with grade 3B FL, primary gastrointestinal or cutaneous FL, and composite lymphoma were not included. Information on relapse, causes of death, and HT was collected and analysed. Baseline patient and disease characteristics, type of frontline treatment, PFS and OS, and risk of HT and SM were assessed and compared between patients with negative (−sIFE) and positive sIFE (+sIFE). In addition, data concerning all types of malignant tumours (and of multiple myeloma in particular) appearing after the diagnosis of FL were collected (including non-melanoma skin cancer). Time from the diagnosis of FL to the second neoplasm was registered. The study was conducted according to the Hospital Clínic de Barcelona Institutional Review Board and in accordance with the Declaration of Helsinki.

Protein and immunochemical parameters

Serum protein electrophoresis (sPEP) was performed at diagnosis in all patients, using capillary electrophoresis (Capillarys 2, Sebia, Lisses, France). When available, the sPEP plot was visually reviewed in search of an identifiable monoclonal spike and, when found, it was quantitatively measured. A non-measurable monoclonal component was defined as an absent identifiable monoclonal spike in the sPEP plot. Serum Ig levels were measured using immunoassay and categorized as decreased, normal, or elevated, according to our laboratory reference ranges (IgG: 6·8–15·3 g/L; IgA: 0·66–3·65 g/L; IgM: 0·36–2·61 g/L). sIFE (Hydrasys, Sebia) was also performed at diagnosis in all patients to identify the monoclonal heavy and light chains. A biclonal sIFE was defined as the presence of two heavy or light chains in the sIFE of the same sample. Immunoparesis was defined as a decreased level of at least one of the non-involved Ig (for patients with a +sIFE), and of any Ig for patients with a −sIFE. When a reassessment of the sIFE was available during follow-up, it was categorized as being of the same isotype as the initial sample, or a different isotype, when a change in the heavy and/or light chain was detected. For the evaluation of the concordance between sIFE and light-chain restriction in the lymph node biopsy (by immunohistochemistry or flow cytometry of the lymph node suspension), only the light-chain isotype was considered.

Response assessment and survival endpoints

An initial wait-and-watch approach was defined as a time interval between the date of diagnosis and that of treatment initiation longer than six months. Computed tomography, with or without positron emission tomography, and unilateral bone marrow biopsy, were used as staging and response assessment tools. Definitions of complete response (CR), partial response (PR), and treatment failure were the standard.[19] PFS was calculated from the date of the first dose of frontline treatment to the date of progression (if a PR was achieved after frontline treatment), relapse (if a CR was achieved after frontline treatment), or death of any cause. Hence, early progressors (POD24) were defined as patients experiencing progressive disease in the first 24 months after the initiation of frontline therapy. This analysis only included patients who received treatment and had a follow-up longer than two years. All patients were included for OS analysis, which was calculated from the date of diagnosis to the date of last follow-up or death from any cause. We considered the following causes of death: progressive FL, treatment-related toxicities, neoplasms different from FL, and other/unknown causes, in line with previous studies.[20]

Statistical analysis

The χ^2 or Fisher's exact test were used for comparison of categorical variables between patients with a −sIFE and +sIFE. Kaplan–Meier survival curves were plotted according to the sIFE and compared for statistical differences by use of the log-rank test. To estimate the risk of HT and SM, in which death without the event is possible, cumulative incidence was calculated (cmprsk package, R software, Vienna,

Austria) and compared by use of Gray's test.[21] Considering clinically relevant variables that did not overlap with the Follicular Lymphoma International Prognostic Index (FLIPI) score, parsimonious multivariate Cox regression models were designed, for all patients and for patients >60 years specifically, after ruling out interaction between age and the sIFE on survival. The multivariate model for OS in patients >60 years was then validated by means of 1000-sample bootstrapping (validate function, rms package, R Foundation for Statistical Computing, Vienna, Austria). $P < 0{\cdot}05$ was considered to indicate statistical significance.

Results

Baseline characteristics

Baseline characteristics of the 311 patients, and according to the sIFE, are shown in Table I. Eighty-two patients (26%) had a +sIFE at diagnosis. The Eastern Cooperative Oncology Group performance status (ECOG PS), the frequency of B symptoms, the Ann-Arbor stage, and tumour burden features, such as the presence of a bulky mass or an elevated lactate dehydrogenase (LDH) level, were comparable between groups. However, an elevated β_2-microglobulin (B2M) level was more common among patients with a +sIFE (62 vs. 42%, $P = 0{\cdot}002$). The presence of a +sIFE was associated with a slightly older age (median age 61·5 vs. 60 years for patients with a +sIFE and −sIFE, respectively, $P = 0{\cdot}007$). Indeed, when patients were categorized into six age groups, the prevalence of a +sIFE at diagnosis increased from 12% in patients <40 years to 48% in those ≥ 80 years ($P = 0{\cdot}021$, Figure S1).

Protein and immunochemical features

When categorizing Ig levels into decreased, normal, and elevated according to the laboratory reference ranges, no differences were seen in the IgG or IgA levels between patients with a −sIFE and +sIFE. However, patients with a +sIFE had a higher proportion of elevated IgM levels (10 vs. 3%, $P = 0{\cdot}03$). Immunoparesis was associated with a more advanced stage in all patients [77% vs. 56% of stage IV disease for patients with and without immunoparesis ($P = 0{\cdot}002$)]. According to the sIFE, no differences were seen in the proportion of patients with immunoparesis. Data on Ig levels for the 311 patients of the series can be found in Table SI.

Among the 82 patients with a +sIFE at diagnosis, IgG was the most common heavy-chain isotype, with a similar distribution between IgG-κ (25%) and IgG-λ (24%). Other isotypes were, by frequency: IgM-κ (15%), IgM-λ (11%), only free λ light chains (11%), IgA-κ (5%), and biclonal (9%). No significant differences were seen in the Ann-Arbor stage according to the heavy or light chain. The sPEP was available for review in 39 patients with a +sIFE: 33 cases had no monoclonal spike in the serum, and for the remaining six cases, the mean MC concentration was 2·7 g/L (range, 1·8–3·9). We had information about a subsequent sIFE performed during follow-up in 19 cases (mostly at relapse): although the most common finding was a +sIFE for the same isotype (11 cases), some cases were negative (5), and others were positive for a different isotype (3). In 38 cases with a +sIFE, the light chain of the serum Ig and the light-chain restriction in the lymph node biopsy were compared: 26 cases were concordant (including the four biclonal cases for which lymph node information was available) and 12 cases were discordant. Protein and immunochemical features of the 82 patients with a +sIFE can be found in Table II.

Treatment, response, and survival

Frontline treatment, responses and outcomes are detailed in Table III. In the entire series, 78 patients (25%) underwent an initial watch-and-wait approach, and this proportion was similar in the two sIFE groups. Treatment strategies were comparable between patients with −sIFE and +sIFE, as was the proportion of patients achieving a CR after frontline treatment.

Median follow-up for the entire series (calculated only for surviving patients) was 4·6 years (range, 7 months to 23·7 years). Considering only those who received treatment, 84 patients (30%) experienced relapse or progression during follow-up, with a median PFS for the entire cohort of 10 years. Compared with those with a −sIFE, patients with a +sIFE had a significantly shorter PFS (median PFS, 5·5 vs. 11 years; 10-year PFS, 42% vs. 52%, $P = 0{\cdot}008$, Fig 1A). Likewise, patients with a +sIFE were at a higher risk of POD24 (27% vs. 15%, $P = 0{\cdot}02$).

Fifty-five patients (18%) died during the time of follow-up. Median OS for the entire cohort was not reached. As seen for PFS, patients with a +sIFE had a shorter OS (59% vs. 77% at 10 years, $P = 0{\cdot}046$, Fig 1B). When causes of death were evaluated, no significant disparities were found according to the sIFE. Considering the differences in age distribution between patients with a +sIFE and −sIFE, and to avoid attributing the adverse prognostic impact of a +sIFE solely to the older age, we performed stratified Kaplan–Meier estimates of PFS and OS for patients ≤ and >60 years. The presence of a +sIFE retained its adverse prognostic impact for PFS and OS in the older ($P = 0{\cdot}02$ for both PFS and OS, Fig 2B, D), but not in the younger age group (Fig 2A, C).

When categorizing patients with a +sIFE according to the heavy-chain isotype, biclonal cases had a shorter OS ($P = 0{\cdot}003$ when compared with IgG cases, Fig 3A), whereas the light-chain isotype did not have a significant impact on OS (Fig 3B). The presence of immunoparesis was evaluated in patients with +sIFE and −sIFE, and it was not an adverse prognostic factor for OS in either of the groups (Figure S2).

We constructed multivariate models using Cox proportional hazards regression (Table SIII). In the entire series, the sIFE did not retain an independent prognostic impact for PFS or OS. However, when we applied the model only to

Table I. Baseline characteristics of the 311 patients of the series, globally and according to the sIFE.

		Serum immunofixation		
	All patients	Negative	Positive	*P*
Number of patients, *n* (%)	311 (100)	229 (74)	82 (26)	–
Age >60 years, *n* (%)	161 (52)	115 (50)	46 (56)	0·4
Female sex, *n* (%)	169 (54)	129 (56)	40 (49)	0·2
ECOG PS ≥2, *n* (%)	19 (6)	13 (6)	6 (7)	0·6
B symptoms, *n* (%)	41 (13)	30 (13)	11 (13)	0·9
Ann-Arbor stage III/IV, *n* (%)	236 (76)	169 (74)	67 (82)	0·2
Secondary extranodal involvement, *n* (%)	200 (64)	140 (61)	60 (73)	0·05
Bone marrow involvement, *n* (%)	168 (57)	120 (55)	48 (62)	0·3
Five or more lymph node areas, *n* (%)	91 (29)	63 (28)	28 (35)	0·2
Bulky mass*, *n* (%)	60 (19)	43 (19)	17 (21)	0·7
Histologic grade 1–2, *n* (%)	231 (74)	172 (75)	59 (72)	0·1
Ki67 index >30%, *n* (%)	115 (52)	91 (56)	24 (42)	0·08
Elevated serum LDH†, *n* (%)	53 (17)	37 (16)	16 (20)	0·5
Elevated β2-microglobulin†, *n* (%)	145 (47)	95 (42)	50 (62)	**0·002**
Haemoglobin <120 g/L, *n* (%)	63 (20)	43 (19)	20 (24)	0·3
High-risk FLIPI score, *n* (%)	73 (24)	48 (21)	25 (31)	0·08

P values in bold indicate statistical significance. ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; FLIPI, Follicular Lymphoma International Prognostic Index; sIFE, serum immunofixation electrophoresis.
*Bulky disease was defined as a mediastinal mass larger than 1/3 of the mediastinal width, or a lymph node mass larger than 10 cm in the horizontal section of the computed tomography scan.
†Above the upper normal limit for the laboratory of Hospital Clínic de Barcelona.

Table II. Immunochemical features for the 82 patients with a +sIFE.

Immunoglobulin isotype (serum), *n* (%)	
IgG-κ	21 (25)
IgG-λ	20 (24)
IgM-κ	12 (15)
IgM-λ	9 (11)
IgA-κ	4 (5)
IgA-λ	0
Only free κ light chains	0
Only free λ light chains	9 (11)
Biclonal*	7 (9)
Serum monoclonal component, *n* (%)	
Information not available	43 (52)
Non-measurable	33 (40)
Measurable†	6 (8)
Light-chain concordance with lymph node‡, *n* (%)	
Information not available	44 (54)
Concordant	26 (32)
Discordant	12 (14)

sIFE, serum immunofixation electrophoresis; Ig, immunoglobulin.
*Biclonal cases were: IgG-κ + free λ light chains (3), IgM-κ + IgG-λ (2), IgA-κ + IgG-κ (1), IgM-κ + IgM-λ (1).
†Cases with a measurable serum monoclonal component (sMC) had a median sMC concentration of 2·7 g/L (range, 1·8–3·9 g/L).
‡Four biclonal cases (IgG-κ + free λ light chains, IgM-κ + IgG-λ, IgA-κ + IgG-κ, and IgG-κ + free λ light chains) had lymph node information, and they were all considered concordant.

patients >60 years, we found that a +sIFE was an independent predictor of OS (HR = 2·4, 95% CI: 1·2–5·0, $P = 0·02$), along with the presence of B symptoms and the ECOG PS.

Risk of histological transformation and of second malignancies

Histological transformation to DLBCL was seen in 19 patients (6%). For the entire series, the median time to HT was 1·8 years (range, 3 months–14·3 years), and the 10-year risk of HT was 8% (95% CI, 4–13%). The cumulative incidence of HT was not significantly different according to the sIFE (Figure S3A).

A second tumour was identified after the diagnosis of FL in 33 patients (11%). Haematological malignancies were the most frequent type of SM, which accounted for 20% of cases [five patients, treated with fludarabine (1), R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; 2), R-CVP (rituximab, cyclophosphamide, vincristine, and prednisone; 1), and FCM (fludarabine, cyclophosphamide and mitoxantrone; 1)]. No patient developed an overt multiple myeloma or other malignant monoclonal gammopathy during the time of follow-up. The median time to the development of a SM was 4·5 years (range, 1 month–16·5 years), with a 10-year risk of developing a SM of 16% for all patients. Patients with a −sIFE and +sIFE had similar rates of SM (Figure S3B).

Discussion

Considering the heterogeneous clinical behaviour of FL, and with the intention of better predicting early relapse, HT and, eventually, lymphoma-related death, many clinical and biological variables have been studied. In this regard, the presence of a sMC in chronic lymphoproliferative disorders has

Table III. Treatment, response, and outcomes for the 311 patients of the series.

	All patients	Serum immunofixation		P
		Negative	Positive	
Watchful waiting for >6 months, *n* (%)	78 (25)	56 (25)	22 (27)	0·7
Received treatment during follow-up, *n* (%)	279 (89)	203 (89)	73 (89)	0·9
Rituximab-containing frontline regimen, *n* (%)	239 (87)	173 (85)	66 (90)	0·3
Single-agent rituximab, *n* (%)	47 (15)	33 (14)	14 (17)	0·7
Frontline treatment with R-CHOP, *n* (%)	129 (47)	95 (47)	34 (47)	
CR rate, *n* (%)	197 (71)	147 (72)	50 (69)	0·5
POD24, *n* (%)	51 (19)	31 (15)	20 (27)	**0·02**
PFS, % at 10 years (95% CI)	49 (40–57)	52 (42–63)	42 (29–61)	**0·008**
OS, % at 10 years (95% CI)	72 (65–80)	77 (69–85)	59 (45–78)	**0·045**
HT, % at 10 years (95% CI)	8 (4–13)	7 (3–13)	11 (4–21)	0·2
SM, % at 10 years (95% CI)	16 (11–23)	16 (10–24)	16 (6–28)	0·9

R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; POD24, progression of disease within 24 months of initiating frontline therapy; PFS, progression-free survival; OS, overall survival; HT, histological transformation; SM, second malignancies; CI, confidence interval. *P* values in bold indicate statistical significance.

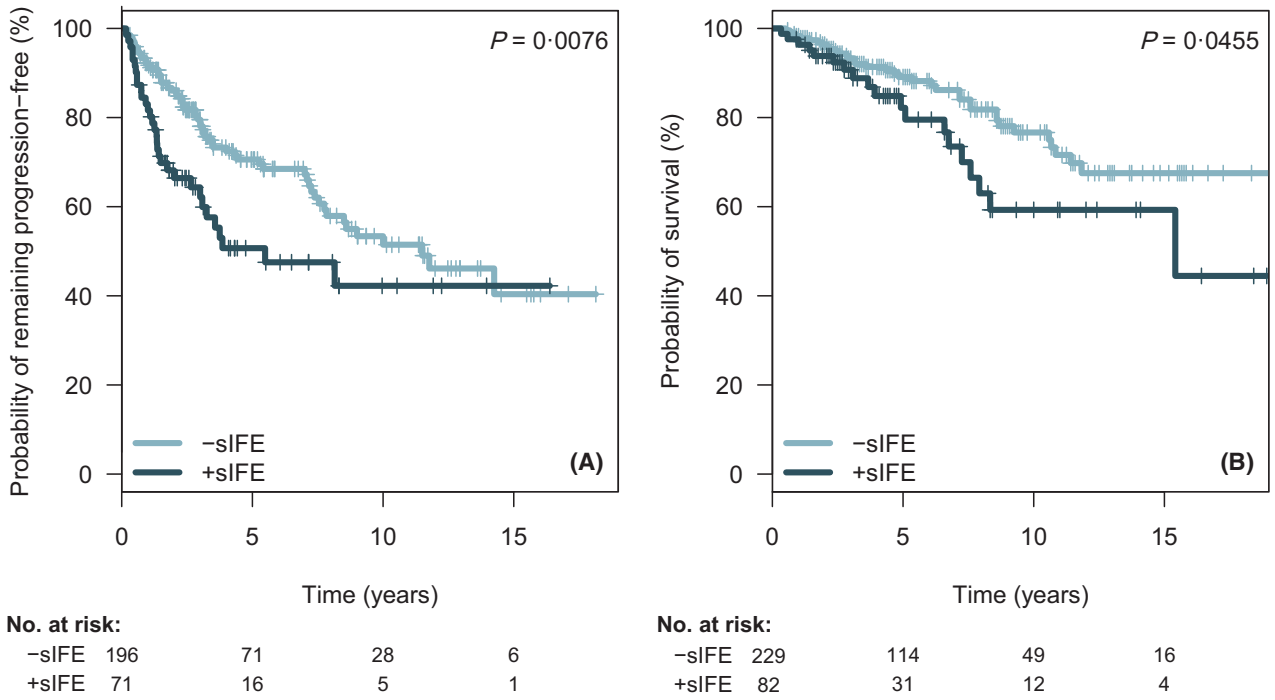

Fig 1. Progression-free (A) and overall survival (B) according to the serum immunofixation electrophoresis (sIFE). [Colour figure can be viewed at wileyonlinelibrary.com]

been identified as an adverse prognostic factor, notably in DLBCL.[14,16,17]

We studied data on sIFE at diagnosis in our cohort of 311 patients with FL and found a sMC in 26% of them, a similar prevalence to that reported for other indolent lymphomas.[18] This subset of patients exhibits comparable clinical and biological baseline features to patients with a −sIFE (including FLIPI score), with the exception of a higher frequency of elevated B2M levels. In our series, IgG constituted the most commonly detected heavy-chain isotype (49%), with a similar distribution of κ and λ light chains, which contrasts with previous reports studying other indolent lymphomas, where IgM was the most frequent isotype,[13,22] although the majority of cases in those series were aggressive NHLs. Most cases where the serum concentration of the monoclonal protein was available did not produce an identifiable spike on the sPEP plot, while all the quantifiable cases had a low concentration sMC (<4 g/L).

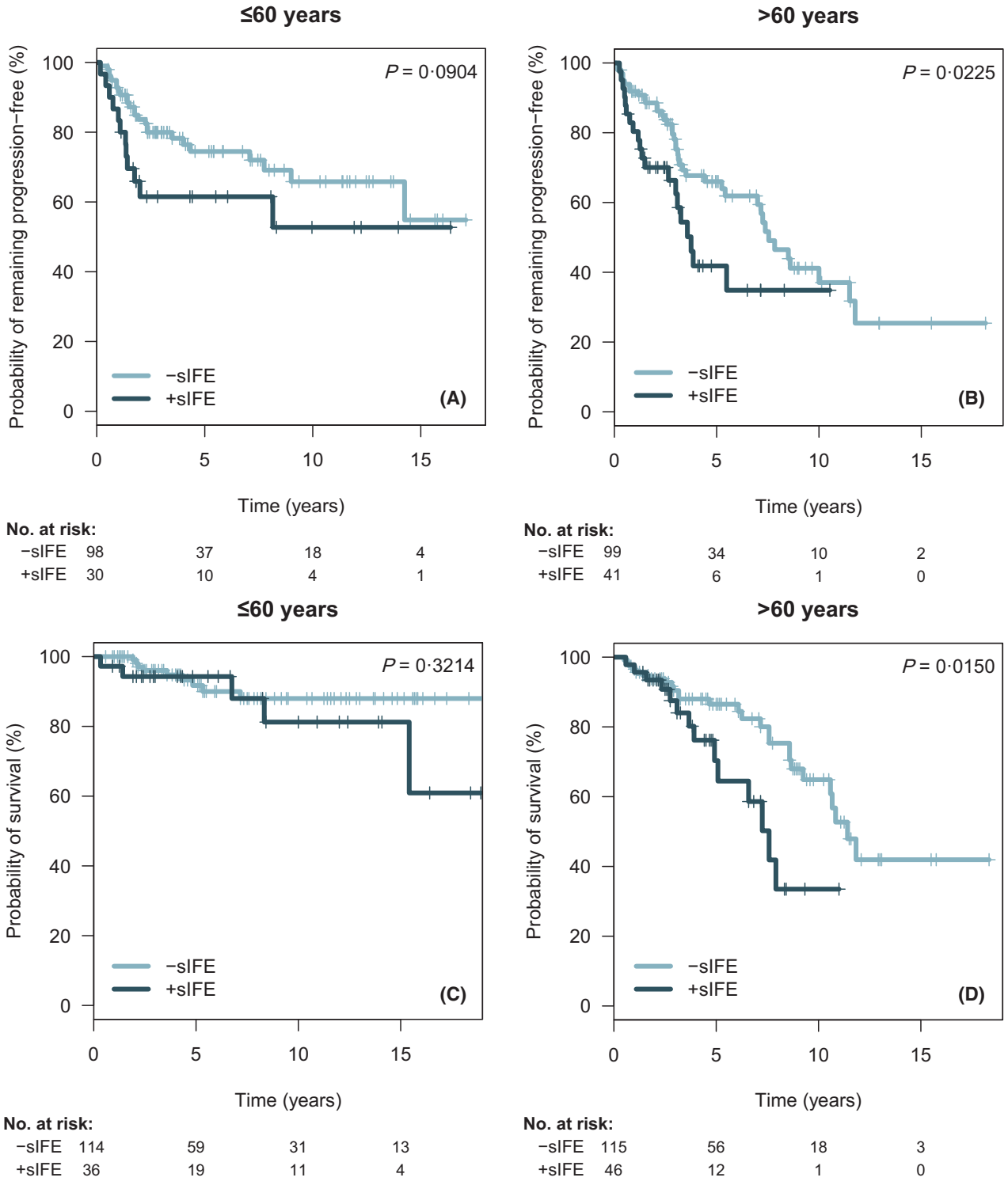

Fig 2. Progression-free according to serum immunofixation electrophoresis (sIFE) for patients younger (A) and older than 60 years (B). Overall survival according to sIFE for patients younger (C) and older than 60 years (D). [Colour figure can be viewed at wileyonlinelibrary.com]

We found that a non-negligible proportion of patients who had a repeated sIFE during follow-up had a serum Ig different from that identified at diagnosis. Likewise, 32% of cases had a light-chain restriction in the lymph node biopsy different from the circulating light chain by sIFE. When studying survival according to the Ig isotypes, we could not identify significant differences in outcome between IgG and IgM cases, in contrast to the unfavourable impact of an IgM

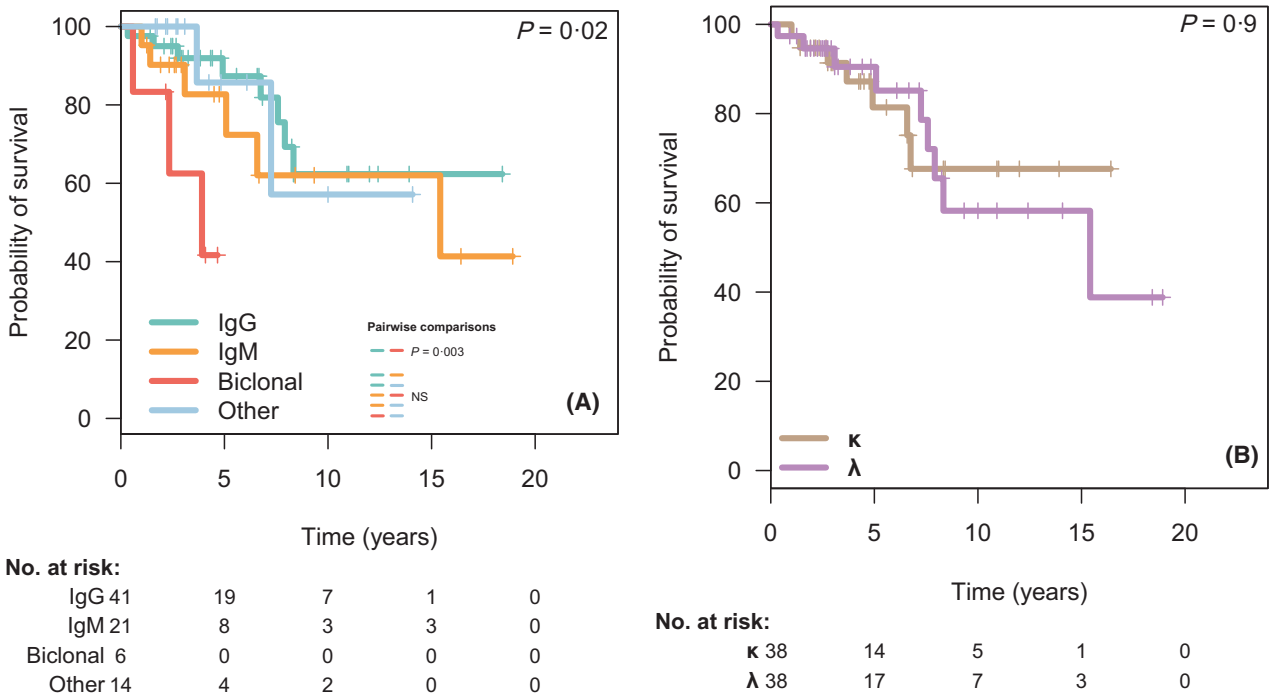

Fig 3. Overall survival of the patients with a positive serum immunofixation electrophoresis (sIFE), according to the heavy- (A), and light-chain isotype (B). [Colour figure can be viewed at wileyonlinelibrary.com]

sMC on PFS found by Maiolo and colleagues in DLBCL.[23] However, cases with a biclonal serum Ig did have a poorer OS compared with IgG cases.

Although the biological explanation of these findings remains elusive and no previous study has addressed this point, it is reasonable to hypothesize that the immunological processes that promote tumour development in B cells, as well as the interactions with the tumour microenvironment (TME),[24] might induce a state of immune deregulation, which could explain the presence of more than one single Ig isotype at diagnosis (biclonal cases), the discordant light-chain restriction between the serum and the lymph node, and the emergence of a different Ig isotype during follow-up.

In contrast with previous reports in DLBCL patients,[17] in our series, patients with a +sIFE achieved a comparable rate of CR after similar frontline treatment regimens. Preceding studies reported a trend towards a better survival for indolent lymphoma patients with a sMC,[13] whereas we identified an impact in the opposite direction concerning the fraction of POD24 patients, PFS, and OS, which is in line with recent studies recognizing the +sIFE as an adverse prognostic factor in DLBCL.[15–17,23] This apparent discrepancy with the findings by Economopoulos and colleagues might be explained by the low number of patients with indolent lymphoma included in that study, the histological heterogeneity within the indolent lymphoma group, and the absence of rituximab treatment for historical reasons.

No previous study had described a differential distribution of a +sIFE according to age in patients with lymphoma. Besides identifying an increasing proportion of patients with a +sIFE in consecutive age groups, we observed that the impact of the sIFE on PFS and OS remained only for older patients, likely due to the lower number of +sIFE cases in the younger subgroup. Moreover, in a multivariate model, a +sIFE was found to be an independent predictor of OS in patients >60 years. Thus, the effect of a +sIFE on OS is not exclusively attributable to the older age.

In conclusion, our study represents the largest series investigating the prevalence and prognostic impact of a sMC in FL. A +sIFE is found in approximately one quarter of patients at diagnosis. Despite comparable baseline, treatment, and response features, patients with a +sIFE have shorter PFS and OS, and this impact is stronger in older patients. With the limitations inherent to single-centre, observational studies, which warrant validation in external cohorts, our findings support further investigation of the relationship between the sMC, B-cell biology, immune deregulation, and the tumour microenvironment.

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PM designed the study, collected the data, performed the analysis, and wrote the paper. AR, ARD, AF, JAP, JGC, FN, AO, AB, EG, JD, NV, MTC, CFL, LR, EC, JIA, JB, LM and ALG contributed to data collection and reviewed the final manuscript. This study was supported by 'Becas de Investigación de la FEHH' (Fundación Española de Hematología y Hemoterapia) to PM, the PI19/00925 grant (Instituto de

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Supporting Information

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

Table SI. Immunochemical features of the 311 patients of the series, globally and according to serum immunofixation electrophoresis (sIFE).

Table SII. Univariate analyses for progression-free survival (PFS) and overall survival (OS), for all patients, and for patients older than 60 years, using Cox proportional hazards regression.

Table SIII. Multivariate analyses for progression-free survival (PFS) and overall survival (OS), for all patients, and for patients older than 60 years, using Cox proportional hazards regression.

Fig S1. Prevalence of a positive serum immunofixation electrophoresis (sIFE) according to age.

Fig S2. Overall survival according to the presence of immunoparesis in patients with a negative (A) and positive (B) serum immunofixation electrophoresis.

Fig S3. Cumulative incidence of histological transformation (A), and of second malignancies (B), according to the serum immunofixation electrophoresis (sIFE).

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. France: Lyon; 2017.
2. Bachy E, Seymour JF, Feugier P, Offner F, López-Guillermo A, Belada D, et al. Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: long-term results of the PRIMA study. *J Clin Oncol*. 2019;**37**: 2815–24.
3. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: an analysis from the National LymphoCare Study. *J Clin Oncol.* 2015;**33**:2516–22.
4. Montoto S, Davies AJ, Matthews J, Calaminici M, Norton AJ, Amess J, et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol.* 2007;**25**:2426–33.
5. Alonso-Álvarez S, Magnano L, Alcoceba M, Andrade-Campos M, Espinosa-Lara N, Rodríguez G, et al. Risk of, and survival following, histological transformation in follicular lymphoma in the rituximab era. A retrospective multicentre study by the Spanish GELTAMO group. *Br J Haematol.* 2017;**178**:699–708.
6. Federico M, Caballero Barrigón MD, Marcheselli L, Tarantino V, Manni M, Sarkozy C, et al. Rituximab and the risk of transformation of follicular lymphoma: a retrospective pooled analysis. *Lancet Haematol*. 2018;**5**:e359–e367.
7. Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, et al. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. *J Clin Oncol.* 2009;**27**:4555–62.
8. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol.* 2015;**16**:1111–22.
9. Solal-Céligny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. *Blood*. 2004;**104**(5):1258–65.
10. Azar HA, Hill WT, Osserman EF. Malignant lymphoma and lymphatic leukemia associated with myeloma-type serum proteins. *Am J Med*. 1957;**23**:239–49.
11. Dimopoulos MA, Kastritis E. How I treat Waldenström macroglobulinemia. *Blood*. 2019;**134**:2022–35.
12. Alexanian R. Monoclonal gammopathy in lymphoma. *Arch Intern Med.* 1975;**135**:62–6.
13. Economopoulos T, Papageorgiou S, Pappa V, Papageorgiou E, Valsami S, Kalantzis D, et al. Monoclonal gammopathies in B-cell non-Hodgkin's lymphomas. *Leuk Res.* 2003;**27**:505–8.
14. Cox MC, Di Napoli A, Scarpino S, Salerno G, Tatarelli C, Talerico C, et al. Clinicopathologic characterization of diffuse-large-B-cell lymphoma with an associated serum monoclonal IgM component. *PLoS One*. 2014;**9**: e93903.
15. Zhang Y, Wei Z, Li J, Gao R, Liu P. Monoclonal gammopathies regardless of subtypes are associated with poor prognosis of diffuse large B-cell lymphoma A STROBE-compliant article. *Medicine*. 2018;**97**:e11719.
16. Li Y, Wang L, Zhu HY, Liang JH, Wu W, Wu JZ, et al. Prognostic significance of serum immunoglobulin paraprotein in patients with diffuse large B cell lymphoma. *Br J Haematol.* 2018;**182**:131–4.
17. Papageorgiou SG, Thomopoulos TP, Spathis A, Bouchla A, Glezou I, Stavroulaki G, et al. Prognostic significance of monoclonal gammopathy in diffuse large B-cell lymphoma. *Hematol Oncol.* 2019;**37**(5):634–7.
18. Asatiani E, Cohen P, Ozdemirli M, Kessler CM, Mavromatis B, Cheson BD. Monoclonal gammopathy in extranodal marginal zone lymphoma (ENMZL) correlates with advanced disease and bone marrow involvement. *Am J Hematol.* 2004;**77**(2):144–6.
19. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol.* 2007;**25**:579–86.
20. Sarkozy C, Maurer MJ, Link BK, Ghesquieres H, Nicolas E, Thompson CA, et al. Cause of death in follicular lymphoma in the first decade of the rituximab era: a pooled analysis of French and US cohorts. *J Clin Oncol*. 2019;**37**:144–52.
21. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;**16**(3):1141–54.
22. Wöhrer S, Streubel B, Bartsch R, Chott A, Raderer M. Monoclonal immunoglobulin production is a frequent event in patients with mucosa-associated lymphoid tissue lymphoma. *Clin Cancer Res.* 2004;**10**:7179–81.
23. Maiolo E, Alma E, Napodano C, Gulli F, Bellesi S, Cuccaro A. The prognostic impact of monoclonal immune globulin and free light chain secretion in diffuse large B cell lymphoma (DLBCL). *Leuk Lymphoma*. 2020;**61** (5):1133–9.
24. Huet S, Sujobert P, Salles G. From genetics to the clinic: a translational perspective on follicular lymphoma. *Nat Rev Cancer*. 2018;**18**(4):224–39.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Immunochemical features of the 311 patients of the series, globally and according to the sIFE.

