

UNIVERSIDAD DE SALAMANCA

Facultad de Medicina



**VNiVERSiDAD
D SALAMANCA**

TESIS DOCTORAL

**Predicción personalizada del pronóstico de la leucemia
mieloblástica aguda del adulto mediante la integración de datos de
secuenciación masiva y técnicas de inteligencia artificial**

Con la aprobación del Programa de Doctorado “Biomedicina clínica y experimental. Salud, enfermedad y sociedad”, esta tesis será defendida el 12 de mayo de 2025 en el Salón de Grados de la Facultad de Medicina, Universidad de Salamanca

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Durante parte del desarrollo de la presente tesis doctoral, Alberto Hernández Sánchez recibió apoyo en forma de un Contrato Río Hortega (**CM23/00101**) del Instituto de Salud Carlos III, cofinanciado por la Unión Europea.

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CERTIFICAN

Que D. Alberto Hernández Sánchez, graduado en Medicina por la Universidad de Salamanca, ha realizado bajo nuestra dirección el trabajo de Tesis Doctoral titulado “Predicción personalizada del pronóstico de la leucemia mieloblástica aguda del adulto mediante la integración de datos de secuenciación masiva y técnicas de inteligencia artificial”, y que este reúne, a nuestro juicio, las condiciones de originalidad y calidad científica para su presentación y defensa ante el tribunal correspondiente para optar al grado de Doctor por la Universidad de Salamanca.

Y para que así conste a los efectos oportunos, firmamos el presente certificado en Salamanca, a 10 de abril de 2025.

Fdo:

A handwritten signature in blue ink, consisting of a stylized 'J' followed by a horizontal line and a small flourish.

Prof. Dr. Jesús M.^a Hernández Rivas

A handwritten signature in blue ink, featuring a stylized 'L' and 'B' followed by a long, sweeping horizontal line.

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LISTA DE ABREVIATURAS

abn	Anomalía
alo-TPH	Trasplante alogénico de progenitores hematopoyéticos
CBF	Factor de unión al núcleo
CID	Coagulación intravascular diseminada
CIR	Incidencia acumulada de recaída
CMF	Citometría de flujo multiparamétrica
del	Deleción
dPCR	PCR digital
EFS	Supervivencia libre de evento
EMR	Enfermedad medible residual
GO	Gemtuzumab ozogamicina
HMA	Agentes hipometilantes
inv	Inversión
ICC	Clasificación Consenso Internacional
ITD	Duplicación interna en tándem
LAL	Leucemia linfoblástica aguda
LMC	Leucemia mieloide crónica
LMA	Leucemia mieloblástica aguda
LMA-s	Leucemia mieloblástica aguda secundaria
LMA-t	Leucemia mieloblástica aguda relacionada con terapia
LMMC	Leucemia mielomonocítica crónica
LNH	Linfoma no Hodgkin
LPA	Leucemia promielocítica aguda
NGS	Secuenciación de nueva generación
NMPc	Neoplasias mieloproliferativas crónicas
OMS	Organización Mundial de la Salud
PR	Respuesta parcial
RC	Respuesta completa
RC-c	Respuesta completa compuesta
RC-h	Respuesta completa con recuperación hematológica parcial
RC-i	Respuesta completa con recuperación hematológica incompleta
RC1	Primera respuesta completa

RFS	Supervivencia libre de recaída
SMD	Síndrome mielodisplásico
SNC	Sistema nervioso central
SG	Supervivencia global
t	Traslocación
VAF	Frecuencia alélica de la variante

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INTRODUCCIÓN

1. LEUCEMIA MIELOBLÁSTICA AGUDA: VISIÓN GENERAL

La leucemia mieloblástica aguda (LMA) es una patología maligna clonal, caracterizada por la proliferación de blastos mieloides, que presentan un bloqueo en la capacidad de diferenciación, dando lugar a una reducción significativa o ausencia de hematopoyesis normal¹. Esto se traduce en citopenias graves, que pueden poner en peligro la vida del paciente y condicionar una dependencia transfusional. En general, la sintomatología se desarrolla de forma rápida (días o semanas) y está asociada al fallo medular secundario a la infiltración por los blastos. Los pacientes pueden presentar astenia y disnea de esfuerzo (en relación con la anemia), infecciones de repetición (asociado a la neutropenia) y una tendencia al sangrado (en contexto de la trombopenia)². La LMA constituye una enfermedad grave, debido a la cual en 2016 se estimaron 85.000 fallecimientos y 2,6 millones de años de vida perdidos, y se prevé que en el año 2040 estas cifras asciendan a 150.000 fallecimientos y 3,8 millones de años de vida perdidos³.

1.1 Epidemiología

La LMA es el subtipo de leucemia aguda más frecuente en adultos, aunque su incidencia es inferior a la de otras neoplasias sólidas como el cáncer colorrectal o el linfoma no Hodgkin². En Estados Unidos en el año 2024 se diagnosticaron aproximadamente 20.800 pacientes de LMA, lo cual supone el 1% de todos los nuevos diagnósticos de cáncer⁴. Además, se estima que más de 11.000 personas fallecieron en el mismo año a causa de la enfermedad en dicho país, por lo que continúa siendo una de las neoplasias con mayor mortalidad. Aunque la enfermedad puede aparecer a cualquier edad, la incidencia aumenta de forma considerable a partir de la sexta década de la vida, con la máxima incidencia en personas entre 75 y 85 años⁵. La mediana de edad al diagnóstico varía en función de la región geográfica, pero habitualmente se sitúa entre los 69 y 72 años^{4,6-9}. De forma global, la incidencia se sitúa entre 2,5 y 4,4 nuevos diagnósticos por cada 100.000 habitantes y año, siendo más frecuente en hombres (la ratio oscila desde 1,1:1 en países nórdicos a 1,5:1 en Estados Unidos)^{4,6-10}. No obstante, hay determinados subtipos de LMA que

son más frecuentes en mujeres, como la LMA con mutación en *NPM1* y/o mutación en *FLT3* con duplicación interna en tándem (ITD, *internal tandem duplication*)¹¹.

1.2 Etiología y factores de riesgo

La inestabilidad genómica es uno de los rasgos distintivos del cáncer¹². La razón de dicha inestabilidad es desconocida, aunque generalmente se asume que las alteraciones surgen de forma estocástica y se van acumulando a lo largo del tiempo⁵. Por ello, la patogénesis de la LMA se caracteriza por la sucesiva adquisición de mutaciones. Aunque la mayoría no tenga una consecuencia funcional, ciertas mutaciones pueden conferir una ventaja proliferativa en una célula madre hematopoyética, ocasionando que una proporción considerable de las células circulantes deriven de una única célula madre con la mutación. Este fenómeno se denomina hematopoyesis clonal y es muy prevalente en población de edad avanzada^{13,14}. Numerosos estudios han demostrado que la hematopoyesis clonal incrementa de forma significativa el riesgo de desarrollar LMA, habitualmente tras la adquisición de eventos genéticos adicionales¹⁵⁻¹⁹. Además de los cambios genéticos, las alteraciones epigenéticas también podrían desempeñar un papel en la leucemogénesis mediante la modificación de la regulación genética, de la accesibilidad de la cromatina y la alteración de la arquitectura nuclear^{13,20}.

Se han descrito múltiples factores que aumentan el riesgo de desarrollar LMA. La exposición a ciertos agentes químicos, como el benceno (presente en el humo del tabaco) constituye uno de los carcinógenos más potentes asociados con la leucemogénesis²¹⁻²³. Asimismo, la exposición a ciertos pesticidas, especialmente en interiores y durante el embarazo o la infancia, también se ha asociado a un mayor riesgo de LMA²⁴. No obstante, la exposición a nivel laboral profesional ha disminuido de forma significativa en los países desarrollados durante las últimas décadas, a causa de una mayor concienciación sobre los químicos y mutagénicos y de las nuevas normativas⁵. La administración de agentes quimioterápicos y/o radiación ionizante para el tratamiento de neoplasias previas constituye un importante factor de riesgo para el posterior desarrollo de síndrome mielodisplásico (SMD) o LMA, descrito hace varias décadas^{25,26}. Los agentes alquilantes (ej. melfalán o ciclofosfamida) y los inhibidores de la topoisomerasa II (ej. etopósido o

doxorubicina), así como la administración de radioterapia, son los agentes que más se han asociado a este fenómeno. Las leucemias agudas mieloblásticas relacionadas con terapia previa (LMA-t) asociadas a agentes alquilantes se desarrollan generalmente entre 3 y 5 años tras la administración del tratamiento para la neoplasia previa, lo cual indica que probablemente haya múltiples eventos mutacionales involucrados en su desarrollo²⁷. No obstante, algunos estudios sugieren que la quimioterapia podría ejercer una presión selectiva al otorgar una ventaja proliferativa a un pequeño clon preexistente, incluso antes del inicio del tratamiento para la neoplasia primaria^{28,29}. De forma característica, estas LMA-t suelen presentar anomalías citogenéticas que afectan a los cromosomas 5 y 7. Por otra parte, el empleo de inhibidores de topoisomerasa II se asocia con un menor tiempo de latencia hasta el desarrollo de la LMA-t (1-2 años), que suele presentar reordenamiento del gen *KMT2A*. El riesgo de presentar LMA-t tras este tratamiento es variable en función de la dosis de quimioterapia, el tratamiento concomitante, las características de la enfermedad de base y la presencia de determinados polimorfismos que puedan afectar a la metabolización de fármacos, aunque de forma global se estima que es inferior al 5% en pacientes tratados con inhibidores de topoisomerasa II. No obstante, son fármacos que se emplean con relativa frecuencia para el tratamiento del cáncer de mama, lo cual contribuye a que la LMA-t sea más frecuente en mujeres³⁰. De forma global, la exposición a quimioterapia que daña el ADN conlleva un riesgo de aproximadamente un 1% de riesgo de desarrollar LMA-t, pero la presencia de hematopoyesis clonal antes del inicio de la quimioterapia de la neoplasia primaria incrementa este riesgo de forma significativa³¹⁻³³. Por otra parte, ciertas mutaciones genéticas de origen germinal también predisponen al desarrollo de neoplasias mieloides, con un patrón clínico característico en función del gen afectado. Por ejemplo, las mutaciones en el gen *CEBPA* se heredan de forma autosómica dominante y presentan una alta penetrancia, de tal forma que la mediana de edad para el desarrollo de edad se sitúa en torno a los 25 años^{34,35}. Por el contrario, las mutaciones en el gen *DDX41* tienen una penetrancia más variable y presentan un largo periodo de latencia hasta el desarrollo de la neoplasia mieloide, con una mediana de edad alrededor de los 62 años, similar a la de la LMA esporádica^{36,37}. Los portadores de mutaciones germinales en *RUNX1* también

presentan un riesgo incrementado para el desarrollo de LMA (35-40% a lo largo de toda la vida), con una mediana de edad al diagnóstico de 35 años³⁸. Otras mutaciones de origen germinal que pueden favorecer el desarrollo de neoplasias mieloides afectan a los genes *TP53*, *ANKRD26*, *ETV6*, *GATA2*, *TERT*, *TERC*, *NF1*, *PTPN11*, *SAMD9* y *SAMD9L*, entre otros³⁹. Como se ha comentado previamente, la presencia de hematopoyesis clonal en individuos sanos incrementa el riesgo de desarrollo de LMA. Las mutaciones se presentan habitualmente en genes involucrados en la regulación epigenética (*DNMT3A*, *ASXL1*, *TET2*) y su prevalencia aumenta de forma considerable con la edad. El diagnóstico de una enfermedad hematológica mieloide previa, fundamentalmente SMD o neoplasias mieloproliferativas crónicas (NMPc), también incrementa el riesgo de desarrollar LMA secundaria (LMA-s) a lo largo de la evolución de la enfermedad. El riesgo de transformación a LMA depende en gran medida de las características clínicas y biológicas de la neoplasia primaria, aunque se estima que aproximadamente un tercio de los pacientes con SMD presentarán LMA-s, mientras que el riesgo se reduce al 1,1% por año de evolución en pacientes con mielofibrosis y aproximadamente 0,4% por año en pacientes con policitemia vera y trombocitemia esencial^{5,40}. Históricamente, los pacientes con leucemia mieloide crónica (LMC) presentaban la mayor incidencia de LMA-s, con una tasa de aproximadamente 20% por año, pero el tratamiento de esta patología mediante inhibidores de la tirosina quinasa (ITKs) ha reducido de forma drástica dicha incidencia⁴¹.

1.3 Manifestaciones clínicas

Los pacientes con LMA habitualmente presentan síntomas y signos relacionados con el fallo medular, los blastos circulantes en SP y la infiltración de órganos por las células leucémicas⁴². En pacientes jóvenes la sintomatología se desarrolla rápidamente (días), mientras los pacientes de edad avanzada pueden referir un cuadro de varias semanas de evolución o incluso algunos meses, lo cual sugiere la presencia de una hemopatía no diagnosticada previamente, como un SMD⁴³.

El síntoma más frecuente de la anemia es la astenia, aunque los pacientes también pueden referir debilidad, cefalea, mareos, disnea, palpitaciones o dolor

torácico. Los pacientes pueden presentar complicaciones cardíacas graves como síntoma inicial de LMA, especialmente aquellos de edad avanzada o con antecedentes de patología cardiovascular. El nivel de neutropenia se correlaciona con el riesgo de infección, por lo que es frecuente que los pacientes tengan fiebre al diagnóstico, incluso sin foco clínico aparente. La trombopenia produce equimosis y sangrado de mucosas, especialmente a nivel nasal y gingival. En ocasiones, los pacientes pueden presentar coagulación intravascular diseminada (CID), lo cual aumenta de forma significativa el riesgo de hemorragias a nivel digestivo, pulmonar o cerebral, que pueden poner en peligro la vida del paciente. En el contexto de una LMA de nuevo diagnóstico, la CID se observa de forma característica en la leucemia promielocítica aguda (LPA). La presencia de blastos circulantes en SP puede traducirse en un incremento de los leucocitos circulantes. Alrededor de un 10% de pacientes con LMA tiene hiperleucocitosis al diagnóstico (cifra de leucocitos $>100 \times 10^9/L$) que puede ocasionar síntomas de leucostasis: mareo, visión borrosa, cefalea, desorientación o disnea⁴². Sin el tratamiento adecuado, los pacientes con leucostasis pueden presentar complicaciones respiratorias o neurológicas graves, por lo que se considera una emergencia médica. La infiltración extramedular por la LMA puede presentarse en cualquier localización, aunque los sitios más frecuentes son el bazo, el hígado, las encías y la piel. La infiltración del sistema nervioso central (SNC) es rara en adultos, aunque se suele manifestar en forma de cefalea, cambios visuales o parálisis de nervios craneales.

En la exploración física, los pacientes suelen presentar palidez cutánea, conjuntival y de mucosa oral. Los hallazgos más frecuentes a nivel orofaríngeo son las úlceras, hipertrofia gingival, gingivitis o gingivorragia. A nivel cutáneo pueden aparecer petequias, equimosis o lesiones en forma de pápulas, placas o nódulos. En algunos pacientes, también es posible objetivar esplenomegalia, hepatomegalia o incluso linfadenopatías periféricas.