	All patients	Serum immunofixation		
		–sIFE	+sIFE	*P*
Serum IgG levels, n (%)				
Decreased	45 (15)	32 (15)	13 (17)	NS
Normal	240 (82)	177 (82)	63 (79)	
Elevated	10 (3)	6 (3)	3 (4)	
Serum IgA levels, n (%)				
Decreased	18 (6)	11 (5)	7 (9)	NS
Normal	256 (87)	193 (90)	63 (80)	
Elevated	20 (7)	11 (5)	9 (11)	
Serum IgM levels, n (%)				
Decreased	36 (12)	28 (13)	8 (10)	0.03
Normal	243 (83)	180 (84)	63 (80)	
Elevated	14 (5)	6 (3)	8 (10)	
Immunoparesis, n (%)	69 (24)	51 (24)	18 (23)	NS

Ig, immunoglobulin; NS, not statistically significant; sIFE, serum immunofixation electrophoresis. Immunoparesis was defined as a decreased level of at least one of the non-involved Ig (for patients with +sIFE), and of any Ig for patients with –sIFE.

Supplementary Table 2. Univariate and multivariate analyses for PFS and OS using Cox proportional hazards regression, for all patients of the study.

Parameter	Risk category	PFS (264 cases, 94 events)				OS (308 cases, 53 events)			
		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex	Male	1.4	NS	NI[a]		1.6	NS	NI[a]	
Age	>60 years	1.7	0.01	NI[b]		3.8	<0.0001	NI[b]	
Ann–Arbor stage	III–IV	1.5	NS	NI[a,b]		2.3	0.049	NI[b]	
B symptoms	Present	2.6	0.0001	2.2	0.003	3	0.0002	1.5	NS
ECOG PS	≥2	4.4	<0.0001	3.5	<0.001	5	<0.0001	3.4	0.001
Bulky mass	Present	1.3	NS	NI[a]		1.3	NS	NI[a]	
No. of nodal sites	>4	1.6	0.049	NI[b]		2.3	0.003	NI[b]	
FLIPI score	Intermediate risk	1.8	0.02	1.4	NS	3.9	0.0004	3.7	0.001
	High risk	2.3 (1.4-3.9)	0.001	1.3		5.8	<0.0001	4.3	
Haemoglobin	<120 g/L	0.9	NS	NI[a,b]		1.1	NS	NI[a,b]	
LDH	Above ULN	1.5	NS	NI[a,b]		1.2	NS	NI[a,b]	
B2M	Above ULN	1.8	0.007	1.4	NS	2.6	0.001	1.6	NS
sIFE	Positive	1.8	0.009	1.7	0.02	1.8	0.049	1.4	NS

ECOG, Eastern Cooperative Oncology Group Performance Status; FLIPI, Follicular Lymphoma International Prognostic Index; LDH, lactate dehydrogenase; B2M, β2-microglobulin; NI, not included in the multivariate model; CI, confidence interval; HR, hazard ratio; NS, not statistically significant; PFS, progression-free survival; OS, overall survival; ULN, upper limit of normal; sIFE, serum immunofixation electrophoresis.

[a] Due to absence of statistical significance in the univariate analysis. [b] To avoid redundancy with the FLIPI score.

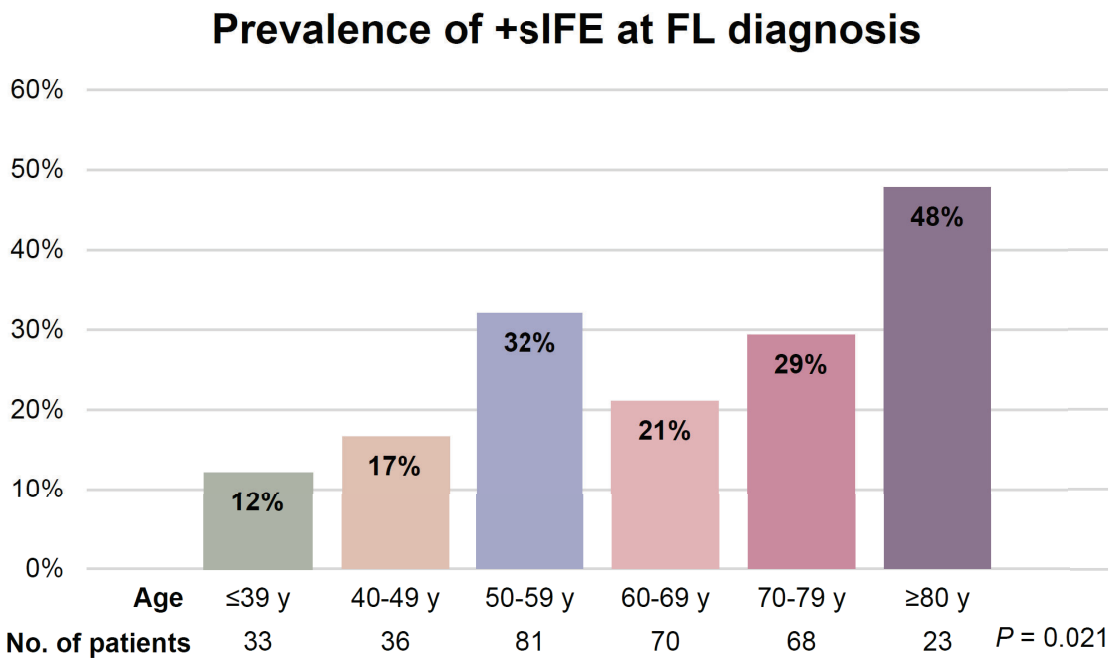
Supplementary Table 3. Univariate and multivariate analyses for PFS and OS using Cox proportional hazards regression, only for patients >60 years.

Parameter	Risk category	PFS (137 cases, 58 events)				OS (159 cases, 40 events)			
		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex	Male	1.3	NS	NI[a]		1.3	NS	NI[a]	
Ann–Arbor stage	III–IV	1.6	NS	NI[a,b]		1.5	NS	NI[a,b]	
B symptoms	Present	5.3	<0.0001	3.7	0.0006	7.4	<0.0001	4.8	0.0006
ECOG PS	≥2	4.4	0.0002	2.3	NS	10.8	<0.0001	6	0.001
Bulky mass	Present	1.3	NS	NI[a]		1.8	NS	NI[a]	
No. of nodal sites	>4	1.4	NS	NI[a,b]		2	0.04	NI[b]	
FLIPI score	Intermediate risk	1.9	NS	1.6	NS	1.8	NS	1.6	NS
	High risk	2.5	0.02	1.4		3.1	0.02	1.3	
Haemoglobin	<120 g/L	1.1	NS	NI[a,b]		1.2	NS	NI[a,b]	
LDH	Above ULN	1.3	NS	NI[a,b]		0.8	NS	NI[a,b]	
B2M	Above ULN	2	0.02	1.5	NS	2.8	0.003	2	NS
sIFE	Positive	1.9	0.02	1.7	NS	2.3	0.02	2.7	0.007

ECOG, Eastern Cooperative Oncology Group Performance Status; FLIPI, Follicular Lymphoma International Prognostic Index; LDH, lactate dehydrogenase; B2M, β2-microglobulin; NI, not included in the multivariate model; CI, confidence interval; HR, hazard ratio; NS, not statistically significant; PFS, progression-free survival; OS, overall survival; ULN, upper limit of normal; sIFE, serum immunofixation electrophoresis.

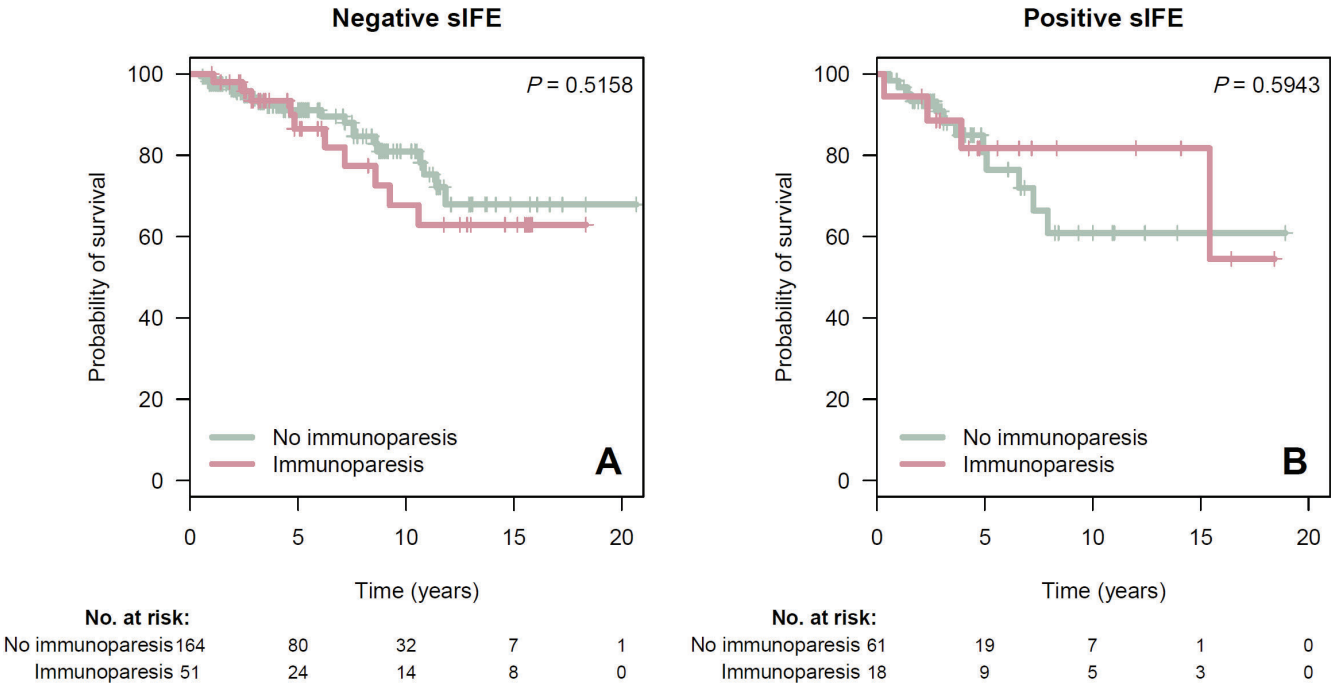
[a] Due to absence of statistical significance in the univariate analysis. [b] To avoid redundancy with the FLIPI score.

Supplementary Figure 1. Prevalence of a +sIFE according to age.



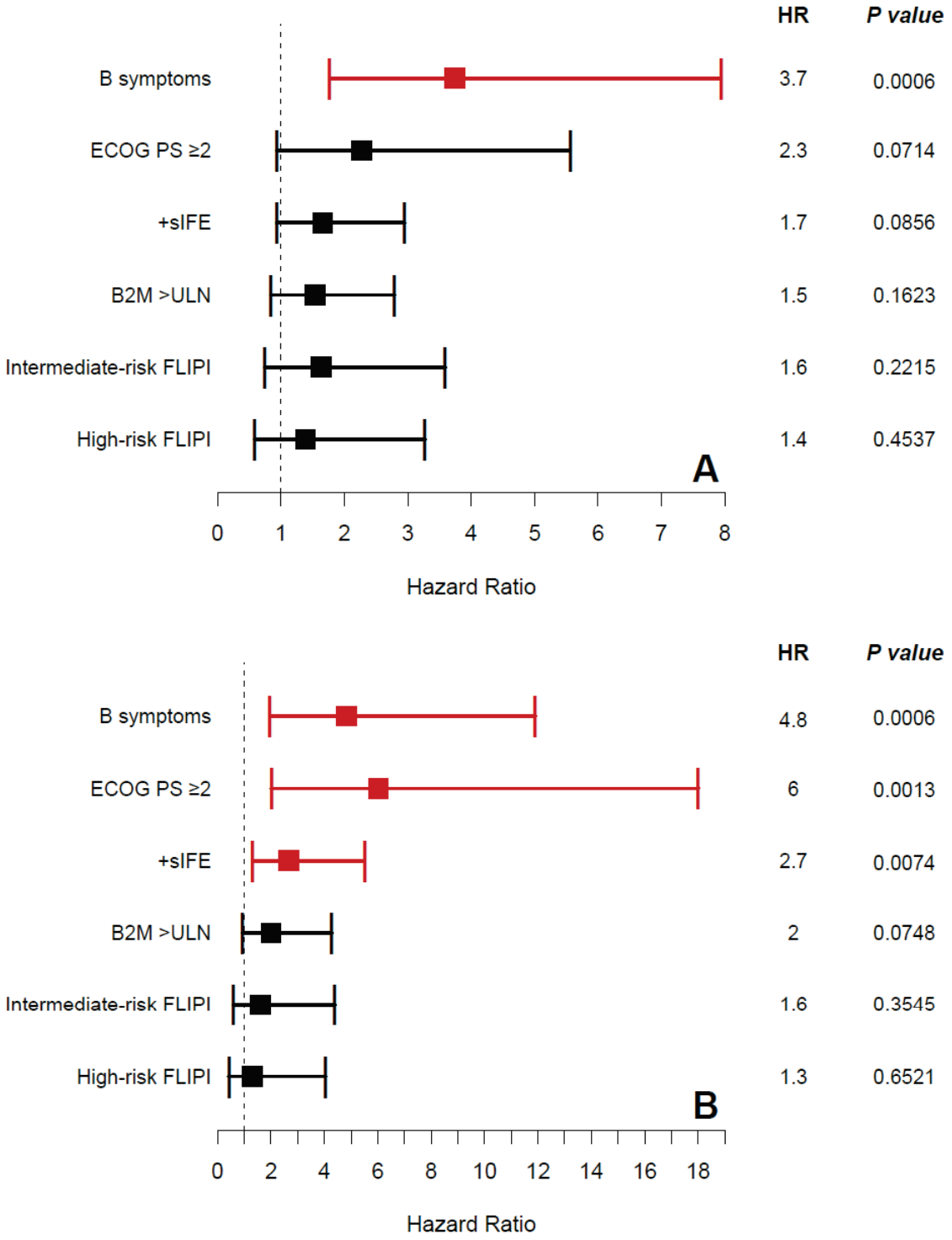
sIFE, serum immunofixation electrophoresis; FL, follicular lymphoma; y, years.

Supplementary Figure 2. Overall survival according to the presence of immunoparesis in patients with a negative (A) and positive (B) serum immunofixation electrophoresis.



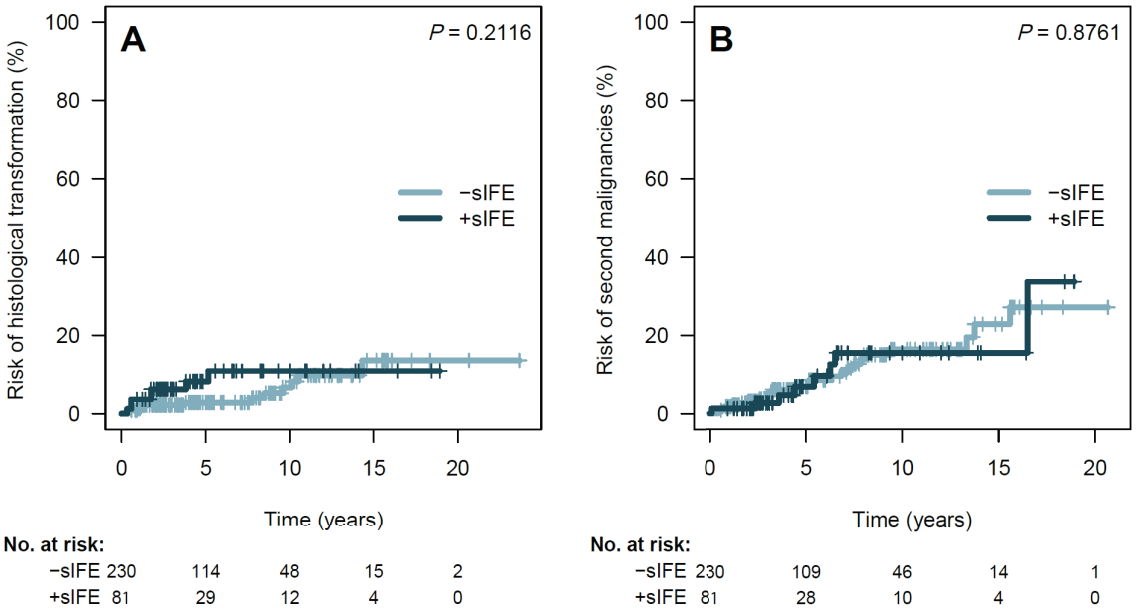
sIFE, serum immunofixation electrophoresis. Immunoparesis was defined as a decreased level of at least one of the non-involved Ig (for patients with a +sIFE), and of any Ig for patients with a –sIFE.

Supplementary Figure 3. Forest plots of the Cox regression models for PFS (A) and OS (B) in patients >60 years.



PFS, progression-free survival; OS, overall survival; HR, hazard ratio; ECOG PS, Eastern Cooperative Oncology Group Performance Status; sIFE, serum immunofixation electrophoresis; B2M, β_2-microglobulin; ULN, upper limit of normal; FLIPI, Follicular Lymphoma International Prognostic Index.

Supplementary Figure 4. Cumulative incidence of histological transformation (A), and of second malignancies (B), according to the sIFE.



sIFE, serum immunofixation electrophoresis.

2.5. Quinto trabajo

Mutational Profile and Copy Number Alterations of Follicular Lymphoma Patients with Different Clinical Behavior

Pablo Mozas, Cristina López, Marta Grau, Ferran Nadeu, Guillem Clot, Sara Valle, Alba Navarro, Marta Kulis, Blanca González-Farré, Alfredo Rivas-Delgado, Andrea Rivero, Juan Gonzalo Correa, Olga Balagué, Eva Giné, Julio Delgado, Neus Villamor, Magda Pinyol, Anna Enjuanes, Laura Magnano, Robert B. Russell, Elías Campo, Sílvia Beà, Armando López-Guillermo

In preparation

Perfil mutacional y alteraciones en el número de copias en pacientes con linfoma folicular y diferente comportamiento clínico

Nota

Hemos considerado pertinente añadir al conjunto de la tesis los resultados de este quinto estudio, todavía no publicado, pero que complementa los otros cuatro desde una vertiente más biológica. Así pues, presentamos los datos del presente artículo, que se encuentra en proceso de preparación.

Resumen

Los pacientes diagnosticados de linfoma folicular (LF) se caracterizan por un comportamiento clínico variable: algunos no requieren tratamiento o presentan respuestas duraderas, mientras que otros recaen de manera precoz o experimentan una transformación histológica. Aunque la información sobre la genómica de la enfermedad es extensa, poco se conoce sobre las alteraciones genéticas específicas de pacientes con un comportamiento clínico concreto. En nuestro estudio, seleccionamos 56 pacientes diagnosticados de LF grado 1-3A según su necesidad de tratamiento o duración de la respuesta: nunca tratados (7), no recaídos (19), recaídos tardíos (14), recaídos precoces o POD24 (11) y primariamente refractarios (5). Extrajimos DNA de 56 muestras de ganglio linfático al diagnóstico y de 12 a la recaída, y realizamos estudio de alteraciones en el número de copias y secuenciación masiva. Las regiones o genes más frecuentemente alterados fueron *KMT2D* (79%), *CREBBP* (67%), *TNFRSF14* (46%), *BCL2* (40%), y la pérdida de heterocigosidad de 1p36.33 (27%). Identificamos *hotspots* mutacionales conocidos y otros descritos por primera vez y establecimos una jerarquía cronológica para algunas alteraciones. *CARD11* y *JUNB* estaban más frecuentemente alterados en pacientes que nunca requirieron tratamiento, y *PIM1* se asoció con una duración de la respuesta más corta. Finalmente, llevamos a cabo un modelado proteico de ciertos genes (*CD79B*, *PLCG2*, *PIM1*, *MCL1*, and *IRF8*). Estos hallazgos profundizan en el conocimiento de la genómica que subyace a la heterogeneidad de pacientes con LF. Si se replican en cohortes amplias de pacientes, podrían contribuir a la estratificación pronóstica y al desarrollo de terapias dirigidas.

Mutational Profile and Copy Number Alterations of Follicular Lymphoma Patients with Different Clinical Behavior

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Abstract

Patients with follicular lymphoma (FL) exhibit a variable clinical behavior. While some of them do not require treatment or experience prolonged responses, others relapse early or undergo histological transformation. Although extensive data exist on the genomics of the disease, little is known about genetic alterations specific to patient groups with a particular clinical behavior. In our study, we carefully selected 56 grade 1-3A FL patients according to their need of treatment or timing of relapse: never treated (7), non-relapsed (19), late relapse (14), early relapse or POD24 (11), and primary refractory (5). We extracted DNA from 56 diagnostic and 12 relapse lymph node biopsies, and performed copy number alteration (CNA) analysis and next generation sequencing (NGS). The most frequently altered genes or regions were *KMT2D* (79%), *CREBBP* (67%), *TNFRSF14* (46%), *BCL2* (40%), and 1p36.33 copy-neutral loss of heterozygosity (27%). We identified known and novel mutation hotspots, and we established a chronological hierarchy for some alterations. *CARD11* and *JUNB* were more frequently altered in patients who never needed treatment, and *PIM1* alterations were associated with a shorter duration of response. Finally, we performed protein modeling of selected genes, including *CD79B*, *PLCG2*, *PIM1*, *MCL1*, and *IRF8*. These data expand the knowledge on the genomics behind the heterogeneous FL population. Upon replication in larger cohorts, they could contribute to risk stratification and the development of targeted therapies.

Introduction

Survival for patients diagnosed with follicular lymphoma (FL), the most common indolent B-cell lymphoma, is now prolonged[1]. However, the continuous pattern of relapses[2], early relapse[3], and histological transformation (HT)[4] remain challenges which compromise patients' life expectancy and quality of life.

FL is characterized by the genetic hallmark t(14;18)(q32;q21), involving the *BCL2* oncogene and the immunoglobulin heavy chain (IGH) locus. Deregulation of *BCL2* is an early but not sufficient event to drive FL lymphomagenesis[5]. Additional genetic abnormalities, such as somatic mutations in chromatin-modifying genes *KMT2D, CREBBP*, and *EZH2* are subsequently acquired, with a prominent role in the development, progression, relapse, and HT of FL[6–11]. Concerning copy number alterations (CNA), losses in 1p36, 6q, 10q, 13p, 17p, and gains in 1q, 2p, 7, 8, 12q, 18q, and trisomy X have been described and correlated with prognosis[12–14]. In an effort to identify higher-risk patients, prognostic indexes have been developed[15], including clinical[16], molecular[17,18], or both types of data[17]. Pastore and colleagues recently developed a prognostic score, the m7-FLIPI, integrating the mutational status of seven genes (*EHZ2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP*, and *CARD11*), along with the Follicular Lymphoma International Prognostic index (FLIPI), and Eastern Cooperative Oncology Group (ECOG) performance status. Nonetheless, the m7-FLIPI did not integrate CNA information, which has recently been used to risk-stratify patients independently of clinical parameters[19]. Additionally, the gene expression profile of 23 genes has also been shown to predict the outcomes of FL patients. To date, however, frontline treatment strategies are not tailored to the result of these scores.

Despite the existence of genomic data on FL, the underrepresentation of specific prognostic groups of patients in unselected cohorts hampers the identification of clinically relevant genetic alterations. Therefore, herein we investigated the genomic abnormalities, using targeted next generation sequencing (NGS) and copy number analysis, of a total of 56 FL patients categorized into five groups according to their clinical behavior.

Patients and Methods

Patients

We selected 56 grade 1-3A FL patients diagnosed at a single institution between 1997 and 2015 who met prespecified criteria concerning their need of treatment and timing of relapse (Supplementary Table 1). Patients with the following entities were not included in the study: grade 3B FL, primary gastrointestinal or cutaneous FL, composite lymphoma (FL/ diffuse large B-cell lymphoma −DLBCL−), or patients developing HT during follow-up. The never treated (NT) group was composed of seven patients who did not require treatment with a minimum follow-up of 5 years (range, 5.4−14.2 years). Nineteen patients were treated with immunochemotherapy (ICT), achieved a complete response, and did not relapse (NR) during at least 10 years of

follow-up (range, 11.5−17.8 years). The late relapse (LR) group was made up of 14 patients treated with ICT, who achieved a complete or partial response, and progressed or relapsed beyond two years after frontline treatment (range, 2.1−7 years). Eleven patients were treated with ICT, achieved a complete or partial response, and progressed or relapsed within two years of frontline treatment initiation (early relapse -ER- or POD24[3]), and five patients were primary refractory (PR) to frontline (immuno)chemotherapy. All patients had an available lymphoid tissue biopsy from the time of diagnosis. Additionally, six patients from the LR and six from the ER group had an available biopsy from the first relapse. The study was designed according to the Declaration of Helsinki and was approved by our Institutional Review Board.

We extracted DNA and RNA from 54 formalin-fixed, paraffin-embedded (FFPE) and two fresh frozen (FF) diagnostic samples, and from 11 FFPE and one FF relapse samples using the AllPrep DNA/RNA FFPE Kit (Qiagen, Germany), according to the manufacturer's instructions.

Clinical endpoints and statistical methods

Computed tomography, with or without positron emission tomography, and unilateral bone marrow biopsy were used for staging and response assessment purposes. Definitions of response to frontline treatment were the standard[20]. Progression-free survival (PFS) was calculated from the initiation of frontline treatment to relapse or death from any cause, only for treated patients. Overall survival (OS) was calculated from diagnosis to last follow-up or death from any cause. Quantitative variables were compared among groups by means of ANOVA or Kruskal-Wallis tests. Fisher's exact test was used to compare categorical variables. Statistical significance was defined as a *P* value <0.05.

Histological review

The diagnosis of grade 1-3A FL had been established at the time of consultation by experienced hematopathologists and underwent histological review upon inclusion in the study according to the 2017 World Health Organization classification[21]. Morphology, growth pattern, cytology, and immunohistochemical staining for CD20, CD79a, BCL6, BCL2, CD10, CD3, and Ki67 were evaluated in FFPE tissue sections.

BCL2 translocation

The *BCL2* rearrangement was assessed by polymerase chain reaction (PCR, especially when performed during the diagnostic workup), or by fluorescence *in situ* hybridization (FISH) using locus-specific identifier (LSI) *BCL2* dual color, break apart and/or dual color, dual fusion IGH-*BCL2* probes (Supplementary Table 2 and Supplementary Figure 1). Additionally, FISH studies were performed using LSI *BCL6* break apart and LSI *MYC* break apart probes in the 12 relapse samples and their respective diagnostic samples when breaks in *BCL6* or *MYC* were detected in relapse samples. All the FISH probes were provided by Abbott Molecular and/or MetaSystems. At least 100 nuclei were examined for each probe whenever possible. Digital

image acquisition, processing, and evaluation were performed using ISIS digital image analysis version 5.0 (MetaSystems).

Copy number analysis and next generation sequencing

CNA were analyzed in 56 diagnostic and 12 relapse samples using the Oncoscan CNV FFPE assay. Nexus version 9.0 Discovery Edition software (Biodiscovery, El Segundo, CA) was used for analysis and visualization of results. GISTIC algorithm (2.0.23) was used to identify driver CNA[22]. The cancer cell fraction (CCF) was calculated from the B-allele frequency (BAF) and corrected for the purity of the sample obtained using ASCAT (Supplementary Methods).

The mutational status of 121 genes recurrently altered in B-cell lymphoma was examined in 55 of 56 diagnostic and 10 of 12 relapse samples (NGS data for diagnostic sample FL027, and relapse samples FL027e and FL034e were not available) by means of a custom targeted NGS panel (Supplementary Table 3). Libraries were generated from 150 ng of DNA, using molecular-barcoded library adapters (ThruPLEX Tag-seq kit; Takara) coupled with a custom hybridization capture-based method (SureSelectXT Target Enrichment System Capture strategy, Agilent Technologies) and sequenced in a MiSeq instrument (Illumina, 2x150 bp). The bioinformatic analysis was performed using an updated version of our in-house pipeline, described in previous publications[23] (Supplementary Methods). The CCF of the single nucleotide variants (SNVs) and indels was calculated as described previously[24].

To perform an integrative genomic analysis, selected genes and genomic regions were considered altered if they harbored single nucleotide variants (SNVs)/indels (small insertions and deletions), and/or CNA (except copy-neutral losses of heterozygosity −CN-LOH−, which were not integrated with mutational data). Moreover, the chronological hierarchy of the genomic aberrations was calculated from the clonality of each aberration as described previously[24,25].

U1 mutations

We investigated the g.3A>C mutations in U1 using a custom rhAMP SNP assay (Integrated DNA Technology)[26]. The assay was run in technical duplicates on a StepOnePlus instrument (Applied Biosystems). Primer sequences are depicted in Supplementary Table 4.

Protein modeling

Selected variants were subjected to the Mechismo[27] system, which identifies putative structures or interaction interfaces affected by the corresponding protein changes. Where possible, we considered available 3D structures[28] for the precise protein; otherwise, we constructed models using Modeler[29]. We assessed sequence conservation across orthologues using BLAST[30] searches against sequences for the closest matching orthologous group in the vertebrate clade of the EggNOG database[31]. For conservation across paralogues, we considered searches against human sequences from Uniprot Sprot[32]. We predicted unstructured (disordered) protein

regions using IUPred[33]. Domain figures were constructed using the Domain Diagram generator from Pfam[34], with domains assigned according to a consensus of Pfam-, Uniprot- or 3D structure-based assignments. Sequence alignments were performed using MUSCLE[35] and structural alignments and superimpositions by STAMP[36]. Gene enrichment was done using the GetGo systems[37].

Results

Baseline features

Baseline and follow-up data of the patients can be found in Supplementary Table 5. Thirty-one patients were female and 25 were male, and median age was 57 years (range, 26−79). Seventy-five percent of patients (42/56) had advanced-stage disease, and 25% had a high-risk FLIPI score. Histological grade was 1 or 2 in 78% of the patients. We detected *BCL2* rearrangements in 87% (41/47) of the cases. Among the 48 patients who received treatment during follow-up, 82% were treated with R-CHOP, and the complete response rate was 83%. With a median follow-up of 12.9 years, 10-year OS was estimated at 79% (95% CI, 69−91). Since patients were specifically selected according to their clinical behavior, OS was markedly different among groups.

Copy number profile

We analyzed CNA using OncoScan arrays in 56 diagnostic (D) and 11 relapse (R) samples, and obtained results in 64 samples (53 D and 11 R). CNA were detected in 97% (62/64) of the samples, with a median of 5 CNA (range 0−26) for D samples, and of 5 (range 2−14) for R samples. Considering only samples at D, we identified a total of 324 alterations, including 154 gains, 134 losses, 18 high copy gains, and 18 homozygous deletions (Figure 1). In addition, a total of 89 copy-neutral losses of heterozygosity (CN-LOH) were detected.

The most recurrently gained regions (≥3 cases) were 1q, 2p16, 12q13-q15, 13q31-q32, 17q22.-q24, 18p11-q21, as well as trisomy 2, 7, 8, 12, 18 and X. Recurrent losses were observed in 1p36, 6p21, 6q14, 6q23, 9p21, 10q23, 13q14, and 22q13. In addition, recurrent CN-LOH were detected in 1p36, 6p25-p21, 12q13, and 16p13. Using GISTIC we identified 5 driver CNA (q-value <0.05) corresponding to losses on 1p36.32 (harboring *TNFRSF14*), 6p21.32 (*HLA*), 6q14.1 (*TMEM30A*), 6q23.3 (*TNFAIP3*), 9p21.3 (*CDKN2A/B*), and 10q23.33 (*PTEN*) (Supplementary Table 6). We identified a chromothripsis-like pattern in four patients at diagnosis (3/53, 6%) involving chromosomes 2, 6 and 12. We also detected this finding in one patient who acquired the chromothripsis-like pattern upon relapse, affecting chromosome 1.

In addition to determining the *BCL2* rearrangement by FISH and/or PCR, we mined the CN data looking for the der(18)t(14;18). We identified gains of 18p11.32-q21.33, suggesting a gain of der(18)t(14;18) in 8/53 (15%) samples. Mapping the breakpoints, we identified four cases

harboring the breakpoint within *BCL2*, three cases downstream, and one case upstream of *BCL2*.

Targeted sequencing and integrative analysis

A 121-gene custom targeted sequencing panel was used to analyze 65 FL samples (55 D and 10 R) at a median coverage of 220x (range 12.8x−1417x, Supplementary Table 7), and 98% of the targeted regions at a median coverage of at least 100 reads. We aimed for a minimum coverage of 50x per patient, which led to the exclusion of FL052 from the analysis. Overall, the median number of SNV/indels per case was 13 (range 1−32) for diagnostic samples (n=54), and 17 (range 9−22) for relapse samples (n=10). We then integrated the CNA, SNVs, and indels, and identified that the genes or regions with an alteration rate greater than 20% at diagnosis were *KMT2D* (79%), *CREBBP* (67%), *TNFRSF14* (46%), *BCL2* (40%), 1p36.33 CN-LOH (27%), *ARID1A* (25%), *TNFAIP3* (23%), *EP300* (21%), trisomy 7 (21%), 1q gain (21%), and 16p13.3-16p13.2 CN-LOH (21%) (Supplementary Table 8 and Figure 2). We identified hotspot mutations in epigenetic modifier genes (*MEF2B, EZH2, CREBBP* and *BCL7A*)[8,17,38,39], genes related to signaling and B-cell differentiation (*STAT6* and *POU2F2*)[40,41], and the BCR/NF-κB pathway (*RRAGC*).[42].

To gain a deeper understanding on the evolutionary history of FL, we examined the temporal order of genomic alterations in diagnostic samples of FL. We found that the majority of early mutations corresponded to genes related to epigenetic/transcription and apoptosis/proliferation, such as *KMT2D*, *EP300*, *CREBBP, HIST1H1E*, *BCL7A,* and *TNFRSF14* (Supplementary Table 9). In addition, 16p13.3-p13.2 CN-LOH was also identified as an early event. In contrast, late aberrations involved genes related to the two abovementioned pathways, such as *BCL2*, together with genes related to ubiquitination and signaling like *SOCS1*, *CARD11*, *CD79B*, and *PIM1.* Moreover, chromosomal gains in 1q, 2p16.1 (*REL*) and 18p11.32-q21.33, trisomy 7, and trisomy X were also acquired late in the FL evolution.

We then explored co-occurrence and mutual exclusivity of genetic alterations within the 52 diagnostic samples which had available NGS and CNA information (Figure 3). Aberrations such as *FAS* with *PTEN*, and 12q13.12-q13.13 CN-LOH with 12q21.33-q24.33 CN-LOH co-occurred significantly (Q <0.05). We also identified a significant co-occurrence between *PIM1*, *CD79B,* and *BTG2* (Q <0.1), especially in cases that relapsed early (Supplementary Figure 2). Moreover, we detected a significant co-occurrence of other several alterations (Q <0.2), including *TNFAIP3* and 1q gain, 1q gain and 12q21.33-q24.33 CN-LOH, 6p25.3-p21.33 CN-LOH and *MEF2B*, *HIST1HD* and *EZH2*, and 12q21.33-q24.33 and *CD79B*. We investigated the aberrations affecting the genomic region 6q, and identified co-occurrence between *PRDM1* and *SGK1* (Q <0.05), as well as between *SGK1* and *TNFAIP3*, *TNFAIP3* and *TMEM30A*, and *TMEM30A* and *PRDM1* (Q <0.2) (Supplementary Figure 3). Combining SNVs, indels and CNA, we identified that all the genes located in the 6q region (which were typically deleted) were also

altered by SNVs, with the exception of *TMEM30A*. Interestingly, we identified biallelic inactivation of *TNFAIP3* in three cases, and of the *SGK1* locus in one case.

Alterations according to the clinical group, need of treatment and timing of relapse

To elucidate the role of genomic aberrations in FL, we analyzed the prevalence and distribution of altered genes according to the clinical group. Although no significant differences were found in the number of SNV/indels or CNA among the five groups, we found that *CARD11* was more frequently altered in patients who never required treatment (NT) compared to those from the other four groups (43 vs. 9%, *P* = 0.043), as was *JUNB* (29 vs. 0%, *P*=0.016). All the genomic variants identified in *CARD11* where located in the coiled-coil protein domain, essential for the interaction of this cytoplasmic scaffolding protein relevant for NF-κB activation[43] with the paracaspase domain of MALT1[44].

PIM1 mutations were more frequently found in ER/PR cases (n=5, 33%) than in in NT/NR/LR cases (n=1, 3%, *P*=0.006). When only the 48 treated patients were considered, *FOXO1* mutations were associated with a decreased PFS [HR 5.5 (95% CI 1.6−19.4), *P*=0.008]. Univariable Cox regression showed a negative impact on OS for *FOXO1* mutations [HR 4.9 (95% CI 1.1−22.8), *P*=0.042], as well as a trend towards poorer OS for the 8 cases with *TMEM30A* alteration [HR 2.9 (95% CI 0.9−9.5), *P*=0.076]. Our dataset was not powered to perform multivariable analyses, due to the number of cases and heterogeneity of baseline features.

Clonal evolution: analysis of paired samples

To investigate the clonal evolution of FL, we studied the presence of genetic alterations in the 11 patients with paired samples between diagnosis and relapse, using NGS and/or CNA analysis. No significant differences were found in the number of SNV/indels or CNA, or in the alteration rate of specific genes or regions between diagnosis and relapse. We detected a total of 146 shared aberrations. Relapse samples harbored a median of 70% of shared aberrations. All sample pairs were characterized by the presence of an ancestral common precursor cell (CPC), pointing towards a clonal relationship between the initial and the relapse FL clones (Figure 4).

Considering all paired samples analyzed by NGS and CNA (n=9), apart from the shared alterations, we detected some other ones that were unique either to the diagnosis or the relapse, indicating a divergent evolution. We also investigated the presence in the CPC of early alterations identified in our study. In all the paired samples the CPC harbored early oncogenic alterations including *KMT2D* (7/9), 16p13.3-p13.2 CN-LOH (5/7), *CREBBP* (5/7), *TNFRSF14* (4/9), and *EP300* (3/9) (Supplementary Figure 4). Moreover, when we analyzed samples according to the timing of relapse, we identified that the late relapse (LR) samples were characterized by the presence of *IRF8* mutations in the CPC, which were not detected in the

CPC of early relapse (ER) samples. We also found that the ER group had a percentage of shared aberrations, on average, 13.61 points higher than the LR group (IC 95%: 0.14−27.07, *P*=0.048) (Supplementary Figure 5).

We assessed the presence of translocations affecting *MYC* and *BCL6* using FISH in relapse samples from 10 patients. *BCL6* rearrangements were identified in two relapse samples (FL029 and FL047). In patient FL029, the *BCL6* translocation was already present at diagnosis (FISH for the diagnostic sample of patient FL047 was not available). In contrast, *MYC* rearrangement was acquired upon relapse in one patient (FL051).

U1 mutations

We explored the presence of somatic mutations in the third base (g.3A>C) of the U1 small nuclear RNAs (snRNAs) in 51 diagnostic and 11 relapse samples. We identified an acquired g.3A>C mutation only in the relapse sample of patient FL034 (Supplementary Table 2).

Protein modeling of selected mutations

We modeled proteins encoded by various genes relevant to B-cell biology. Concerning CD79B, a protein responsible for mediating immune signals, three different variants were detected, p.Ile54Arg in 4 cases, and p.As118Thr and p.Ala206fs in one case each. The frameshift variant p.Ala206fs lies in immune receptor tyrosine-based activation motifs (ITAM) and is phosphorylated by SRC-family kinases, including LYN, FYN and BLK[45]. This deletion will likely ablate this interaction, thus leading to a receptor that is no longer signaling normally. On the other hand, the two missense variants, p.Ile54Arg and p.As118Thr, are likely to disrupt the structure of the extracellular domain that is responsible for binding to CD79A and thus the formation of the signaling complex.

We identified three missense variants in *PLCG2*, a gene encoding a protein that is crucial for antigen-stimulated BCR signaling through BTK activation. Variants involved the EF-hand region (p.Leu163Phe and p.Pro236Leu), and C2 domain (p.Thr1152Pro) (Figure 5A).

Regarding cytokine signaling, *PIM1* is a proto-oncogene encoding a serine/threonine kinase that has been implicated in many cancers, by promoting cell survival through transcriptional activation of genes involved in cell proliferation[46]. We identified a total of three different missense variants located in the kinase catalytic domain (p.Thr114Ile in four cases, p.Ser188Asn and p.Gln218Glu in one case each), suggesting an activating effect of the PIM1 protein.

On the other hand, MCL1 is a member of the Bcl-2 family of proteins involved in the regulation of apoptosis. Interestingly, both variants identified in our study, p.Glu110Gly (three cases) and p.Leu160Ile (one case), lie in the PEST region and are close to the phosphorylation sites

involved in the stability of the protein[47] (Supplementary Figure 6). This argues that both positions likely disrupt the local structure.

Finally, we detected a total of six mutations in *IRF8*, corresponding to four frameshift and two missense variants. The frameshift variants were clustered on the C-terminal region of the IRF8 protein, a region known to be responsible for binding to the SPRY domain of TRIM21[48] (Figure 5C). In contrast, missense variants were located in the SMAD domain. Frameshift in the C-terminal region have been seen previously[49], and in our study were identified in the LR group. The frameshifts at this specific position argue an importance for the loss of the IRF4-TRIM21 interaction. Regarding the two SNVs in *IRF8*, the p.Val287Met and p.Glu335Lys, disturbed the IRF4 protein affecting the structure conformation and disrupting the charge balance, respectively.

Discussion

Patients diagnosed with FL, the most common indolent B-cell lymphoma, usually have prolonged survival. However, the population is highly heterogeneous, with some patients exhibiting aggressive and chemotherapy-resistant behavior or histological transformation, and others achieving durable remissions after treatment. The ability to stratify FL patients according to their risk at diagnosis is becoming increasingly important for predicting their outcome and selecting the most appropriate therapy. Here, we assessed the genomic alterations (gene mutations, CNA, and translocations) of a series of FL patients selected according to their clinical behavior, specifically, the need of treatment and duration of response to frontline therapy.

The copy number profile that we identified was typical for FL, including gains in 1q, 2p16.1 (*BCL11A*), 12q13.13-q15, 13q31.3-q32.3 (*MIR17HG*), and 18p11.32-q21.33, trisomies of chromosomes 2, 7, 8, 12, 18, and X as well as losses in 1p36.32 (*TNFRSF14*), 6q14.1 (*TMEM30A*), 6q23.3 (*TNFAIP3*), 9p21.3 (*CDKN2A/B*), 10q23 (*PTEN*), and 13q14.2-q14.3 (*DLEU1/2*). Although recent studies based on arrays described that an increased genomic complexity was associated with poor outcome in FL[19], we could not demonstrate a significantly different copy number profile or genomic complexity according to the clinical behavior. Nonetheless, we determined 5 driver CNA affecting genomic regions relevant to the pathogenesis of FL, namely 1p36.32 (*TNFRSF14*), 6p21.32 (*HLA*), 6q14.1 (*TMEM30A*), 6q23.3 (*TNFAIP3*), 9p21.3 (*CDKN2A/B*), and 10q23.33 (*PTEN*).

In line with previous studies[50], we observed that the genetic landscape of FL is characterized by alterations in epigenetic modifiers (*KMT2D*, *EP300*, *CREBBP*, *EZH2*, *ARID1A*), proliferation and apoptosis (*BCL2, TNFRSF14*), and BCR signaling (*TNFAIP3*). We have determined that recurrent alterations in epigenetic modifiers such *KMT2D, EP300, CREBBP, HIST1HE, BCL7A*, and apoptosis (*TNFRSF14)*, are early events in the evolution of the disease, in contrast to alterations in ubiquitination and signaling (*SOCS1*, *CARD11*, *CD79B*, and *PIM1*), which emerge

later. We thus corroborated the findings by previous studies reporting that somatic mutations in *CREBBP* and *KMT2D* are enriched in the common precursor cell (CPC) and are early driver events[9,51,52]. Although alterations in epigenetic modifiers are early and very prevalent events in FL, they are not sufficient to give rise to a lymphoma[53–55]. Interestingly, in the present study we detected the co-occurrence of relevant alterations like *PIM1*, *CD79B* and *BTG2*. This putative cooperation might be relevant, since they involve different pathways [JAK-STAT signaling (*PIM1*), BCR (*CD79B*), and proliferation (*BTG2*)] and were more prevalent in cases experiencing an early relapse, indicating a possible role in the aggressive clinical behavior of those patients.

Despite the limited number of cases included in our study, we found that some genetic alterations were associated with different clinical patterns. Indeed, *CARD11* and *JUNB* alterations were more frequent in patients who never required treatment, and cases with a short duration of response were enriched in *PIM1* mutations. Furthermore, *FOXO1* and *TMEM30A* deletions were associated with decreased OS. These findings are in line with observations in other settings[56–58] and, though preliminary, pave the way for further exploration in larger cohorts of patients.

Phylogenetic studies of paired diagnostic/relapse samples showed distinct patterns, including linear/sequential, divergent, and complex evolution[7]. Despite the low number of cases with paired samples in our study, we have identified the presence of an ancestral CPC, in addition to genomic alterations that were specific to diagnosis or relapse, suggesting a divergent evolution in all patients. Moreover, we revealed that the cases relapsing early harbored a higher number of shared aberrations than those with a late relapse, corroborating the findings reported by Kridel and colleagues[6] describing that early relapses are caused by clones already detected at diagnosis, with only slightly clonal dynamics.

Although NGS technologies have increased the knowledge about the genetic landscape of FL, non-coding mutations remain poorly understood. Recurrent variants in noncoding splicing factors, such as *SF3B1* and *SRSF2* have been previously described across different types of cancers[59]. Mutations in the small nuclear RNA U1, specifically at the third base (g.3A<C), have been recently reported in solid tumors and CLL[26,60]. We did not find this mutation in any sample at diagnosis, and only in one sample at relapse, suggesting a possible role in the progression of the disease.

Selected genes playing a relevant role in immune biology, like *IRF8, PLCG2, CD79B, PIM1* and *MCL1,* were more thoroughly investigated in order to understand the molecular consequences of the genomic aberrations identified. The *PIM1* variants detected in our study clustered in the kinase domain, as described by other authors[61]. Kuo and colleagues described that mutations in *PIM1* seem to stabilize the protein and enhance NF-κB signaling, as well as being associated

with intrinsic resistance to ibrutinib[61]. Taken together, it seems reasonable to further investigate *PIM1* mutations in FL.

CD79B mutations have been previously reported in FL, although their prevalence is lower than in other germinal center-derived B-cell lymphomas, mainly DLBCL[62]. The variants herein identified affect the ITAM domain of the CD79B protein abolishing its normal signaling function, thus preventing the interaction with the SRC kinase family. This is in line with previous studies in ABC-DLBCL, which described that *CD79B* mutations decrease the negative autoregulation by the specific SRC tyrosine kinase LYN, during chronic active BCR signaling, and increase the expression of the BCR[63].

The Bruton tyrosine kinase (BTK) is essential for antigen-stimulated BCR signaling through the activation of PLCG2[64]. An activating mutation (p.Met1141Lys) within the PLCG2 C-terminal C2-domain and other gain-of-function mutations have been previously described[65]. Although the prevalence of *PLCG2* mutations in our study is only 6%, we have predicted that the molecular consequence of these variants is a gain of function leading to an activated PLCG2 protein, and suggesting a potential role in the response to ibrutinib, similar to that previously described in CLL[66,67].

More controversial is the impact of the mutations coding for the member of the BCL2 antiapoptotic family, MCL1. Two isoforms have been described, isoform 1 (longer, which inhibits apoptosis), and isoform 2 (shorter, which promotes it). The variants determined here clustered in the PEST region and likely disrupt the local structure of the protein. Interestingly, it seems that these changes could go either way in terms of promoting or suppressing apoptosis, depending on which isoform is expressed. Nevertheless, previous studies show that MCL1 is highly expressed in DLBCL, due to *MCL1* gain/amplification and constitutive activation of the STAT3 pathway[68].

In the protein modeling of *IRF8* mutations, we identified a cluster of variants lying in the C-terminal region, which were present in the CPC of cases with a late relapse. This protein region showed a significant predicted match to the surface of the TRIM21 SPRY domain[69] using PepSite. TRIM21 is a ubiquitin-ligase and it could be suggested that the loss of this IRF8 interaction could lead to a concomitant loss of ubiquitin-mediated degradation. However, the bulk of evidence argues that ubiquitination is activating for IRF8 function[70] meaning that the loss of this C-terminus would lead to a lowering of IRF8 activity.

In conclusion, in this comprehensive genetic analysis of samples from FL patients with distinct clinical behavior, we confirmed the frequency of CNA and mutations that had been previously described, and identified five CNA as drivers. Moreover, we established the temporal order of recurrent alterations in the FL pathogenesis, and observed co-occurrence among some of them.

Mutations of *CARD11* and *JUNB* were associated with a delayed need of treatment, while *PIM1* predicted a shorter response. Finally, we performed a thorough characterization of the functional impact of genes central to B-cell biology. These data expand the knowledge on FL pathogenesis, although larger studies are required to corroborate our findings.

Acknowledgments

This study was supported by Instituto de Salud Carlos III: PI17/01061 (SB), PI19/00925 (LM), PI19/00887 (ALG and EG). Fundación AECC/CIBER: PROYE18020BEA (SB). FEHH: “Becas de Investigación 2018” (PM). Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR): CL. CL is also supported by postdoctoral Beatriu de Pinós from Secretaria d’Universitats I Recerca del Departament d’Empresa i Coneixement de la Generalitat de Catalunya and by Marie Sklodowska-Curie COFUND program from H2020 (2018-BP-00055).

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FIGURES

Figure 1. Copy number alterations (CNA) of the 53 FL patients with data at diagnosis.

A. Copy number gains and losses.

B. Copy-neutral losses of heterozygosity (CN-LOH). Each probe is aligned from chromosome 1 to X and p to q (chromosome Y was excluded). Gains are depicted in blue and losses are depicted in red. Altered genomic regions and genes relevant for FL pathogenesis are indicated. Driver CNA as per GISTIC are depicted in bold.

CNA, copy number alteration; CN-LOH, copy-neutral loss of heterozygosity; FL, follicular lymphoma.

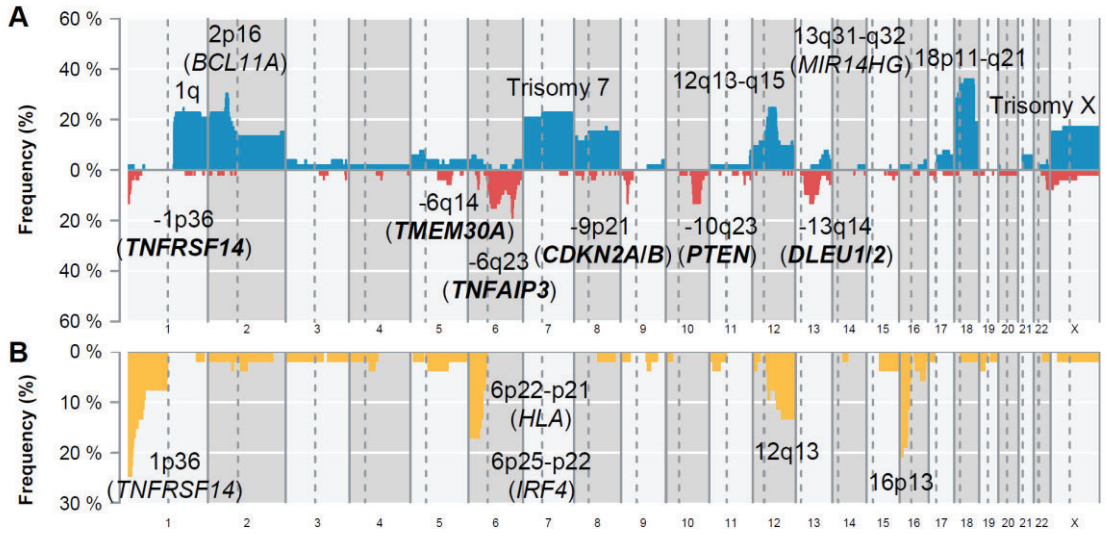


Figure 2. Oncoprint representing single nucleotide variants (SNVs), insertions/deletions (indels) and copy number alterations (CNAs) involving specific genes (upper block), CNAs and copy neutral loss of heterozigosity (CN-LOH) (middle block) and baseline features (lower block) of the 52 diagnostic samples from the five different clinical groups, analyzed using next generation sequencing and copy number analysis. Altered genes and genomic regions are ordered by frequency. Cases are annotated with sex, *BCL2* rearrangement (*BCL2* rearr.), histological grade, Follicular Lymphoma International Prognostic Index (FLIPI), and treatment.

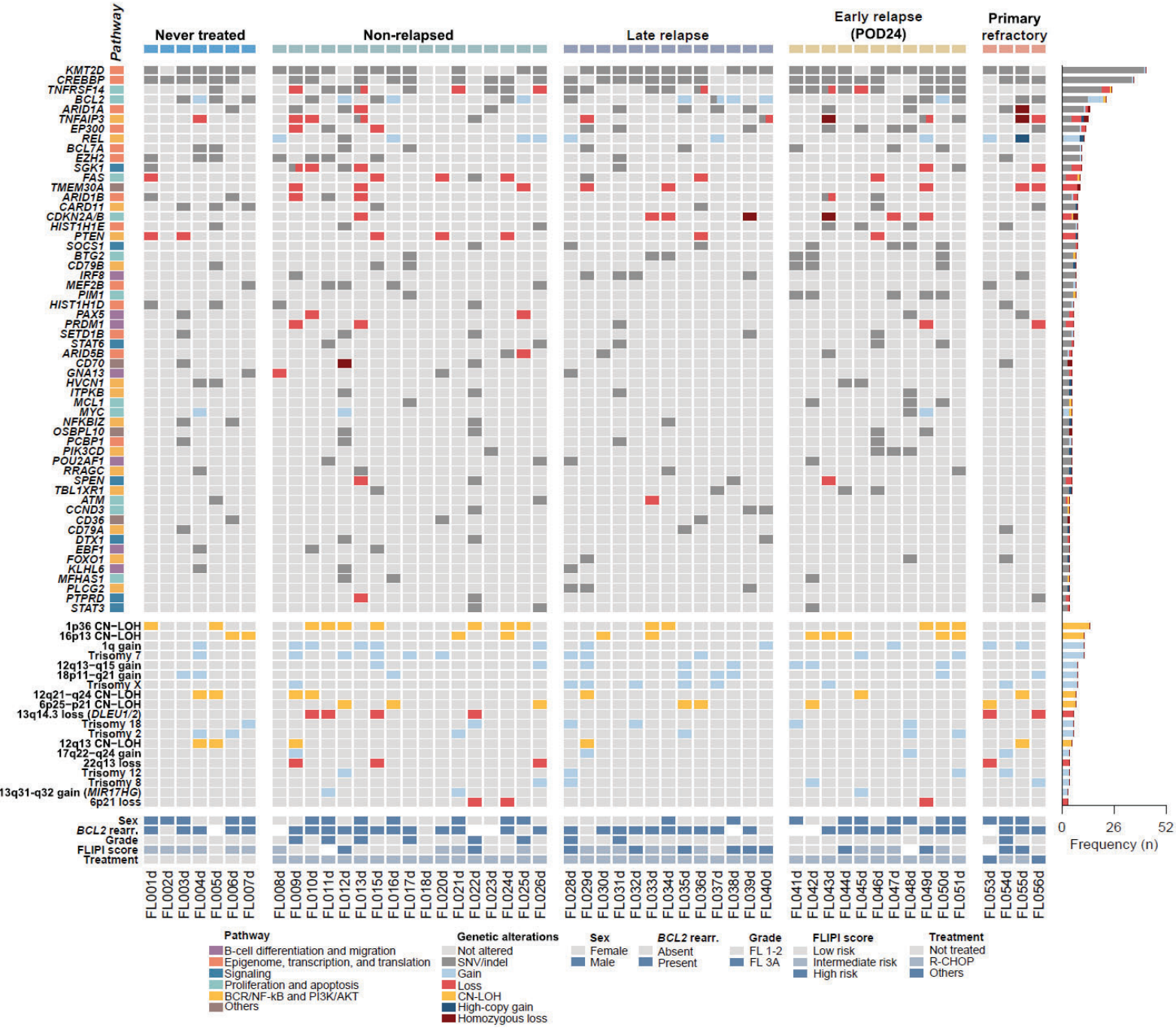


Figure 3. **Mutually exclusive and co-occurring genomic aberrations.** The color range indicates the odds ratio of the genomic aberration distribution from blue, depicting co-occurrence, to red, showing mutual exclusivity. The asterisks illustrate the adjusted *P*-values (*Q*).

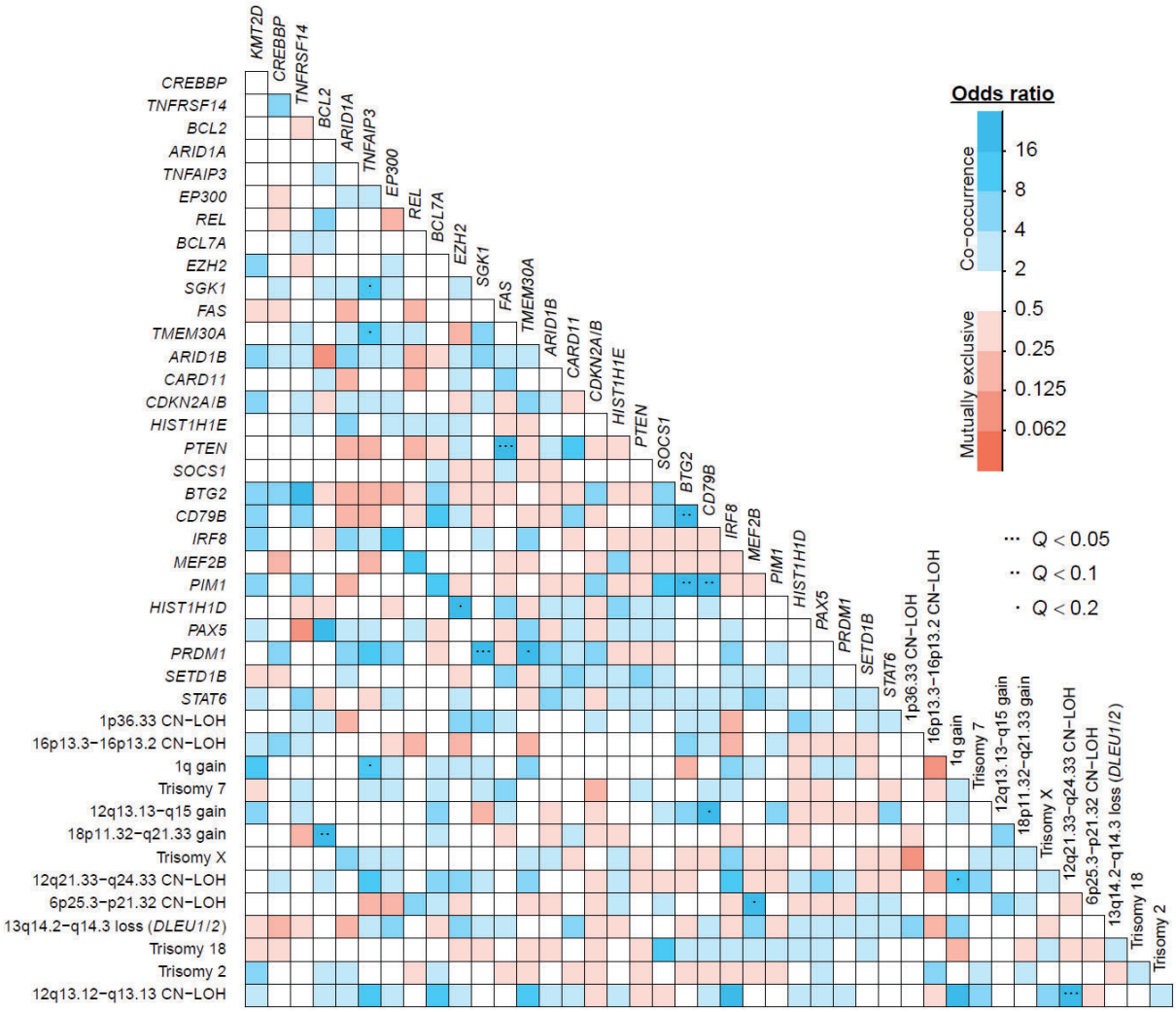


Figure 4. Total number of shared and unique genomic aberrations identified in FL patients with paired diagnostic/relapse samples, using NGS and CNA analysis.

A. Graph showing the overall shared aberrations in each case, including single nucleotide variants (SNVs), indels and copy number aberrations (CNAs).

B. Unique genomic aberrations identified at diagnosis and relapse in each case. Note that cases FL027 and FL028 lack NGS and CNA data, respectively, and thus information from those techniques is not displayed.