1.4 Diagnóstico

El análisis morfológico de la sangre periférica (SP) y la médula ósea (MO) es la piedra angular para el diagnóstico de la LMA. Desde 2001, de acuerdo con la Organización Mundial de la Salud (OMS), el diagnóstico de LMA generalmente

requiere la identificación de un 20% o más de blastos de estirpe mieloide, en SP o MO⁴⁴. Los avances en el conocimiento de la biología de la enfermedad han permitido reducir o incluso eliminar dicho umbral, ya que ciertas anomalías citogenéticas o mutaciones se consideran definitorias de LMA. No obstante, este es un tema de debate y las dos clasificaciones vigentes actualmente, OMS y Clasificación Consenso Internacional (ICC), discrepan sobre el porcentaje mínimo de blastos para clasificar una hemopatía maligna como LMA en presencia de determinadas alteraciones genómicas^{45,46}. El inmunofenotipo mediante citometría de flujo multiparamétrica (CMF) es una herramienta fundamental para caracterizar los marcadores de superficie y citoplasmáticos de las células neoplásicas, lo cual resulta de gran utilidad para el diagnóstico de LMA y la filiación de algunos subtipos. Por último, los estudios citogenéticos y moleculares permiten identificar anomalías citogenéticas y mutaciones genéticas recurrentes, con importantes implicaciones tanto diagnósticas como pronósticas. Por ello, aunque las clasificaciones iniciales de LMA estaban basadas en criterios morfológicos⁴⁷, se ha producido un cambio progresivo hacia las clasificaciones actuales, que se fundamentan en perfiles genómicos^{45,46}. Probablemente el ejemplo más paradigmático sea la LPA, que se caracteriza por la traslocación t(15;17)(q24.1;q21.2), que origina el gen de fusión *PML::RARA*. Este subtipo representa aproximadamente el 5-8% de las LMAs y presenta características distintivas a nivel clínico, analítico, biológico y terapéutico⁴⁸, por lo que en adelante será excluido de la presente tesis doctoral.

1.5 Tratamiento

La LMA es una enfermedad que precisa tratamiento. Sin embargo, es necesario adaptar la intensidad del mismo a las características del paciente, con el objetivo de obtener el máximo beneficio clínico posible y minimizar la mortalidad precoz relacionada con complicaciones derivadas del tratamiento. En general, los pacientes jóvenes son candidatos a recibir esquemas basados en quimioterapia intensiva, con el objetivo de alcanzar la remisión y potencial curación de la enfermedad. Por el contrario, en los pacientes de edad avanzada o con comorbilidades graves, el objetivo suele ser la reducción de la carga de la enfermedad y prolongar la supervivencia con la mejor calidad de vida posible.

1.5.1 Tratamiento con quimioterapia intensiva

La primera publicación sobre un tratamiento que demostrara alguna eficacia para el tratamiento de la LMA se remonta a 1968, con la citarabina en monoterapia⁴⁹. Sin embargo, solo conseguía unas tasas de respuesta completa (RC) del 17-24%. En un corto intervalo de tiempo se empleó por primera vez la daunorrubicina, que permitió alcanzar RC en el 55% de pacientes⁵⁰. Posteriormente se reportó que el tratamiento combinado de ambos fármacos podía tener incluso mejores resultados, sentando las bases del tratamiento que hoy se conoce como “3+7”, ya que emplea 3 días de daunorrubicina y 7 días de citarabina^{51,52}. Durante décadas se ha intentado mejorar la eficacia del esquema “3+7” mediante el cambio de dosis de los fármacos o la sustitución de daunorrubicina por otros agentes. Quizás el cambio más significativo con respecto a la pauta inicial fue el aumento de dosis de daunorrubicina, inicialmente al doble de dosis (90mg/m²/día) y posteriormente a una dosis intermedia (60mg/m²/día), con similares tasas de RC y supervivencia global^{53,54}. El cambio de antraciclina por idarrubicina o mitoxantrona no se asoció a cambios significativos en la tasa de respuestas o supervivencia, al menos en comparación con dosis intermedias o altas de idarrubicina^{55,56}. La incorporación de otros agentes quimioterápicos como el etopósido tampoco se asoció con una mayor supervivencia⁵⁷. Por ello, existen varias modificaciones sobre el esquema habitual del “3+7”, con resultados similares, que se emplean de forma preferente en función del centro.

En los pacientes que alcanzan RC es preciso administrar tratamiento de consolidación para reducir la probabilidad de recaída. En términos generales, los pacientes de riesgo favorable suelen recibir varios ciclos de consolidación con dosis altas de citarabina, con la opción de realizar un trasplante autólogo de progenitores hematopoyéticos⁵⁸. Por el contrario, los pacientes de riesgo adverso se benefician de la realización de un trasplante alogénico de progenitores hematopoyéticos (allo-TPH)⁵⁹.

En la última década se han producido avances importantes en el tratamiento de la LMA, que han permitido la incorporación de varios fármacos a la primera línea de tratamiento (**Figura 1**). El ensayo clínico RATIFY demostró que la midostaurina

(inhibidor de tirosina quinasa de primera generación), en combinación con el tratamiento estándar de inducción y consolidación, mejoraba la supervivencia global (SG) de pacientes con mutación en el gen *FLT3*, por lo que ha sido incorporado a la práctica clínica habitual en este subgrupo de pacientes⁶⁰. Por otra parte, gemtuzumab ozogamicina (anticuerpo monoclonal anti-CD33 conjugado con calicheamicina), igualmente en combinación con la quimioterapia estándar, mejora las respuestas de pacientes con LMA de riesgo favorable y, en menor medida, de riesgo intermedio, sin aportar beneficio en pacientes de riesgo adverso^{61,62}. La formulación liposomal de citarabina y daunorrubicina (CPX-351) demostró superioridad frente al “3+7” tradicional en pacientes con edad entre 60-75 años y LMA-s o con cambios relacionados con la mielodisplasia, especialmente en aquellos que recibieron tratamiento de consolidación con alo-TPH, por lo que también ha sido aprobada en primera línea para este perfil de pacientes⁶³. A pesar de que el esquema “3+7” ha sido durante décadas la piedra angular del tratamiento de la LMA, los avances en el conocimiento de la biología de la enfermedad están permitiendo que la medicina de precisión se convierta progresivamente en una realidad en esta enfermedad⁶⁴.

1.5.2 Tratamientos menos intensivos

Los pacientes no candidatos a recibir quimioterapia intensiva históricamente solo podían recibir terapia de soporte, con el objetivo de mejorar la sintomatología asociada a la enfermedad.

La citarabina a dosis bajas se empleó durante décadas en pacientes no candidatos a recibir quimioterapia intensiva, con unas tasas de respuesta completa compuesta (es decir, RC o respuesta completa con recuperación hematológica incompleta [RC-i]) en torno al 20% y una mediana de supervivencia de tan solo 5 meses⁶⁵. Se realizaron numerosos intentos de asociar otros fármacos a la citarabina, como gemtuzumab ozogamicina (GO), trióxido de arsénico, tipifarnib o vosaroxin, pero no lograron demostrar un beneficio en la SG con respecto a la citarabina en monoterapia⁶⁶⁻⁶⁹.

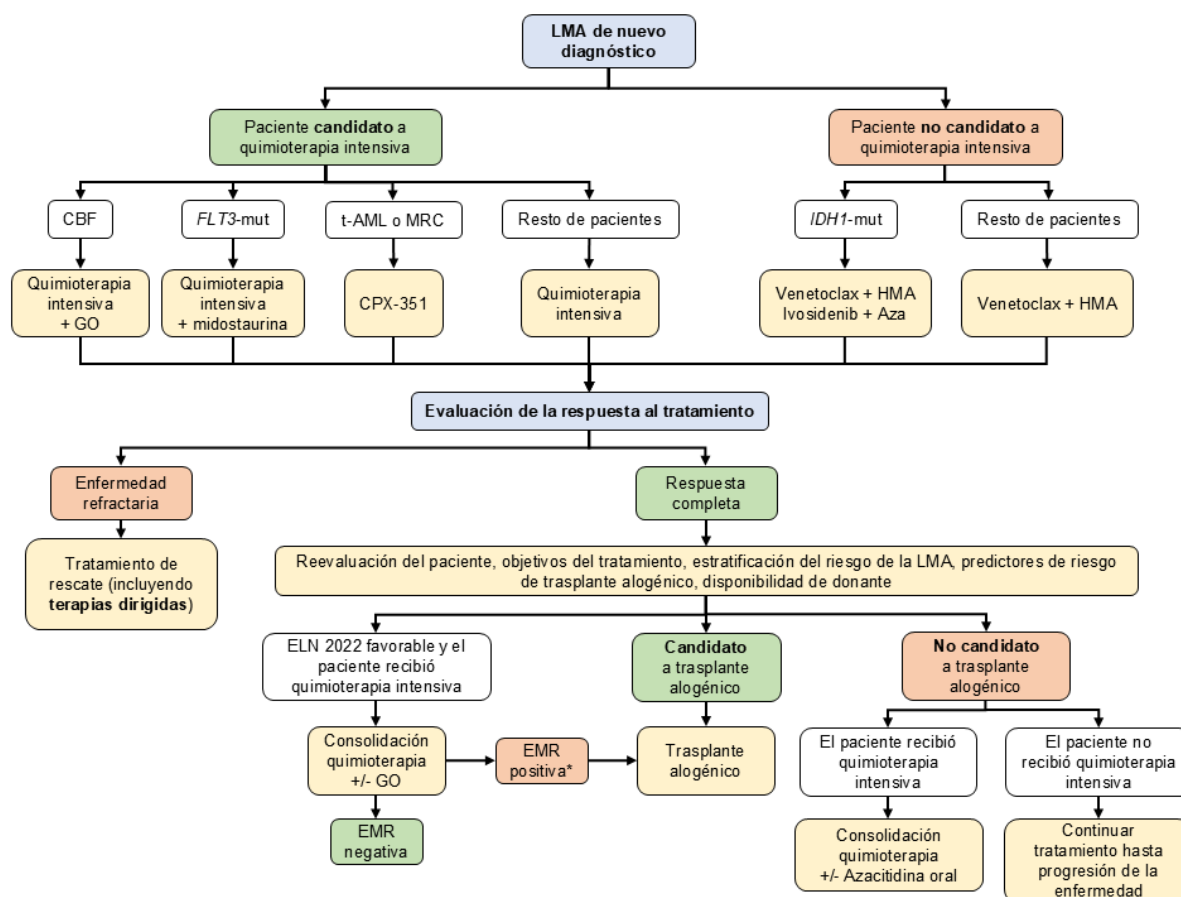


Figura 1. Propuesta de algoritmo terapéutico para pacientes con LMA de nuevo diagnóstico, teniendo en cuenta las características de la enfermedad, del paciente y la respuesta al tratamiento. *En los momentos validados según las recomendaciones de la ELN en EMR⁷⁰. Aza, azacitidina; CBF, factor de unión al núcleo (*core binding factor*); ELN 2022, clasificación de riesgo de la *European LeukemiaNet* de 2022; EMR, enfermedad medible residual; GO, gemtuzumab ozogamicina; HMA, agentes hipometilantes; MRC, cambios relacionados con la mielodisplasia; t-AML, LMA relacionada con terapia (*modificado de Hernández-Sánchez y Bullinger, 2023*)⁷¹.

El empleo de fármacos hipometilantes (5-azacitidina, decitabina) supuso un primer avance en el tratamiento de estos pacientes. A pesar de que no consiguió mejorar las tasas de respuesta completa (RC-c), el tratamiento con 5-azacitidina se asoció a un discreto incremento en la SG, con una mediana de 10 meses⁷². De forma similar, los pacientes tratados con decitabina presentaron un aumento numérico (aunque no estadísticamente significativo) de la SG, con una mediana de 8 meses⁷³. Estos resultados dieron lugar a la aprobación de ambos fármacos en Europa, aunque no en Estados Unidos.

La incorporación del inhibidor de BCL2 venetoclax a la azacitidina ha permitido incrementar la tasa de RC-c al 66%, con una mediana de SG de 15 meses, por lo que

actualmente se considera el estándar de tratamiento para pacientes no candidatos a recibir quimioterapia intensiva^{1,74}. Además, hay varios ensayos clínicos en desarrollo que estudian la incorporación de un tercer fármaco como gilteritinib o cladribina a esta base de tratamiento^{75,76}. Asimismo, el empleo de terapias dirigidas, como el inhibidor de *IDH1* ivosidenib, en combinación con fármacos hipometilantes, supone una alternativa terapéutica en pacientes no candidatos a quimioterapia intensiva que presenten mutación en dicho gen, con una mediana de supervivencia de hasta 2 años⁷⁷. No obstante, los pacientes con mutación en *IDH1* también presentan una alta tasa de respuestas a venetoclax y azacitidina, por lo que actualmente se desconoce qué combinación podría ser más beneficiosa en estos pacientes o si la administración de los tres fármacos se podría asociar a una mayor supervivencia^{78,79}.

1.6 Seguimiento

Uno de los aspectos fundamentales en el seguimiento de los pacientes con LMA es la evaluación de la respuesta al tratamiento. Habitualmente se realiza un estudio de la médula ósea 21 días después del inicio del tratamiento, siendo los criterios de respuesta los siguientes, según las recomendaciones de 2022 de la *European LeukemiaNet* (ELN2022)³⁹:

- **Respuesta completa (RC):** se deben cumplir todos los siguientes requisitos:
 - <5% de blastos en MO
 - Ausencia de blastos circulantes en SP
 - Ausencia de enfermedad extramedular
 - Cifra de neutrófilos $\geq 1,000/\mu\text{L}$
 - Cifra de plaquetas $\geq 100,000/\mu\text{L}$
- **Respuesta completa con recuperación hematológica parcial (RC-h):** cifra de neutrófilos $\geq 500/\mu\text{L}$ y de plaquetas $\geq 50,000/\mu\text{L}$, pero por lo demás se cumple el resto de criterios de RC.
- **Respuesta completa con recuperación hematológica incompleta (RC-i):** cifra de neutrófilos $< 1,000/\mu\text{L}$ o de plaquetas $< 100,000/\mu\text{L}$, cumpliendo el resto de criterios de RC.

- **Estado libre de leucemia a nivel morfológico (MLFS):** cumple criterios de RC, sin recuperación de cifra de neutrófilos ni de plaquetas.
- **Respuesta parcial (PR):** se cumplen los criterios de recuperación hematológica de la RC, pero persisten blastos en MO entre el 5 y 25% (con descenso del porcentaje de blastos con respecto a la determinación previa al inicio de tratamiento de al menos el 50%).
- **Enfermedad refractaria:** ausencia de RC, RC-h o RC-i en el punto de referencia de evaluación del tratamiento. Aunque no existe consenso al respecto, las guías actuales recomiendan establecer dicho punto tras la administración de dos ciclos de quimioterapia intensiva. En pacientes que reciben tratamiento no intensivo, el punto de referencia suele ser posterior, tras la administración de varios ciclos de tratamiento.
- **Respuesta no evaluable:** incluye pacientes que fallecen de forma prematura (muerte precoz), aquellos que abandonan el tratamiento antes de evaluar la respuesta o en los que no se puede valorar por muestra subóptima.
- **Enfermedad refractaria:** se define para aquellos pacientes que obtuvieron RC, RC-h o RC-i en los que se objetivan $\geq 5\%$ de blastos en MO, reaparición de blastos en SP en al menos dos determinaciones separadas por al menos una semana, o el desarrollo de enfermedad extramedular.

En los pacientes que alcanzan RC, RC-h o RC-i, resulta de gran utilidad la evaluación de la enfermedad medible residual (EMR) mediante técnicas más sensibles. Actualmente, las dos metodologías que han sido más evaluadas son la CMF y la reacción en cadena de la polimerasa cuantitativa en tiempo real (RT-qPCR), aunque se están desarrollando otras técnicas como la secuenciación de nueva generación (NGS) y la PCR digital (dPCR)⁷⁰. La CMF es aplicable a la gran mayoría de las LMA, pero es menos sensible y el análisis puede ser más subjetivo. Por otra parte, la RT-qPCR es más sensible pero solo es aplicable a LMA que presenten determinadas alteraciones citogenéticas o ciertas mutaciones, lo cual reduce su aplicabilidad³⁹.