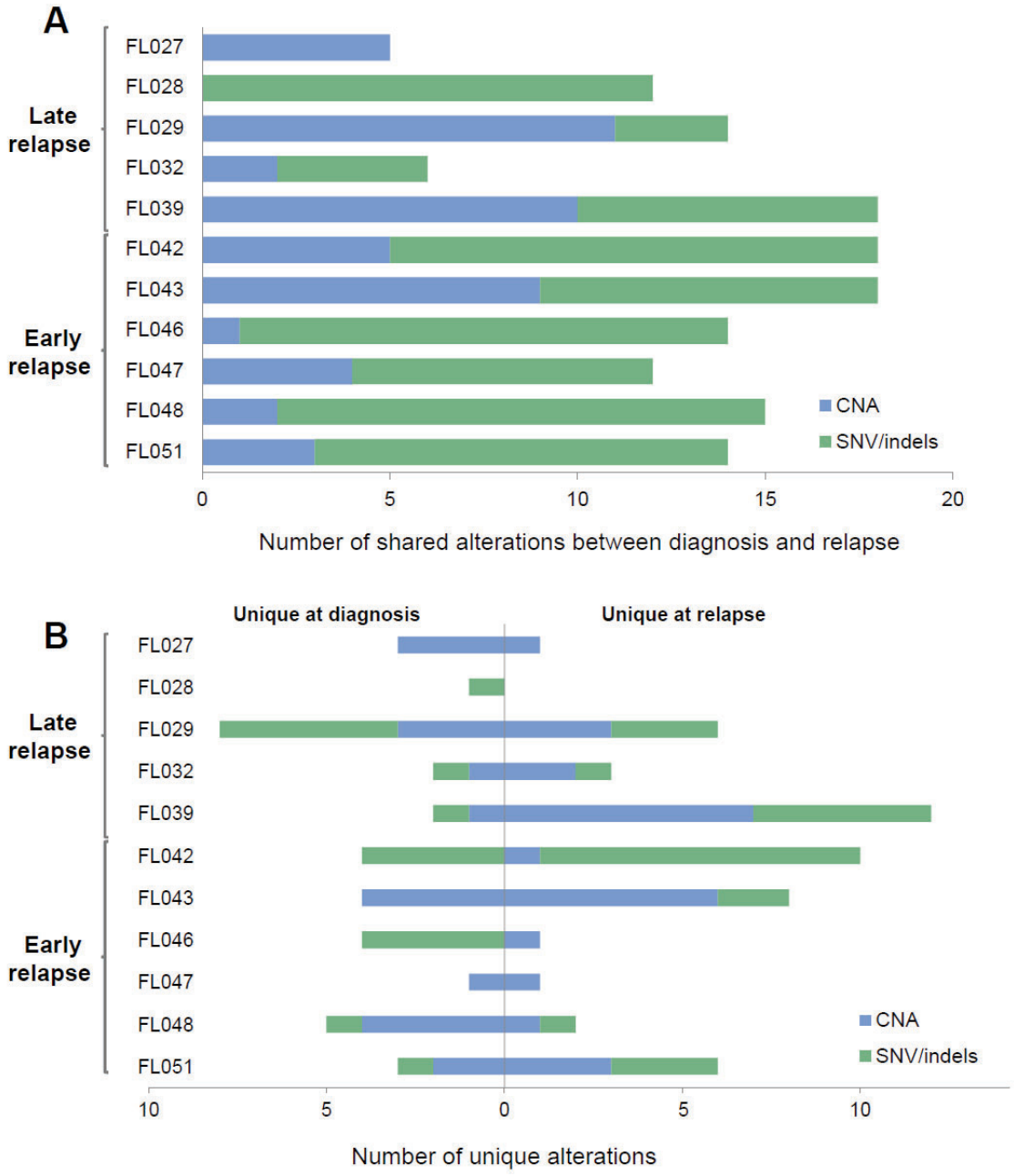
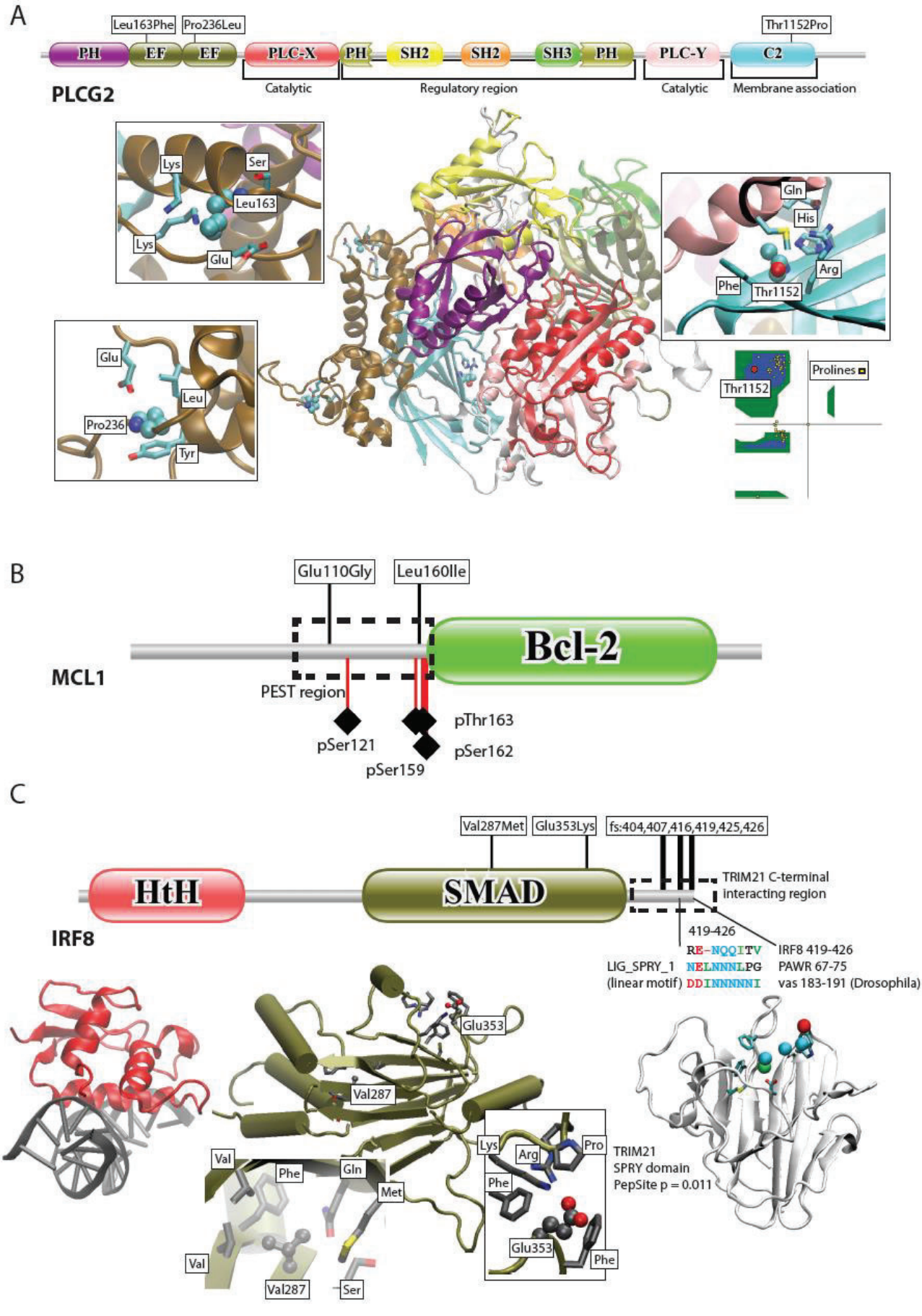


Figure 5. Protein modeling.

A. Domain schema (top) of human PLCG2 showing the location of variants uncovered. The complete structure of PLCG2 (modelled based on the PLCG1 structure PDB:6pbc) also with variants labeled. The inset structures zoom in on the location of specific mutated amino acids (labeled with numbers) and their interacting residues (labeled without numbers). The figure bottom right is a Psi/Phi (Ramachandran) plot showing how the Thr1152 backbone conformation (red circle) compares to those of other prolines in the structure (yellow squares).

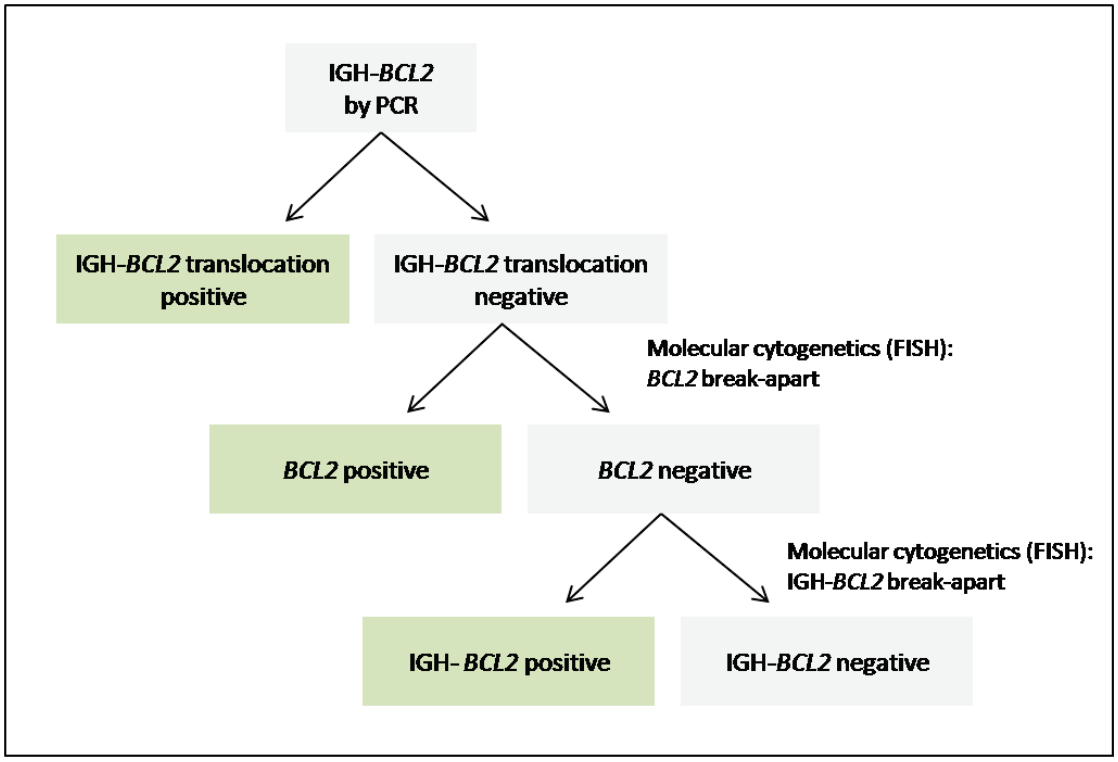
B. Domain schema (top) of human MCL1 showing the location of variants uncovered. The schema also shows the location of phosphosites below the domains.

C. Domain schema (top) of human IRF8 above structures showing the location of variants uncovered. The structure below left is the N-terminal helix-turn-helix (HtH) domain bound to DNA (modeled on mouse IRF1 PDB:1if1 selected to view the bound DNA). The center structure shows the structure of the SMAD domain (modelled using human IRF4 PDB:5bvi) and the location of Val287, which sits in a beta-strand region (which is less favored by Met) making mostly hydrophobic contacts, and Glu353, which sits in a pocket close to two positive amino acids (Lys/Arg) making a substitution to Lys unfavorable. The right shows how the C-terminal 8 amino acids (either containing or near to the six observed frameshifts, fs) are similar to two other peptides known to bind SPRY domains (blue = Asn/Gln; red = Asp/Glu, green = hydrophobic) according to the ELM database. The structure below is the TRIM21 SPRY domain (PDB:2iwg) including the prediction from PepSite[71] where the spheres show the predicted location of each amino acid.

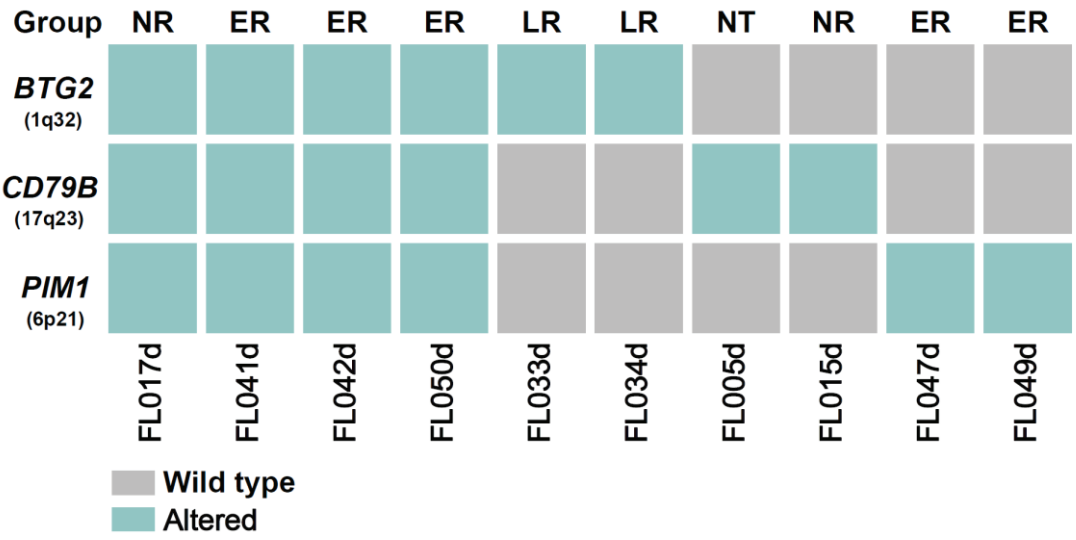

A
Leu163Phe
Pro236Leu
Thr1152Pro
PH
EF
EF
PLC-X
PH
SH2
SH2
SH3
PH
PLC-Y
C2
Catalytic
Regulatory region
Catalytic
Membrane association
PLCG2
Lys
Ser
Leu163
Lys
Glu
Glu
Leu
Pro236
Tyr
Gln
His
Arg
Phe
Thr1152
Thr1152
Prolines
B
Glu110Gly
Leu160Ile
Bcl-2
MCL1
PEST region
pSer121
pSer159
pThr163
pSer162
C
Val287Met
Glu353Lys
fs:404,407,416,419,425,426
HtH
SMAD
TRIM21 C-terminal interacting region
IRF8
419-426
RE-NQQITV
IRF8 419-426
LIG_SPRY_1
NELNNNLPG
PAWR 67-75
(linear motif)
DDINNNNNI
vas 183-191 (Drosophila)
Glu353
Val287
Val
Phe
Gln
Met
Val
Val287
Ser
Lys
Arg
Pro
Phe
Glu353
Phe
TRIM21
SPRY domain
PepSite p = 0.011

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Workflow of *BCL2* rearrangement analysis.



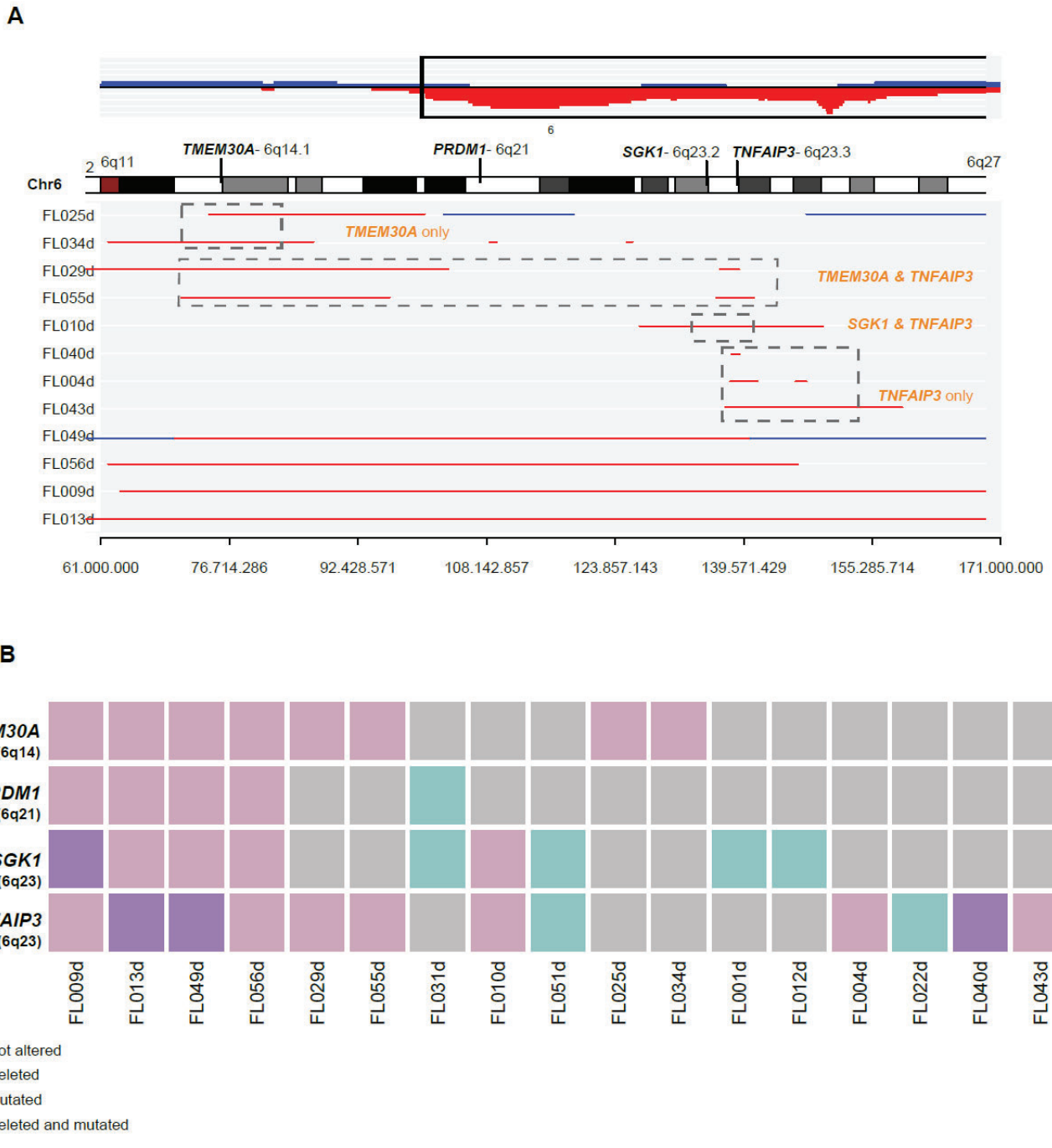
Supplementary Figure 2. Co-occurrence of genomic aberrations in *BTG2*, *CD79B* and *PIM1*. NT, never treated; NR, non-relapsed; LR, late relapse; ER, early relapse.



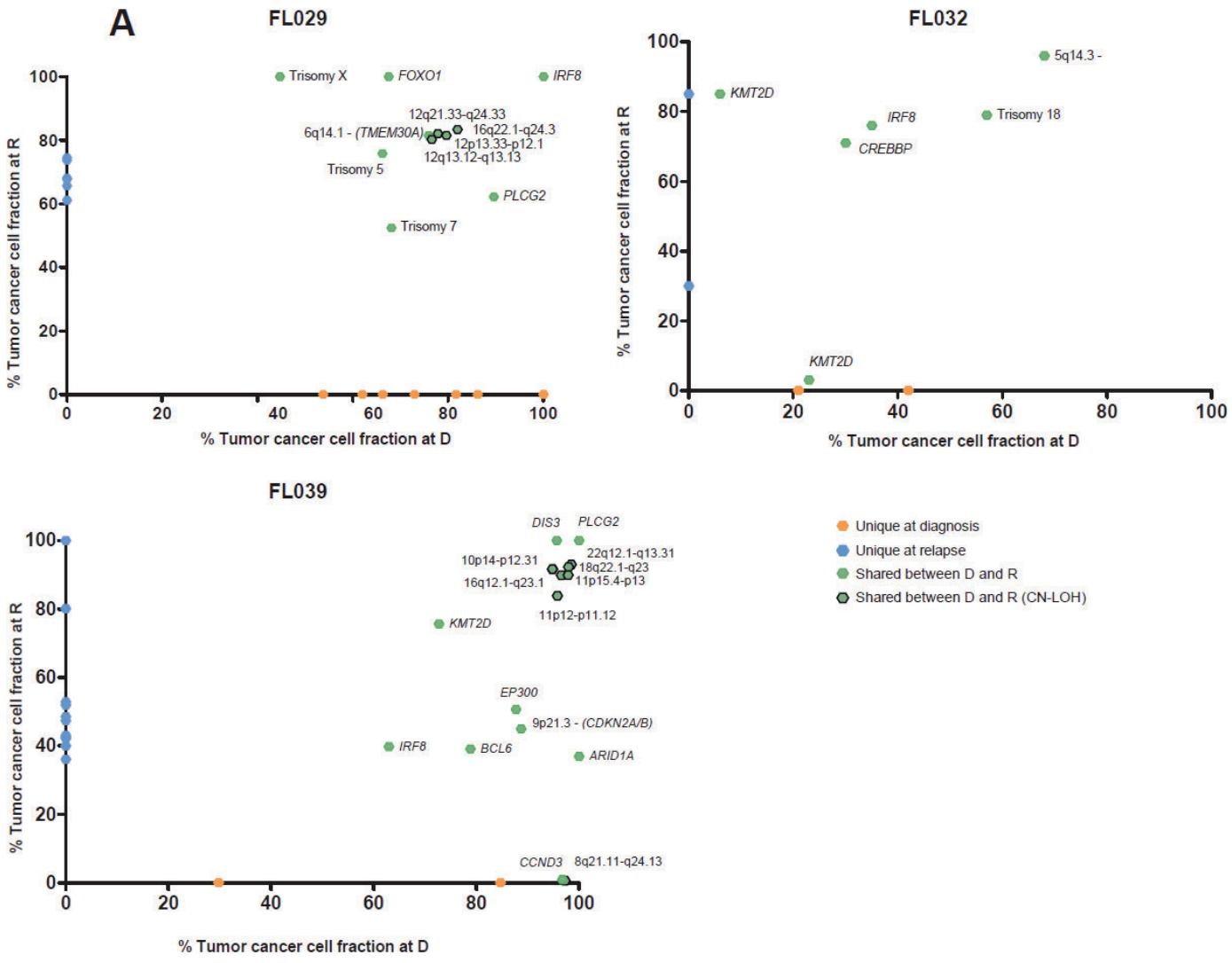
Supplementary Figure 3. Genomic alterations of *TMEM30A*, *PRDM1*, *SGK1*, and *TNFAIP3*.

A. Display of the copy number aberrations detected in the 6q genomic region. Each line is a case and the color indicates the CNA, gains in blue and losses in red, respectively. The genomic location of the tumor suppressor genes in 6q region is highlighted, as well as the co-deletion pattern observed in the FL cohort.

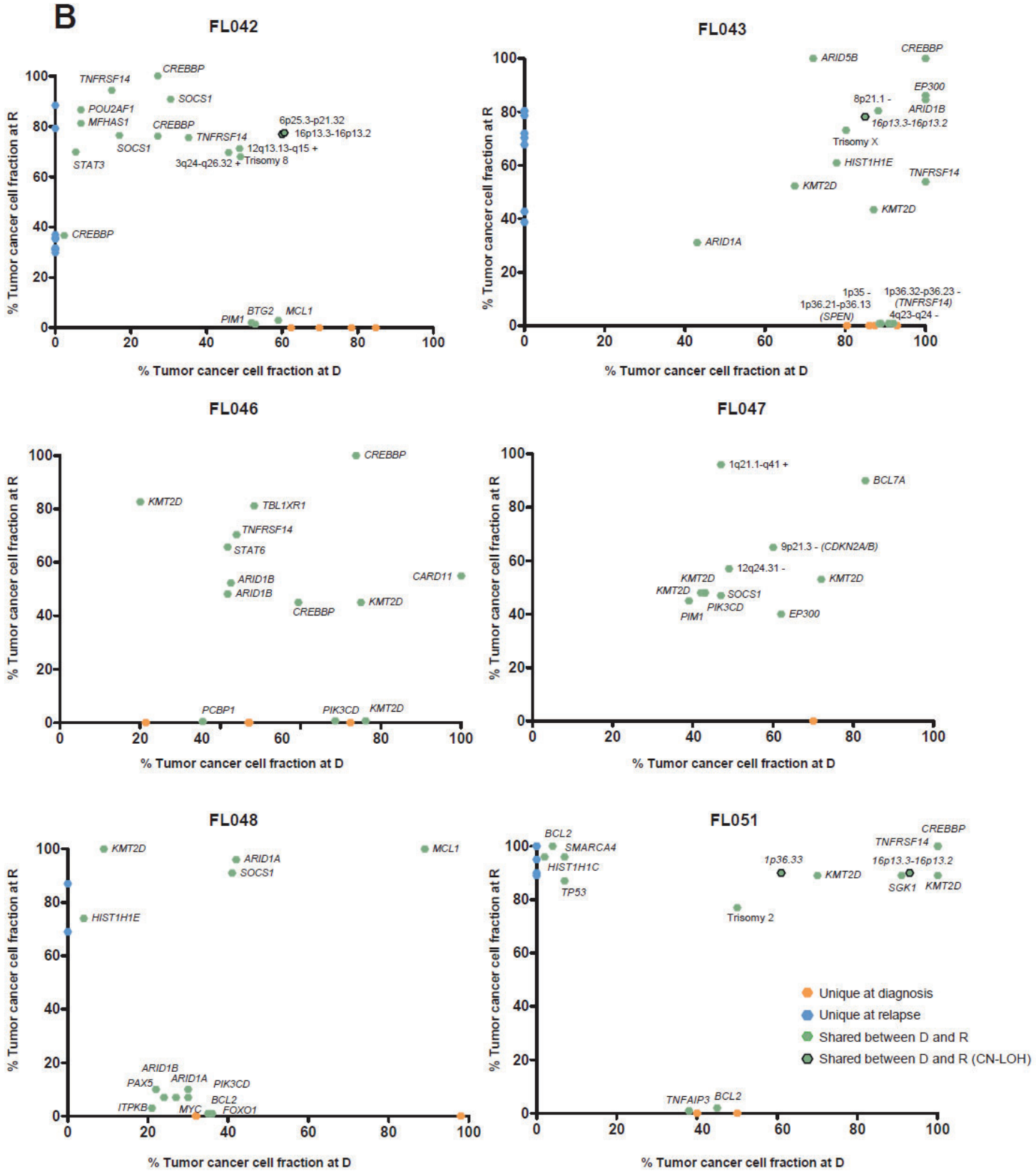
B. Genomic aberrations detected in *TMEM30A*, *PRDM1*, *SGK1*, and *TNFAIP3*, integrating SNVs, indels and CNA. Genes are sorted according to their genomic location, from centromer to telomer.



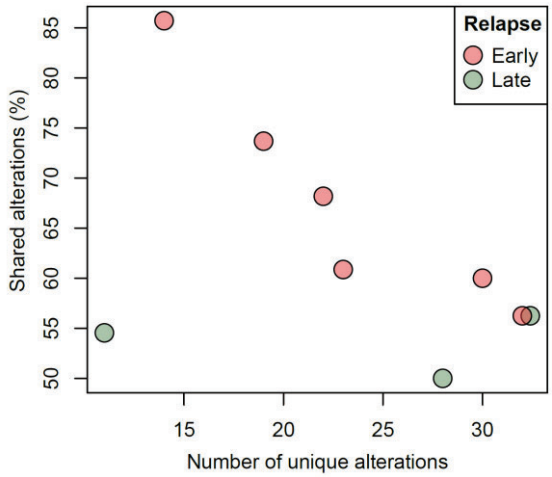
Supplementary Figure 4. Dynamics of genomic aberrations during disease progression. Tumor cancer cell fraction (expressed in percentage) of each genomic aberration detected at diagnosis (D) and relapse (R). The color code indicates unique aberrations observed at diagnosis (orange), unique aberrations at relapse (blue), and shared aberrations (green). Copy-neutral losses of heterozigosity (CN-LOH) are depicted as dots with a black outline. Note that genomic aberrations without available tumor cell content are not depicted, or samples FL027 and F028, which lacked NGS data. **A. Cases from the late relapse group.**



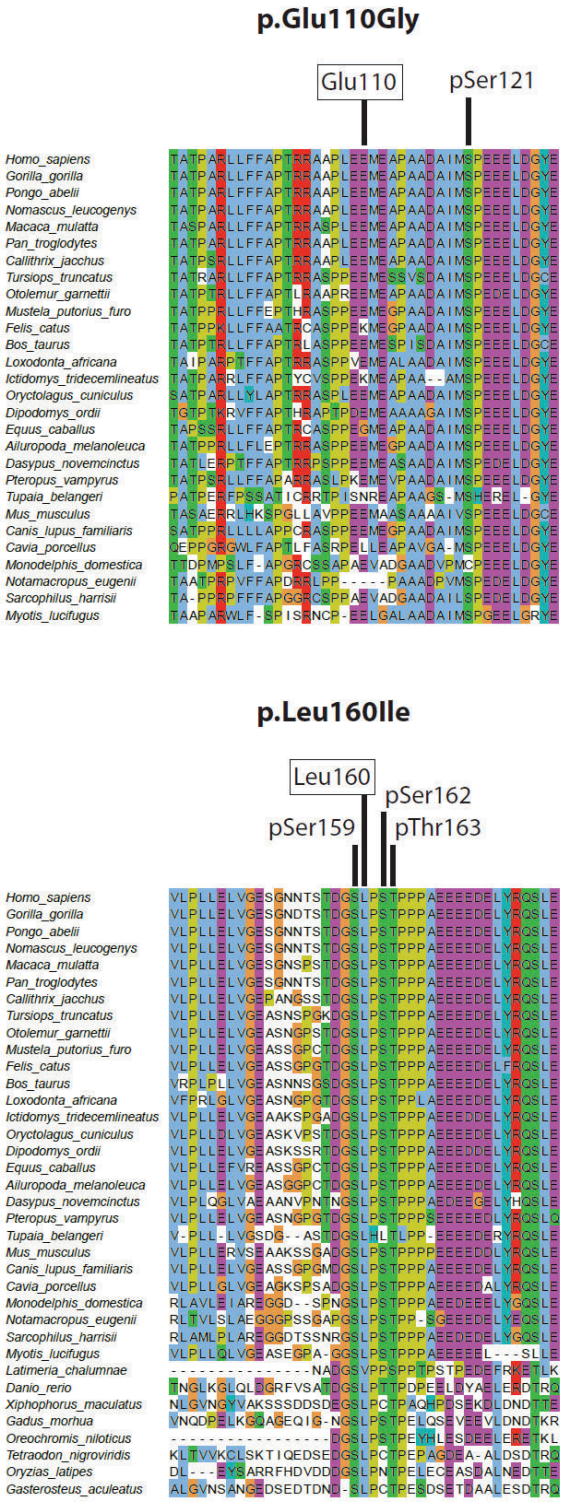
B. Cases from the early relapse group.



Supplementary Figure 5. Plot of shared aberrations in paired samples. The plot displays the percentage of shared aberrations (Y axis) versus the total number or alterations unique at diagnosis or relapse (X axis). The colors indicate the two different clinical groups: early relapse (red) and late relapse (green).



Supplementary Figure 6. Conservation of MCL1 variants among vertebrates. Alignments showing conservation of the two variants, p.Glu110Gly (upper block) and p.Leu160Ile (lower block) across vertebrates are depicted below. Amino acids are colored only if conserved across the position: blue=aliphatic; red=positive; magenta=negative; yellow=Pro; green=small; aquamarine = aromatic.