Los resultados a largo plazo de los pacientes con LMA se pueden evaluar de varias formas³⁹:

- **Supervivencia global (SG):** tiempo desde el diagnóstico de LMA (o fecha de aleatorización en ensayo clínico) hasta la fecha de fallecimiento por cualquier causa. Los pacientes que no han fallecido a la fecha de último seguimiento se censuran en dicha fecha.
- **Supervivencia libre de recaída (*relapse-free survival, RFS*):** se define únicamente para pacientes que alcanzan RC, RC-h o RC-i, como el tiempo desde la fecha de remisión hasta la fecha de recaída hematológica o fallecimiento por cualquier causa.
- **Incidencia acumulada de recaída (*cumulative incidence of relapse, CIR*):** también es aplicable solo a pacientes que obtienen RC, RC-h o RC-i, y se define como el tiempo desde la fecha de respuesta hasta la recaída de la enfermedad, pero teniendo en cuenta que la muerte sin recaída es un evento competitivo.
- **Supervivencia libre de evento (*event-free survival, EFS*):** tiempo desde el diagnóstico de LMA (o fecha de aleatorización en ensayo clínico) hasta la fecha de fracaso del tratamiento, recaída o muerte por cualquier causa, lo que suceda primero. Los pacientes refractarios al tratamiento o que fallecen de forma precoz antes de la reevaluación se consideran como evento a tiempo cero.

1.7 Pronóstico

A pesar de los avances en el tratamiento de la enfermedad, el pronóstico de los pacientes con LMA continúa siendo desfavorable. La SG a los 5 años del diagnóstico se sitúa en torno al 32% en Estados Unidos (**Figura 2**)⁴. La mayor parte de los fallecimientos ocurre durante los primeros años tras el diagnóstico, de tal forma que los pacientes que sobreviven al primer año tienen un 56% de probabilidades de vivir 5 años más, lo cual se incrementa al 83% y al 91% si sobreviven al tercer y al quinto año, respectivamente.

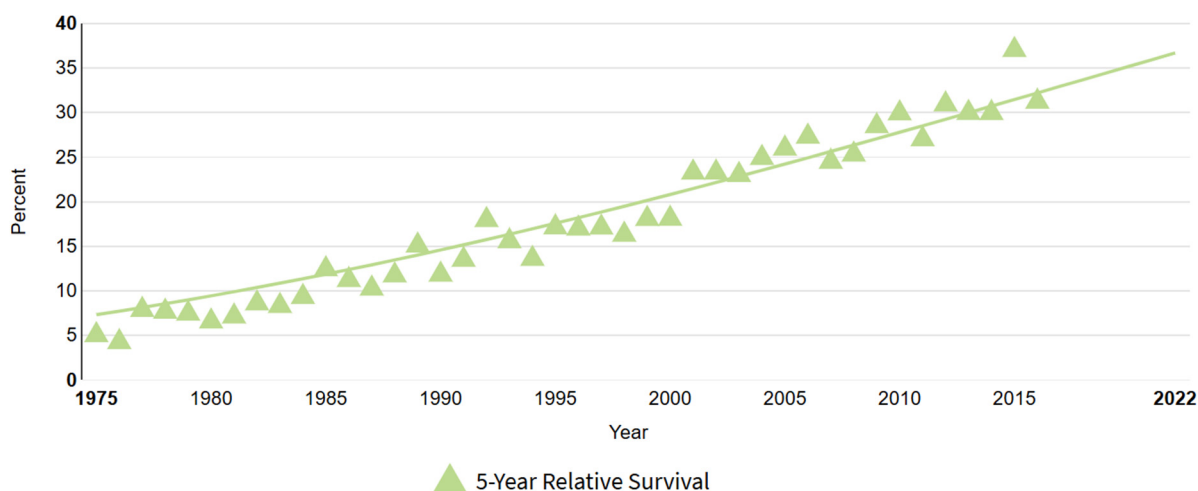


Figura 2. Evolución de la supervivencia a 5 años en pacientes con LMA desde 1975 hasta 2016, según el registro SEER 8 (tomado del registro Surveillance, Epidemiology, and End Results Program del National Cancer Institute de Estados Unidos)⁴.

No obstante, existen numerosos factores que influyen de forma determinante en el pronóstico de los pacientes con LMA.

1.7.1 Factores relacionados con el paciente

La edad del paciente al diagnóstico de la LMA es uno de los principales factores que determina el pronóstico, por varias razones. En primer lugar, las características genéticas de la enfermedad cambian, de tal forma que las alteraciones citogenéticas asociadas a pronóstico adverso son casi diez veces más frecuentes en pacientes de edad avanzada con respecto a aquellos con edad <60 años⁸⁰. Asimismo, las mutaciones genéticas de alto riesgo también son más frecuentes⁸¹. Por otra parte, la edad avanzada se asocia con un peor estado funcional y una mayor incidencia de fragilidad y comorbilidades, lo cual puede limitar las alternativas terapéuticas⁹.

La forma de presentación de la enfermedad al diagnóstico también se relaciona con el pronóstico. La hiperleucocitosis se relaciona con un mayor riesgo de mortalidad precoz debido a las complicaciones previamente mencionadas. Se asocia a ciertas alteraciones citogenéticas, como los reordenamientos de *KMT2A* o la inv(16), así como a determinadas mutaciones genéticas, como *FLT3*-ITD.

Las complicaciones que se desarrollan en los primeros días tras el diagnóstico o el inicio de tratamiento de la enfermedad también pueden condicionar la supervivencia de los pacientes con LMA. Las principales complicaciones

potencialmente mortales son las infecciones, las alteraciones de la coagulación (incluyendo CID), la leucostasis y el síndrome de lisis tumoral⁸².

1.7.2 Origen de la leucemia mieloblástica aguda

Las LMA-s presentan un peor pronóstico que las LMA primarias o *de novo*. Aunque no existe consenso con respecto a la definición de LMA-s, habitualmente se consideran como tal aquellas que presentan un antecedente de enfermedad hematológica (SMD, NMP o SMD/NMP incluyendo la leucemia mielomonocítica crónica), síndrome de fallo medular, o LMA-t. Además, ciertas alteraciones citogenéticas y moleculares podrían ser sugerentes de LMA-s a pesar de no presentar ninguno de los antecedentes previamente mencionados⁸³.

1.7.3 Anomalías genómicas

Las alteraciones citogenéticas están presentes en aproximadamente la mitad de los pacientes con LMA y son fundamentales tanto para la clasificación de la enfermedad como para la estratificación pronóstica³⁹.

Por otra parte, gracias al desarrollo de las técnicas de secuenciación masiva de nueva generación (NGS) y a un mejor conocimiento de la biología de la enfermedad, las mutaciones genéticas se han identificado como otro factor pronóstico de gran relevancia en la LMA^{81,84-86}.

Entre todas las variables que se determinan al momento del diagnóstico, las anomalías genómicas constituyen el factor predictivo más determinante en la supervivencia de los pacientes con LMA (**Figura 3**). En aquellos que reciben tratamiento intensivo, las anomalías genómicas representan casi dos tercios de la variación explicada en la SG, mientras que el otro tercio corresponde a variables demográficas (edad y género), clínicas, analíticas y relacionadas con el tratamiento⁸⁴. Por ello, se detallarán en la siguiente sección.

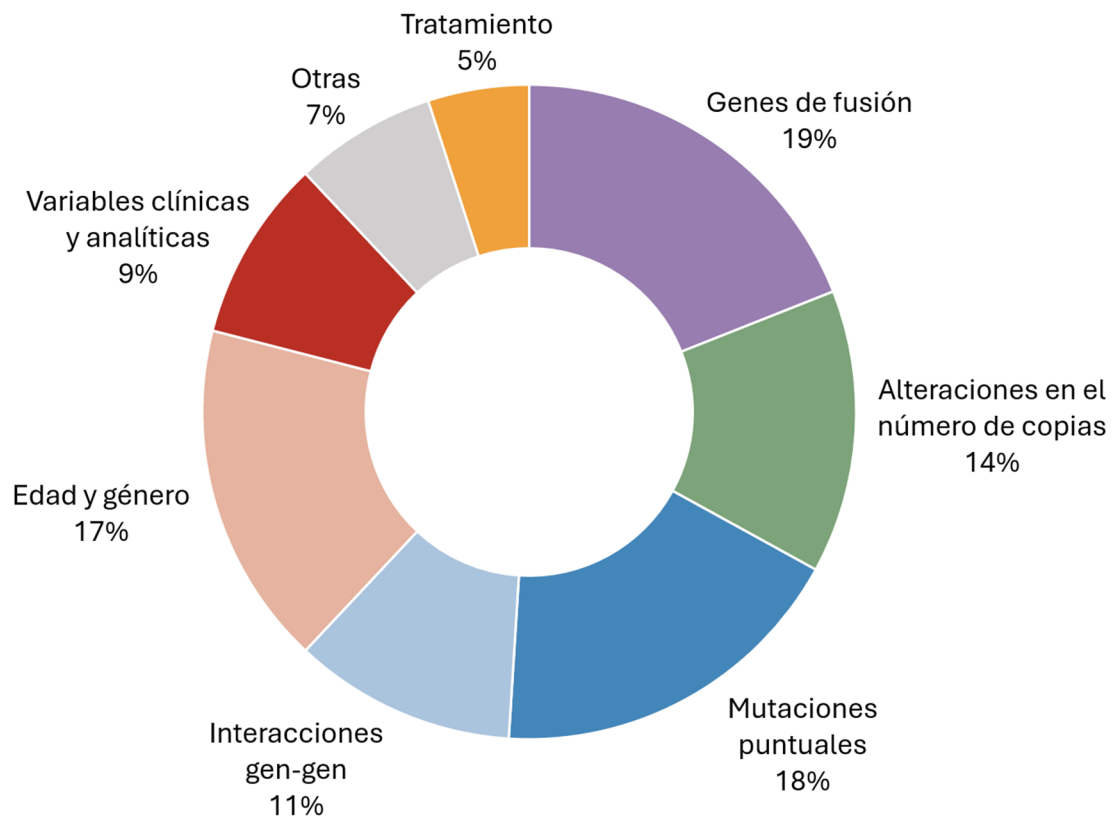


Figura 3. Proporción relativa de variabilidad explicada en la predicción de la supervivencia global, en pacientes con LMA que reciben tratamiento con esquemas de quimioterapia intensiva (modificado de Papaemmanuil et al. 2016)⁸⁴

2. PERFIL GENÓMICO DE LA LMA DEL ADULTO

La LMA se desarrolla como una expansión clonal de precursores mieloides indiferenciados tras la adquisición de determinadas alteraciones citogenéticas y/o moleculares. De hecho, las clasificaciones de la enfermedad han ido evolucionando progresivamente desde criterios exclusivamente morfológicos a centrarse principalmente en criterios genómicos^{47,87,88}. Las dos propuestas vigentes en la actualidad son la 5.^a edición de la clasificación de la OMS y la clasificación ICC. A pesar de que existen algunas diferencias entre ellas, la concordancia entre ambas es superior al 85%⁸⁹⁻⁹¹. Dado que la propuesta de la ICC está íntimamente relacionada con la clasificación pronóstica ELN2022, será la que se desarrollará a continuación.

2.1 Clasificación Consenso Internacional (ICC 2022)

La ICC mantiene muchos de los subtipos reconocidos en clasificaciones previas e incluye nuevas entidades definidas genéticamente. Además, considera el antecedente de una neoplasia hematológica o el tratamiento con quimioterapia o radioterapia previos como “calificativo” del diagnóstico, en lugar de una categoría en sí misma. Por otra parte, elimina los criterios morfológicos para definir la LMA con cambios relacionados con la mielodisplasia, en favor de las alteraciones citogenéticas y las mutaciones, incrementando el peso de la genómica en la clasificación de la LMA.

2.1.1 LMA con t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*

La traslocación da lugar a la formación del gen de fusión *RUNX1::RUNX1T1*, que produce la alteración de la función del factor de unión al núcleo (CBF, *core binding factor*), que es un complejo transcripcional clave para la diferenciación mieloide normal. Este subtipo representa aproximadamente el 4-6% de la LMA del adulto y es más frecuente en pacientes jóvenes^{84,86}. Presenta una elevada tasa de afectación extramedular (sarcoma granulocítico) y suele tener rasgos morfológicos característicos, como la alta frecuencia de displasia en la serie granulocítica o el incremento de precursores de basófilos y eosinófilos⁹².

Las alteraciones citogenéticas concomitantes más comunes son la pérdida del cromosoma sexual (especialmente en varones) y la pérdida del brazo largo del cromosoma 9 – del(9q). Por otra parte, las mutaciones más frecuentes afectan a los genes *KIT*, *NRAS*, *ASXL2*, *ZBTB7A* y a los genes del complejo cohesina. Además, aproximadamente el 5-10% puede presentar mutación en *FLT3*^{86,93}.

Este subtipo se considera de pronóstico favorable, ya que presenta una alta tasa de respuesta al tratamiento con quimioterapia intensiva³⁹. Sin embargo, la proporción de recaídas puede llegar hasta el 40% y no existe consenso sobre si la presencia de determinadas mutaciones adicionales (*KIT*, *FLT3*-ITD) podrían aumentar el riesgo en pacientes con este subtipo de LMA⁹².

2.1.2 LMA con inv(16)(p13.1q22) o t(16;16)(p13.1;q22)/CBFB::MYH11

La traslocación da lugar a la formación del gen de fusión *CBFB::MYH11*, por lo que también se produce una alteración del CBF. Esta alteración está presente en aproximadamente el 5-8% de las LMA de nuevo diagnóstico; también es más frecuente en pacientes jóvenes y suele asociarse a eosinofilia. Aunque en varios estudios tanto la t(8;21) como la inv(16) se han analizado de forma conjunta, presentan características clínicas y biológicas diferentes⁹⁴⁻⁹⁷.

Las alteraciones citogenéticas concomitantes más frecuentes son las trisomías, especialmente del cromosoma 8 y del 22. Esta última parece conferir un mejor pronóstico, incluso en presencia de *FLT3*-ITD⁹⁸. Las mutaciones suelen afectar a los genes *NRAS*, *KIT*, *FLT3* en dominio tirosina quinasa (*FLT3*-TKD) o *KRAS*, sin clara modificación del buen pronóstico de la enfermedad.

Ambos subtipos se consideran de pronóstico favorable y las recomendaciones de la ELN2022 mantienen esta estratificación pronóstica, incluso en presencia de alteraciones citogenéticas o mutaciones concomitantes³⁹. Por ello, se considera un subtipo potencialmente curable con quimioterapia intensiva, especialmente si se añade GO al esquema terapéutico^{61,62}.

2.1.3 LMA con t(9;11)(p21.3;q23.3)/MLLT3::KMT2A

Los reordenamientos del gen *lisina metiltransferasa 2a* (*KMT2A*) están presentes en aproximadamente el 3-6% de la LMA *de novo* del adulto⁹⁹⁻¹⁰¹. No

obstante, una proporción significativa de pacientes desarrolla este subtipo de LMA tras la administración de quimioterapia con inhibidores de la topoisomerasa II por otras neoplasias. Se han documentado más de 100 reordenamientos diferentes que implican a *KMT2A*, pero en aproximadamente la mitad de los casos se produce el gen de fusión *MLLT3::KMT2A*¹⁰². Varios estudios han reportado que este reordenamiento se asocia a un mejor pronóstico¹⁰³⁻¹⁰⁵, aunque otros trabajos no pudieron confirmar estos resultados¹⁰⁶⁻¹⁰⁸. En cualquier caso, la LMA con *MLLT3::KMT2A* constituye actualmente un subtipo independiente y es el único reordenamiento de *KMT2A* que se considera de riesgo intermedio³⁹.

2.1.4 LMA con otros reordenamientos de *KMT2A*

Todos los demás reordenamientos de *KMT2A* se asocian a pronóstico adverso, independientemente del gen de fusión que se genere. Las alteraciones más frecuentes dentro de esta categoría en la LMA del adulto son:

- t(6;11)(q27;q23.3)/*AFDN::KMT2A*
- t(11;19)(q23.3;p13.1)/*KMT2A::ELL*
- t(10;11)(p12.3;q23.3)/*MLLT10::KMT2A*
- t(11;19)(q23.3;p13.3)/*KMT2A::MLLT1*

La anomalía citogenética concomitante más común es la trisomía del cromosoma 8, mientras que las mutaciones son menos frecuentes y afectan fundamentalmente a la vía de RAS (*NRAS*, *KRAS*) y al gen *FLT3*⁸⁶.