Supplementary Table 1. Inclusion criteria for the patients of the study

<table>
<tr><th>Group</th><th>Relapse</th><th>PFS</th><th>OS</th><th>Treatment</th><th>Response</th><th>Histology</th><th>Tissue</th></tr>
<tr><td>Never treated</td><td>–</td><td>NA</td><td>>5 y</td><td>None</td><td>NA</td><td rowspan="5">FL123A

Exclusion criteria:
• grade 3B FL
• primary gastrointestinal or cutaneous FL
• composite (FL/DLBCL) lymphoma
• HT during follow-up</td><td rowspan="5">Lymphoid tissue (lymph node, mass, tonsil, parotid gland)</td></tr>
<tr><td>Non-relapsed</td><td>No</td><td>>10 y</td><td>>10 y</td><td rowspan="3">R-CHOP or GA-CHOP (+/- ASCT)</td><td>CR</td></tr>
<tr><td>Late relapse</td><td>Yes</td><td>>2 y (all >33 months)</td><td>>2 y</td><td>PR/CR</td></tr>
<tr><td>Early relapse</td><td>Yes</td><td>5 months – 2 y</td><td rowspan="2">Irrelevant</td><td>PR/CR</td></tr>
<tr><td>Primary refractory</td><td>Absence of initial response</td><td><2 months</td><td>R-CHOP, BR, CHOP, FCM (any curative-intent treatment)</td><td>Refractory</td></tr>
<tr><td colspan="8">PFS, progression-free survival; OS, overall survival; NA, not available; y, years; (R-)CHOP, (rituximab), cyclophosphamide, doxorubicin, vincristine, and prednisone; GA-CHOP, obinutuzumab, cyclophosphamide, doxorubicin, vincristine, and prednisone; ASCT, autologous stem cell transplantation; BR, bendamustine-rituximab; FCM, fludarabine, cyclophosphamide, mitoxantrone; CR, complete response; PR, partial response; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; HT, histological transformation.</td></tr>
</table>

Supplementary Table 2. Biological characteristics of the cohort and genomic techniques applied to each sample

Patient ID	Sample ID	Clinical Group	Age	Sex	CN-Array	NGS-panel	g.3A>C U1	Ploidy	Purity	*BCL2* status	*BCL2* analysis			Additional FISH	
											BCL2 FISH	IGH-*BCL2* FISH	IGH-*BCL2* PCR	*BCL6* FISH	*MYC* FISH
FL001	FL001d	NT	61	male	yes	yes	n.a.	1.95	1	positive	–	–	positive	–	–
FL002	FL002d	NT	72	male	yes	yes	wt	1.95	1	negative	negative	negative	–	–	–
FL003	FL003d	NT	65	male	yes	yes	wt	1.96	1	positive	–	–	positive	–	–
FL004	FL004d	NT	79	female	yes	yes	wt	1.75	0.46	positive	positive	–	–	–	–
FL005	FL005d	NT	67	female	yes	yes	wt	2.00	1	n.a.	n.a.	n.a.	n.a.	–	–
FL006	FL006d	NT	65	male	yes	yes	wt	1.96	0.61	positive	positive	–	–	–	–
FL007	FL007d	NT	72	male	yes	yes	n.r.	1.98	1	positive	positive	–	–	–	–
FL008	FL008d	NR	71	female	yes	yes	n.r.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	–	–
FL009	FL009d	NR	73	female	yes	yes	wt	2.00	1	positive	positive	–	–	–	–
FL010	FL010d	NR	36	male	yes	yes	wt	1.98	0.59	positive	positive	–	–	–	–
FL011	FL011d	NR	53	male	yes	yes	wt	1.92	0.4	positive	positive	–	–	–	–
FL012	FL012d	NR	59	female	yes	yes	wt	2.10	0.86	positive	–	–	positive	–	–
FL013	FL013d	NR	47	male	yes	yes	n.r.	1.91	0.72	positive	–	–	positive	–	–
FL014	FL014d	NR	60	female	n.r.	yes	n.r.	n.a.	n.a.	n.r.	n.r.	n.r.	n.a.	–	–
FL015	FL015d	NR	59	female	yes	yes	n.r.	2.07	0.55	positive	–	–	positive	–	–
FL016	FL016d	NR	54	male	yes	yes	wt	3.56	0.36	positive	–	–	positive	–	–
FL017	FL017d	NR	26	female	yes	yes	n.r.	2.07	1	positive	positive	–	–	–	–
FL018	FL018d	NR	48	female	yes	yes	wt	2.00	1	negative	negative	negative	–	–	–
FL019	FL019d	NR	37	female	n.r.	yes	n.a.	n.a.	n.a.	positive	–	–	positive	–	–
FL020	FL020d	NR	62	female	yes	yes	wt	2.00	1	positive	–	–	positive	–	–
FL021	FL021d	NR	27	male	yes	yes	n.r.	2.07	1	positive	positive	–	–	–	–
FL022	FL022d	NR	62	female	yes	yes	wt	1.93	0.82	n.a.	n.a.	n.a.	n.a.	–	–
FL023	FL023d	NR	52	female	yes	yes	n.a.	2.00	1	n.r.	n.r.	n.r.	n.a.	–	–
FL024	FL024d	NR	68	male	yes	yes	wt	1.94	0.62	positive	positive	–	–	–	–
FL025	FL025d	NR	74	male	yes	yes	wt	2.10	0.67	negative	negative	negative	negative	–	–
FL026	FL026d	NR	54	female	yes	yes	wt	2.02	0.58	positive	–	–	positive	–	–
FL027	FL027d	LR	51	male	yes	no	n.r.	2.05	0.99	positive	positive	–	–	–	–
	FL027e	LR			yes	no	wt	1.95	1	–	–	–	–	negative	negative
FL028	FL028d	LR	68	female	yes	yes	n.a.	2.18	1	positive	–	–	positive		
	FL028e	LR			n.r.	yes	n.a.	n.a.	n.a.	positive	–	–	positive	negative	negative
FL029	FL029d	LR	73	female	yes	yes	n.r.	2.26	0.48	negative	negative	n.r.	–	break	–
	FL029e	LR			yes	yes	wt	2.08	0.54	–	–	–	–	break	negative
FL030	FL030d	LR	58	female	yes	yes	wt	2.01	0.92	positive	–	–	positive	–	–

FL031	FL031d	LR	26	female	yes	yes	wt	n.a.	n.a.	positive	positive	–	–	–	–
FL032	FL032d	LR	66	female	yes	yes	wt	2.07	1	positive	–	–	positive	–	–
	FL032e	LR			yes	yes	wt	2.01	0.79	–	–	–	–	negative	negative
FL033	FL033d	LR	59	female	yes	yes	wt	1.99	1	positive	positive	–	–		
FL034	FL034d	LR	63	male	yes	yes	wt	1.94	0.53	positive	positive	–	–	–	–
	FL034e	LR			yes	no	heterozygous mutation	2.00	1	–		–	–	–	–
FL035	FL035d	LR	40	female	yes	yes	wt	2.15	1	positive	positive	–	–	–	–
FL036	FL036d	LR	78	female	yes	yes	wt	1.95	0.49	positive	positive	–	–	–	–
FL037	FL037d	LR	52	female	yes	yes	n.r.	2.05	1	positive	positive	–	–	–	–
FL038	FL038d	LR	48	male	yes	yes	wt	2.08	1	n.a.	n.a.	n.a.	n.a.	–	–
FL039	FL039d	LR	65	female	yes	yes	n.r.	1.99	0.88	positive	positive	–	–	–	–
	FL039e	LR			yes	yes	wt	2.05	0.92	positive	positive	–	–	negative	negative
FL040	FL040d	LR	66	female	yes	yes	wt	2.00	0.45	negative	negative	negative	–	–	–
FL041	FL041d	ER	54	male	yes	yes	n.r.	1.95	0.79	n.r.	n.r.	n.r.	n.r.	–	–
FL042	FL042d	ER	52	female	yes	yes	n.r.	2.02	0.59	n.r.	n.r.	n.r.	negative	–	–
	FL042e	ER			yes	yes	wt	2.05	0.67	–	–	–	negative	negative	negative
FL043	FL043d	ER	54	female	yes	yes	wt	2.04	0.65	positive	positive	–	–		
	FL043e	ER			yes	yes	wt	2.06	0.65	–	–	–	–	negative	negative
FL044	FL044d	ER	46	male	yes	yes	n.r.	1.99	0.66	positive	–	–	positive	–	–
FL045	FL045d	ER	68	male	yes	yes	n.r.	2.01	0.75	positive	positive	–	–	–	–
FL046	FL046d	ER	35	female	yes	yes	n.r.	1.95	1	positive	positive	–	negative	–	–
	FL046e	ER			yes	yes	wt	1.94	0.83	positive	positive	–	–	negative	negative
FL047	FL047d	ER	42	male	yes	yes	wt	1.96	0.78	positive	positive	–	negative	n.a.	
	FL047e	ER			yes	yes	n.r.	1.98	0.88	–	–	–	–	break	negative
FL048	FL048d	ER	29	male	yes	yes	n.r.	1.99	1	negative	negative	negative	–	–	–
	FL048e	ER			yes	yes	wt	2.05	0.58	positive	positive	–	–	negative	negative
FL049	FL049d	ER	52	female	yes	yes	n.r.	2.02	0.63	positive	positive	–	–	–	–
FL050	FL050d	ER	57	male	yes	yes	wt	1.98	0.74	positive	positive	–	–	–	–
FL051	FL051d	ER	50	male	yes	yes	wt	1.99	0.55	positive	positive	–	–	–	negative
	FL051e	ER			yes	yes	wt	2.04	0.85	–	–	–	–	negative	break
FL052	FL052d	PR	54	female	n.r.	n.r.	n.a.	n.a.	n.a.	positive	positive	–	–	–	–
FL053	FL053d	PR	37	male	yes	yes	n.a.	2.06	1	n.a.	n.a.	n.a.	n.a.	–	–
FL054	FL054d	PR	42	male	yes	yes	wt	2.01	1	positive	–	–	positive	–	–
FL055	FL055d	PR	73	male	yes	yes	wt	2.08	1	positive	positive	–	–	–	–
FL056	FL056d	PR	53	female	yes	yes	n.r.	2.07	1	positive	–	–	positive	–	–

NT, never treated; NR, non-relapsed; LR, late relapse; ER, early relapse; PR, primary refractory, n.r., no result; wt, wildtype; n.a. not available

Supplementary Table 3. Genes and genomic regions included in the B-cell malignancy-oriented targeted sequencing panel.

Gene	Exons	Genomic region	Transcripts
ACTB	Exons 2-6	All CDS	NM_001101.3
ARID1A	Exons 1-20	All CDS	NM_006015.4
ARID1B	Exons 1-20	All CDS	NM_020732
ARID5B	Exons 1-10	All CDS	NM_032199.3
ATM	Exons 2-63	All CDS	NM_000051.3
ATP10A	Exons 1	All CDS	NM_024490.3
B2M	Exons 1-3	All CDS	NM_004048.2
BCL10	Exons 1-3	All CDS	NM_003921.4
BCL2	Exons 1-2	All CDS + CNV	NM_000633.2
BCL6	Exons 3-10	All CDS + CNV	NM_001706.4
BCL7A	Exons 1-5	All CDS	NM_020993.5
BCOR	Exons 2-15	All CDS	NM_001123385
BRAF	Exons 11-15	–	NM_004333.4
BTG1	Exons 1-2	All CDS	NM_001731.2
BTG2	Exons 1-2	All CDS	NM_006763.2
BTK	Exons 2-19	All CDS	NM_000061.2
CARD11	Exons 2-25	All CDS	NM_032415.4
CCND3	Exons 1-5	All CDS	NM_001760.3
CD274	Exons 2-7	All CDS	NM_014143
CD36	Exons 3-14	All CDS	NM_001001548.2
CD58	Exons 1-6	All CDS	NM_001779.2
CD70	Exons 1-3	All CDS + CNV	NM_001252.3
CD79A	Exons 1-5	All CDS	NM_001783.3
CD79B	Exons 1-6	All CDS	NM_001039933.2
CD83	Exons 1-5	All CDS	NM_001040280.1
CDKN2A	Exons 1-3	All CDS + CNV	NM_001195132.1
CDKN2B	Exons 1-2	All CDS	NM_004936.3
CIITA	Exons 1-19	All CDS + CNV	NM_000246.3
CREBBP	Exons 1-31	All CDS	NM_004380.2
CSF1R	Exons 2-24	All CDS	NM_005211.3
CXCR4	Exons 1-2	All CDS	NM_001008540.2
DDX3X	Exons 1-17	All CDS	NM_001356.3
DIS3	Exons 1-22	hotspot	NM_014953.3
DTX1	Exons 1-9	All CDS	NM_004416
DUSP2	Exons 1-4	All CDS	NM_004418.4
EBF1	Exons 1-16	hotspot	NM_001290360
EP300	Exons 1-31	All CDS	NM_001429.3
ETS1	Exons 2-10	All CDS	NM_001143820.1
ETV6	Exons 1-8	All CDS + CNV	NM_001987
EZH2	Exons 10-20	–	NM_004456.4
FAS	Exons 1-9	–	NM_000043
FBXW7	Exons 2-12	All CDS	NM_033632.3

FOXO1	Exons 1-2	All CDS	NM_002015.3
GNA13	Exons 1-4	All CDS	NM_006572.4
GNAI2	Exons 1-8	All CDS	NM_002070
HIST1H1B	Exons 1	All CDS	NM_005322
HIST1H1C	Exons 1	All CDS	NM_005319
HIST1H1D	Exons 1	All CDS	NM_005320.4
HIST1H1E	Exons 3-8	All CDS + CNV	NM_005321
HIST1H3G	Exon 1	All CDS	NM_003534
HVCN1	Exons 2	–	NM_001040107
ID3	Exons 1-2	All CDS	NM_002167.4
IRF2BP2	Exons 1-2	All CDS	NM_182972
IRF4	Exons 2-9	All CDS	NM_002460.3
IRF8	Exons 2-9	All CDS	NM_002163.2
ITPKB	Exons 2-8	All CDS	NM_002221
JUNB	Exon 1	All CDS	NM_002229.3
KLHL14	Exons 2-9	All CDS	NM_020805
KLHL6	Exons 1-7	All CDS	NM_130446.2
KMT2D	Exons 1-54	All CDS	NM_003482.3
KRAS	Exons 2-4	All CDS	NM_033360.2
LPHN2	Exons 2-22	All CDS	NM_012302
MALT1	Exons 1-17	All CDS + CNV	NM_006785
MAML1	Exons 1-4	All CDS	NM_014757
MAP2K1	Exons 1-11	All CDS	NM_002755.3
MAPK1	Exons 1-8	All CDS	NM_002745.4
MCL1	Exons 1-3	All CDS	NM_021960
MEF2B	Exons 2-9	All CDS	NM_001145785.1
MFHAS1	Exons 2-3	All CDS	NM_004225
MYC	Exons 2-3	All CDS + intron 1 + translocation region	NM_002467.4
MYD88	Exons 1-5	All CDS	NM_002468.4
NFKBIA	Exons 2-6	All CDS	NM_020529
NFKBIE	Exons 1-6	All CDS	NM_004556.2
NFKBIZ	Exons 2-12	All CDS + amplification	NM_031419.3
NOTCH1	Exon 26, 27, 34, 3'UTR	–	NM_017617.3
NOTCH2	Exon 26, 27, 34,	All CDS	NM_024408.3
NRAS	Exons 2-5	–	NM_002524.4
OSBPL10	Exons 1-12	All CDS	NM_017784
P2RY8	Exons 2	–	NM_178129
PARP2	Exons 1-16	All CDS	NM_005484
PAX5	Exons 1-10	–	NM_016734.3
PCBP1	Exons 1	–	NM_006196
PIK3CA	Exons 2-21	All CDS	NM_006218
PIK3CD	Exons 3-24	–	NM_005026.3
PIK3R1	Exon 2	–	NM_001243186.1

PIM1	Exons 1-6	All CDS	NM_002661.5
PLCG2	Exons 2-33	All CDS	NM_006235.3
POU2AF1	Exon 1-5	All CDS	NM_001207025.2
POU2F2	Exons 1-14	All CDS	NM_001198.3
PRDM1	Exons 1-7	All CDS	NM_002738
PRKCB	Exons 1-17	All CDS	NM_000314
PTEN	Exons 1-9	All CDS + CNV	NM_002839
PTPRD	Exons 15-46	–	NM_002908
REL	Exons 1-11	All CDS + CNV	NM_001313941
RHOA	Exons 3-6	All CDS + CNV	NM_001271851
RRAGC	Exons 1-7	All CDS	NM_001320730
S1PR1	Exons 2	All CDS	NM_004230
S1PR2	Exons 2	All CDS	NM_015048
SETD1B	Exons 1-16	All CDS	NM_014159.6
SETD2	Exons 1-21	All CDS	NM_012433
SF3B1	Exons 14-18	–	NM_001143676.1
SGK1	Exons 1-14	All CDS	NM_003072
SMARCA4	Exons 2-35	All CDS	NM_003745.1
SOCS1	Exons 2	All CDS	NM_015001
SPEN	Exons 1-16	All CDS	NM_003121
SPIB	Exons 1-6	All CDS + CNV	NM_139276.2
STAT3	Exons 2-24	All CDS	NM_001178078.1
STAT6	Exons 2-22	All CDS	NM_024665.4
TBL1XR1	Exons 3-16	All CDS + CNV	NM_003200.3
TCF3	Exons 2-19	All CDS	NM_001127208.2
TET2	Exons 3-11	–	NM_018247.3
TMEM30A	Exons 1-7	All CDS	NM_021109
TMSB4X	Exons 2-3	All CDS	NM_006290.3
TNFAIP3	Exons 2-9	All CDS+CNV	NM_003820.2
TNFRSF14	Exons 1-8	All CDS	NM_001252391
TNIP1	Exons 2-18	All CDS + CNV	NM_014729
TOX	Exons 1-9	All CDS + CNV	NM_000546.5
TP53	Exons 2-11	All CDS	NM_005080
XBP1	Exons 1-5	All CDS	NM_003400
XPO1	Exons 15-16	–	NM_014795
ZEB2	Exons 2-10	All CDS	NM_181523

CDS, coding sequence; CNV, copy number variant

Supplementary Table 4. Primers used in rhAMP SNP assays to interrogate the g.3A>C U1 mutations.

Gene/Assay	Forward Primer	Reverse Primer
RNU1_Batch SNP Assay (WT)	/rhAmp-F/TCCCCTGCCAGGTAAGTrATGAG	GCGCAAGTGACCGTGTGTGTAArAGAGT
RNU1_Batch SNP Assay (Mut)	/rhAmp-Y/CCCCTGCCAGGTAAGGrATGAG	GCGCAAGTGACCGTGTGTGTAArAGAGT
RNU1_Pseudo SNP Assay (WT)	/rhAmp-F/TCCCCTGCCAGGTAAGTrATGGA	GCAAGTGACCGTGTGTTGAGAGrGAGTG
RNU1_Pseudo SNP Assay (Mut)	/rhAmp-Y/CCCCTGCCAGGTAAGGrATGGA	GCAAGTGACCGTGTGTTGAGAGrGAGTG

Supplementary Table 5. Baseline features and outcomes of the 56 patients included in the study.

	All	Never treated	Non-relapsed	Late relapse	Early relapse	Primary refractory
Number of patients and diagnostic samples (with both CNA & NGS info)	56 (52)	7 (7)	19 (17)	14 (13)	11 (11)	5 (4)
Number of relapse samples (with both CNA & NGS info)	12 (9)	0	0	6 (3)	6 (6)	0
Female sex, n (%)	31 (55)	2 (29)	12 (63)	11 (79)	4 (36)	2 (40)
Median age (range)	57 (26–79)	67 (61–79)	54 (26–74)	61 (26–78)	52 (29–68)	48 (37–73)
ECOG PS ≥2, n (%)	4 (7)	1 (14)	0	1 (7)	0	2 (40)
Ann-Arbor stage III-IV, n (%)	41 (75)	6 (86)	10 (53)	14 (100)	8 (73)	3 (60)
High-risk FLIPI score, n (%)	13 (25)	0	2 (11)	7 (50)	2 (20)	2 (40)
Histological grade 1-2, n (%)	43 (78)	7 (100)	13 (68)	10 (71)	10 (91)	3 (60)
***BCL2* rearrangement*, n (%) [n=47, 84%]**	41 (87)	4 (67)	14 (87)	12 (85)	8 (89)	3 (100)
Frontline treatment with R-CHOP, n (%)	45 (82)	–	19 (100)	14 (100)	11 (100)	1 (20)
CR rate, n (%)	40 (83)	–	19 (100)	12 (86)	9 (82)	0
OS at 10 y, % (95% CI)	79 (69-91)	71** (45-100)	100	74 (51-100)	82 (62-100)	0

**BCL2/IGH* rearrangement by PCR and/or rearranged *BCL2* gene by dual-color, break-apart FISH probe. *BCL2/IGH* dual color, dual fusion FISH study pending.

**3 patients from this group died, none of them of lymphoma-related causes (1 unknown cause, 1 lung cancer, 1 hip prosthetic infection)

CNA, copy number alterations; NGS, next generation sequencing; ECOG PS, Eastern Cooperative Oncology Group Performance Status; FLIPI, Follicular Lymphoma International Prognostic Index; R-CHOP, rituximab, cyclophosphamide, vincristine, and prednisone; CR, complete response; OS, overall survival; y, years; CI, confidence interval.

Supplementary Table 6. Minimal regions defined in at least three cases

Event	Chromosomal region	Chromosome	Start	End	Candidate genes	Gistic (q-value)
Loss	1p36.32	1	1	8497319	*TNFRSF14*	0.005341
Loss	6p21.32	6	31083401	32192238	*HLA*	5.40E-07
Loss	6q14.1	6	74201303	87070445	*TMEM30A*	0.031931
Loss	6q23.3	6	136595753	140897287	*TNFAIP3*	0.0012615
Loss	9p21.3	9	21352378	22735072	*CDKN2A/B*	0.00000843
Loss	10q23.33	10	85032997	109373558	*PTEN*	0.0065932
Loss	13q14.2-q14.3	13	47460442	55056195	*DLEU1, DLEU2, RB1*	n.a.
Gain	2p16.1	2	57854369	63237116	*REL, BCL11A*	0.066872
Gain	12q13.13-q15	12	52677677	71278552	-	n.a.
Gain	13q31.3-q32.3	13	92000000	101321244	*MIR17HG*	n.a.
Gain	17q22-q24.2	17	51382417	64376856	*RAD51C, CD79B, MAP3K3*	n.a.
Loss	22q13.2-q13.32	22	42545221	49080367	*TAFA5*	n.a.
Gain	1q	1	-	-	-	n.a.
Gain	18p11.32-q21.33	18	12842	60418491	-	n.a.
CN-LOH	1p36.33-p36.22	1	754192	10650344	*TNFRSF14*	n.a.
CN-LOH	6p25.3-p21.32	6	204909	33595399	*IRF4, HLA, HISTI1H* cluster	n.a.
CN-LOH	12q13.12-q13.13	12	49342939	52031943	-	n.a.
CN-LOH	12q21.33-q24.33	12	92043449	133776400	-	n.a.
CN-LOH	16p13.3-p13.2	16	83887	8717907	-	n.a.

CN-LOH: copy-neutral loss heterozigosity; n.a., not available.

Supplementary Table 7. Statistics of sample subjected to target sequencing

Sample	Group	Median Target Coverage
FL001d	Never treated	65x
FL002d	Never treated	228x
FL003d	Never treated	92x
FL004d	Never treated	489x
FL005d	Never treated	1083x
FL006d	Never treated	373x
FL007d	Never treated	124x
FL008d	Non-relapsed	285x
FL009d	Non-relapsed	127x
FL010d	Non-relapsed	193x
FL011d	Non-relapsed	276x
FL012d	Non-relapsed	360x
FL013d	Non-relapsed	232x
FL014d	Non-relapsed	319x
FL015d	Non-relapsed	238x
FL016d	Non-relapsed	342x
FL017d	Non-relapsed	161x
FL018d	Non-relapsed	220x
FL019d	Non-relapsed	456x
FL020d	Non-relapsed	269x
FL021d	Non-relapsed	112x
FL022d	Non-relapsed	1123x
FL023d	Non-relapsed	100x
FL024d	Non-relapsed	84x
FL025d	Non-relapsed	151x
FL026d	Non-relapsed	257x
FL028d	Late relapse	
FL028e	Late relapse	445x
FL029d	Late relapse	97x
FL029e	Late relapse	169x
FL030d	Late relapse	209x
FL031d	Late relapse	87x
FL032d	Late relapse	277x
FL032e	Late relapse	647x
FL033d	Late relapse	1039x
FL034d	Late relapse	160x
FL035d	Late relapse	403x
FL036d	Late relapse	226x
FL037d	Late relapse	143x
FL038d	Late relapse	108x
FL039d	Late relapse	179x
FL039e	Late relapse	595x
FL040d	Late relapse	261x
FL041d	Early relapse	428x
FL042d	Early relapse	135x
FL042e	Early relapse	116x
FL043d	Early relapse	140x
FL043e	Early relapse	246x
FL044d	Early relapse	129x

FL045d	Early relapse	
FL046d	Early relapse	55x
FL046e	Early relapse	261x
FL047d	Early relapse	179x
FL047e	Early relapse	225x
FL048d	Early relapse	76x
FL048e	Early relapse	360x
FL049d	Early relapse	105x
FL050d	Early relapse	299x
FL051d	Early relapse	200x
FL051e	Early relapse	1417x
FL052d	Primary refractory	12.8X
FL053d	Primary refractory	182x
FL054d	Primary refractory	262x
FL055d	Primary refractory	192x
FL056d	Primary refractory	132x

Supplementary Table 8. Gene/region alterations by frequency

Gene or region	No. of diagnostic samples with alteration	Frequency of alteration
KMT2D	41	79%
CREBBP	35	67%
TNFRSF14	24	46%
BCL2	21	40%
1p36.33 CN-LOH	14	27%
ARID1A	13	25%
TNFAIP3	12	23%
EP300	11	21%
16p13.3-16p13.2 CN-LOH	11	21%
1q gain	11	21%
Trisomy 7	11	21%
REL	10	19%
BCL7A	9	17%
EZH2	9	17%
SGK1	9	17%
FAS	8	15%
TMEM30A	8	15%
12q13.13-q15 gain	8	15%
18p11.32-q21.33 gain	8	15%
Trisomy X	8	15%
ARID1B	7	13%
CARD11	7	13%
CDKN2A/B	7	13%
HIST1H1E	7	13%
PTEN	7	13%
SOCS1	7	13%
12q21.33-q24.33 CN-LOH	7	13%
6p25.3-p21.32 CN-LOH	7	13%
BTG2	6	12%
CD79B	6	12%
IRF8	6	12%
MEF2B	6	12%
PIM1	6	12%
13q14.2-q14.3 loss (***DLEU1/2***)	6	12%
Trisomy 18	6	12%
Trisomy 2	6	12%
HIST1H1D	5	10%
PAX5	5	10%
PRDM1	5	10%
SETD1B	5	10%
STAT6	5	10%
12q13.12-q13.13 CN-LOH	5	10%
ARID5B	4	8%
CD70	4	8%
GNA13	4	8%
HVCN1	4	8%
ITPKB	4	8%
MCL1	4	8%
MYC	4	8%
NFKBIZ	4	8%
OSBPL10	4	8%
PCBP1	4	8%
PIK3CD	4	8%
POU2AF1	4	8%
RRAGC	4	8%
SPEN	4	8%

TBL1XR1	4	8%
17q22-q24.2 gain	4	8%
22q13.2-q13.32 loss	4	8%
Trisomy 12	4	8%
Trisomy 8	4	8%
ATM	3	6%
CCND3	3	6%
CD36	3	6%
CD79A	3	6%
DTX1	3	6%
EBF1	3	6%
FOXO1	3	6%
KLHL6	3	6%
MFHAS1	3	6%
PLCG2	3	6%
PTPRD	3	6%
STAT3	3	6%
13q31.3-q32.3 gain (*MIR17HG*)	3	6%
6p21.32 loss	3	6%

Supplementary Table 9. Temporal order of acquisition of genomic alterations

Gene / Genomic Alteration	*P* value	*Q* value	Out degrees	In degrees	Neutral	Temporal order
KMT2D	0.000	0.000	130	31	183	Early
TNFRSF14	0.000	0.000	75	10	117	Early
SOCS1	0.000	0.000	2	32	50	Late
Gain 1q	0.000	0.000	7	41	66	Late
CARD11	0.001	0.003	12	37	25	Late
CN-LOH 16p13.3-p13.2	0.001	0.003	17	2	57	Early
Trisomy X	0.001	0.003	2	17	39	Late
CD79B	0.002	0.005	5	22	40	Late
HIST1H1E	0.003	0.009	31	11	38	Early
18p11.32-q21.33 +	0.004	0.010	6	22	29	Late
BCL7A	0.012	0.029	29	12	68	Early
Trisomy 7	0.014	0.032	9	24	62	Late
BCL2	0.016	0.035	19	38	76	Late
EP300	0.020	0.040	26	11	40	Early
CREBBP	0.027	0.050	77	51	169	Early
2p16.1+ (*REL*)	0.029	0.051	7	19	58	Late
PIM1	0.031	0.051	4	14	40	Late
EZH2	0.099	0.154	13	24	50	Not powered
TNFAIP3	0.115	0.170	14	6	46	Not powered
ARID1A	0.233	0.326	18	27	48	Not powered
6q14.1- (*TMEM30A*)	0.302	0.402	5	10	44	Not powered
CN-LOH 6p25.3-p21.32	0.424	0.540	10	15	56	Not powered
MEF2B	0.458	0.558	17	12	35	Not powered
13q14.2-q14.3-	0.503	0.586	12	8	45	Not powered
12q13.13-q15+	0.524	0.586	9	13	45	Not powered
CN-LOH 1p36.33	0.552	0.594	20	25	80	Not powered
1p36.32- (*TNFRSF14*)	1.000	1.000	6	6	52	Not powered
ARID1B	1.000	1.000	9	10	40	Not powered

SUPPLEMENTARY METHODS

DNA extraction

We extracted DNA and RNA from 53 FFPE and two fresh frozen (FF) diagnostic samples, and from 11 FFPE and one FF relapse samples using five 10 µm-thick sections per sample and the AllPrep DNA/RNA FFPE Kit (Qiagen, Germany), according to the manufacturer's instructions.

Copy number analysis

Copy number aberrations (CNA) were analyzed using the Affymetrix Genome wide/Oncoscan CNV FFPE assay. Gains, losses and copy neutral loss of heterozygosity (CN-LOH) were evaluated and visually inspected using Nexus version 9.0 Discovery Edition software (Biodiscovery, El Segundo, CA). Human reference genome was GRCh37/hg19. Gains and losses with minimum size of 100kb and CN-LOH larger than 10Mb were considered. Exceptionally, focal copy number alterations smaller than 100 kb or CN-LOH smaller than 10Mb were recognized if they included a driver gene or recurrent altered region previously described in follicular lymphoma (FL). Chromothripsis and chromothripsis-like patterns have been defined when at least 10 and 7 switches between two or more CN states were detected on an individual chromosome, respectively[72]. Furthermore, information originated from CNA was merged with SNV/indels from the targeted sequencing data to classify 44 genes as normal/altered.

Next generation sequencing

Single nucleotide variants (SNVs) and insertions/deletions (indels) were analyzed using a B-cell malignancy-oriented targeted sequencing panel containing 121 genes. The NGS panel is an updated version of our B-cell next generation sequencing (NGS) panel previously described[73]. Mutation profiles were generated using a custom hybridization capture-based panel strategy and subsequent sequenced in a MiSeq instrument (Illumina). Libraries were performed using 15-30 ng of cfDNA and 150 ng of gDNA from FFPE samples, following the procedure indicated by the manufacturer. Targeted sequencing was performed using molecular-barcoded library adapters using the ThruPLEX Tag-seq kit (Takara) and a hybridization capture based method (SureSelectXT-Agilent Technologies). The quality of the libraries was determined using the Bioanalyzer high sensitivity DNA kit (Agilent) and quantified by PCR using the KAPA library quantification kit (KAPA Biosystems). Finally, the libraries were pooled and sequenced 2x130 bp in the MiSeq instrument.

Bioinformatic analyses

Variant calling was performed using an updated version of our in-house pipeline[23]. Briefly, raw reads were trimmed using the SurecallTrimmer (v4.0.1, AGeNT, Agilent). Alignment of the trimmed reads was performed using minimap2 algorithm[74], PCR or optical duplicates were marked using MarkDuplicates from Picard (RRID: SCR_006525), and the base quality score recalibration was performed using GATK's BaseRecalibrator and ApplyBQSR functions (RRID:

SCR_001876 v4.0). Variant calling was performed in parallel using VarScan2 (v2.4.3)[75], Mutect2, VarDictJava (v1.4)[75], LoFreq (v2.1.3.1)[76], outLyzer (v1.0)[77], and freebayes (v1.1.0)[78]. Variants identified were annotated using snpEff/snpSift (v4.3t). Only variants that were identified as "PASS" by at least 4 of the algorithms and with variant allele frequency (VAF) > 10 were considered. Particularly, we manually retrieved SNVs and indels with VAF smaller than 10 by visually inspected on Integrative Genomics Viewer (IGV) the genomic aberrations of the paired samples.

Variants reported in the 1000 Genome Project, ExAC and/or gnomAD with a population frequency >1% were considered polymorphisms and therefore removed from the analysis. To further filter out non-recurrent polymorphisms, variants were only considered somatic if 1) they were not reported as germ line in our custom ICGC data base of 506 WGS/WES[79]; and were 2) reported as somatic in lymphoid neoplasm in COSMIC database, 3) truncating, or 4) predicted as potentially damaging by at least one of the following algorithms: CADD (phred score > 10), PolyPhen2 (score > 0.9), SIFT (score < 0.1), MutationAssessor (score > 2) and Provean (deleterious).

Cancer cell fraction and temporal order of genetic alterations

The cancer cell fraction (CCF) CCF from CNA was retrieved from the OncoScan data as previously described[80]. The CCF was calculated using the corresponding formula in which BAF is the mean B-allele frequency of the CN locus, minor is the minor number of copies of the least frequent allele, and major the major number of copies of the most frequent allele. The major and minor information were retrieved from ASCAT (R package v2.5.2). The predicted CCF were corrected by the tumor purity of the respective samples obtained by ASCAT (R package v2.5.2). CCF were only considered for those samples with predicted diploid genotype. The CCF of the SNVs and indels was calculated integrating read counts, copy-number status of each locus and tumor purity, as previously described. To explore the temporal order of the genomic aberrations we used the recently published approached[24,25]. In brief, we analyzed for each alteration the enrichment of out-going edges compared to in-going edges, where out-going edges indicates the number of instances where the alteration was detected at higher CCF than other alteration in the same tumor sample, and in-going when it was present at lower CCF. Genomic aberrations were classified as early, late (false discovery rate <0.1) or not powered.

U1 mutations using PCR-based SNP assay

The g.3A>C mutation in U1 was evaluated using custom rhAMP SNP assays (Integrating DNA technology) as previously reported. Locus and allele-specific primers were designed for RNU1-3 position. The assay was run in technical duplicates in 5µL volume with a DNA concentration of at least 10ng, with control gBlocks for wild-type, mutants and heterozygous genotypes. Reporter mix were labeled with Yakima Yellow, FAM and ROX dyes for mutant, wild-type, and passive

reference, respectively. Plates were analyzed on the StepOnePlus (Applied Biosystems) RT-PCR machine, and genotypes called using the StepOne v.2.3 software.

REFERENCES

1. Bachy E, Seymour JF, Feugier P, Offner F, López-Guillermo A, Belada D, et al. Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: Long-term results of the PRIMA study. J Clin Oncol. 2019;37:2815-24.
2. Rivas-Delgado A, Magnano L, Moreno-Velázquez M, García O, Nadeu F, Mozas P, et al. Response duration and survival shorten after each relapse in patients with follicular lymphoma treated in the rituximab era. Br J Haematol. 2019;184:753-9.
3. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: An analysis from the National LymphoCare Study. J Clin Oncol. 2015;33:2516-22.
4. Alonso-Álvarez S, Magnano L, Alcoceba M, Andrade-Campos M, Espinosa-Lara N, Rodríguez G, et al. Risk of, and survival following, histological transformation in follicular lymphoma in the rituximab era. A retrospective multicentre study by the Spanish GELTAMO group. Br J Haematol. 2017;178:699-708.
5. Limpens J, Stad R, Vos C, De Vlaam C, De Jong D, Van Ommen GJB, et al. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood. 1995;85:2528-36.
6. Kridel R, Chan FC, Mottok A, Boyle M, Farinha P, Tan K, et al. Histological Transformation and Progression in Follicular Lymphoma: A Clonal Evolution Study. PLoS Med. 2016;13:1-25.
7. Okosun J, Bödör C, Wang J, Araf S, Yang CY, Pan C, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. Nat Genet. 2014;46:176-81.
8. Pasqualucci L, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, et al. Genetics of Follicular Lymphoma Transformation. Cell Rep. 2014;6:130-40.
9. Green MR, Kihira S, Liu CL, Nair R V., Salari R, Gentles AJ, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. Proc Natl Acad Sci U S A. 2015;112:E1116-25.
10. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011;471:189-96.
11. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42:181-5.
12. Bouska A, McKeithan TW, Deffenbacher KE, Lachel C, Wright GW, Iqbal J, et al. Genome-wide copy-number analyses reveal genomic abnormalities involved in transformation of follicular lymphoma. Blood. 2014;123:1681-90.
13. Cheung KJJ, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, et al. Genome-

wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. Blood. 2009;113:137-48.

14. Johnson NA, Al-Tourah A, Brown CJ, Connors JM, Gascoyne RD, Horsman DE. Prognostic significance of secondary cytogenetic alterations in follicular lymphomas. Genes Chromosom Cancer. 2008;47:1038-48.
15. Mozas P, Rivero A, López-Guillermo A. Past, present and future of prognostic scores in follicular lymphoma. Blood Rev. 2021:100865.
16. Solal-Céligny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. Blood. 2004;104:1258-65.
17. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: A retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16:1111-22.
18. Huet S, Tesson B, Jais JP, Feldman AL, Magnano L, Thomas E, et al. A gene-expression profiling score for prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts. Lancet Oncol. 2018;19:549-61.
19. Qu X, Li H, Braziel RM, Passerini V, Rimsza LM, His ED, et al. Genomic alterations important for the prognosis in patients with follicular lymphoma treated in SWOG study S0016. Blood. 2019;133:81-93.
20. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25:579-86.
21. Swerdlow S, Campo E, Harris N, Jaffe E, Pileri S, Stein H. WHO Classification of Tumours of Haematopoietic and Lymphoid tissues. Revised 4t. 2017.
22. Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhim R, Getz G. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. Genome Biol. 2011;12.
23. Nadeu F, Delgado J, Royo C, Baumann T, Stankovic T, Pinyol M, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood. 2016;127:2122-30.
24. Nadeu F, Martin-Garcia D, Clot G, Díaz-Navarro A, Duran-Ferrer M, Navarro A, et al. Genomic and epigenomic insights into the origin, pathogenesis, and clinical behavior of mantle cell lymphoma subtypes. Blood. 2020;136:1419-32.
25. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015;526:525-30.
26. Shuai S, Suzuki H, Diaz-Navarro A, Nadeu F, Kumar SA, Gutierrez-Fernandez A, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature. 2019;574:712-6.
27. Betts MJ, Lu Q, Jiang Y, Drusko A, Wichmann O, Utz M, et al. Mechismo: Predicting the

mechanistic impact of mutations and modifications on molecular interactions. Nucleic Acids Res. 2015;43:e10.

28. Burley SK, Berman HM, Bhikadiya C, Bi C, Chen L, Di Costanzo L, et al. RCSB Protein Data Bank: Biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic Acids Res. 2019;47:D464-74.
29. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen M, et al. Comparative Protein Structure Modeling Using MODELLER. Curr Protoc Protein Sci. 2007;50.
30. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389-402.
31. Powell S, Szklarczyk D, Trachana K, Roth A, Kuhn M, Muller J, et al. eggNOG v3.0: Orthologous groups covering 1133 organisms at 41 different taxonomic ranges. Nucleic Acids Res. 2012;40.
32. Bateman A. UniProt: A worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47:D506-15.
33. Dosztányi Z, Csizmok V, Tompa P, Simon I. IUPred: Web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content. Bioinformatics. 2005;21:3433-4.
34. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, et al. The Pfam protein families database: Towards a more sustainable future. Nucleic Acids Res. 2016;44:D279-85.
35. Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792-7.
36. Russell RB, Barton GJ. Multiple protein sequence alignment from tertiary structure comparison: Assignment of global and residue confidence levels. Proteins Struct Funct Bioinforma. 1992;14:309-23.
37. Boldt K, Van Reeuwijk J, Lu Q, Koutroumpas K, Nguyen TMT, Texier Y, et al. An organelle-specific protein landscape identifies novel diseases and molecular mechanisms. Nat Commun. 2016;7.
38. Green MR. Chromatin modifying gene mutations in follicular lymphoma. Blood. 2018;131:595-604.
39. Baliñas-Gavira C, Rodríguez MI, Andrades A, Cuadros M, Álvarez-Pérez JC, Álvarez-Prado ÁF, et al. Frequent mutations in the amino-terminal domain of BCL7A impair its tumor suppressor role in DLBCL. Leukemia. 2020;34:2722-35.
40. Yildiz M, Li H, Bernard D, Amin NA, Ouillette P, Jones S, et al. Lymphoid neoplasia: Activating stat6 mutations in follicular lymphoma. Blood. 2015;125:668-79.
41. Krysiak K, Gomez F, White BS, Matlock M, Miller CA, Trani L, et al. Recurrent somatic mutations affecting B-cell receptor signaling pathway genes in follicular lymphoma.

Blood. 2017;129:473-83.
42. Okosun J, Wolfson RL, Wang J, Araf S, Wilkins L, Castellano BM, et al. Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. Nat Genet. 2016;48:183-8.
43. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science (80-). 2008;319:1676-9.
44. Che T, You Y, Wang D, Tanner MJ, Dixit VM, Lin X. MALT1/Paracaspase Is a Signaling Component Downstream of CARMA1 and Mediates T Cell Receptor-induced NF-κB Activation. J Biol Chem. 2004;279:15870-6.
45. Saijo K, Schmedt C, Su I hsin, Karasuyama H, Lowell CA, Reth M, et al. Essential role of Src-family protein tyrosine kinases in NF-κB activation during B cell development. Nat Immunol. 2003;4:274-9.
46. Mary Photini S, Chaiwangyen W, Weber M, Al-Kawlani B, Favaro RR, Jeschke U, et al. PIM kinases 1, 2 and 3 in intracellular LIF signaling, proliferation and apoptosis in trophoblastic cells. Exp Cell Res. 2017;359:275-83.
47. Maurer U, Charvet C, Wagman AS, Dejardin E, Green DR. Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. Mol Cell. 2006;21:749-60.
48. Kong HJ, Anderson DE, Lee CH, Jang MK, Tamura T, Tailor P, et al. Cutting Edge: Autoantigen Ro52 Is an Interferon Inducible E3 Ligase That Ubiquitinates IRF-8 and Enhances Cytokine Expression in Macrophages. J Immunol. 2007;179:26-30.
49. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: Somatic cancer genetics at high-resolution. Nucleic Acids Res. 2017;45:D777-83.
50. Kumar E, Pickard L, Okosun J. Pathogenesis of follicular lymphoma: genetics to the microenvironment to clinical translation. Br J Haematol. 2021:1-12.
51. Pickard L, Palladino G, Okosun J. Follicular lymphoma genomics. Leuk Lymphoma. 2020;61:2313-23.
52. Korfi K, Ali S, Heward JA, Fitzgibbon J. Follicular lymphoma, a B cell malignancy addicted to epigenetic mutations. Epigenetics. 2017;12:370-7.
53. Zhang J, Vlasevska S, Wells VA, Nataraj S, Holmes AB, Duval R, et al. The CREBBP acetyltransferase is a haploinsufficient tumor suppressor in B-cell lymphoma. Cancer Discov. 2017;7:323-37.
54. Zhang J, Dominguez-Sola D, Hussein S, Lee JE, Holmes AB, Bansal M, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. Nat Med. 2015;21:1190-8.
55. Béguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, et al. EZH2 Is Required for Germinal Center Formation and Somatic EZH2 Mutations Promote Lymphoid Transformation. Cancer Cell. 2013;23:677-92.
56. Hsi ED, Jung SH, Lai R, Johnson JL, Cook JR, Jones D, et al. Ki67 and PIM1 expression predict outcome in mantle cell lymphoma treated with high dose therapy,

stem cell transplantation and rituximab: A Cancer and Leukemia Group B 59909 correlative science study. Leuk Lymphoma. 2008;49:2081-90.

57. Rossi C, Tosolini M, Gravelle P, Pericart S, Kanoun S, Evrard S, et al. Baseline SUVmax is related to tumor cell proliferation and patient outcome in follicular lymphoma. Haematologica. 2020;Online ahe.
58. Ennishi D, Healy S, Bashashati A, Saberi S, Hother C, Mottok A, et al. TMEM30A loss-of-function mutations drive lymphomagenesis and confer therapeutically exploitable vulnerability in B-cell lymphoma. Nat Med. 2020;26:577-88.
59. Saez B, Walter MJ, Graubert TA. Splicing factor gene mutations in hematologic malignancies. Blood. 2017;129:1260-9.
60. Suzuki H, Kumar SA, Shuai S, Diaz-Navarro A, Gutierrez-Fernandez A, De Antonellis P, et al. Recurrent noncoding U1 snRNA mutations drive cryptic splicing in SHH medulloblastoma. Nature. 2019;574:707-11.
61. Kuo HP, Ezell SA, Hsieh S, Schweighofer KJ, Cheung LWK, Wu S, et al. The role of PIM1 in the ibrutinib-resistant ABC subtype of diffuse large B-cell lymphoma. Am J Cancer Res. 2016;6:2489-501.
62. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476:298-303.
63. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature. 2010;463:88-92.
64. Jones D, Woyach JA, Zhao W, Caruthers S, Tu H, Coleman J, et al. PLCG2 C2 domain mutations co-occur with BTK and PLCG2 resistance mutations in chronic lymphocytic leukemia undergoing ibrutinib treatment. Leukemia. 2017;31:1645-7.
65. Novice T, Kariminia A, Del Bel KL, Lu H, Sharma M, Lim CJ, et al. A Germline Mutation in the C2 Domain of PLCγ2 Associated with Gain-of-Function Expands the Phenotype for PLCG2-Related Diseases. J Clin Immunol. 2020;40:267-76.
66. Liu TM, Woyach JA, Zhong Y, Lozanski A, Lozanski G, Dong S, et al. Hypermorphic mutation of phospholipase C, γ2 acquired in ibrutinib-resistant CLL confers BTK independency upon B-cell receptor activation. Blood. 2015;126:61-8.
67. Burger JA, Landau DA, Taylor-Weiner A, Bozic I, Zhang H, Sarosiek K, et al. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. Nat Commun. 2016;7.
68. Wenzel SS, Grau M, Mavis C, Hailfinger S, Wolf A, Madle H, et al. MCL1 is deregulated in subgroups of diffuse large B-cell lymphoma. Leukemia. 2013;27:1381-90.
69. James LC, Keeble AH, Khan Z, Rhodes DA, Trowsdale J. Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. Proc Natl Acad Sci U S A. 2007;104:6200-5.
70. Oke V, Wahren-Herlenius M. The immunobiology of Ro52 (TRIM21) in autoimmunity: A critical review. J Autoimmun. 2012;39:77-82.

71. Trabuco LG, Lise S, Petsalaki E, Russell RB. PepSite: Prediction of peptide-binding sites from protein surfaces. Nucleic Acids Res. 2012;40.
72. Edelmann J, Holzmann K, Miller F, Winkler D, Bühler A, Zenz T, et al. High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. Blood. 2012;120:4783-94.
73. Rivas-Delgado A, Nadeu F, Enjuanes A, Casanueva-Eliceiry S, Mozas P, Magnano L, et al. Mutational landscape and tumor burden assessed by cell-free DNA in diffuse large B-cell lymphoma in a population-based study. Clin Cancer Res. 2021;27:513-21.
74. Li H. Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34:3094-100.
75. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012;22:568-76.
76. Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, et al. LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res. 2012;40:11189-201.
77. Muller E, Goardon N, Brault B, Rousselin A, Paimparay G, Legros A, et al. OutLyzer: Software for extracting low-allele-frequency tumor mutations from sequencing background noise in clinical practice. Oncotarget. 2016;7:79485-93.
78. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing 2012.
79. Puente XS, Beà S, Valdés-Mas R, Villamor N, Gutiérrez-Abril J, Martín-Subero JI, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature. 2015;526:519-24.
80. Ramis-Zaldivar JE, Gonzalez-Farre B, Nicolae A, Pack S, Clot G, Nadeu F, et al. MAP-kinase and JAK-STAT pathways dysregulation in plasmablastic lymphoma. Haematologica. 2021.

Discusión

La población de pacientes con LF presenta una marcada heterogeneidad biológica y clínica. Mientras que en algunos casos el diagnóstico se realiza de manera incidental, el paciente permanece asintomático y sin requerir tratamiento durante años, en otros el inicio de la enfermedad se produce con marcadores analíticos de alta carga tumoral, afectación adenopática y extraganglionar extensa y necesidad de tratamiento sin demora. Por otra parte, una vez completado el tratamiento, algunos pacientes presentan una duración de la respuesta muy prolongada sin requerir ninguna otra intervención para su linfoma, otros presentan recaídas tardías durante el seguimiento, en torno a un 20% de los pacientes presenta una recaída precoz y una minoría son primariamente refractarios al esquema de primera línea.

Tal y como se ha expuesto, durante las últimas décadas se han llevado a cabo esfuerzos para identificar factores individuales o diseñar índices pronósticos combinados que permitan refinar la estimación pronóstica de los pacientes con LF e, idealmente, seleccionar el régimen terapéutico que permita maximizar las opciones de una respuesta duradera y minimizar el riesgo de toxicidad asociada con el tratamiento. Actualmente, y con contadas excepciones, todos los pacientes con LF en estadio avanzado y alta carga tumoral se tratan con regímenes de IQT, sin considerar el riesgo estimado según factores o índices pronósticos. Esto puede ser explicado por varias razones: 1) que la supervivencia global de la mayoría de los pacientes con LF es muy prolongada, lo que hace difícil justificar opciones terapéuticas más intensivas en primera línea, 2) que son escasos los estudios aleatorizados enfocados a subgrupos de riesgo específicos que permitan evaluar la utilidad de intervenciones dirigidas y 3) que la multitud de índices y factores pronósticos sin clara superioridad entre ellos hace difícil su conocimiento y aplicación por el hematólogo general, con la consiguiente dificultad de que lleguen al terreno de los ensayos clínicos.

Sin embargo, con el avance de los datos clínicos, analíticos, genómicos, de imagen y de la inteligencia artificial, parece cada vez más realista caracterizar de manera precisa la población de pacientes con LF y generar una predicción individualizada del riesgo de cada paciente para adaptar la estrategia terapéutica y mejorar los resultados. En los estudios que agrupa la presente tesis se han analizado cinco tipos de elementos (históricos, demográficos, hematimétricos, inmunoquímicos y genómicos) con el objetivo de establecer su relevancia pronóstica en LF y contribuir al citado proceso.

En el análisis histórico de nuestra cohorte de 727 pacientes con LF diagnosticados a lo largo de las últimas cuatro décadas observamos que los pacientes diagnosticados en el último periodo tenían una edad más avanzada, pero presentaban una mejor situación funcional. Esta tendencia refleja probablemente cambios en la población de referencia de nuestra institución, además de la realización de pruebas diagnósticas por otros motivos a individuos asintomáticos de edad avanzada, que podrían ayudar a identificar la enfermedad en pacientes mayores. En lo referente al tratamiento, la introducción de la inmunoterapia ha causado que el esquema R-CHOP se convierta en el más empleado en nuestra serie durante las últimas dos décadas del estudio. Este cambio en el tratamiento explica, con toda probabilidad, junto a la evolución del tratamiento de soporte, la mejoría de los resultados.

La proporción de pacientes en RC después del tratamiento aumentó sustancialmente a lo largo de las décadas. Aunque es indiscutible que la inmunoterapia ha contribuido a estos resultados, la generalización en el uso del PET-TC puede haber constituido un factor adicional, considerando como RC con masa residual aquellas lesiones morfológicamente patológicas, pero sin actividad metabólica. La profundización de las respuestas se tradujo en una mejoría de la SLP y SG, como ya han apuntado estudios multicéntricos en la era rituximab[15]. Algunos estudios unicéntricos y de registro habían evidenciado ya una mejoría de la supervivencia de los pacientes con LF respecto a la de la población general[7,9]. Estos resultados se confirman en nuestra serie, con una mejoría de la supervivencia relativa a 5 años de 0.83 a 0.94. Resultados similares son los publicados en un reciente estudio poblacional con más de 12 000 pacientes con LF[193], en el que se evidencia una mejoría en la supervivencia durante los últimos años, especialmente en pacientes mayores con LF en estadio avanzado.

En cuanto a las causas de muerte de los pacientes con LF, aunque la proporción de pacientes que fallecen por progresión del linfoma ha disminuido a lo largo de las décadas, la mayoría (55%) muere por causas relacionadas con la enfermedad (progresión y toxicidad del tratamiento), algo que se alinea con los hallazgos de estudios en amplias series de pacientes[191]. A pesar de lo reportado en un gran estudio multicéntrico reciente[44], en nuestro estudio no pudimos identificar diferencias significativas en la incidencia de TH entre las cuatro décadas, probablemente debido a la baja frecuencia de eventos de este tipo en cada uno de los periodos.

La aparición de SN es una de las principales preocupaciones en el empleo de la quimioterapia, que se añade a la potencial situación de inmunodepresión conferida por la propia neoplasia. En la era del rituximab, la incidencia acumulada de SN se ha situado en torno al 3% a 10 años[191],

mientras que en nuestra serie es de un 10% a 10 años, una variabilidad que puede ser explicada por el pequeño número de eventos.

Debemos reconocer algunas limitaciones de nuestro estudio. En primer lugar, las características iniciales no fueron totalmente equiparables entre las décadas, lo que en sí mismo ya constituye un resultado relevante. En efecto, el hecho de que los pacientes de la última década tuvieran una edad más avanzada subraya aún más la mejoría en los resultados a lo largo del tiempo, puesto que se esperaría que los individuos más mayores tuvieran una supervivencia inferior. En segundo lugar, por razones históricas, el número de pacientes incluidos en la primera década, la disponibilidad de muestra histológica y la causa de muerte fue menor. Por último, teniendo en cuenta la historia natural de la enfermedad, sería de utilidad un análisis de la segunda y posteriores líneas de tratamiento para caracterizar los patrones de recaída y los resultados. Como fortalezas del estudio cabe destacar, sin embargo, el gran número de pacientes de un solo centro incluidos en el estudio, diagnosticados y tratados de manera homogénea en cada década, el grado de detalle de los datos clínicos y de seguimiento, así como el uso de herramientas estadísticas robustas (riesgos competitivos y supervivencia relativa).

En nuestro segundo trabajo quisimos explorar el impacto de la edad, la comorbilidad y de la interacción entre ellas, en la supervivencia de los pacientes con LF. La mayoría de los índices pronósticos consideran la edad como uno de los elementos de riesgo adverso[56,60–62], mientras que el reciente PRIMA-PI[67] solo tiene en cuenta los niveles de B2M y la infiltración de MO. Cuando analizamos la distribución de los índices pronósticos según franjas de edad, los pacientes mayores presentaban índices FLIPI y FLIPI2 de más alto riesgo, y lo siguieron siendo cuando no consideramos la edad para el cálculo, lo que refleja una biología tumoral más agresiva en pacientes de edad avanzada, algo que ya ha sido descrito[79].

Consideramos particularmente interesante el análisis de los diferentes *endpoints* relacionados con la duración de la respuesta en base a las franjas de edad. Por convenio, la SLP se calcula como el tiempo transcurrido entre la primera dosis del tratamiento de primera línea y la progresión, recaída o muerte por cualquier causa. La incidencia acumulada de recaída es un *endpoint* que solo considera como evento la progresión o recaída, mientras que la incidencia acumulada de muerte sin recaída (*non-relapse mortality*) toma como evento la muerte por cualquier causa. Al analizar la SLP en base a la edad, los pacientes más mayores presentaban una SLP inferior, pero este hallazgo se debía a una mayor incidencia acumulada de muerte sin recaída, con una incidencia acumulada de recaída similar. Estos resultados ponen de manifiesto que la duración de la

respuesta en los pacientes mayores no es inferior, y que por lo tanto pueden beneficiarse de recibir tratamiento activo independientemente de su edad.

Como era esperable y se había publicado previamente[77], la edad más avanzada se relaciona con una SG inferior. Al analizar las causas de muerte, la proporción de pacientes fallecidos por causas relacionadas con el linfoma (progresión y toxicidad) fue mayor entre los pacientes más jóvenes. Además, el exceso de mortalidad respecto a la población general en el global de pacientes fue del 15%, pero este impacto fue más marcado en pacientes mayores (30%), lo que subraya la necesidad de diseñar una estrategia terapéutica adaptada al riesgo y a la esperanza de vida para todos los pacientes diagnosticados de LF, como se ha propuesto por otros autores[194]. Estos hallazgos se alinean con el estudio recientemente publicado por Dinnessen y colaboradores[193], en el que se evidencia un exceso de mortalidad más marcado entre los pacientes con LF en estadio avanzado y, especialmente, los mayores de 70 años.

El segundo estudio que compone la presente tesis es, hasta donde sabemos, el más amplio de los que analizan la comorbilidad en pacientes con LF. La mediana de edad avanzada de los pacientes diagnosticados con esta enfermedad hace que la fragilidad y la comorbilidad sean elementos claves en su manejo. Más de la mitad de los pacientes de nuestra serie no presentaba ninguna comorbilidad relevante, algo que va en la línea de lo publicado en el único antecedente sobre el tema[65]. La presencia de comorbilidad moderada-grave condicionó la elección de tratamientos menos intensivos y, a pesar de una incidencia acumulada de recaída similar, la presencia de comorbilidad supuso una reducción de la SG, que se mantuvo en el análisis multivariado. A pesar de que creemos en el impacto pronóstico de la comorbilidad y en su importancia en la elección del tratamiento, consideramos que su evaluación debería ser estandarizada y validada antes de su inclusión en nuevos índices pronósticos.

La comorbilidad también tiene un impacto relevante sobre las causas de muerte. Entre los pacientes con comorbilidad, la mayoría de los pacientes murió por causas no relacionadas con el linfoma, mientras que esta proporción se invirtió entre aquellos sin comorbilidad o en los que esta era leve. El exceso de mortalidad respecto a la población general también fue mayor en los pacientes comórbidos. Por último, y probablemente debido a la existencia de factores de riesgo comunes entre la comorbilidad y las segundas neoplasias, la incidencia acumulada de segundas neoplasias fue significativamente mayor entre los pacientes que tenían otras enfermedades aparte del linfoma.

Si las características demográficas del paciente y genéticas del tumor son factores relevantes en el desarrollo y progresión del LF, tanto o más lo es la configuración del microambiente tumoral, compuesto principalmente por linfocitos, macrófagos y células estromales. Los linfocitos y monocitos de la sangre periférica se han considerado por algunos autores los representantes circulantes de las células del microambiente[195] y, atendiendo a su accesibilidad, ha crecido el interés en estudiar parámetros hematimétricos con potencial impacto pronóstico en linfomas, como la ratio linfocito/monocito (LMR)[196–202]. Los tres estudios publicados previamente sobre el LMR en LF no evaluaban su impacto sobre la SG o no mostraron diferencias en este *endpoint*, por lo que nuestro objetivo fue explorar dicho parámetro en una serie amplia de pacientes diagnosticados en la época del rituximab.

El grupo de pacientes con un LMR por debajo del punto de corte [(2.5) calculado mediante la técnica de *maximally selected rank statistics* y validado mediante *bootstrapping*] constituía un 20% de la población del estudio y presentaba una edad más avanzada y marcadores de más alta carga tumoral. Tras recibir esquemas de tratamiento similares, los pacientes con un LMR bajo presentaron una inferior tasa de respuestas completas y una SLP más corta, en línea con estudios previos[170–172]. Sin embargo, el impacto pronóstico era aún más evidente sobre la SG: los pacientes con un LMR bajo tenían una SG a 10 años de menos de la mitad que la de los pacientes con un LMR >2.5. Para evitar atribuir el impacto pronóstico de este parámetro exclusivamente a la edad más avanzada de los pacientes con LMR bajo, realizamos un análisis por subgrupos etarios y un análisis multivariado, que confirmaron nuestros hallazgos. Un LMR bajo fue también capaz de predecir un mayor riesgo de TH y de SN.

Dada la importancia de los parámetros hematimétricos en la evolución de los pacientes con LF, se han realizado intentos de incorporar dicha información a índices pronósticos establecidos: el AMC-FLIPI[168] consideraba la monocitosis (≥5.7 $x10^9$/L) como factor de riesgo añadido a los del FLIPI, mientras que el FLIPI-L[70] atribuía un punto adicional a los pacientes con linfopenia (<1$x10^9$/L). Hasta la fecha ningún estudio ha combinado índices pronósticos clásicos con el LMR. Si bien consideramos que un estudio unicéntrico sin cohorte de validación no debería proponer otro nuevo índice pronóstico, hicimos el ejercicio de combinar el FLIPI (bajo-intermedio-alto) con el LMR (≤/>2.5) para generar seis categorías de pacientes y analizar su SG. Observamos que el LMR aportaba información pronóstica en las tres categorías del FLIPI, por lo que la exploración de este parámetro en series externas de pacientes podría ser de interés. En este sentido, un grupo egipcio ha publicado recientemente un estudio[203] sobre el impacto pronóstico del recuento linfocitario, recuento monocitario y LMR en una serie de 100 pacientes con LF. A pesar de que el

punto de corte (1.63) fue obtenido mediante una curva ROC, que no son idóneas en la categorización de variables cuantitativas para predecir supervivencia, un LMR bajo se asoció con una SG disminuida en el análisis uni y multivariado.

Por el momento, no comprendemos por completo las interacciones biológicas que subyacen a este claro impacto pronóstico. Algunos grupos han propuesto que la secreción de citocinas por parte del microambiente podría generar un aumento en el número de monocitos, lo que a su vez podría comprometer la inmunidad antitumoral y favorecer la progresión de la enfermedad[167]. En este sentido, será de especial importancia establecer la correspondencia entre las poblaciones circulantes y las del ganglio de pacientes con LF. Por otra parte, la linfopenia y, por ende, un LMR bajo podrían reflejar un repertorio disminuido de linfocitos T específicos contra el tumor y una inmunidad antitumoral deficiente. Además, puesto que el rituximab depende de los linfocitos para ejercer citotoxicidad celular dependiente de anticuerpo, la linfopenia también podría comprometer la eficacia de la inmunoterapia antiCD20.

En un artículo de correspondencia[204] publicado en respuesta al artículo que forma parte de la tesis, Sorigué y Sancho sugirieron que el impacto pronóstico y, por lo tanto, la aplicabilidad clínica del LMR podría depender del régimen terapéutico, puesto que de él depende también la composición del microambiente[205]. Cuando analizamos por separado los pacientes tratados con R-CHOP/R-COP y aquellos que recibieron R-bendamustina o R-FCM[206], se mantuvo el impacto del LMR sobre la SG. Esto ha sido recientemente validado en una serie japonesa de 87 pacientes con LF tratados con R-bendamustina[207]. En cambio, entre los pacientes que recibieron un esquema sin quimioterapia, no hubo diferencias significativas en la SG. Una explicación para este hallazgo, aparte del número relativamente bajo de pacientes en este grupo, es que, en nuestro medio, los pacientes tratados con rituximab en monoterapia tienen por lo general baja carga tumoral, y solo siete pacientes de este grupo tenían un LMR ≤2.5, disipando así el potencial impacto que podría tener el LMR en el contexto de un ensayo clínico aleatorizado.

Otra de las cuestiones sugeridas por Sorigué y Sancho fue la potencial capacidad del LMR de predecir el tiempo hasta el primer tratamiento (THPT) en aquellos pacientes inicialmente considerados candidatos a un manejo expectante. Entre los 119 pacientes que iniciaron abstención terapéutica, la mediana de THPT fue de 2.5 años, y el LMR no fue útil en su predicción. Una vez más, entre los pacientes inicialmente considerados candidatos a abstención, solo una minoría tendrán un LMR bajo, por lo que este parámetro no es un buen discriminador dentro del grupo.

También en la línea del estudio del microambiente desde un punto de vista clinicoanalítico se sitúa la hipótesis del cuarto trabajo. Es bien conocido que algunos síndromes linfoproliferativos B como la leucemia linfocítica crónica o ciertos linfomas pueden presentar en suero una inmunoglobulina monoclonal[208]. De hecho, la presencia de una inmunoglobulina monoclonal IgM en suero es uno de los datos característicos y criterios diagnósticos de la macroglobulinemia de Waldenström[209]. Décadas más tarde de la primera descripción de un componente monoclonal sérico (CMs) en neoplasias linfoides B, estudios posteriores han corroborado su existencia en varios tipos de neoplasias e impacto pronóstico adverso en algunas de ellas, particularmente en LBDCG[210–216] pero, hasta donde sabemos, esta característica no se había estudiado en pacientes con LF. La presencia de una proteína monoclonal circulante en el suero de los pacientes con LF podría traducir un mayor grado de actividad de las células tumorales o un estado de diferenciación particular del linfocito B neoplásico. Nuestro objetivo fue explorar la prevalencia de una inmunofijación sérica (IFs) positiva medida por electroforesis y describir su correlación con características iniciales y potencial impacto pronóstico.

En primer lugar, evidenciamos que en torno a un cuarto de los pacientes con LF presentaba una IFs positiva (IFs+) en el momento del diagnóstico del LF, lo cual supone una prevalencia similar a las observadas en otros linfomas indolentes, como el de la zona marginal[216]. Los pacientes que presentaban una IFs+ presentaban características iniciales similares a las de aquellos con una IFs negativa (IFs−), salvo una edad más avanzada y niveles más elevados de B2M. En lo que respecta al análisis inmunoquímico, el isotipo de cadena pesada más frecuente fue IgG, con una distribución similar entre cadenas ligeras κ y λ, algo que contrasta con el predominio del isotipo IgM en otros estudios de linfoma indolente, probablemente por la inclusión en ellos de un número significativo de linfomas linfoplasmocíticos[211], o por el número reducido de pacientes[217]. Algo que distingue la proteína secretada por este subgrupo de LF de otras gammapatías monoclonales es su escasa cantidad: en la mayoría de los casos no se evidenció un CMs medible, y aquellos en los que sí lo era, se encontraba en baja concentración (<4 g/L).