2.1.5 LMA con t(6;9)(p22.3;q34.1)/*DEK::NUP214*

Es un subtipo poco frecuente, ya que únicamente está presente en el 1% de las LMA del adulto. La mayor parte de los pacientes (70%) presenta mutación en *FLT3*-ITD de forma concomitante⁸⁴. Se asocia a riesgo adverso, por lo que se recomienda la realización de trasplante alogénico en primera respuesta completa¹⁰⁹.

2.1.6 LMA con inv(3)(q21.3q26.2) o t(3;3)(q21.3;q26.2)/*GATA2::MECOM*

La LMA con reordenamiento *GATA2::MECOM* constituye aproximadamente el 1,5% de la LMA del adulto. El reordenamiento también se ha descrito en otras neoplasias mieloides como SMD, LMMC o NMPc. Habitualmente la cifra de

plaquetas es normal e incluso una pequeña proporción de pacientes puede presentar trombocitosis al diagnóstico¹¹⁰.

La presencia de alteraciones citogenéticas adicionales es frecuente, especialmente la monosomía del cromosoma 7 (70-85%)^{84,86}. Las mutaciones concomitantes más frecuentes son *NRAS*, *KRAS*, *PTPN11* y *SF3B1*, siendo esta última más prevalente en pacientes de edad avanzada y un posible marcador de un SMD previo¹¹¹.

El pronóstico en pacientes tratados con quimioterapia intensiva es infausto, con tasas de RC en torno al 30% y supervivencia a los 4 años del 10-15%¹¹⁰. Incluso los pacientes que reciben trasplante alogénico también presentan muy mal pronóstico, formando parte de un subgrupo que presenta una supervivencia significativamente inferior que otros subgrupos considerados de riesgo adverso¹¹². Por tanto, es necesario el desarrollo de nuevas estrategias terapéuticas para este subtipo de LMA^{113,114}.

2.1.7 LMA con otros reordenamientos de *MECOM*

Tradicionalmente se ha diferenciado el subtipo previo del resto de reordenamientos de *MECOM*, lo cual se mantiene en las clasificaciones actuales. Sin embargo, estudios recientes sugieren que probablemente se trate de genocopias, por lo que es posible que puedan considerarse como un único subtipo en el futuro¹¹⁵.

Los reordenamientos más frecuentes dentro de esta categoría son:

- t(3;21)(q26.2;q22.1)/*MECOM*::*RUNX1*
- t(3;8)(q26.2;q24.2)/*MYC*::*MECOM*
- t(2;3)(p11~23;q26.2)/*MECOM*::?

2.1.8 LMA con otras traslocaciones recurrentes menos frecuentes

Incluye las siguientes alteraciones cromosómicas, algunas de las cuales ocurren principalmente en LMA pediátricas:

- LMA con t(1;3)(p36.3;q21.3)/*PRDM16*::*RPN1*

- LMA con t(3;5)(q25.3;q35.1)/*NPM1::MLF1*
- LMA con t(8;16)(p11.2;p13.3)/*KAT6A::CREBBP*
- LMA (megacarioblástica) con t(1;22)(p13.3;q13.1)/*RBM15::MRTF1*
- LMA con t(5;11)(q35.2;p15.4)/ *NUP98::NSD1*
- LMA con t(11;12)(p15.4;p13.3)/*NUP98::KMD5A*
- LMA con reordenamiento de *NUP98* y otros genes
- LMA con t(7;12)(q36.3;p13.2)/*ETV6::MNX1*
- LMA con t(10;11)(p12.3;q14.2)/*PICALM::MLLT10*
- LMA con t(16;21)(p11.2;q22.2)/*FUS::ERG*
- LMA con t(16;21)(q24.3;q22.1)/*RUNX1::CBFA2T3*
- LMA con inv(16)(p13.3q24.3)/*CBFA2T3::GLIS2*

2.1.9 LMA con t(9;22)(q34.1;q11.2)/*BCR::ABL1*

Es el único subtipo de LMA con alteración citogenética recurrente que mantiene el umbral de $\geq 20\%$ de blastos para ser considerado como leucemia aguda, para evitar el solapamiento con la LMC⁴⁶. Hasta el 60% de los pacientes presenta alteraciones citogenéticas adicionales, lo cual también es frecuente en pacientes con LMC que progresan a crisis blástica¹¹⁶. Por ello, el diagnóstico diferencial entre ambas entidades puede resultar complejo.

2.1.10 LMA con mutación en *NPM1*

Las mutaciones en el gen de la nucleofosmina (*NPM1*) se han descrito en aproximadamente el 30% de los adultos < 60 años con LMA y definen el subtipo más frecuente en este rango de edad. La nucleofosmina es una proteína que se desplaza entre el núcleo y el citoplasma, siendo más prominente en el nucleolo. Sin embargo, la mutación provoca que la proteína se localice en el citoplasma de forma anómala. Por ello, el descubrimiento de esta mutación se realizó hace dos décadas mediante técnicas de inmunohistoquímica, antes de la era de la NGS¹¹⁷.

Las mutaciones en *NPM1* son siempre heterocigotas y en la inmensa mayoría de los casos (99%) afectan al exón 12 del gen. Habitualmente consisten en inserciones de cuatro pares de bases, que producen un cambio en el marco de

lectura en los últimos aminoácidos del extremo C-terminal, provocando tanto la pérdida del triptófano del codón 288 y/o 290, como la generación de una señal de exportación nuclear aberrante, responsables de la localización citoplasmática de la nucleofosmina mutada¹¹⁸.

Este subtipo de LMA se asocia de forma característica con un cariotipo normal, ya que las alteraciones citogenéticas solo están presentes en un 10-20% de los casos¹¹⁹⁻¹²³. Por el contrario, la presencia de co-mutaciones es frecuente, especialmente en genes como *DNMT3A* (50%) y *FLT3*-ITD (40%)⁸⁶.

Varios estudios apoyan que este subtipo de leucemia se desarrolla a partir de una hematopoyesis clonal preexistente, especialmente en los casos con mutaciones en *DNMT3A* (*DNMT3A*-mut) o *IDH2*-mut¹²⁴⁻¹²⁷. La posterior adquisición de un “segundo evento” (*NPM1*-mut) daría lugar al desarrollo de la LMA¹²⁸. Esto explicaría la persistencia de mutaciones en *DNMT3A* en pacientes con LMA y *NPM1*-mut que alcanzan respuesta completa y mantenida en el tiempo, en los que se cree que la terapia podría “restituir” el estado previo de hematopoyesis clonal¹²⁹⁻¹³¹. Sin embargo, la persistencia de dicha hematopoyesis clonal podría predisponer a los pacientes a desarrollar una segunda neoplasia hematológica. De hecho, aunque la mayor parte de las recaídas presentan la *NPM1*-mut, aproximadamente en el 5-10% de los casos no se detecta la mutación¹³². Por otra parte, las mutaciones en *FLT3*-ITD se consideran eventos tardíos, pero que confieren una ventaja proliferativa y pueden convertirse rápidamente en el clon predominante (**Figura 4**).

La LMA con mutación en *NPM1* es el paradigma que pone de manifiesto la importancia pronóstica de las alteraciones genómicas concomitantes en esta patología. En general se considera de riesgo favorable, pero la presencia de *FLT3*-ITD empeora significativamente el pronóstico, pasando a considerarse una LMA de riesgo intermedio³⁹. Actualmente constituye el único ejemplo recogido en la clasificación ELN2022 en el que la presencia de dos co-mutaciones influye en la estratificación pronóstica. No obstante, algunos estudios han reportado que las co-mutaciones de *NRAS*, *RAD21* o *FLT3*-TKD podrían asociarse a un riesgo más favorable^{84,133}, mientras que los pacientes con *WT1*-mut o con la triple mutación de *NPM1*, *FLT3*-ITD y *DNMT3A* tendrían un peor pronóstico^{134,135}.

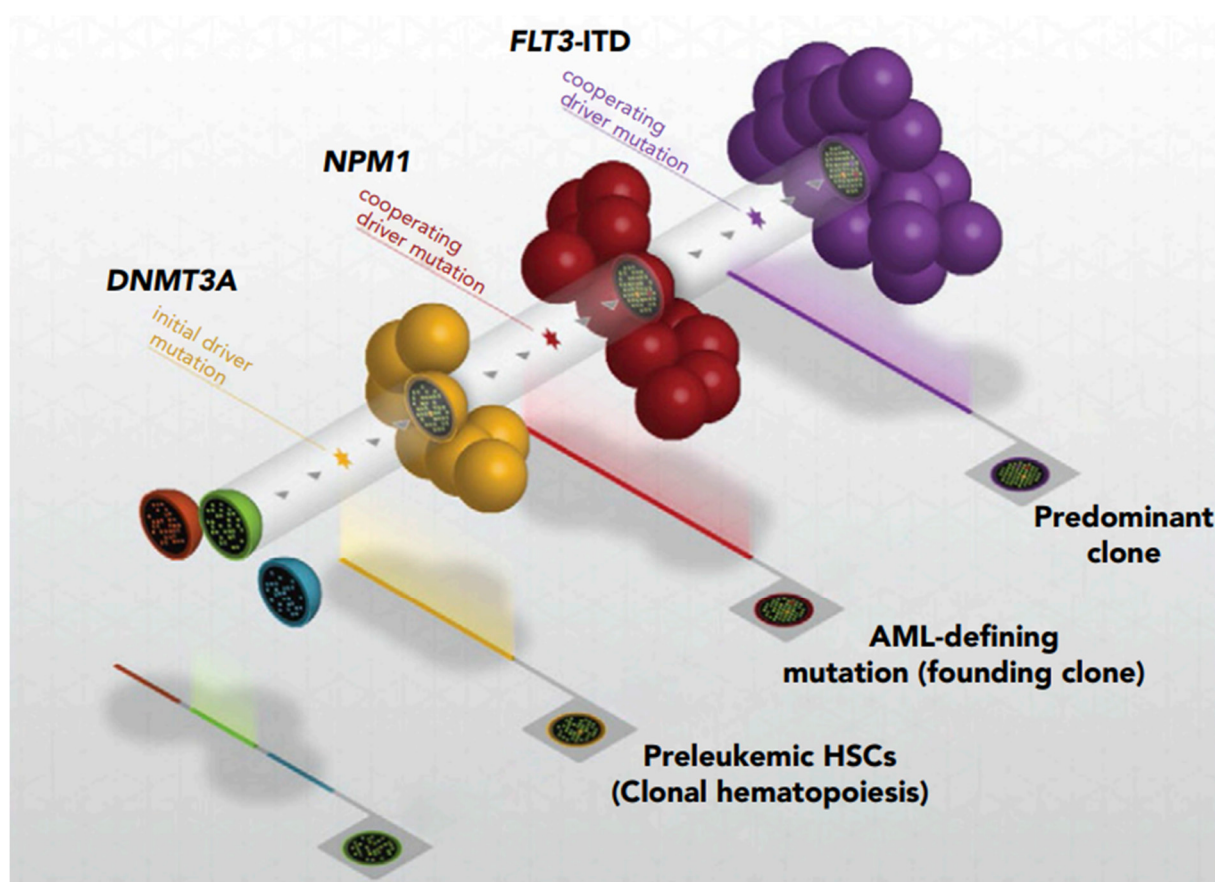


Figura 4. Propuesta de evolución clonal en la LMA con mutación en *NPM1*. Las mutaciones en *DNMT3A* son eventos genéticos iniciales, responsables de la generación de hematopoyesis clonal. Las mutaciones en *NPM1* son las que definen la enfermedad y actúan como “guardianes” de la LMA, mientras que las mutaciones en *FLT3-ITD* son eventos tardíos (tomado de Fallini et al. 2020)¹²⁸

2.1.11 LMA con mutación *in-frame* en el dominio bZIP de *CEBPA*

Inicialmente se consideró la mutación bialélica como la definitoria de este subtipo de LMA^{136,137}. En esos casos, una de las mutaciones se localiza en el dominio N-terminal, mientras que la otra afecta al extremo C-terminal. Sin embargo, estudios recientes demostraron que la mayor parte de las mutaciones que afectan al dominio C-terminal se localizan en la región de la cremallera básica de leucina (bZIP) y es dicha alteración la que se asocia con un perfil biológico diferencial^{138,139}. De forma característica, estas mutaciones son inserciones o duplicaciones en múltiplos de 3 pares de bases, por lo que no se altera el marco de lectura de la proteína (*in-frame*). Las mutaciones *CEBPA* bZIP se encuentran en el 90% de los casos con afectación bialélica, pero también en el 35% de los que presentan alteración monoalélica, por

lo que representa un buen ejemplo de cómo los grandes estudios colaborativos pueden mejorar la estratificación pronóstica de los pacientes con LMA^{39,138,139}.

El perfil mutacional de este subtipo presenta notables diferencias con respecto a la LMA con mutación en otras regiones de *CEBPA*, ya que son más frecuentes las mutaciones en *GATA2* (37% vs 7%, respectivamente) y más raras las mutaciones concomitantes en *NPM1* (8% vs 38%, respectivamente)¹³⁹.

Actualmente se considera que las LMA con mutación en el dominio bZIP de *CEBPA* tienen menor riesgo, mientras que el resto de mutaciones en este gen no se asocia con el pronóstico, que depende de las alteraciones citogenéticas y mutaciones concomitantes.

2.1.12 LMA con mutación en *TP53*

Constituye un subtipo descrito recientemente, dentro del espectro de las neoplasias mieloides con mutación en *TP53*. De forma característica, se asocia con alteraciones citogenéticas complejas, incluyendo monosomías del cromosoma 5 y/o 7, anomalías en el brazo corto del cromosoma 17, cariotipo monosomal o complejo y cromotripsis¹⁴⁰⁻¹⁴³.

La clasificación de la ICC ha establecido una categoría de SMD/LMA para pacientes con mutación en *TP53* y 10-19% de blastos, reflejando la continuidad entre SMD y LMA en este subtipo de neoplasia mieloide⁴⁶. Sin embargo, un estudio reciente ha demostrado que no hay diferencias clínicas, analíticas, genómicas ni pronósticas entre pacientes que tienen SMD con exceso de blastos (SMD-EB) con mutación de *TP53* y este subtipo de LMA, por lo que es posible que se puedan considerar como una única entidad en el futuro¹⁴¹.

El pronóstico de los pacientes con esta alteración es infausto, con una supervivencia a 2 años de tan solo el 10-15%¹⁴¹. En pacientes que consiguen someterse a trasplante alogénico la supervivencia es significativamente inferior que otros subtipos considerados de riesgo adverso según la ELN2022, similar a la LMA con reordenamiento de *MECOM*¹¹².

2.1.13 LMA con mutaciones relacionadas con la mielodisplasia (MRG)

Se define por la presencia de mutaciones en alguno de los siguientes genes: *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1* o *ZRSR2*, en ausencia de todas las alteraciones citogenéticas y mutaciones mencionadas en los apartados previos. Este nuevo subtipo se fundamenta en varios estudios que demuestran que estas mutaciones se relacionan con la LMA-s tras una neoplasia mieloide previa, incluso sin antecedente clínico de dicha patología¹⁴⁴⁻¹⁴⁷.

La clasificación de la ELN2022 considera este subtipo de riesgo adverso, independientemente de cuál de los nueve genes presente la mutación o del número de co-mutaciones concomitantes³⁹. Sin embargo, un estudio revela que aproximadamente el 60% de los pacientes de este subtipo presenta dos o más mutaciones de genes relacionados con la mielodisplasia¹⁴⁸. Ese subgrupo presenta una SG similar a la de otras categorías ELN2022 de riesgo adverso, pero los pacientes que solo presentan una mutación aislada tienen un SG similar a la categoría de la ELN2022 de riesgo intermedio. Por ello, es posible que el pronóstico de este subtipo de LMA pueda ser modificado en el futuro, teniendo en cuenta el perfil de las co-mutaciones.