Un fenómeno que invita a la reflexión es la discordancia, tanto topográfica como cronológica, evidenciada en algunos casos. Por la primera entendemos una cadena ligera diferente entre la inmunoglobulina sérica (evaluada por inmunofijación) y aquella expresada por las células tumorales en el ganglio (evaluada por inmunohistoquímica). Por la segunda, en cambio, la positividad para una inmunoglobulina monoclonal diferente (en su cadena ligera y/o pesada) en el momento del diagnóstico y durante el seguimiento (ya sea en la reevaluación tras el tratamiento

o en el momento de la recaída). Además, una proporción de pacientes presentaba una IFs positiva para más de un isotipo de inmunoglobulina (cadena pesada y/o ligera). Aunque no pudimos evaluar el impacto pronóstico de la discordancia topográfica o cronológica por un pequeño número de casos y la presencia de sesgos inaceptables, observamos que los casos con una IFs biclonal presentaban un peor pronóstico. Las tres situaciones comentadas anteriormente podrían formar parte de un espectro de desregulación inmunológica asociado a la biología de los linfocitos tumorales y a su interacción con el microambiente, aunque no existen argumentos a favor de esta hipótesis.

Aunque los estudios realizados en pacientes con linfoma indolente en la época previa al rituximab atribuyeron a la presencia de un CMs un impacto pronóstico favorable[211], las últimas publicaciones en LBDCG[213–215,218] y la nuestra lo sitúan como un factor de mal pronóstico. En nuestra serie, los pacientes con una IFs+ presentaron mayor riesgo de recaída precoz y una SLP y SG inferior. Esta discrepancia con el estudio de Economopoulos y colaboradores[211] podría explicarse por la heterogeneidad histológica de los pacientes incluidos en el estudio y por un posible papel predictivo (además de pronóstico) de la presencia de una IFs+ en pacientes tratados con regímenes que incluyen rituximab.

Como se ha comentado previamente, no conocemos ningún estudio que haya descrito previamente el aumento en la prevalencia de una IFs+ con la edad en pacientes con linfoma. Sin embargo, es bien conocido que la incidencia de gammapatías (y en especial las de significado incierto, o GMSI) es más alta entre individuos de edad más avanzada[219]. La frecuencia claramente superior en nuestra serie a la de la GMSI en la población general[220], sumada al hecho de que ningún caso de nuestro estudio haya presentado una progresión a mieloma múltiple hacen descartar razonablemente la hipótesis de que se trate de un diagnóstico concomitante de un LF y una GMSI y apoyan la idea de que la inmunoglobulina monoclonal sea secretada por las células tumorales, posiblemente con la cooperación del microambiente.

Cuando evaluamos de la IFs+ en la SLP y SG por subgrupos de edad, observamos que este se mantenía solo en los pacientes mayores, posiblemente debido a la baja frecuencia de una IFs+ entre los pacientes jóvenes. Este impacto pronóstico se mantuvo además en un análisis multivariado en pacientes mayores de 60 años que incluía otros factores clínicos relevantes. Con estos datos podemos concluir que existe una asociación entre la edad y la IFs, pero el impacto pronóstico adverso de la IFs+ no se debe únicamente a una edad más avanzada de los pacientes.

Nuestro trabajo presenta las limitaciones inherentes a un estudio unicéntrico, observacional y retrospectivo. Aunque estos hallazgos apuntan a un posible papel de la IFs como marcador fácilmente accesible de la biología del linfocito B, la desregulación inmune y el microambiente, se requieren más estudios que validen los resultados y profundicen en aspectos concretos como la caracterización de la población que secreta la inmunoglobulina, la evolución de la IFs tras el tratamiento y en la recaída, así como su papel predictivo en la respuesta a nuevos tratamientos como la lenalidomida.

El quinto y último de los trabajos que componen la tesis se centra en el estudio genómico de pacientes diagnosticados de LF con un comportamiento clínico concreto. Como se ha comentado previamente, se recomienda la abstención terapéutica para los pacientes en estado avanzado sin criterios de alta carga tumoral[27,32], la recaída precoz o la refractariedad primaria comprometen drásticamente la supervivencia de los pacientes[35,183], mientras que una respuesta prolongada proporciona una esperanza de vida similar a la de la población general[184]. Sin embargo, la identificación de alteraciones genéticas en los estudios de cohortes no seleccionadas está limitada por la pequeña representación de pacientes con comportamientos clínicos concretos.

En nuestro estudio, efectuamos un análisis de alteraciones en el número de copias (CNA) y de mutaciones (secuenciación masiva) sobre muestras de ganglio linfático obtenidas al diagnóstico y en la recaída. El perfil de CNA identificado fue el típico de LF, siendo las alteraciones más frecuentes las de 1p36.33, 16p13, 1q y la trisomía 7. Aunque estudios recientes han descrito que una mayor complejidad genómica se asocia con un peor pronóstico en LF[131], no pudimos demostrar diferencias en el perfil del número de copias o en la complejidad genética en base al comportamiento clínico. Sin embargo, en la búsqueda de CNA *driver* identificamos cinco de ellas que eran importantes en la patogenia del LF: 1p36.32 (*TNFRSF14*), 6p21.32 (*HLA*), 6q14.1 (*TMEM30A*), 6q23.3 (*TNFAIP3*), 9p21.3 (*CDKN2A/B*) y 10q23.33 (*PTEN*).

En el perfil mutacional evaluado mediante secuenciación masiva, observamos que los casos incluidos en el estudio presentaban alteraciones en modificadores epigenéticos (*KMT2D*, *EP300*, *CREBBP*, *EZH2*, *ARID1A*), proliferación y apoptosis (*BCL2, TNFRSF14*), y señalización del (*TNFAIP3*), lo que concuerda con estudios previos[134,221,222]. Observamos también que las alteraciones en modificadores epigenéticos como *KMT2D, EP300, CREBBP, HIST1HE, BCL7A*, y en genes relacionados con la apoptosis (*TNFRSF14)*, son eventos precoces en la evolución de la enfermedad, mientras que las alteraciones en la ubiquitinación y señalización (*SOCS1*, *CARD11*, *CD79B* y *PIM1*) aparecen de manera más tardía. Esto corrobora los hallazgos de estudios previos

que evidencian que las células precursoras comunes (CPC) están enriquecidas en mutaciones de *CREBBP* y *KMT2D*, que por lo tanto son consideradas como eventos precoces *driver*. Aunque las alteraciones en modificadores epigenéticos son precoces y muy prevalentes en LF, no son suficientes para dar lugar a un linfoma[223–225]. Como hallazgo destacable, detectamos la coocurrencia de ciertas alteraciones genéticas, como la de *PIM1*, *CD79B* y *BTG2*. Esta potencial cooperación puede ser de relevancia, puesto que afecta a varias vías de señalización [JAK-STAT (*PIM1*), BCR (*CD79B*) y proliferación (*BTG2*)] y es más frecuente en casos con una recaída precoz, lo que podría indicar un posible papel en el comportamiento clínico de este grupo de pacientes.

A pesar del número reducido de casos incluidos en nuestro estudio, observamos que ciertas alteraciones genéticas (mutaciones o CNA) estaban asociadas con un comportamiento clínico concreto. Así, encontramos más frecuentemente las alteraciones de *CARD11* y *JUNB* en pacientes que nunca requirieron tratamiento, mientras que las de *PIM1* se asociaron con una duración de la respuesta más corta (casos recaídos precoces o primariamente refractarios. Además, las mutaciones en *FOXO1* y en *TMEM30A* se asociaron con una inferior SG. Estos hallazgos son compatibles con observaciones en otros contextos[226–228] y, aunque preliminares, abren una vía para su mayor exploración en grandes cohortes de pacientes.

Los estudios filogenéticos de muestras pareadas entre el diagnóstico y la recaída han mostrado diferentes patrones (lineal/secuencial, divergente y complejo)[133]. A pesar del número reducido de casos con muestras pareadas en nuestro estudio, identificamos la presencia de CPC ancestrales en todos los casos, además de alteraciones genómicas específicas del diagnóstico o de la recaída, lo que sugiere una evolución divergente en todos los pacientes analizados. Además, los casos que presentaban una recaída precoz mostraban un número más alto de alteraciones compartidas que aquellos con una recaída tardía, lo que corrobora los hallazgos de Kridel y colaboradores[138], quienes describieron que las recaídas precoces están causadas por clones ya detectables en le momento del diagnóstico, con una dinámica clonal poco marcada.

Aunque las tecnologías de secuenciación masiva han permitido ampliar el conocimiento sobre el perfil genético del LF, las mutaciones no codificantes siguen siendo poco conocidas. Recientemente se ha descrito que las mutaciones en el RNA nuclear pequeño U1 (específicamente, en la tercera base: g.3A<C) están presentes en ciertos tumores sólidos y en la leucemia linfocítica crónica (LLC)[229,230]. En nuestro estudio no observamos esta mutación en ninguna muestra correspondiente al diagnóstico, y solamente en una muestra a la recaída, lo que

sugiere un escaso papel de la mutación en LF, pero una potencial relevancia en la progresión de la enfermedad.

Finalmente, en el estudio del posible impacto funcional de las mutaciones de *PIM1*, *CD79B, PLCG2*, *MCL1* e *IRF8*, observamos ciertas alteraciones que ya habían sido previamente descritas en neoplasias linfoides, mientras que otras fueron novedosas en este campo, en lo que respecta a una posible ganancia o pérdida de función o mecanismos de resistencia a tratamientos dirigidos.

Conclusiones

1. A lo largo de las últimas cuatro décadas, los pacientes con LF han experimentado un aumento en la tasa de respuestas completas tras el tratamiento de primera línea.
2. Se ha evidenciado una clara mejoría de la supervivencia libre de progresión, global y relativa, así como de la incidencia acumulada de muerte por progresión del linfoma.
3. La proporción de muertes debidas al linfoma y la incidencia de transformación histológica y segundas neoplasias ha permanecido estable en los últimos tiempos.

4. La edad avanzada y la comorbilidad moderada-grave predicen una supervivencia libre de progresión y global inferiores, a pesar de una incidencia acumulada de recaída similar.
5. La proporción de muertes relacionadas con el linfoma (progresión o toxicidad) es más alta entre los pacientes jóvenes o aquellos sin comorbilidad o con comorbilidad leve.
6. El exceso de mortalidad respecto a la población general es mayor en pacientes mayores o comórbidos.

7. Los pacientes con una ratio linfocito-monocito (LMR) ≤2.5 al diagnóstico presentan una edad más avanzada, peor situación funcional, marcadores de más alta carga tumoral y un índice FLIPI de más alto riesgo.
8. Un LMR bajo se asocia con una tasa más baja de respuestas completas y predice una supervivencia libre de progresión y global inferiores.
9. Los pacientes con LMR bajo tienen mayor riesgo de sufrir una transformación histológica y segundas neoplasias.

10. Alrededor de un cuarto de los pacientes con LF presenta una inmunofijación sérica positiva al diagnóstico, más frecuentemente IgG-κ o IgG-λ, con un componente monoclonal no medible o de escasa cuantía.
11. La prevalencia de una inmunofijación sérica positiva aumenta con la edad y se asocia con niveles elevados de β_2-microglobulina.
12. Los pacientes con una inmunofijación sérica positiva presentan una supervivencia libre de progresión y global inferiores, y este impacto es más marcado en los pacientes mayores.

13. Las alteraciones genéticas más frecuentes en nuestra serie de pacientes con LF fueron las de *KMT2D*, *CREBBP*, *TNFRSF14*, *BCL2* y 1p36.33.
14. Ciertas alteraciones genéticas se asociaron con la necesidad de tratamiento (*CARD11* y *JUNB*), mientras que otras con la duración de la respuesta (*PIM1*) o con la supervivencia (*FOXO1* y *TMEM30A*).
15. Identificamos *hotspots* mutacionales conocidos y otros no descritos previamente en el estudio de impacto funcional (modelado proteico) de genes como *CD79B*, *PLCG2*, *PIM1*, *MCL1* e *IRF8*, lo cual puede explicar la ganancia o pérdida de función y la posible resistencia a terapias dirigidas.

Bibliografía

1. Bastos-Oreiro M, Muntañola A, Panizo C, Gonzalez-Barca E, de Villambrosia SG, Córdoba R, et al. RELINF: prospective epidemiological registry of lymphoid neoplasms in Spain. A project from the GELTAMO group. Ann Hematol. 2020;99:799-808.
2. Mounier M, Bossard N, Belot A, Remontet L, Iwaz J, Dandoit M, et al. Trends in excess mortality in follicular lymphoma at a population level. Eur J Haematol. 2015;94:120-9.
3. Horning SJ. Natural history of and therapy for the indolent non-Hodgkin's lymphomas. Semin Oncol. 1993;20:75-88.
4. Fisher RL, LeBlanc M, Press OW, Maloney DG, Unger JM, Miller TP. New treatment options have changed the survival of patients with follicular lymphoma. J Clin Oncol. 2005;23:8447-52.
5. Swenson WT, Wooldridge JE, Lynch CF, Forman-Hoffman VL, Chrischilles E, Link BK. Improved survival of follicular lymphoma patients in the United States. J Clin Oncol. 2005;23:5019-26.
6. Liu Q, Fayad L, Cabanillas F, Hagemeister FB, Ayers GD, Hess M, et al. Improvement of overall and failure-free survival in stage IV follicular lymphoma: 25 years of treatment experience at the University of Texas M.D. Anderson Cancer Center. J Clin Oncol. 2006;24:1582-9.
7. Tan D, Horning SJ, Hoppe RT, Levy R, Rosenberg SA, Sigal BM, et al. Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. Blood. 2013;122:981-7.
8. Shi Q, Flowers CR, Hiddemann W, Marcus R, Herold M, Hagenbeek A, et al. Thirty-month complete response as a surrogate end point in first-line follicular lymphoma therapy: An individual patient-level analysis of multiple randomized trials. J Clin Oncol. 2017;35:552-60.
9. Junlén HR, Peterson S, Kimby E, Lockmer S, Lindén O, Nilsson-Ehle H, et al. Follicular lymphoma in Sweden: nationwide improved survival in the rituximab era, particularly in elderly women: a Swedish Lymphoma Registry Study. Leukemia. 2015;29:668-76.
10. Herold M, Haas A, Srock S, Neser S, Al-Ali KH, Neubauer A, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: An East German study group hematology and oncology study. J Clin Oncol. 2007;25:1986-92.
11. Hiddemann W, Kneba M, Dreyling M, Schmitz N, Lengfelder E, Schmits R, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin,

vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: R. Blood. 2005;106:3725-32.

12. Marcus R, Imrie K, Belch A, Cunningham D, Flores E, Catalano J, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood. 2005;105:1417-23.
13. Marcus R, Imrie K, Solal-Celigny P, Catalano J V., Dmoszynska A, Raposo JC, et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. J Clin Oncol. 2008;26:4579-86.
14. Salles G, Mounier N, De Guibert S, Morschhauser F, Doyen C, Rossi JF, et al. Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: Results of the GELA-GOELAMS FL2000 study. Blood. 2008;112:4824-31.
15. Bachy E, Seymour JF, Feugier P, Offner F, López-Guillermo A, Belada D, et al. Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: Long-term results of the PRIMA study. J Clin Oncol. 2019;37:2815-24.
16. Rivas-Delgado A, Magnano L, Moreno-Velázquez M, García O, Nadeu F, Mozas P, et al. Response duration and survival shorten after each relapse in patients with follicular lymphoma treated in the rituximab era. Br J Haematol. 2019;184:753-9.
17. Mozas P, Sorigué M, López-Guillermo A. Follicular lymphoma: an update on diagnosis, prognosis, and management. Med Clin (Barc). 2021.
18. Roulland S, Navarro JM, Grenot P, Milili M, Agopian J, Montpellier B, et al. Follicular lymphoma-like B cells in healthy individuals: A novel intermediate step in early lymphomagenesis. J Exp Med. 2006;203:2425-31.
19. Roulland S, Kelly RS, Morgado E, Sungalee S, Solal-Celigny P, Colombat P, et al. t(14;18) translocation: A predictive blood biomarker for follicular lymphoma. J Clin Oncol. 2014;32:1347-55.
20. Basso K, Dalla-Favera R. Germinal centres and B cell lymphomagenesis. Nat Rev Immunol. 2015;15:172-84.
21. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science (80-). 1985;228:1440-3.
22. Green MR. Chromatin modifying gene mutations in follicular lymphoma. Blood. 2018;131:595-604.
23. De Jong D, Fest T. The microenvironment in follicular lymphoma. Best Pract Res Clin Haematol. 2011;24:135-46.

24. Carbone A, Roulland S, Gloghini A, Younes A, von Keudell G, López-Guillermo A, et al. Follicular lymphoma. Nat Rev Dis Prim. 2019;5.
25. Nathan AJ, Scobell A. How China sees America. vol. 91. 2012.
26. Huet S, Salles G. Potential of Circulating Tumor DNA for the Management of Patients With Lymphoma. JCO Oncol Pract. 2020;16:561-8.
27. Dreyling M, Ghielmini M, Rule S, Salles G, Ladetto M, Tonino SH, et al. Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2021;32:298-308.
28. Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol. 1989;7:1630-6.
29. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of hodgkin and non-hodgkin lymphoma: The lugano classification. J Clin Oncol. 2014;32:3059-67.
30. Link BK, Friedberg JW, Taylor MD, Cerhan JR, Flowers CR, Dillon H, et al. Follicular lymphoma in the United States: First report of the national LymphoCare study. J Clin Oncol. 2009;27:1202-8.
31. Brady JL, Binkley MS, Hajj C, Chelius M, Chau K, Balogh A, et al. Definitive radiotherapy for localized follicular lymphoma staged by18F-FDG PET-CT: A collaborative study by ILROG. Blood. 2019;133:237-45.
32. Brice P, Bastion Y, Lepage E, Brousse N, Haïoun C, Moreau P, et al. Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: A randomized study from the Groupe d'Etude des Lymphomes Folliculaires. J Clin Oncol. 1997;15:1110-7.
33. Ardeshna KM, Qian W, Smith P, Braganca N, Lowry L, Patrick P, et al. Rituximab versus a watch-and-wait approach in patients with advanced-stage, asymptomatic, non-bulky follicular lymphoma: An open-label randomised phase 3 trial. Lancet Oncol. 2014;15:424-35.
34. Police RL, Trask PC, Wang J, Olivares R, Khan S, Abbe A, et al. Randomized controlled trials in relapsed/refractory follicular lymphoma: A systematic review and meta-analysis. J Oncol Pharm Pract. 2016;22:666-78.
35. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: An analysis from the National LymphoCare Study. J Clin Oncol. 2015;33:2516-22.

36. Montoto S, Matthews J, Greaves P, Lillington D, Anderson D, Gribben JG, et al. Myeloablative chemotherapy for chemo-sensitive recurrent follicular lymphoma: Potential benefit in second relapse. Haematologica. 2013;98:620-5.
37. Jurinovic V, Metzner B, Pfreundschuh M, Schmitz N, Wandt H, Keller U, et al. Autologous Stem Cell Transplantation for Patients with Early Progression of Follicular Lymphoma: A Follow-Up Study of 2 Randomized Trials from the German Low Grade Lymphoma Study Group. Biol Blood Marrow Transplant. 2018;24:1172-9.
38. le Gouill S, de Guibert S, Planche L, Brice P, Dupuis J, Cartron G, et al. Impact of the use of autologous stem cell transplantation at first relapse both in naïve and previously rituximab exposed follicular lymphoma patients treated in the GELA/GOELAMS FL2000 study. Haematologica. 2011;96:1128-35.
39. Leonard JP, Trneny M, Izutsu K, Fowler NH, Hong X, Zhu J, et al. AUGMENT: A Phase III study of lenalidomide plus rituximab versus placebo plus rituximab in relapsed or refractory indolent lymphoma. J. Clin. Oncol., vol. 37, 2019, p. 1188-99.
40. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kδ Inhibition by Idelalisib in Patients with Relapsed Indolent Lymphoma. N Engl J Med. 2014;370:1008-18.
41. Morschhauser F, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S, et al. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. Lancet Oncol. 2020;21:1433-42.
42. Fowler NH, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler JP, et al. Efficacy and Safety of Tisagenlecleucel in Adult Patients with Relapsed/Refractory Follicular Lymphoma: Interim Analysis of the Phase 2 Elara Trial. Blood. 2020;136:1-3.
43. Montoto S, Davies AJ, Matthews J, Calaminici M, Norton AJ, Amess J, et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. J Clin Oncol. 2007;25:2426-33.
44. Federico M, Caballero Barrigón MD, Marcheselli L, Tarantino V, Manni M, Sarkozy C, et al. Rituximab and the risk of transformation of follicular lymphoma: a retrospective pooled analysis. Lancet Haematol. 2018;5:e359-67.
45. Alonso-Álvarez S, Magnano L, Alcoceba M, Andrade-Campos M, Espinosa-Lara N, Rodríguez G, et al. Risk of, and survival following, histological transformation in follicular lymphoma in the rituximab era. A retrospective multicentre study by the Spanish GELTAMO group. Br J Haematol. 2017;178:699-708.
46. Casulo C. Treatment of Histologic Transformation. Hematol Oncol Clin North Am. 2020;34:785-94.

47. Oldenhuis CNAM, Oosting SF, Gietema JA, de Vries EGE. Prognostic versus predictive value of biomarkers in oncology. Eur J Cancer. 2008;44:946-53.
48. Mozas P, Rivero A, López-Guillermo A. Past, present and future of prognostic scores in follicular lymphoma. Blood Rev. 2021:100865.
49. Gospodarowicz MK, Bush RS, Brown TC, Chua T. Prognostic factors in nodular lymphomas: A multivariate analysis based on the Princess Margaret Hospital experience. Int J Radiat Oncol Biol Phys. 1984;10:489-97.
50. Coiffier B, Gisselbrecht C, Vose JM, Tilly H, Herbrecht R, Bosly A, et al. Prognostic factors in aggressive malignant lymphomas: Description and validation of a prognostic index that could identify patients requiring a more intensive therapy. J Clin Oncol. 1991;9:211-9.
51. Romaguera BJE, Mclaughlin P, North L, Dixon D, Silvermintz KB, Garnsey LA, et al. Follicular Low-Grade Lymphoma : A Risk Model. J Clin Oncol. 1991;9:762-9.
52. Leonard RCF, Hayward RL, Prescott RJ, Wang JX. Original article: The identification of discrete prognostic groups in low grade non-Hodgkin's lymphoma. Ann Oncol. 1991;2:655-62.
53. Soubeyran P, Eghbali H, Bonichon F, Trojani M, Richaud P, Hœrni B. Low-grade follicular lymphomas: Analysis of prognosis in a series of 281 patients. Eur J Cancer Clin Oncol. 1991;27:1606-13.
54. Cameron DA, Leonard RCF, Mao JH, Prescott RJ. Identification of prognostic groups in follicular lymphoma. Leuk Lymphoma. 1993;10:89-99.
55. International Non-Hodgkin's Lymphoma Prognostic Factors Project. A Predictive Model for Aggressive Non-Hodgkin's Lymphoma. N Engl J Med. 1993;329:987-94.
56. López-Guillermo A, Montserrat E, Bosch F, Terol MJ, Campo E, Rozman C. Applicability of the International Index for aggressive lymphomas to patients with low-grade lymphoma. J Clin Oncol. 1994;12:1343-8.
57. Denham JW, Denham E, Dear KB, Hudson G V. The follicular non-Hodgkin's lymphomas - II. Prognostic factors: What do they mean? Eur J Cancer. 1996;32:480-90.
58. López-Guillermo A, Cabanillas F, McLaughlin P, Smith T, Hagemeister F, Rodríguez MA, et al. The clinical significance of molecular response in indolent follicular lymphomas. Blood. 1998;91:2955-60.
59. Decaudin D, Lepage E, Brousse N, Brice P, Haroussеau JL, Belhadj K, et al. Low-grade stage ILL-IV follicular lymphoma: Multivariate analysis of prognostic factors in 484 patients - A study of the groupe d'etude des lymphomes de l'adulte. J Clin Oncol. 1999;17:2499-505.
60. Federico M, Vitolo U, Zinzani PL, Chisesi T, Clò V, Bellesi G, et al. Prognosis of follicular lymphoma: A predictive model based on a retrospective analysis of 987 cases. Blood.

2000;95:783-9.

61. Solal-Céligny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. Blood. 2004;104:1258-65.
62. Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, et al. Follicular lymphoma international prognostic index 2: A new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol. 2009;27:4555-62.
63. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: A retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16:1111-22.
64. Jurinovic V, Kridel R, Staiger AM, Szczepanowski M, Horn H, Dreyling MH, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. Blood. 2016;128:1112-20.
65. Mihaljevic B, Jelicic J, Andjelic B, Antic D, Markovic O, Petkovic I, et al. FCG (FLIPI, Charlson comorbidity index, and histological grade) score is superior to FLIPI in advanced follicular lymphoma. Int J Hematol. 2016;104:692-9.
66. Meignan M, Cottereau AS, Versari A, Chartier L, Dupuis J, Boussetta S, et al. Baseline metabolic tumor volume predicts outcome in high-tumor-burden follicular lymphoma: A pooled analysis of three multicenter studies. J Clin Oncol. 2016;34:3618-26.
67. Bachy E, Maurer MJ, Habermann TM, Gelas-Dore B, Maucort-Boulch D, Estell JA, et al. A simplified scoring system in de novo follicular lymphoma treated initially with immunochemotherapy. Blood. 2018;132:49-58.
68. Cottereau AS, Versari A, Luminari S, Dupuis J, Chartier L, Casasnovas RO, et al. Prognostic model for high-tumor-burden follicular lymphoma integrating baseline and end-induction PET: a LYSA/FIL study. Blood. 2018;131:2449-53.
69. Huet S, Tesson B, Jais JP, Feldman AL, Magnano L, Thomas E, et al. A gene-expression profiling score for prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts. Lancet Oncol. 2018;19:549-61.
70. Yang G, Mills M, Kim Y, Figura NB, Doyle C, Oliver D, et al. Enhancement of the Follicular Lymphoma International Prognostic Index (FLIPI) with lymphopenia (FLIPI-L): a predictor for overall survival and histologic transformation. Blood Cancer J. 2019;9:104.
71. Mir F, Mattiello F, Grigg A, Herold M, Hiddemann W, Marcus R, et al. Follicular Lymphoma Evaluation Index (FLEX): A new clinical prognostic model that is superior to existing risk

scores for predicting progression-free survival and early treatment failure after frontline immunochemotherapy. Am J Hematol. 2020;95:1503-10.

72. Sorigue M, Sancho JM. Current prognostic and predictive factors in follicular lymphoma. Ann Hematol. 2018;97:209-27.
73. Nabhan C, Aschebrook-Kilfoy B, Chiu BCH, Kruczek K, Smith SM, Evens AM. The impact of race, age, and sex in follicular lymphoma: A comprehensive SEER analysis across consecutive treatment eras. Am J Hematol. 2014;89:633-8.
74. Maartense E, Le Cessie S, Kluin-Nelemans HC, Kluin PM, Snijder S, Wijermans PW, et al. Age-related differences among patients with follicular lymphoma and the importance of prognostic scoring systems: Analysis from a population-based non-Hodgkin's lynphoma registry. Ann Oncol. 2002;13:1275-84.
75. Nabhan C, Raca G, Lynn Wang Y. Predicting prognosis in chronic lymphocytic leukemia in the contemporary era. JAMA Oncol. 2015;1:965-74.
76. Hester LL, Park SI, Wood WA, Stürmer T, Brookhart MA, Lund JL. Cause-specific mortality among Medicare beneficiaries with newly diagnosed non-Hodgkin lymphoma subtypes. Cancer. 2019;125:1101-12.
77. Flowers C, Ou F-S, Shi Q, Hoster E, Peterson BA, Hochster HS, et al. Outcomes for Elderly Patients (pts) with Follicular Lymphoma (FL) Using Individual Patient Data (IPD) from 5922 Pts in 18 Randomized Controlled Trials (RCTs): a Follicular Lymphoma Analysis of Surrogate Hypothesis (FLASH) Group Study. Blood. 2016;128:1102-1102.
78. Conconi A, Lobetti-Bodoni C, Montoto S, Lopez-Guillermo A, Coutinho R, Matthews J, et al. Life expectancy of young adults with follicular lymphoma. Ann Oncol. 2015;26:2317-22.
79. Alig S, Jurinovic V, Pastore A, Bararia D, Häbe S, Hellmuth JC, et al. Impact of age on genetics and treatment efficacy in follicular lymphoma. Haematologica. 2018;103:e364-7.
80. Cordoba R, Luminari S, Eyre TA. The use of frailty assessments in treating older adults with aggressive lymphomas. Br J Haematol. 2021.
81. Kobayashi Y, Miura K, Hojo A, Hatta Y, Tanaka T, Kurita D, et al. Charlson Comorbidity Index is an independent prognostic factor among elderly patients with diffuse large B-cell lymphoma. J Cancer Res Clin Oncol. 2011;137:1079-84.
82. Lin TL, Kuo MC, Shih LY, Dunn P, Wang PN, Wu JH, et al. The impact of age, Charlson comorbidity index, and performance status on treatment of elderly patients with diffuse large B cell lymphoma. Ann Hematol. 2012;91:1383-91.
83. Van Spronsen DJ, Janssen-Heijnen MLG, Breed WPM, Coebergh JWW. Prevalence of co-morbidity and its relationship to treatment among unselected patients with Hodgkin's disease and non Hodgkin's lymphoma, 1993-l996. Ann Hematol. 1999;78:315-9.

84. Wieringa A, Boslooper K, Hoogendoorn M, Joosten P, Beerden T, Storm H, et al. Comorbidity is an independent prognostic factor in patients with advanced-stage diffuse large B-cell lymphoma treated with R-CHOP: A population-based cohort study. Br J Haematol. 2014;165:489-96.

85. Saygin C, Jia X, Hill B, Dean R, Pohlman B, Smith MR, et al. Impact of comorbidities on outcomes of elderly patients with diffuse large B-cell lymphoma. Am J Hematol. 2017;92:989-96.

86. Antic D, Jelicic J, Trajkovic G, Balint MT, Bila J, Markovic O, et al. Is it possible to improve prognostic value of NCCN-IPI in patients with diffuse large B cell lymphoma? The prognostic significance of comorbidities. Ann Hematol. 2018;97:267-76.

87. Glimelius I, Smedby KE, Eloranta S, Jerkeman M, Weibull CE. Comorbidities and sex differences in causes of death among mantle cell lymphoma patients – A nationwide population-based cohort study. Br J Haematol. 2020;189:106-16.

88. Mozessohn L, Zhang L, Odejide OO, Chen R, Buckstein R, Soiffer RJ, et al. Prognostic value of disease risk score versus gait speed in older adults with lymphoma. Leuk Lymphoma. 2021;0:1-8.

89. Bittenbring JT, Neumann F, Altmann B, Achenbach M, Reichrath J, Ziepert M, et al. Vitamin D deficiency impairs rituximab-mediated cellular cytotoxicity and outcome of patients with diffuse large B-cell lymphoma treated with but not without rituximab. J Clin Oncol. 2014;32:3242-8.

90. Tracy SI, Maurer MJ, Witzig TE, Drake MT, Ansell SM, Nowakowski GS, et al. Vitamin D insufficiency is associated with an increased risk of early clinical failure in follicular lymphoma. Blood Cancer J. 2017;7.

91. Goldstein JS, Nastoupil LJ, Han X, Jemal A, Ward E, Flowers CR. Disparities in survival by insurance status in follicular lymphoma. Blood. 2018;132:1159-66.

92. El-Galaly TC, Gormsen LC, Hutchings M. PET/CT for Staging; Past, Present, and Future. Semin Nucl Med. 2018;48:4-16.

93. Jurisic V, Radenkovic S, Konjevic G. The actual role of LDH as tumor marker, biochemical and clinical aspects. Adv Exp Med Biol. 2015;867:115-224.

94. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8:519-30.

95. Yoo C, Yoon DH, Suh C. Serum beta-2 microglobulin in malignant lymphomas: An old but powerful prognostic factor. Blood Res. 2014;49:148-53.

96. Delfau-Larue MH, Van Der Gucht A, Dupuis J, Jais JP, Nel I, Beldi-Ferchiou A, et al. Total metabolic tumor volume, circulating tumor cells, cell-free DNA: Distinct prognostic value

in follicular lymphoma. Blood Adv. 2018;2:807-16.