2.1.14 LMA con alteraciones citogenéticas relacionadas con la mielodisplasia (MRCA)

Se define por la presencia de alguna de las siguientes alteraciones citogenéticas: cariotipo complejo (≥ 3 alteraciones cromosómicas no relacionadas, en ausencia de una alteración citogenética recurrente definitoria de otro subtipo de LMA), *del(5q)/t(5q)/add(5q)*, *-7/del(7q)*, *+8*, *del(12p)/t(12p)/add(12p)*, *i(17q)*, *-17/add(17p)/del(17p)*, *del(20q)*, y/o *idic(X)(q13)* clonal, en ausencia de todas las alteraciones definitorias de otros subtipos de LMA expuestas con anterioridad.

Hay que tener en cuenta que una proporción significativa de pacientes puede presentar varias de las alteraciones de los tres últimos subtipos, dando lugar a cierto grado de solapamiento. Sin embargo, la clasificación de la ICC es jerárquica, de tal forma que las mutaciones de *TP53* predominan sobre las mutaciones con genes relacionados con la mielodisplasia y MRCA (**Figura 5**).

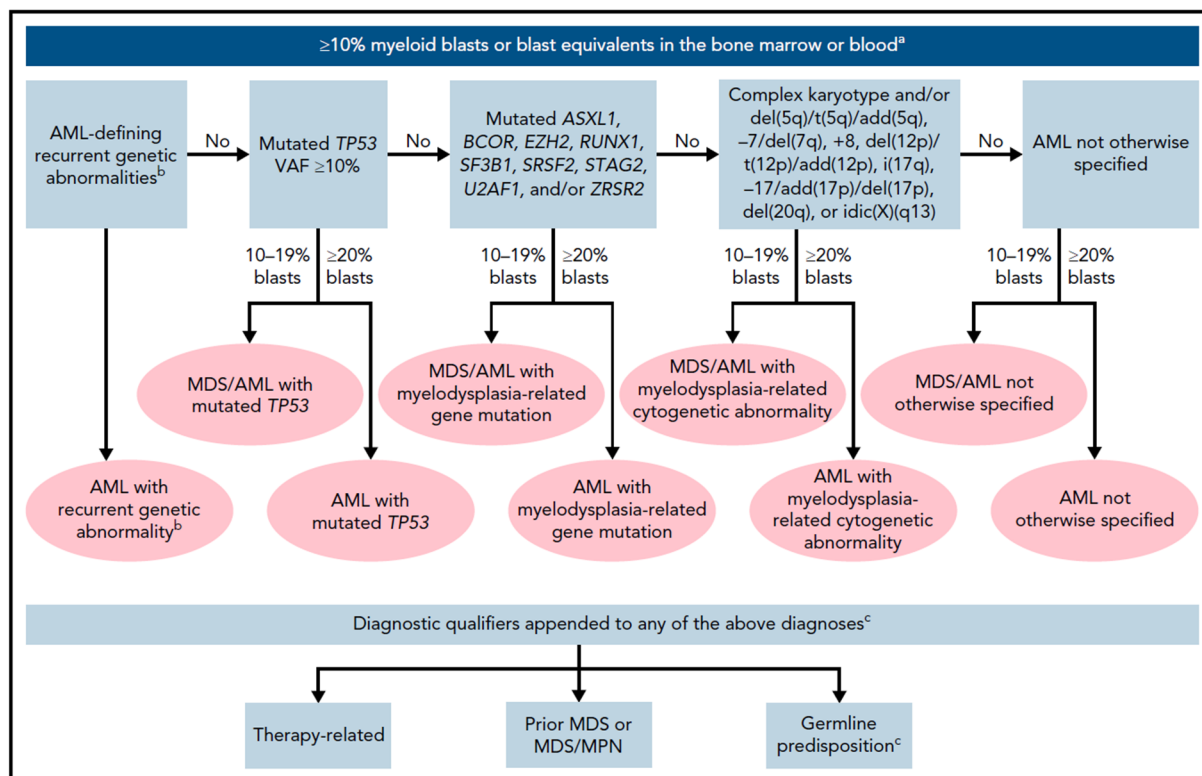


Figura 5. Clasificación jerárquica de LMA según la Clasificación Consenso Internacional (ICC). Las alteraciones genéticas recurrentes toman prioridad sobre cualquier otra categoría, y de las restantes la LMA con mutación de TP53 prevalece sobre la LMA con mutaciones en genes relacionados con la mielodisplasia, y a su vez esta prevalece sobre la LMA con alteraciones citogenéticas relacionadas con la mielodisplasia (tomado de Döhner et al. 2022)³⁹

El pronóstico de este subtipo de LMA se establece en función de cada alteración citogenética, considerándose la mayoría de ellas de riesgo adverso y algunas de riesgo intermedio, como se detallará más adelante³⁹.

2.1.15 LMA no especificada de otra manera (NOS)

Esta categoría engloba a los casos que no pueden clasificarse en ninguno de los subtipos previamente mencionados. En clasificaciones anteriores, este subgrupo representaba una proporción significativa de pacientes con LMA. Sin embargo, con la propuesta de la ICC se estima que aproximadamente el 15% de los pacientes pertenecería a este subtipo^{84,149}.

2.2 Clasificación pronóstica ELN2022

Las implicaciones pronósticas de las alteraciones genómicas de la LMA del adulto se sintetizan en la clasificación de la ELN, que en su última versión (ELN2022)

estratifica el riesgo de los pacientes en tres categorías: favorable, intermedio y adverso (**Tabla 1**)³⁹.

Cabe destacar que con cada actualización de la clasificación se ha concedido una mayor importancia a las mutaciones, ya que en la versión de 2010 solo se consideraban 3 genes para la estratificación pronóstica¹⁵⁰. Este número aumentó a 6 en la edición de 2017 y en la versión actual se consideran hasta 13 genes (*NPM1*, *FLT3*-ITD, *CEBPA* en dominio bZIP, *TP53*, *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1* y *ZRSR2*)^{39,136}. Además, se incluyen algunas indicaciones sobre la concurrencia de ciertas co-mutaciones o la presencia de mutaciones genéticas en determinados subtipos citogenéticos. La LMA con mutación en *NPM1* se considera de riesgo favorable únicamente en ausencia de *FLT3*-ITD, pero se clasifica como riesgo adverso si presenta anomalías citogenéticas de riesgo adverso. Por otra parte, la t(9;11)(p21.3;q23.3)/*MLL3::KMT2A* se considera de riesgo intermedio incluso en presencia de mutaciones concomitantes de riesgo adverso.

La clasificación ELN2022 establece tres categorías pronósticas claramente diferenciadas, con una supervivencia a 5 años de 53%, 32% y 13% para pacientes con LMA de riesgo favorable, intermedio y adverso, respectivamente¹⁵¹. Por ello, la clasificación sirve como referencia para el desarrollo de guías y protocolos de tratamiento en esta patología. En términos generales, se considera que un paciente de riesgo favorable no debería recibir trasplante alogénico en primera respuesta completa, salvo si presenta una respuesta subóptima al tratamiento administrado con persistencia de la enfermedad residual medible. Por otra parte, los pacientes de riesgo adverso que sean candidatos se benefician de la realización de un trasplante alogénico en primera línea¹⁵².

No obstante, algunos estudios recientes sugieren que se podría mejorar la estratificación pronóstica de la ELN2022 modificando algunos criterios, como el de las mutaciones en genes relacionados con la mielodisplasia, ya que algunos pacientes con estas alteraciones podrían tener una supervivencia similar al subgrupo de riesgo intermedio^{148,151}.

Tabla 1. Clasificación de riesgo de la leucemia mieloblástica aguda del adulto según las recomendaciones de 2022 de la *European LeukemiaNet*. La clasificación está basada en el riesgo genético al diagnóstico en pacientes tratados con quimioterapia intensiva, pero el riesgo puede modificarse en función de la enfermedad medible residual tras la administración de tratamiento (*modificado de Döhner et al. 2022*)³⁹

Categoría de riesgo	Anomalía genética	Consideraciones
Favorable	t(8;21)(q22;q22.1)/ <i>RUNX1::RUNX1T1</i>	La mutación concurrente de <i>KIT</i> o <i>FLT3</i> no cambia la categoría de riesgo
	inv(16)(p13.1q22) o t(16;16)(p13.1;q22)/ <i>CBFB::MYH11</i>	
	Mutación de <i>NPM1</i> , sin <i>FLT3</i> -ITD	Si presenta anomalías citogenéticas de riesgo adverso se clasifica como riesgo adverso
	Mutación <i>in-frame</i> en bZIP de <i>CEBPA</i>	
Intermedio	Mutación de <i>NPM1</i> , con <i>FLT3</i> -ITD	Si presenta anomalías citogenéticas de riesgo adverso se clasifica como riesgo adverso
	<i>FLT3</i> -ITD, sin mutación en <i>NPM1</i> ni alteraciones de riesgo adverso	
	t(9;11)(p21.3;q23.3)/ <i>MLLT3::KMT2A</i>	La t(9;11) tiene prioridad sobre las mutaciones de riesgo adverso
	Otras anomalías citogenéticas o moleculares no clasificadas como riesgo favorable o adverso	
Adverso	t(6;9)(p23.3;q34.1)/ <i>DEK::NUP214</i>	
	t(v;11q23.3)/reordenamiento <i>KMT2A</i>	Excluye <i>KMT2A</i> -PTD
	t(9;22)(q34.1;q11.2)/ <i>BCR::ABL1</i>	
	t(8;16)(p11.2;p13.3)/ <i>KAT6A::CREBBP</i>	
	inv(3)(q21.3q26.2) o t(3;3)(q21.3;q26.2)/ <i>GATA2, MECOM</i>	
	t(3q26.2;v)/reordenamiento <i>MECOM</i>	
	-5 o del(5q); -7; -17/abn(17p)	
	Cariotipo complejo* o cariotipo monosomal**	Las definiciones se especifican en el pie de tabla
	Mutación de <i>ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1</i> y/o <i>ZRSR2</i>	No se consideran de riesgo adverso en subtipos de LMA de riesgo favorable
	Mutación de <i>TP53</i>	La mutación debe presentar una VAF >10%

* Cariotipo complejo: ≥3 anomalías cromosómicas no relacionadas, en ausencia de otras alteraciones definitorias de subtipo; excluye los cariotipos hiperdiploides con tres o más trisomías (o polisomías) sin alteraciones estructurales.

** Cariotipo monosomal: presencia de dos o más monosomías distintas (excluyendo la pérdida de los cromosomas X o Y), o una monosomía autosómica en combinación con al menos una anomalía cromosómica estructural (excluyendo LMA CBF).

PTD: duplicación parcial en tándem; VAF: frecuencia alélica de la variante.

2.3 Mutaciones genéticas no definitorias de subtipo de LMA

El proyecto del Atlas del Genoma del Cáncer (TCGA) analizó 200 pacientes con LMA mediante genoma completo o exoma completo y permitió descubrir que la media de mutaciones por paciente es de 13, aunque solo 23 genes presentan mutación de forma recurrente⁸¹. A continuación, se comentarán algunas de las más relevantes por su frecuencia o impacto clínico.

2.3.1 *FLT3*

Las mutaciones en el gen *FLT3* se dividen en función del tipo, ya que presentan características biológicas y pronósticas diferentes:

- ***FLT3*-ITD**: está presente en aproximadamente el 20% de las LMA del adulto, aunque su frecuencia disminuye con la edad⁸⁶. Como se ha comentado previamente, se asocia con las mutaciones en *NPM1* y con la t(6;9). Se asocia con pronóstico adverso, especialmente cuando la ratio alélica es alta¹³⁶. Sin embargo, los problemas metodológicos con la estandarización de las pruebas para medir dicha proporción alélica y el impacto de la terapia dirigida con midostaurina han ocasionado que no se tenga en cuenta en la clasificación ELN2022. De esta forma, los pacientes con *FLT3*-ITD se consideran de riesgo intermedio, independientemente de la ratio alélica o de la presencia de co-mutación de *NPM1*.
- ***FLT3*-TKD**: se encuentra en el 7-8% de las LMA, aunque es más frecuente en algunos subtipos como la inv(16)/t(16;16) o la LMA con mutación de *NPM1*. Su impacto pronóstico es controvertido¹⁵³⁻¹⁵⁶, por lo que esta alteración no forma parte de la propuesta de la ELN2022.

2.3.2 *DNMT3A*

Es una mutación que se relaciona con la hematopoyesis clonal, por lo que se considera un evento temprano en la leucemogénesis. Su frecuencia aumenta con la edad (20-30% en pacientes >60 años) y está asociada a co-mutaciones en *NPM1*, *FLT3*-ITD, *IDH1* e *IDH2*. El impacto pronóstico en la LMA del adulto no está bien definido y parece depender del subtipo o del perfil mutacional concomitante¹⁵⁷.

2.3.3 *TET2*

Es otra mutación asociada a la hematopoyesis clonal, por lo que su frecuencia también aumenta con la edad (10-25% en pacientes >60 años). Se asocia con cariotipo normal y mutación en *NPM1*, y es mutuamente excluyente con mutaciones de *IDH1* e *IDH2*⁸⁶.

2.3.4 *NRAS*

Está presente en aproximadamente el 15% de las LMA del adulto, aunque se asocia con ciertos subtipos, como la mutación de *NPM1* o *CEBPA* bZIP, así como con la inv(16)(t(16;16), los reordenamientos de *KMT2A* y la inv(3)/t(3;3). No tiene impacto pronóstico definido, ya que es posible que dependa del perfil mutacional concomitante⁸⁴.

2.3.5 *IDH1*

Se considera un evento inicial en la leucemogénesis, dado que también se asocia a hematopoyesis clonal en personas de edad avanzada. La frecuencia de la mutación es del 6-10% y su pronóstico también podría depender del contexto^{158,159}.

2.3.6 *IDH2*

Las mutaciones en *IDH2* también se han objetivado en personas de edad avanzada con hematopoyesis clonal. Están presentes en aproximadamente el 10-20% de las LMA del adulto. Las mutaciones se localizan preferentemente en dos posiciones, que se asocian a características biológicas diferentes¹⁶⁰⁻¹⁶³:

- ***IDH2* R140:** es la más frecuente (5-15%) y se asocia con la mutación de *NPM1*. Aunque no existe consenso, algunos estudios han asociado esta mutación a un mejor pronóstico^{161,162}.
- ***IDH2* R172:** está presente únicamente en el 1-4% de las LMA del adulto y es mutuamente excluyente con la mutación de *NPM1*. Algunos estudios sugieren que, en ausencia de otras alteraciones, podría constituir un subtipo propio, con un perfil de expresión génica diferencial⁸⁴. En pacientes de riesgo intermedio o adverso esta mutación podría mejorar el pronóstico¹⁶³.

2.4 Interacciones gen-gen

El perfil mutacional de la LMA del adulto puede ser complejo, con múltiples mutaciones concomitantes, en las que el efecto pronóstico de un gen se modifique de forma significativa, si existe una co-mutación en otro⁸¹. De hecho, el 11% de la variación en la supervivencia global de adultos con LMA tratados de forma intensiva se podría explicar a partir de combinaciones de co-mutaciones, lo cual pone de manifiesto la importancia de evaluar el perfil mutacional completo (**Figura 3**)⁸⁴.

Por ejemplo, el efecto pronóstico adverso de *FLT3*-ITD es mayor cuando están presentes las co-mutaciones de *NPM1* y *DNMT3A*, pero si alguno de los dos no está alterado, la supervivencia global en presencia o ausencia de *FLT3*-ITD es similar (**Figura 6**)^{84,134}. Por el contrario, la mutación en el codón 12 o 13 de *NRAS* solo se asocia con un pronóstico favorable en el contexto de mutación en *NPM1* y *DNMT3A* concomitantes, pero únicamente cuando las tres alteraciones están presentes de forma simultánea⁸⁴.