97. Nagy Á, Bátai B, Balogh A, Illés S, Mikala G, Nagy N, et al. Quantitative analysis and monitoring of ezh2 mutations using liquid biopsy in follicular lymphoma. Genes (Basel). 2020;11:1-12.

98. Kimby E, Lockmer S, Holte H, Hagberg H, Wahlin BE, Brown P, et al. The simplified follicular lymphoma PRIMA-prognostic index is useful in patients with first-line chemo-free rituximab-based therapy. Br J Haematol. 2020;191:738-47.

99. Bastion Y, Berger F, Bryon PA, Felman P, Ffrench M, Coiffier B. Follicular lymphomas: Assessment of prognostic factors in 127 patients followed for 10 years. Ann Oncol. 1991;2:123-9.

100. Bloomfield CD, Goldman A, Dick F, Brunning RD, Kennedy BJ. Multivariate analysis of prognostic factors in the non-Hodgkin's malignant lymphomas. Cancer. 1974;33:870-9.

101. Steward WP, Crowther D, McWilliam LJ, Jones JM, Deakin DP, Todd IDH, et al. Maintenance chlorambucil after CVP in the management of advanced stage, low-grade histologic type non-Hodgkin's lymphoma: A randomized prospective study with an assessment of prognostic factors. Cancer. 1988;61:441-7.

102. Dana BW, Dahlberg S, Nathwani BN, Chase E, Coltman C, Miller TP, et al. Long-term follow-up of patients with low-grade malignant lymphomas treated with doxorubicin-based chemotherapy or chemoimmunotherapy. J Clin Oncol. 1993;11:644-51.

103. Canioni D, Brice P, Lepage E, Chababi M, Meignin V, Salles B, et al. Bone marrow histological patterns can predict survival of patients with grade 1 or 2 follicular lymphoma: A study from the Groupe d'Etude des Lymphomes Folliculaires. Br J Haematol. 2004;126:364-71.

104. Schmidt B, Kremer M, Götze K, John K, Peschel C, Höfler H, et al. Bone marrow involvement in follicular lymphoma: Comparison of histology and flow cytometry as staging procedures. Leuk Lymphoma. 2006;47:1857-62.

105. Sovani V, Harvey C, Haynes AP, McMillan AK, Clark DM, O'Connor SR. Bone marrow trephine biopsy involvement by lymphoma: Review of histopathological features in 511 specimens and correlation with diagnostic biopsy, aspirate and peripheral blood findings. J Clin Pathol. 2014;67:389-95.

106. Cabezas-Quintario MA, Gomez P, Yuste-Del Pozo V, Valencia-Mesa AL, Sosa G, Ricard P, et al. Bone marrow trephine biopsy involvement by lymphoma: pattern of involvement and concordance with flow cytometry, in 10 years from a single institution. Clin Transl Oncol. 2016;18:537-40.

107. Kato D, Yoshioka S, Yabushita T, Shimomura Y, Ono Y, Hiramoto N, et al. The Clinical Impact

of Minimal Bone Marrow Involvement on the Outcome of Patients with Follicular Lymphoma. Blood. 2016;128:2966-2966.

108. Galimberti S, Luminari S, Ciabatti E, Grassi S, Guerrini F, Dondi A, et al. Minimal residual disease after conventional treatment significantly impacts on progression-free survival of patients with follicular lymphoma: The FIL FOLL05 trial. Clin Cancer Res. 2014;20:6398-405.
109. Van Oers MHJ, Tönnissen E, Van Glabbeke M, Giurgea L, Jansen JH, Klasa R, et al. BCL-2/IgH polymerase chain reaction status at the end of induction treatment is not predictive for progression-free survival in relapsed/resistant follicular lymphoma: Results of a prospective randomized EORTC 20981 phase III intergroup study. J Clin Oncol. 2010;28:2246-52.
110. Rambaldi A, Carlotti E, Oldani E, Della Starza I, Baccarani M, Cortelazzo S, et al. Quantitative PCR of bone marrow BCL2/IgH+ cells at diagnosis predicts treatment response and long-term outcome in follicular non-Hodgkin lymphoma. Blood. 2005;105:3428-33.
111. Ladetto M, De Marco F, Benedetti F, Vitolo U, Parti C, Rambaldi A, et al. Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk follicular lymphoma at diagnosis: The superior disease control of R-HDS does not translate into an overall surviva. Blood. 2008;111:4004-13.
112. Ladetto M, Lobetti-Bodoni C, Mantoan B, Ceccarelli M, Boccomini C, Genuardi E, et al. Persistence of minimal residual disease in bone marrow predicts outcome in follicular lymphomas treated with a rituximab-intensive program. Blood. 2013;122:3759-66.
113. Zohren F, Bruns I, Pechtel S, Schroeder T, Fenk R, Czibere A, et al. Prognostic value of circulating Bcl-2/IgH levels in patients with follicular lymphoma receiving first-line immunochemotherapy. Blood. 2015;126:1407-14.
114. Pott C, Hoster E, Kehden B, Unterhalt M, Herold M, van der Jagt RH, et al. Minimal Residual Disease in Patients with Follicular Lymphoma Treated with Obinutuzumab or Rituximab As First-Line Induction Immunochemotherapy and Maintenance in the Phase 3 GALLIUM Study. Blood. 2016;128:613-613.
115. St-Pierre F, Broski SM, LaPlant BR, Ristow K, Maurer MJ, Macon WR, et al. Detection of extranodal and spleen involvement by FDG-PET imaging predicts adverse survival in untreated follicular lymphoma. Am J Hematol. 2019;94:786-93.
116. Ott G, Katzenberger T, Lohr A, Kindelberger S, Rüdiger T, Wilhelm M, et al. Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. Blood. 2002;99:3806-12.
117. Hans CP, Weisenburger DD, Vose JM, Hock LM, Lynch JC, Aoun P, et al. A significant diffuse

component predicts for inferior survival in grade 3 follicular lymphoma, but cytologic subtypes do not predict survival. Blood. 2003;101:2363-7.

118. Sorigue M, Ribera JM, Sancho JM. The clinical dilemma of grade 3 follicular lymphoma. Ann Oncol. 2016;27:1974.
119. Chau I, Jones R, Cunningham D, Wotherspoon A, Maisey N, Norman AR, et al. Outcome of follicular lymphoma grade 3: Is anthracycline necessary as front-line therapy? Br J Cancer. 2003;89:36-42.
120. Ganti AK, Weisenburger DD, Smith LM, Hans CP, Bociek RG, Bierman PJ, et al. Patients with grade 3 follicular lymphoma have prolonged relapse-free survival following anthracycline-based chemotherapy: The Nebraska Lymphoma Study Group Experience. Ann Oncol. 2006;17:920-7.
121. Shustik J, Quinn M, Connors JM, Gascoyne RD, Skinnider B, Sehn LH. Follicular non-Hodgkin lymphoma grades 3A and 3B have a similar outcome and appear incurable with anthracycline-based therapy. Ann Oncol. 2011;22:1164-9.
122. Wahlin BE, Sundström C, Sander B, Christensson B, Jeppsson-Ahlberg Å, Hjalmarsson E, et al. Higher World Health Organization grades of follicular lymphoma correlate with better outcome in two Nordic Lymphoma Group trials of rituximab without chemotherapy. Leuk Lymphoma. 2014;55:288-95.
123. Tomita N, Takasaki H, Fujisawa S, Miyashita K, Ogusa E, Kishimoto K, et al. Standard R-CHOP therapy in follicular lymphoma and diffuse large B-cell lymphoma. J Clin Exp Hematop. 2013;53:121-5.
124. Koch K, Hoster E, Ziepert M, Unterhalt M, Ott G, Rosenwald A, et al. Clinical, pathological and genetic features of follicular lymphoma grade 3A: A joint analysis of the German low-grade and high-grade lymphoma study groups GLSG and DSHNHL. Ann Oncol. 2016;27:1323-9.
125. Mercadal S, Pomares H, Sancho JM, Climent F, García O, Encuentra M, et al. Clinico-biological features, treatment and survival of 457 patients with histological Grades 3A and 1-2 follicular lymphoma mostly treated with immunochemotherapy. Br J Haematol. 2016;172:470-3.
126. Wahlin BE, Yri OE, Kimby E, Holte H, Delabie J, Smeland EB, et al. Clinical significance of the WHO grades of follicular lymphoma in a population-based cohort of 505 patients with long follow-up times. Br J Haematol. 2012;156:225-33.
127. Nann D, Ramis-Zaldivar JE, Müller I, Gonzalez-Farre B, Schmidt J, Egan C, et al. Follicular lymphoma t(14;18)-negative is genetically a heterogeneous disease. Blood Adv. 2020;4:5652-65.

128. Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A, et al. Prognostic value of chromosomal abnormalities in follicular lymphoma. Blood. 1994;84:1043-9.

129. Viardot A, Möller P, Högel J, Werner K, Mechtersheimer G, Ho AD, et al. Clinicopathologic correlations of genomic gains and losses in follicular lymphoma. J Clin Oncol. 2002;20:4523-30.

130. Mitsui T, Yokohama A, Koiso H, Saito A, Toyama K, Shimizu H, et al. Prognostic impact of trisomy 21 in follicular lymphoma. Br J Haematol. 2019;184:570-7.

131. Qu X, Li H, Braziel RM, Passerini V, Rimsza LM, His ED, et al. Genomic alterations important for the prognosis in patients with follicular lymphoma treated in SWOG study S0016. Blood. 2019;133:81-93.

132. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476:298-303.

133. Okosun J, Bödör C, Wang J, Araf S, Yang CY, Pan C, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. Nat Genet. 2014;46:176-81.

134. Green MR, Kihira S, Liu CL, Nair R V., Salari R, Gentles AJ, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. Proc Natl Acad Sci U S A. 2015;112:E1116-25.

135. Bödör C, Grossmann V, Popov N, Okosun J, O'Riain C, Tan K, et al. EZH2 mutations are frequent and represent an early event in follicular lymphoma. Blood. 2013;122:3165-8.

136. Okosun J, Wolfson RL, Wang J, Araf S, Wilkins L, Castellano BM, et al. Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. Nat Genet. 2016;48:183-8.

137. Pasqualucci L, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, et al. Genetics of Follicular Lymphoma Transformation. Cell Rep. 2014;6:130-40.

138. Kridel R, Chan FC, Mottok A, Boyle M, Farinha P, Tan K, et al. Histological Transformation and Progression in Follicular Lymphoma: A Clonal Evolution Study. PLoS Med. 2016;13:1-25.

139. Dheilly E, Battistello E, Katanayeva N, Sungalee S, Michaux J, Duns G, et al. Cathepsin S Regulates Antigen Processing and T Cell Activity in Non-Hodgkin Lymphoma. Cancer Cell. 2020;37:674-689.e12.

140. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011;471:189-96.

141. O'Shea D, O'Riain C, Taylor C, Waters R, Carlotti E, MacDougall F, et al. The presence of

TP53 mutation at diagnosis of Follicular Lymphoma identifies a high-risk group of patients with shortened time to disease progression and poorer overall survival. Blood. 2008;112:3126-9.

142. Davies AJ, Lee AM, Taylor C, Clear AJ, Goff LK, Iqbal S, et al. A limited role for TP53 mutation in the transformation of follicular lymphoma to diffuse large B-cell lymphoma. Leukemia. 2005;19:1459-65.
143. Alhejaily A, Day AG, Feilotter HE, Baetz T, LeBrun DP. Inactivation of the CDKN2A tumor-suppressor gene by deletion or methylation is common at diagnosis in follicular lymphoma and associated with poor clinical outcome. Clin Cancer Res. 2014;20:1676-86.
144. Correia C, Schneider PA, Dai H, Dogan A, Maurer MJ, Church AK, et al. BCL2 mutations are associated with increased risk of transformation and shortened survival in follicular lymphoma. Blood. 2015;125:658-67.
145. Scott DW, Gascoyne RD. The tumour microenvironment in B cell lymphomas. Nat Rev Cancer. 2014;14:517-34.
146. Kumar E, Pickard L, Okosun J. Pathogenesis of follicular lymphoma: genetics to the microenvironment to clinical translation. Br J Haematol. 2021:1-12.
147. Cai G, Freeman GJ. The CD160, BTLA, LIGHT/HVEM pathway: A bidirectional switch regulating T-cell activation. Immunol Rev. 2009;229:244-58.
148. Cheung KJJ, Johnson NA, Affleck JG, Severson T, Steidl C, Ben-Neriah S, et al. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. Cancer Res. 2010;70:9166-74.
149. Hilchey SP, De A, Rimsza LM, Bankert RB, Bernstein SH. Follicular Lymphoma Intratumoral CD4 + CD25 + GITR + Regulatory T Cells Potently Suppress CD3/CD28-Costimulated Autologous and Allogeneic CD8 + CD25 − and CD4 + CD25 − T Cells . J Immunol. 2007;178:4051-61.
150. Yang ZZ, Kim HJ, Villasboas JC, Price-Troska T, Jalali S, Wu H, et al. Mass Cytometry Analysis Reveals that Specific Intratumoral CD4 + T Cell Subsets Correlate with Patient Survival in Follicular Lymphoma. Cell Rep. 2019;26:2178-2193.e3.
151. Amé-Thomas P, Hoeller S, Artchounin C, Misiak J, Braza MS, Jean R, et al. CD10 delineates a subset of human IL-4 producing follicular helper T cells involved in the survival of follicular lymphoma B cells. Blood. 2015;125:2381-5.
152. Mozas P, Rivas-Delgado A, Rivero A, Dlouhy I, Nadeu F, Balagué O, et al. High serum levels of IL-2R, IL-6, and TNF-α are associated with higher tumor burden and poorer outcome of follicular lymphoma patients in the rituximab era. Leuk Res. 2020;94:106371.
153. Laurent C, Müller S, Do C, Al-Saati T, Allart S, Larocca LM, et al. Distribution, function, and

prognostic value of cytotoxic T lymphocytes in follicular lymphoma: A 3-D tissue-imaging study. Blood. 2011;118:5371-9.

154. Álvaro-Naranjo T, Lejeune M, Salvadó MT, Lopez C, Jaén J, Bosch R, et al. Immunohistochemical patterns of reactive microenvironment are associated with clinicobiologic behavior in follicular lymphoma patients. J Clin Oncol. 2006;24:5350-7.
155. Lopez-Guillermo A, Carreras J, Roncador G, Villamor N, Colomo L, Martinez A, et al. High numbers of tumor-infiltrating programmed cell death 1-positive regulatory lymphocytes are associated with improved overall survival in follicular lymphoma. J Clin Oncol. 2009;27:1470-6.
156. Tobin JWD, Keane C, Gunawardana J, Mollee P, Birch S, Hoang T, et al. Progression of disease within 24 months in follicular lymphoma is associated with reduced intratumoral immune infiltration. J Clin Oncol. 2019;37:3300-9.
157. Mondello P, Fama A, Larson MC, Feldman AL, Villasboas JC, Yang ZZ, et al. Lack of intrafollicular memory CD4 + T cells is predictive of early clinical failure in newly diagnosed follicular lymphoma. Blood Cancer J. 2021;11.
158. Nath K, Law SC, Sabdia MB, Gunawardana J, De Long LM, Sester D, et al. Intratumoral T cells have a differential impact on FDG-PET parameters in follicular lymphoma. Blood Adv. 2021;5:2644-9.
159. Guilliams M, Mildner A, Yona S. Developmental and Functional Heterogeneity of Monocytes. Immunity. 2018;49:595-613.
160. Amé-Thomas P, Tarte K. The yin and the yang of follicular lymphoma cell niches: Role of microenvironment heterogeneity and plasticity. Semin Cancer Biol. 2014;24:23-32.
161. Manches O, Lui G, Chaperot L, Gressin R, Molens JP, Jacob MC, et al. In vitro mechanisms of action of rituximab on primary non-Hodgkin lymphomas. Blood. 2003;101:949-54.
162. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of Survival in Follicular Lymphoma Based on Molecular Features of Tumor-Infiltrating Immune Cells. N Engl J Med. 2004;351:2159-69.
163. Kridel R, Xerri L, Gelas-Dore B, Tan K, Feugier P, Vawda A, et al. The prognostic impact of CD163-positive macrophages in follicular Lymphoma: A study from the BC cancer agency and the lymphoma study association. Clin Cancer Res. 2015;21:3428-35.
164. Stevens WBC, Mendeville M, Redd R, Clear AJ, Bladergroen R, Calaminici M, et al. Prognostic relevance of CD163 and CD8 combined with EZH2 and gain of chromosome 18 in follicular lymphoma: A study by the Lunenburg Lymphoma Biomarker Consortium. Haematologica. 2017;102:1413-23.
165. Wahlin BE, Sander B, Christensson B, Østenstad B, Holte H, Brown PD, et al. Entourage: The

immune microenvironment following follicular lymphoma. Blood Cancer J. 2012;2.

166. Siddiqui M, Ristow K, Markovic SN, Witzig TE, Habermann TM, Colgan JP, et al. Absolute lymphocyte count predicts overall survival in follicular lymphomas. Br J Haematol. 2006;134:596-601.

167. Marcheselli L, Bari A, Anastasia A, Botto B, Puccini B, Dondi A, et al. Prognostic roles of absolute monocyte and absolute lymphocyte counts in patients with advanced-stage follicular lymphoma in the rituximab era: An analysis from the FOLL05 trial of the Fondazione Italiana Linfomi. Br J Haematol. 2015;169:544-51.

168. Wilcox RA, Ristow K, Habermann TM, Inwards DJ, Micallef INM, Johnston PB, et al. The absolute monocyte count is associated with overall survival in patients newly diagnosed with follicular lymphoma. Leuk Lymphoma. 2012;53:575-80.

169. Watanabe R, Tomita N, Kishimoto K, Koyama S, Ogusa E, Ishii Y, et al. Absolute monocyte count in follicular lymphoma patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. Leuk Res. 2013;37:1208-12.

170. Kumagai S, Tashima M, Fujikawa J, Iwasaki M, Iwamoto Y, Sueki Y, et al. Ratio of peripheral blood absolute lymphocyte count to absolute monocyte count at diagnosis is associated with progression-free survival in follicular lymphoma. Int J Hematol. 2014;99:737-42.

171. Belotti A, Doni E, Bolis S, Rossini F, Casaroli I, Pezzatti S, et al. Peripheral blood lymphocyte/monocyte ratio predicts outcome in follicular lymphoma and in diffuse large b-cell lymphoma patients in the rituximab era. Clin Lymphoma, Myeloma Leuk. 2015;15:208-13.

172. Lee SF, Luque-Fernandez MA. Prognostic value of lymphocyte-To-monocyte ratio and neutrophil-To-lymphocyte ratio in follicular lymphoma: A retrospective cohort study. BMJ Open. 2017;7:1-8.

173. Lobetti Bodoni C, Mantoan B, Monitillo L, Genuardi E, Drandi D, Barbero D, et al. Clinical implications and prognostic role of minimal residual disease detection in follicular lymphoma. Ther Adv Hematol. 2013;4:189-98.

174. Weinberg OK, Ai WZ, Mariappan MR, Shum C, Levy R, Arber DA. «Minor» BCL2 breakpoints in follicular lymphoma: Frequency and correlation with grade and disease presentation in 236 cases. J Mol Diagnostics. 2007;9:530-7.

175. Schüler F, Dölken L, Hirt C, Kiefer T, Berg T, Fusch G, et al. Prevalence and frequency of circulating (14;18)-MBE translocation carrying cells in healthy individuals. Int J Cancer. 2009;124:958-63.

176. Trotman J, Luminari S, Boussetta S, Versari A, Dupuis J, Tychyj C, et al. Prognostic value of PET-CT after fi rst-line therapy in patients with follicular lymphoma: A pooled analysis of

central scan review in three multicentre studies. Lancet Haematol. 2014;1:e17-27.

177. Marcus R, Davies A, Ando K, Klapper W, Opat S, Owen C, et al. Obinutuzumab for the First-Line Treatment of Follicular Lymphoma. N Engl J Med. 2017;377:1331-44.

178. Trotman J, Barrington SF, Belada D, Meignan M, MacEwan R, Owen C, et al. Prognostic value of end-of-induction PET response after first-line immunochemotherapy for follicular lymphoma (GALLIUM): secondary analysis of a randomised, phase 3 trial. Lancet Oncol. 2018;2045:1-13.

179. Maurer MJ, Bachy E, Ghesquières H, Ansell SM, Nowakowski GS, Thompson CA, et al. Early event status informs subsequent outcome in newly diagnosed follicular lymphoma. Am J Hematol. 2016;91:1096-101.

180. Moccia AA, Schär S, Hayoz S, Pirosa MC, Taverna C, Novak U, et al. Prognostic value of POD24 validation in follicular lymphoma patients initially treated with chemotherapy-free regimens in a pooled analysis of three randomized trials of the Swiss Group for Clinical Cancer Research (SAKK). Br J Haematol. 2021;192:1031-4.

181. Cheson BD, Chua N, Mayer J, Dueck G, Trněný M, Bouabdallah K, et al. Overall survival benefit in patients with rituximab-refractory indolent non-hodgkin lymphoma who received obinutuzumab plus bendamustine induction and obinutuzumab maintenance in the GADOLIN study. J Clin Oncol. 2018;36:2259-66.

182. Mozessohn L, Cheung MC, Crump M, Buckstein R, Berinstein N, Imrie K, et al. Chemoimmunotherapy resistant follicular lymphoma: Predictors of resistance, association with transformation and prognosis. Leuk Lymphoma. 2014;55:2502-7.

183. Sorigue M, Mercadal S, Alonso S, Fernández-Álvarez R, García O, Moreno M, et al. Refractoriness to immunochemotherapy in follicular lymphoma: Predictive factors and outcome. Hematol Oncol. 2017;35:520-7.

184. Magnano L, Alonso-Alvarez S, Alcoceba M, Rivas-Delgado A, Muntañola A, Nadeu F, et al. Life expectancy of follicular lymphoma patients in complete response at 30 months is similar to that of the Spanish general population. Br J Haematol. 2019;185:480-91.

185. Montoto S, López-Guillermo A, Altés A, Perea G, Ferrer G, Camós M, et al. Predictive value of Follicular Lymphoma International Prognostic Index (FLIPI) in patients with follicular lymphoma at first progression. Ann Oncol. 2004;15:1484-9.

186. Buske C, Hoster E, Dreyling M, Hasford J, Unterhalt M, Hiddemann W. The Follicular Lymphoma International Prognostic Index (FLIPI) separates high-risk from intermediate- or low-risk patients with advanced-stage follicular lymphoma treated front-line with rituximab and the combination of cyclophosphamide, doxorubicin, vinc. Blood. 2006;108:1504-8.

187. Batlevi CL, Sha F, Alperovich A, Ni A, Smith K, Ying Z, et al. Follicular lymphoma in the modern era: survival, treatment outcomes, and identification of high-risk subgroups. Blood Cancer J. 2020;10.

188. Mozas P, Rivero A, Rivas-Delgado A, Correa JG, Condom M, Nadeu F, et al. Prognostic ability of five clinical risk scores in follicular lymphoma: A single-center evaluation. Hematol Oncol. 2021:hon.2922.

189. McNamara C, Davies J, Dyer M, Hoskin P, Illidge T, Lyttelton M, et al. Guidelines on the investigation and management of follicular lymphoma. Br J Haematol. 2012;156:446-67.

190. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25:579-86.

191. Sarkozy C, Maurer MJ, Link BK, Ghesquieres H, Nicolas E, Thompson CA, et al. Cause of death in follicular lymphoma in the first decade of the rituximab era: A pooled analysis of French and US cohorts. J Clin Oncol. 2019;37:144-52.

192. Gray RJ. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. Ann Stat. 2007;16:1141-54.

193. Dinnessen MAW, van der Poel MWM, Tonino SH, Visser O, Blijlevens NMA, de Jong D, et al. Stage-specific trends in primary therapy and survival in follicular lymphoma: a nationwide population-based analysis in the Netherlands, 1989–2016. Leukemia. 2021;35:1683-95.

194. Albarmawi H, Onukwugha E, Keating KN, Appukkuttan S, Yared J. Survival benefit associated with treating follicular lymphoma in patients 80 years or older. J Geriatr Oncol. 2020;11:55-61.

195. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science (80-). 2010;327:656-61.

196. Li ZM, Huang JJ, Xia Y, Sun J, Huang Y, Wang Y, et al. Blood lymphocyte-to-monocyte ratio identifies high-risk patients in diffuse large B-cell lymphoma treated with R-CHOP. PLoS One. 2012;7:e41658.

197. Rambaldi A, Boschini C, Gritti G, Delaini F, Oldani E, Rossi A, et al. The lymphocyte to monocyte ratio improves the IPI-risk definition of diffuse large B-cell lymphoma when rituximab is added to chemotherapy. Am J Hematol. 2013;88:1062-7.

198. Bento L, Díaz-López A, Barranco G, Martín-Moreno AM, Baile M, Martín A, et al. New prognosis score including absolute lymphocyte/monocyte ratio, red blood cell distribution width and beta-2 microglobulin in patients with diffuse large B-cell lymphoma treated with R-CHOP: Spanish Lymphoma Group Experience (GELTAMO). Br J Haematol. 2020;188:888-97.

199. Watanabe R, Tomita N, Itabashi M, Ishibashi D, Yamamoto E, Koyama S, et al. Peripheral

blood absolute lymphocyte/monocyte ratio as a useful prognostic factor in diffuse large B-cell lymphoma in the rituximab era. Eur J Haematol. 2014;92:204-10.

200. Porrata LF, Ristow K, Colgan JP, Habermann TM, Witzig TE, Inwards DJ, et al. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma. Haematologica. 2012;97:262-9.
201. Reddy JP, Hernandez M, Gunther JR, Dabaja BS, Martin G V., Jiang W, et al. Pre-treatment neutrophil/lymphocyte ratio and platelet/lymphocyte ratio are prognostic of progression in early stage classical Hodgkin lymphoma. Br J Haematol. 2018;180:545-9.
202. Gao F, Hu J, Zhang J, Xu Y. Prognostic value of peripheral blood lymphocyte/ monocyte ratio in lymphoma. J Cancer. 2021;12:3407-17.
203. Mohsen A, Taalab M, Abousamra N, Mabed M. Prognostic Significance of Absolute Lymphocyte Count, Absolute Monocyte Count, and Absolute Lymphocyte Count to Absolute Monocyte Count Ratio in Follicular Non-Hodgkin Lymphoma. Clin Lymphoma, Myeloma Leuk. 2020;20:e606-15.
204. Sorigue M, Sancho JM. The lymphocyte-to-monocyte ratio in follicular lymphoma. Leuk Lymphoma. 2021.
205. Bolen CR, Mattiello F, Herold M, Hiddemann W, Huet S, Klapper W, et al. Treatment dependence of prognostic gene expression signatures in de novo follicular lymphoma. Blood. 2021;137:2704-7.
206. Mozas P, López-Guillermo A. Response to: The lymphocyte-to-monocyte ratio in follicular lymphoma. Leuk Lymphoma. 2021.
207. Shimono J, Izumiyama K, Ito S, Tsutsmi Y, Kondo T, Kakinoki Y, et al. Lymphocyte-monocyte ratio (LMR) can predict bendamustine therapeutic efficacy in low-grade B-cell lymphoma. Int J Lab Hematol. 2020;42:431-8.
208. Azar HA, Hill WT, Osserman EF. Malignant lymphoma and lymphatic leukemia associated with myeloma-type serum proteins. Am J Med. 1957;23:239-49.
209. Dimopoulos MA, Kastritis E. How I treat Waldenström macroglobulinemia. Blood. 2019;134:2022-35.
210. Alexanian R. Monoclonal Gammopathy in Lymphoma. Arch Intern Med. 1975;135:62-6.
211. Economopoulos T, Papageorgiou S, Pappa V, Papageorgiou E, Valsami S, Kalantzis D, et al. Monoclonal gammopathies in B-cell non-Hodgkin's lymphomas. Leuk Res. 2003;27:505-8.
212. Cox MC, Di Napoli A, Scarpino S, Salerno G, Tatarelli C, Talerico C, et al. Clinicopathologic characterization of diffuse-large-B-cell lymphoma with an associated serum monoclonal IgM component. PLoS One. 2014;9.
213. Zhang Y, Wei Z, Li J, Gao R, Liu P. Monoclonal gammopathies regardless of subtypes are

associated with poor prognosis of diffuse large B-cell lymphoma A STROBE-compliant article. Med (United States). 2018;97.

214. Li Y, Wang L, Zhu HY, Liang JH, Wu W, Wu JZ, et al. Prognostic significance of serum immunoglobulin paraprotein in patients with diffuse large B cell lymphoma. Br J Haematol. 2018;182:131-4.
215. Papageorgiou SG, Thomopoulos TP, Spathis A, Bouchla A, Glezou I, Stavroulaki G, et al. Prognostic significance of monoclonal gammopathy in diffuse large B-cell lymphoma. Hematol Oncol. 2019;37:634-7.
216. Asatiani E, Cohen P, Ozdemirli M, Kessler CM, Mavromatis B, Cheson BD. Monoclonal gammopathy in extranodal marginal zone lymphoma (ENMZL) correlates with advanced disease and bone marrow involvement. Am J Hematol. 2004;77:144-6.
217. Wöhrer S, Streubel B, Bartsch R, Chott A, Raderer M. Monoclonal immunoglobulin production is a frequent event in patients with mucosa-associated lymphoid tissue lymphoma. Clin Cancer Res. 2004;10:7179-81.
218. Maiolo E, Alma E, Napodano C, Gulli F, Bellesi S, Cuccaro A, et al. The prognostic impact of monoclonal immune globulin and free light chain secretion in diffuse large B cell lymphoma (DLBCL). Leuk Lymphoma. 2020;61:1133-9.
219. Wadhera RK, Rajkumar SV. Prevalence of monoclonal gammopathy of undetermined significance: A systematic review. Mayo Clin Proc. 2010;85:933-42.
220. Mouhieddine TH, Weeks LD, Ghobrial IM. Monoclonal gammopathy of undetermined significance. Blood. 2019;133:2484-94.
221. Pickard L, Palladino G, Okosun J. Follicular lymphoma genomics. Leuk Lymphoma. 2020;61:2313-23.
222. Korfi K, Ali S, Heward JA, Fitzgibbon J. Follicular lymphoma, a B cell malignancy addicted to epigenetic mutations. Epigenetics. 2017;12:370-7.
223. Zhang J, Vlasevska S, Wells VA, Nataraj S, Holmes AB, Duval R, et al. The CREBBP acetyltransferase is a haploinsufficient tumor suppressor in B-cell lymphoma. Cancer Discov. 2017;7:323-37.
224. Zhang J, Dominguez-Sola D, Hussein S, Lee JE, Holmes AB, Bansal M, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. Nat Med. 2015;21:1190-8.
225. Béguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, et al. EZH2 Is Required for Germinal Center Formation and Somatic EZH2 Mutations Promote Lymphoid Transformation. Cancer Cell. 2013;23:677-92.
226. Hsi ED, Jung SH, Lai R, Johnson JL, Cook JR, Jones D, et al. Ki67 and PIM1 expression predict

outcome in mantle cell lymphoma treated with high dose therapy, stem cell transplantation and rituximab: A Cancer and Leukemia Group B 59909 correlative science study. Leuk Lymphoma. 2008;49:2081-90.

227. Rossi C, Tosolini M, Gravelle P, Pericart S, Kanoun S, Evrard S, et al. Baseline SUVmax is related to tumor cell proliferation and patient outcome in follicular lymphoma. Haematologica. 2020;Online ahe.
228. Ennishi D, Healy S, Bashashati A, Saberi S, Hother C, Mottok A, et al. TMEM30A loss-of-function mutations drive lymphomagenesis and confer therapeutically exploitable vulnerability in B-cell lymphoma. Nat Med. 2020;26:577-88.
229. Suzuki H, Kumar SA, Shuai S, Diaz-Navarro A, Gutierrez-Fernandez A, De Antonellis P, et al. Recurrent noncoding U1 snRNA mutations drive cryptic splicing in SHH medulloblastoma. Nature. 2019;574:707-11.
230. Shuai S, Suzuki H, Diaz-Navarro A, Nadeu F, Kumar SA, Gutierrez-Fernandez A, et al. The U1 spliceosomal RNA is recurrently mutated in multiple cancers. Nature. 2019;574:712-6.