Otro estudio demostró que las mutaciones en *DNMT3A* se asocian a riesgo adverso en la LMA del adulto, pero solo es posible objetivar dicho efecto cuando se estratifica el análisis por el estado mutacional de *NPM1*. Este efecto paradójico se debe a que ambos genes están íntimamente relacionados (el 80% de pacientes con *DNMT3A*-mut presentan también co-mutación en *NPM1*) y uno de ellos se asocia a pronóstico favorable, mientras que el otro implicaría un riesgo adverso¹⁵⁷.

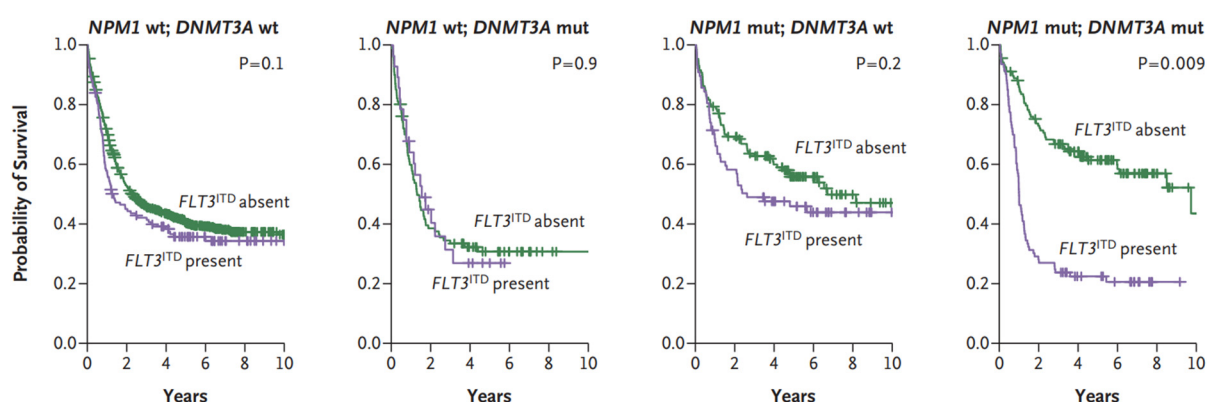


Figura 6. Supervivencia global en función de la presencia o ausencia de *FLT3*-ITD. El efecto pronóstico adverso fue significativamente mayor cuando tanto *NPM1* como *DNMT3A* presentaban mutación concomitante (tomado de Papaemmanuil et al. 2016)⁸⁴

2.5 Relación entre el perfil mutacional de la LMA y la edad

Las alteraciones genómicas que se han objetivado en la LMA guardan una estrecha relación entre ellas, tal y como se ha detallado en el presente capítulo. Además, el perfil mutacional de la enfermedad es diferente en función de la edad del paciente, con un cambio hacia alteraciones de pronóstico desfavorable a medida que se incrementa la edad (**Figura 7**). Esto plantea un reto a nivel terapéutico, pero también es un factor de confusión relevante a la hora de evaluar el impacto pronóstico de las alteraciones genómicas en la LMA del adulto.

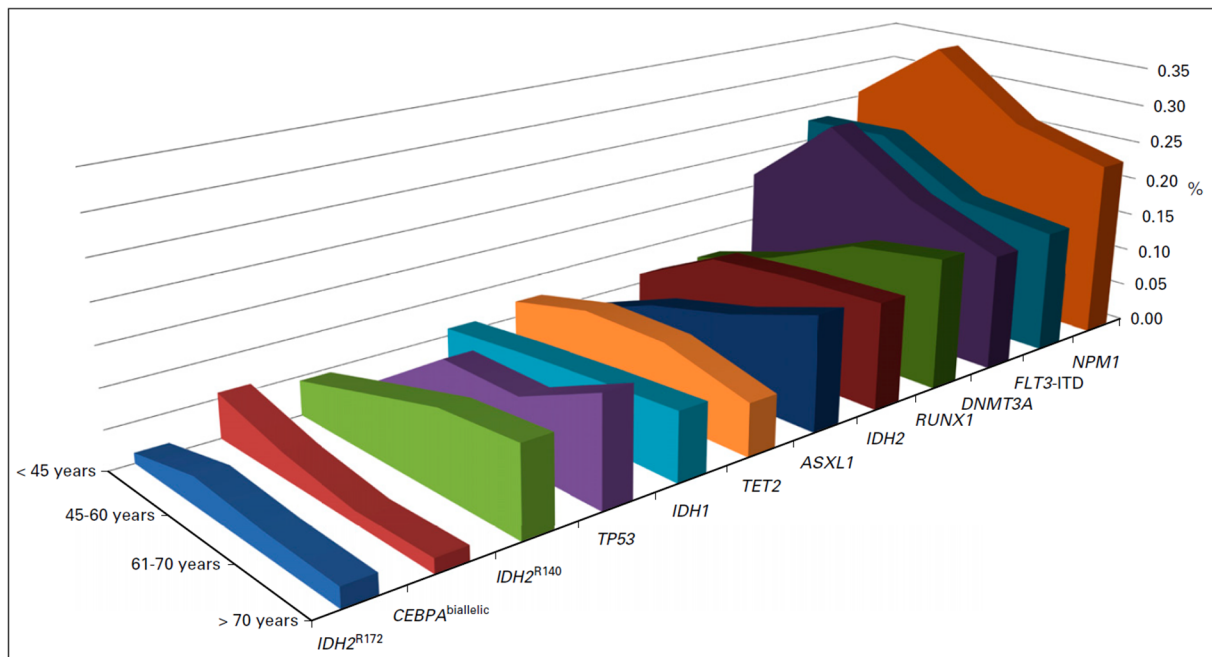


Figura 7. Frecuencia relacionada con la edad de mutaciones genéticas recurrentes seleccionadas en leucemia mielooblástica aguda del adulto. La figura se elaboró con la información de la base de datos del Grupo Germano-Austriaco de Estudio de la LMA (AMLSG), que contenía en 10622 pacientes con LMA tratados mayoritariamente con quimioterapia intensiva (tomado de Bullinger et al. 2017)⁸⁶

3. INTELIGENCIA ARTIFICIAL EN HEMATOLOGÍA

La digitalización de los datos ha transformado muchos de los aspectos de la asistencia sanitaria. Cada interacción con un paciente genera una gran cantidad de datos electrónicos, como constantes vitales, resultados analíticos o pruebas de imagen, que se incorporan a la historia clínica electrónica. Además, en los últimos años se han incorporado también los datos moleculares, dando lugar a una complejidad sin precedentes^{164–166}. Los hematólogos actúan basados en su formación, experiencia e intuición, pero pueden estar limitados por sus percepciones y sesgos. Por otra parte, la tecnología de aprendizaje automático ofrece herramientas para procesar grandes cantidades de datos que exceden la capacidad de comprensión de la mente humana.

En la literatura médica, el término inteligencia artificial se emplea a menudo de forma intercambiable con el de aprendizaje automático (*machine learning*). Sin embargo, son dos conceptos diferentes. La inteligencia artificial describe programas informáticos que simulan e imitan la inteligencia humana, como la resolución de problemas y el aprendizaje. El aprendizaje automático es una rama de la inteligencia artificial implicada en el descubrimiento automático de patrones en los datos^{165,167,168}. Es decir, el modelo “aprende” de ejemplos, en lugar de haber sido programado previamente con reglas que siguen una hipótesis estricta¹⁶⁷.

El aprendizaje automático está profundamente relacionado con los métodos estadísticos tradicionales. Los modelos desarrollados de una u otra manera se podrían representar en un continuo del “espectro del aprendizaje automático”, desde un modelo guiado plenamente por los investigadores hasta uno guiado exclusivamente por la máquina¹⁶⁷. El éxito de los modelos de aprendizaje automático se basa en su habilidad para extraer características informativas de grandes cantidades de datos¹⁶⁹. Sin embargo, requieren una gran cantidad de información para el entrenamiento del modelo¹⁶⁵. Para que un modelo sea útil, debe responder a una pregunta clínica y tener la capacidad de aplicarse en poblaciones que no se incluyeron en el desarrollo del mismo¹⁷⁰.

Las aplicaciones del aprendizaje automático en hematología son muy diversas, pero se pueden agrupar en dos grandes campos: diagnóstico y modelos predictivos.

3.1 Diagnóstico

Durante décadas, el diagnóstico de las neoplasias hematológicas se ha basado casi de forma exclusiva en la valoración de muestras teñidas de MO o SP. La citomorfología todavía constituye el pilar del diagnóstico hematológico y permite guiar otras pruebas como la citogenética, el inmunofenotipo o la genética molecular. Sin embargo, la exactitud de los resultados citomorfológicos depende en gran medida de la experiencia del personal, e incluso con hematólogos o patólogos expertos, la variabilidad inter e intra-observador puede llegar a ser significativa^{171,172}. En cambio, la digitalización de las imágenes de microscopía, en combinación con métodos de aprendizaje automático, permite procesar las imágenes de forma automática y diferenciar cada estirpe celular¹⁷³⁻¹⁷⁵. Los modelos se han evaluado de forma prospectiva para clasificar imágenes de células individuales de extensiones de SP y la concordancia entre las predicciones del modelo y las valoraciones de los hematólogos alcanza el 95% en los casos patológicos^{176,177}.

Algunas características morfológicas se pueden relacionar con anomalías genéticas. Por ejemplo, los reordenamientos de *MYC* pueden aparecer en un subgrupo de linfoma B difuso de célula grande y es posible inferir las muestras que presentarán dicho reordenamiento únicamente mediante la revisión de preparaciones teñidas con hematoxilina-eosina de biopsias de tejido afecto, con una sensibilidad del 93%¹⁷⁸. En el campo de la LMA, los algoritmos publicados son capaces de predecir con gran precisión las mutaciones de *NPM1*¹⁷⁹, *FLT3-ITD*¹⁸⁰ o incluso el gen de fusión *CBFB:MYH11*^{181,182} únicamente a partir de las imágenes, con importantes implicaciones a nivel de tratamiento y pronóstico en esta patología.

Los modelos de inteligencia artificial también se han explorado en el ámbito de la CMF, la citogenética convencional o la NGS, facilitando el proceso diagnóstico y la interpretación de los resultados (**Figura 8**)^{172,183}.

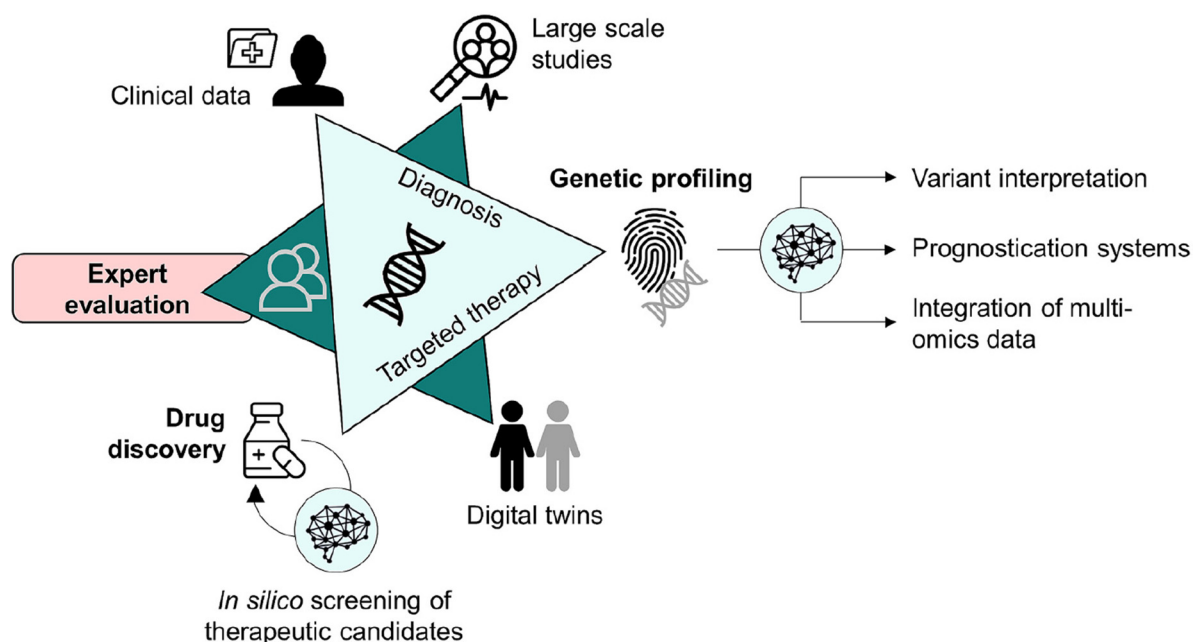


Figura 8. Posibles implementaciones de sistemas basados en inteligencia artificial para complementar la evaluación de los datos genéticos y ayudar en la toma de decisiones médicas (tomado de Walter et al. 2023)¹⁷²

3.2 Modelos predictivos

El desarrollo de la metodología de aprendizaje automático en el análisis de perfiles moleculares no solo es relevante a nivel diagnóstico, sino que también permite realizar estimaciones pronósticas centradas en el paciente. Es posible predecir de forma personalizada la SG y el riesgo de transformación a LMA en pacientes con SMD en diferentes momentos evolutivos de la enfermedad, con una precisión que supera las clasificaciones de riesgo establecidas en dicha patología¹⁸⁴. De forma análoga, un estudio del Grupo Español de Enfermedades Mieloproliferativas Crónicas Filadelfia Negativas (GEMFIN) demostró que la capacidad predictiva de un modelo desarrollado con inteligencia artificial en pacientes con mielofibrosis era superior a la de los índices pronósticos tradicionales^{185,186}.

En el ámbito de la LMA, se han publicado modelos que permiten predecir la probabilidad de muerte precoz tras la administración de quimioterapia intensiva o la probabilidad de obtención de respuesta completa¹⁸⁷⁻¹⁸⁹. Además, la inteligencia artificial también puede ser útil para predecir el pronóstico a largo plazo, pudiendo mejorar la precisión pronóstica de la clasificación de la ELN^{188,190-193}. Sin embargo, algunos de estos modelos precisan datos que actualmente no se obtienen de forma habitual, como

perfiles de expresión génica o marcadores epigenéticos, lo cual limita su aplicación en la práctica asistencial de los pacientes con LMA^{190,191}.

4. EL PROYECTO HARMONY

En 2016, la Iniciativa de Medicamentos Innovadores (IMI) lanzó un programa denominado “Big Data for Better Outcomes”, con el objetivo de explorar nuevas formas de aprovechar el potencial colectivo de compartir datos de salud y usarlos para investigar y mejorar los resultados de este ámbito, conforme con las recomendaciones y principios éticos vigentes. El programa reconocía el potencial de la capacidad transformadora del Big Data en diversas áreas como los sistemas de atención médica, la estratificación de pacientes, el tratamiento de enfermedades, el diseño de ensayos clínicos o el uso de tecnologías médicas por parte de los pacientes. Sin embargo, solo es posible desbloquear este potencial cuando los sistemas sanitarios vayan más allá de la mera recopilación de grandes cantidades de datos. Por ello, es necesario que las bases de datos existentes se vinculen y analicen con técnicas adecuadas. De esta manera, el ámbito sanitario podría tener a su disposición nuevas formas de acelerar la investigación e identificar el tratamiento más adecuado de forma individualizada, en dirección hacia una medicina personalizada. El acceso a grandes conjuntos de datos combinados representa una imagen más completa de los pacientes con una determinada patología, por lo que permitiría identificar subgrupos clínicamente relevantes. El programa se construyó en torno a diferentes proyectos IMI, cada uno centrado en un área terapéutica: Roadmap (Alzheimer), HARMONY y HARMONY PLUS (neoplasias hematológicas), Big Data @ Heart (enfermedades cardiovasculares), Pioneer y OPTIMA (cáncer de próstata) y el programa conjunto EHDEN (*European Health Data Evidence Network*).

El proyecto HARMONY (*Healthcare Alliance for Resourceful Medicine Offensive against Neoplasms in Hematology*) fue lanzado en enero de 2017 con el objetivo de recopilar, armonizar y analizar datos sobre el diagnóstico, tratamiento y pronóstico de pacientes con neoplasias hematológicas, específicamente en leucemia linfoblástica aguda (LAL), LMA, leucemia linfática crónica (LLC), SMD, mieloma múltiple (MM) y linfoma no Hodgkin (LNH). HARMONY se constituyó como una colaboración público-privada multidisciplinar, contando con el apoyo de grupos de trabajo cooperativos, hospitales y universidades, así como de empresas farmacéuticas y organizaciones de

pacientes. Además, el 2019 IMI aprobó HARMONY PLUS, una ampliación del proyecto original, para dar cobertura a las demás neoplasias hematológicas que no pudieron ser incluidas en el primer proyecto.

La alianza HARMONY se ha convertido de una de las iniciativas líderes en Europa para el uso de Big Data en salud, con más de 170.000 pacientes con neoplasias hematológicas identificados por los colaboradores y más de 120.000 registros transferidos a la plataforma¹⁹⁴. La base de datos incluye variables clínicas, analíticas, tratamientos administrados y respuesta a los mismos, evolución y supervivencia de los pacientes, así como datos ómicos procedentes tanto de técnicas tradicionales como de paneles de NGS.

Uno de los aspectos fundamentales es determinar que los datos que se incorporan a la plataforma sean de calidad, para poder asegurar la fiabilidad de la información descriptiva, comparativa y predictiva generada con los análisis realizados en la plataforma. La información se verifica antes de su inclusión en la base de datos, para garantizar que esté estandarizada, anonimizada y sea lo más completa posible. Para facilitar la transmisión con el centro de origen, los datos transferidos a HARMONY se reciben en el mismo formato que en la base original, lo que implica múltiples estructuras y codificaciones de datos, por lo que se requiere una comunicación constante con el proveedor de los datos y un gran conocimiento del campo para garantizar la correcta armonización de los datos.

HARMONY ha desarrollado estándares de seguridad y procesamiento de datos consistentes con las regulaciones nacionales y de la Unión Europea sobre intercambio de datos, privacidad y normativas éticas. Este sistema de pseudonimización doble con garantías, que cumple con el Reglamento General de Protección de Datos, se ha convertido en un modelo para proyectos similares. Además de que el proveedor de datos elimine los identificadores directos y enmascare los identificadores indirectos antes de realizar la transferencia, el Instituto de Epidemiología y Biometría Médica de Ulm actúa como intermediario, verificando la correcta anonimización y asignando un nuevo identificador de HARMONY a los datos.

El siguiente paso del procesamiento es la armonización, que consiste en convertir todos los datos al modelo OMOP (*Observational Medical Outcomes Partnership*), lo cual

resulta crucial para organizar de forma estandarizada la información, permitiendo su posterior análisis junto con otros datos ya armonizados presentes en la plataforma (Figura 9).

La información se almacena en una única base de datos, pero solo están disponibles los subconjuntos de variables esenciales para los análisis diseñados con el fin de responder a preguntas de investigación específicas. Además, solo se otorga acceso a dichos subconjuntos a un grupo limitado de usuarios durante el periodo de tiempo que sea necesario para completar el proyecto. El hecho de que solo ingresen datos anónimos, de que la plataforma cuente con la certificación ISO27001, junto a otras medidas técnicas y de organización, contribuye a garantizar la privacidad y seguridad de los datos en todo momento.

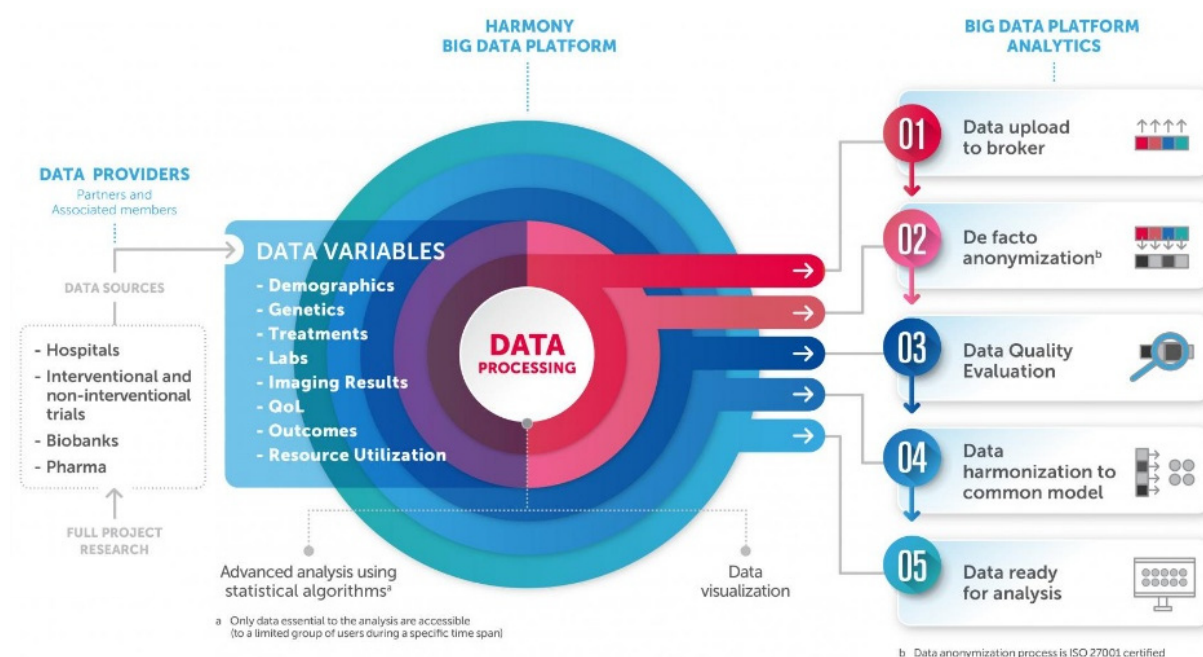


Figura 9. Representación esquemática de las etapas en el procesamiento de los datos para su inclusión en la plataforma HARMONY (modificado de la página web de la Fundación HARMONY)¹⁹⁵

La base de datos de HARMONY contiene información sobre numerosas neoplasias hematológicas, pero ha sido pionera en la incorporación de datos sobre LMA y en el momento actual es la patología con más registros tanto identificados como transferidos¹⁹⁴. Para ello, ha contado con la colaboración de grupos cooperativos como el Grupo Germano-Austriaco de Estudio de la LMA (AMLSG), la Fundación Hemato-Oncológica para Adultos de Países Bajos (HOVON), la Alianza para el Estudio de la

Leucemia (SAL) y Grupo Cooperativo de LMA (AMLCG) de Alemania, el Instituto Nacional de Investigación sobre el Cáncer del Reino Unido (NCRI), el Grupo Cooperativo de Estudio y Tratamiento de las Leucemias Agudas y Mielodisplásicas (CETLAM, España), el registro sueco de LMA, y de centros como el Laboratorio de Leucemia de Múnich (MLL, Alemania), la Universidad de Wrocław (Polonia), el Hospital Universitario 12 de Octubre (España), el Hospital Universitario de Essen (Alemania) y la Universidad Queen's de Belfast (Irlanda del Norte), entre otros.

En definitiva, la plataforma HARMONY representa una excelente oportunidad para profundizar en el conocimiento de la LMA del adulto e intentar responder algunas de las preguntas no resueltas en esta patología.

HIPÓTESIS

La leucemia mieloide aguda (LMA) es una enfermedad heterogénea en relación a sus características clínicas, respuesta al tratamiento y evolución. En pacientes candidatos a recibir esquemas de quimioterapia intensiva, la base del tratamiento apenas se ha visto modificada durante décadas, por lo que el incremento de la supervivencia de los pacientes ha estado condicionado por una terapia de soporte más adecuada y por la optimización del trasplante alogénico de progenitores hematopoyéticos (alo-TPH). Este procedimiento está asociado a múltiples complicaciones potencialmente fatales, por lo que es preciso realizar una cuidadosa valoración del pronóstico de la LMA para seleccionar a los pacientes que se puedan beneficiar de la realización del mismo.

El perfil genómico es uno de los factores pronósticos más importantes en la LMA del adulto, por lo que las alteraciones citogenéticas y moleculares más frecuentes se han ido incorporando progresivamente a las clasificaciones de riesgo y las guías clínicas de esta patología. El desarrollo y la generalización de las técnicas de secuenciación de nueva generación (NGS) ha relevado la existencia de múltiples alteraciones genéticas en cada paciente, desvelando un perfil genómico muy diverso. Además, se han descrito varias interacciones gen-gen, lo que sugiere que el efecto clínico de algunas mutaciones podría depender del contexto genómico global en el que suceden. Sin embargo, la mayor parte de los estudios publicados hasta la fecha evalúan las alteraciones genéticas de forma individual, sin tener en cuenta otras alteraciones concomitantes, probablemente limitados por el tamaño muestral. Es preciso disponer de una gran base de datos para encontrar factores pronósticos que puedan ser relevantes únicamente en ciertos subtipos de LMA o en presencia de determinadas co-mutaciones.

La plataforma HARMONY dispone de datos de miles de pacientes con LMA de varios países europeos, incluyendo resultados de NGS de paneles mieloides, por lo que podría constituir una herramienta de gran utilidad para el estudio de las interacciones entre las anomalías genómicas en estos enfermos, así como el análisis de subtipos de LMA minoritarios.

La LMA con t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A* se ha considerado de riesgo intermedio durante más de una década, mientras que la LMA con cualquier otro reordenamiento de dicho gen se considera de riesgo adverso. Sin embargo, varios

estudios no encontraron diferencias en el pronóstico entre ambos subtipos, por lo que cabría la posibilidad de que la supervivencia de los pacientes estuviera condicionada por otras variables, como las mutaciones genéticas concomitantes.

El estudio de las interacciones gen-gen en la LMA puede ser complejo, ya que la mitad de los pacientes presenta alteraciones citogenéticas concomitantes. Sin embargo, la mutación en *NPM1* se asocia de forma característica a cariotipo normal, por lo que podría constituir el subtipo de LMA idóneo para evaluar el impacto pronóstico de combinaciones de mutaciones y las interacciones entre las mismas.

Uno de los aspectos más difíciles en el manejo de los pacientes con LMA es decidir quién debe someterse a un alo-TPH tras alcanzar la primera respuesta completa (RC1). La estratificación pronóstica de la ELN ofrece una aproximación de gran utilidad y ha sido validada en numerosos estudios, por lo que se ha implementado en los protocolos de tratamiento a nivel nacional e internacional. Sin embargo, es posible que el pronóstico de algunos pacientes sea diferente al de la categoría de riesgo en la que se clasifican. Por ello, sería necesaria una valoración individualizada, que tuviera en cuenta tanto los parámetros clínicos como la interacción entre las alteraciones genómicas de cada paciente.

El aprendizaje automático es una rama de la inteligencia artificial que podría ser de utilidad para integrar los datos de los pacientes con LMA y encontrar asociaciones complejas que resultan difíciles de identificar con métodos estadísticos convencionales. De esta manera, permitiría predecir el riesgo de cada paciente de forma individualizada, lo cual podría contribuir en la toma de decisiones clínicas. Los modelos de aprendizaje automático requieren una gran cantidad de datos para su entrenamiento, lo cual estaría disponible en la plataforma de HARMONY.

En resumen, la hipótesis de esta tesis doctoral es que la integración de las alteraciones citogenéticas y el perfil mutacional de la LMA del adulto requiere una amplia base de datos y herramientas de análisis avanzadas, pero podría proporcionar información clínicamente relevante, contribuyendo a mejorar el diagnóstico y tratamiento de los pacientes con esta patología.

OBJETIVOS

OBJETIVO GENERAL

Analizar el valor pronóstico de las alteraciones genómicas concomitantes en la LMA del adulto con reordenamiento de *KMT2A* o mutación en *NPM1* y explorar si el empleo de técnicas de inteligencia artificial podría mejorar la capacidad predictiva del pronóstico de los pacientes con LMA en primera respuesta completa.

OBJETIVOS ESPECÍFICOS

1. Evaluar el impacto pronóstico de los diferentes reordenamientos de *KMT2A* en la LMA del adulto.
2. Caracterizar el perfil genómico de la LMA con reordenamiento de *KMT2A*, así como sus potenciales implicaciones en la respuesta al tratamiento y la supervivencia global.
3. Identificar las combinaciones de co-mutaciones clínicamente relevantes en la LMA con mutación en *NPM1*.
4. Proponer una nueva clasificación de riesgo en la LMA con mutación en *NPM1* basada en los patrones de co-mutaciones y compararla con la establecida por la *European LeukemiaNet* en 2022 (ELN2022) para ese subtipo.
5. Desarrollar y validar un modelo de aprendizaje automático que genere estimaciones pronósticas personalizadas en adultos con LMA que alcancen la primera respuesta completa tras la administración de esquemas de quimioterapia intensiva, para identificar pacientes en los que se podría valorar la realización de un trasplante alogénico de progenitores hematopoyéticos.
6. Comparar la capacidad predictiva del modelo con respecto a la clasificación de riesgo ELN2022, en términos de supervivencia global (OS), supervivencia libre de recaída (RFS) e incidencia acumulada de recaída (CIR).

RESULTADOS

Esta sección comprende varios estudios realizados a lo largo de la presente tesis doctoral e incluye los apartados de material y métodos, resultados y discusión. Se ha dividido en tres capítulos:

Capítulo 1. Alberto Hernández-Sánchez, Teresa González, Marta Sobas, Eric Sträng, Gastone Castellani, María Abáigar, Peter J M Valk, Ángela Villaverde Ramiro, Axel Benner, Klaus H Metzeler, Raúl Azibeiro, Jesse M Tettero, Joaquín Martínez-López, Marta Pratcorona, Javier Martínez Elicegui, Ken I Mills, Christian Thiede, Guillermo Sanz, Konstanze Döhner, Michael Heuser, Torsten Haferlach, Amin T Turki, Dirk Reinhardt, Renate Schulze-Rath, Martje Barbus, Jesús María Hernández-Rivas, Brian Huntly, Gert Ossenkoppele, Hartmut Döhner, Lars Bullinger. **Rearrangements involving 11q23.3/KMT2A in adult AML: mutational landscape and prognostic implications - a HARMONY study.** *Leukemia*. 2024;38(9):1929-1937.

Capítulo 2. Alberto Hernández-Sánchez, Angela Villaverde Ramiro, Eric Sträng, Amin T. Turki, María Abáigar, Jurjen Versluis, Ian Thomas, Marta Sobas, Javier Martínez Elicegui, Gastone Castellani, Axel Benner, Raúl Azibeiro, Jesse M. Tettero, Rabea Mecklenbrauck, Joaquín Martínez-López, Marta Pratcorona, Ken I Mills, Guillermo Sanz, Maria Teresa Voso, Sören Lehmann, Christoph Röllig, Christian Thiede, Klaus H. Metzeler, Konstanze Döhner, Michael Heuser, Torsten Haferlach, Peter JM Valk, Nigel Russell, Jesús María Hernández-Rivas, Brian Huntly, Gert Ossenkoppele, Hartmut Döhner, Lars Bullinger. **Unravelling Co-Mutational Patterns with Prognostic Implications in *NPM1* Mutated Adult Acute Myeloid Leukemia – A HARMONY Study.** *Submitted manuscript (under review).*

Capítulo 3. Alberto Hernández-Sánchez, Javier Martínez Elicegui, Eric Sträng, Axel Benner, Marta Sobas, Angela Villaverde Ramiro, Amin T. Turki, María Abáigar, Gastone Castellani, Jurjen Versluis, Ian Thomas, Jesse M. Tettero, Rabea Mecklenbrauck, Joaquín Martínez-López, Marta Pratcorona, Guillermo Sanz, Maria Teresa Voso, Sören Lehmann, Christoph Röllig, Klaus H. Metzeler, Konstanze Döhner, Michael Heuser, Torsten

Haferlach, Peter JM Valk, Nigel Russell, Jesús María Hernández-Rivas, Brian Huntly, Gert Ossenkoppele, Hartmut Döhner, Lars Bullinger. **Machine Learning Provides Individualized Prediction of Outcomes after First Complete Remission in Adult Acute Myeloid Leukemia – A HARMONY Study.** *Manuscript in preparation.*

Todos ellos se han desarrollado para cumplir con el objetivo general de este trabajo y dar título a esta tesis doctoral: **“Predicción personalizada del pronóstico de la leucemia mieloblástica del adulto mediante la integración de datos de secuenciación masiva y técnicas de inteligencia artificial”.**

Una sección independiente de esta tesis está dedicada a una discusión general, que incluye datos adicionales en relación a toda la investigación.

Además, al final de la tesis se incluye el material suplementario correspondiente a cada uno de los capítulos mencionados previamente.

Rearrangements Involving 11q23.3/*KMT2A* in Adult AML: Mutational Landscape and Prognostic Implications – A HARMONY Study

Alberto Hernández-Sánchez^{1,2,3}, Teresa González^{2,3}, Marta Sobas⁴, Eric Sträng⁵, Gastone Castellani⁶, María Abáigar^{2,3}, Peter JM Valk⁷, Angela Villaverde Ramiro^{2,3}, Axel Benner⁸, Klaus H. Metzeler⁹, Raúl Azibeiro^{1,2}, Jesse M. Tetters¹⁰, Joaquín Martínez-López¹¹, Marta Pratcorona¹², Javier Martínez Elicegui^{2,3}, Ken I Mills¹³, Christian Thiede¹⁴, Guillermo Sanz^{15,16}, Konstanze Döhner¹⁷, Michael Heuser¹⁸, Torsten Haferlach¹⁹, Amin T. Turki²⁰, Dirk Reinhardt²¹, Renate Schulze-Rath²², Martje Barbus²³, Jesús María Hernández-Rivas^{1,2,3}, Brian Huntly²⁴, Gert Ossenkoppele¹⁰, Hartmut Döhner¹⁷, Lars Bullinger²⁵.

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ARTICLE OPEN



ACUTE MYELOID LEUKEMIA

Rearrangements involving 11q23.3/*KMT2A* in adult AML: mutational landscape and prognostic implications – a HARMONY study

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Balanced rearrangements involving the *KMT2A* gene (*KMT2Ar*) are recurrent genetic abnormalities in acute myeloid leukemia (AML), but there is lack of consensus regarding the prognostic impact of different fusion partners. Moreover, prognostic implications of gene mutations co-occurring with *KMT2Ar* are not established. From the HARMONY AML database 205 *KMT2Ar* adult patients were selected, 185 of whom had mutational information by a panel-based next-generation sequencing analysis. Overall survival (OS) was similar across the different translocations, including t(9;11)(p21.3;q23.3)/*KMT2A::MLL3* ($p = 0.756$). However, independent prognostic factors for OS in intensively treated patients were age >60 years (HR 2.1, $p = 0.001$), secondary AML (HR 2.2, $p = 0.043$), *DNMT3A*-mut (HR 2.1, $p = 0.047$) and *KRAS*-mut (HR 2.0, $p = 0.005$). In the subset of patients with de novo AML < 60 years, *KRAS* and *TP53* were the prognostically most relevant mutated genes, as patients with a mutation of any of those two genes had a lower complete remission rate (50% vs 86%, $p < 0.001$) and inferior OS (median 7 vs 30 months, $p < 0.001$). Allogeneic hematopoietic stem cell transplantation in first complete remission was able to improve OS ($p = 0.003$). Our study highlights the importance of the mutational patterns in adult *KMT2Ar* AML and provides new insights into more accurate prognostic stratification of these patients.

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INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease in terms of clinical features and outcomes, in which the presence of cytogenetic aberrations and gene mutations provides crucial prognostic information that guides clinical decisions [1–5]. Balanced rearrangements involving the *lysine methyltransferase 2a* gene (*KMT2A*, previously known as *MLL*), located on

chromosome band 11q23.3, have been described in 3–6% of adult patients with de novo AML [6–11].

Balanced chromosome rearrangements involving 11q23.3 and *KMT2A* (*KMT2Ar*) are very heterogeneous, as more than 100 fusion partners have already been described in leukemia patients [12]. Among them, the most frequent is the t(9;11)(p21.3;q23.3) resulting in a *KMT2A::MLL3* fusion, which will be referred to as

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t(9;11) hereafter. Other common translocations in adult AML are the t(6;11)(q27;q23.3)/*KMT2A::AFDN* [hereafter t(6;11)], t(11;19)(q23.3;p13.3)/*KMT2A::MLLT1*, t(11;19)(q23.3;p13.1) resulting in a *KMT2A::ELL* fusion, t(ins(10;11)(p12.3;q23.3)/*KMT2A::MLLT10* [hereafter t(10;11)], and t(11;17)(q23.3;q25) resulting in a *KMT2A::SEPTIN9* fusion [hereafter t(11;17)] [7, 13, 14].

There is a controversy in the literature regarding the prognostic impact of different *KMT2Ar* AML. Several studies have reported better outcomes in patients with t(9;11) when compared to the rest of *KMT2Ar* [7, 14–16], although some of them were restricted to specific populations (e.g., patients aged <60 years with de novo AML). On the other hand, these findings could not be confirmed by other studies [6, 17–19]. Moreover, heterogeneous results have also been reported regarding outcome in the post-allogeneic transplantation setting [20–22].

Additional cytogenetic aberrations have been identified in about 40% of *KMT2Ar* adult AML patients, but they were not associated with different patient outcomes [11, 16, 17]. While distinct gene mutations have proven to be excellent prognostic markers in AML [1–4, 23], *KMT2Ar* AML shows a lower incidence of co-occurring gene mutations, although a predominance of *RAS* mutations has been reported. The prognostic impact of these additional gene mutations in *KMT2Ar* adult AML has not been established yet [9, 14, 17, 19].

In order to address these open questions, we analyzed a large cohort of patients with *KMT2Ar* AML included in the Healthcare Alliance for Resourceful Medicine Offensive against Neoplasms in Hematology (HARMONY) AML multicenter database.

METHODS

Patients

At the time of the analysis (December 2022) the HARMONY Alliance AML database contained 6342 AML patient data sets contributed by 11 European centers or cooperative groups (Supplementary Table S1). Conventional karyotype information was available for 6005 patients based on which a total of 205 *KMT2Ar* adult patients could be identified (incidence of *KMT2Ar*: 3.4%), who were diagnosed with AML between December 1996 and January 2020 (Supplementary Fig. S1). Some of the patients included in this analysis were also part of previously published AML cohorts [3, 9, 24–26].

For data upload into the HARMONY Big Data Platform, all patient data went through a robust double brokerage pseudonymization procedure in compliance with the General Data Protection Regulation. Next, data were harmonized and transformed using the Observational Medical Outcomes Partnership (OMOP) Common Data Model [27].

This study was performed in accordance with the Declaration of Helsinki and was approved by the HARMONY steering committee. The HARMONY research project was reviewed and approved by the Medicinal Research Ethics Committee of the University of Salamanca. For its studies, HARMONY provides an ethical and data-protection framework for the secondary use of data including a *de facto* anonymization. Written informed consent had been previously collected from all patients in the respective HARMONY partner institutions.

Cytogenetic and genetic analyses

Cytogenetic analyses were performed using standard G-banding with trypsin-Giemsa or trypsin-Wright staining in approved cytogenetic laboratories. The analysis and nomenclature of the chromosomes were based on International System for Human Cytogenetic Nomenclature (ISCN) [28]. Karyotypes were considered with clonal abnormalities if at least two metaphases showing the same abnormality were detected. The results of cytogenetic analysis of bone marrow were centrally reviewed by experienced cytogeneticists (JMHR and TGM) using standard ISCN-2020 criteria. Although t(11;19)(q23.3;p13.3) and t(11;19)(q23.3;p13.1) are known to be two distinct rearrangements [13], enough information to discriminate was only provided for around half of those patients, so we grouped them together as t(11;19)(q23.3;p13) [hereafter t(11;19)].

KMT2Ar cases were also evaluated by fluorescence in situ hybridization (FISH) with a commercial break apart probe in order to confirm *KMT2Ar*. FISH results were also centrally reviewed and reported using standard

ISCN-2020 nomenclature. For selected cases, the *AF9::KMT2A* fusion transcript was also validated by reverse transcription polymerase chain reaction (RT-PCR) using the generic PCR cyclor program developed by the BIOMED-1 initiative for the standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia [29].

Next-generation sequencing (NGS) data using local custom diagnostic panels were available for 185 patients (90% of our *KMT2Ar* cohort). The different NGS panels overlapped with regard to 40 genes implicated in myeloid malignancies (Supplementary Table S2). Panel sequencing was performed and analyzed according to previous studies [3, 9, 24]. Gene variants were centrally reviewed, only pathogenic and likely pathogenic variants were considered, and data including variant allele frequency were harmonized.

Statistical analysis

Clinical endpoints were defined as recommended by international guidelines [4]. Composite complete remission (CRc) was defined as either complete remission (CR) or CR with incomplete hematologic recovery (CRi). Categorical variables were compared using Pearson's Chi-squared test and Fisher's exact test, while continuous variables were evaluated using Kruskal–Wallis test for multiple comparisons and Wilcoxon test for pairwise comparison. Overall survival (OS) and relapse-free survival (RFS) were estimated using the Kaplan–Meier method and differences between survival distributions were evaluated using the log-rank test. To estimate survival probabilities considering the effect of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in first complete remission (CR1), Simon–Makuch method with clock-back correction was applied as previously reported [30, 31]. Cox proportional hazards model was used for multivariate survival analysis, including variables that were significant in the univariate analysis (age, AML type, *KRAS*-mut, *DNMT3A*-mut, *TP53*-mut and presence of complex cytogenetic aberrations), as well as *KMT2A* fusion partner. To examine the effect of allo-HSCT in CR1, multivariate Cox model with allo-HSCT as a time-dependent intervening event was used. All reported p values are two-sided at the conventional 5% significance level. Data were analyzed as of December 2022 using R software (v3.6.3).

RESULTS

Patient characteristics

The study population of 205 *KMT2Ar* adult AML patients included 54% females and median age at diagnosis was 48 years (range 18–86), while 75% of the patients were younger than 60 years. Most of the patients (73%) had de novo AML and 43% underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT). Median follow-up was 4.9 years for those patients still alive. Patient baseline characteristics are summarized in Table 1 and Supplementary Table S3.

Frequency of specific 11q23.3/*KMT2A* rearrangements

Translocation t(9;11) was the most frequent *KMT2Ar* [$n = 101$, 49%], followed by t(11;19) [$n = 33$, 16%] and t(6;11) [$n = 24$, 12%]. Both t(10;11) and t(11;17) accounted for 5% of patients each ($n = 10$), while the less common translocations grouped together (hereafter “other *KMT2Ar*”) represented 13% of patients. A comparison of basic characteristics among different translocation partners showed differences in age distribution, with the youngest patients being in t(6;11) [median 41 years] and the oldest in the “other *KMT2Ar*” subgroup (median 55.9 years) ($p = 0.004$) (Supplementary Table S4). In addition, the proportion of therapy-related AML was highest in the t(9;11) [30.7%] subgroup, while none was found in the t(6;11) cases ($p = 0.013$). Allo-HSCT rates did not differ significantly among distinct *KMT2Ar* categories ($p = 0.523$).

Additional cytogenetic abnormalities

Additional cytogenetic abnormalities were present in 40% of patients. Complex aberrations (≥ 2 abnormalities in addition to the *KMT2Ar*) were the most frequent (19%), followed by trisomy 8 (18%), derivative 11 (6%) and trisomy 21 (5%). Isolated trisomy 8 as the only additional cytogenetic abnormality (apart from *KMT2Ar*) was found in 19 patients (9%) and was almost exclusively

Table 1. Baseline characteristics of *KMT2Ar* adult AML patients.

	n = 205
Female sex	111 (54.1%)
Median age in years (range)	48.1 (18.6–86.2)
Age ≥ 60 years	51 (24.9%)
AML type	
De novo AML	148 (72.2%)
Secondary AML	57 (27.8%)
AML after MDS	14 (6.8%)
Therapy-related AML	43 (21%)
Hemoglobin (g/dL)	9.5 [Q1 = 8.3, Q3 = 11.3]
WBC ($\times 10^9/L$)	23.1 [Q1 = 4.7, Q3 = 58]
(>100 $\times 10^9/L$)	21 (10.2%)
Platelets ($\times 10^9/L$)	51.5 [Q1 = 29.8, Q3 = 107.5]
Bone marrow % of blasts	83 [Q1 = 70.2, Q3 = 90]
Chromosomal aberrations	
t(9;11)	101 (49%)
t(11;19)	33 (16%)
t(6;11)	24 (12%)
t(10;11)	10 (5%)
t(11;17)	10 (5%)
Other <i>KMT2Ar</i>	27 (13%)
Additional chromosomal aberrations	85 (41.6%)
Complex aberrations (≥2 apart from <i>KMT2Ar</i>)	38 (19%)
Trisomy 8	37 (18%)
Derivative 11	12 (6%)
ELN 2022	
Favorable	–
Intermediate	101 (49.3%)
Adverse	104 (50.7%)
Treatment	
Intensive	195 (95%)
Non-intensive	10 (5%)
Composite complete response	120 (63.8%)
Early death	
30-day mortality	28 (13.6%)
60-day mortality	36 (17.6%)
Allogeneic HSCT	88 (42.9%)
In CR1	48 (23.4%)
In CR2 or later	15 (7.3%)
Unknown response at allo-HSCT	25 (12.2%)
Median survival in years (95% CI)	1.4 (1.1–1.7)

AML acute myeloid leukemia, MDS myelodysplastic syndrome, WBC white blood cell, ELN European LeukemiaNet, HSCT hematopoietic stem cell transplantation, CR1 first complete remission, CR2 second complete remission, CI confidence interval.

found in the t(9;11) subgroup (16 patients, $p < 0.001$). On the other hand, complex aberrations were more frequently associated with “other *KMT2Ar*” ($p = 0.001$).

Mutational landscape

NGS analysis was performed in 185 (90%) cases using a panel of up to 90 genes (Supplementary Table S2). A total of 263 mutations

were found in 44 of the 90 analyzed genes. The median number of gene mutations per patient was 1 (range 0–6).

The most frequent gene mutations co-occurring with a *KMT2Ar* were *NRAS* (21%), *KRAS* (19.5%) and *FLT3*-TKD (13.3%), followed by *TP53* (8.6%), *TET2* (8.1%), *ASXL1* (7%), *WT1* (7%), *DNMT3A* (6.5%) and *FLT3*-ITD (5.8%). When grouped together, mutations in genes that comprise the RAS signaling pathway (*NRAS*, *KRAS*, *PTPN11* and *BRAF*) were present in 42.1% of *KMT2Ar* AML. Of note, *NRAS* and *KRAS* mutations were not mutually exclusive in our cohort, as seven patients harbored mutations in both genes. The distribution of mutations within the different *KMT2Ar* subgroups is shown in Fig. 1. *WT1* mutation was predominant in t(11;19) subgroup ($p < 0.001$), while *TP53* mutation was recurrent in the “other *KMT2Ar*” subgroup ($p = 0.002$).

Patient outcomes

Median OS was 1.4 years for the whole cohort. Notably, OS was similar across the different *KMT2Ar* subgroups and we were not able to find any differences in OS between t(9;11) and the remainder of the *KMT2Ar* patients ($p = 0.756$, Fig. 2A). However, in t(9;11) patients there was a trend for better RFS ($p = 0.054$, Fig. 2B).

Patients harboring concomitant cytogenetic aberrations had a higher rate of early deaths (first 60 days 23.2% vs 7.3%, $p = 0.002$), although those patients were older (median age 52 vs 46 years, $p = 0.008$) and median overall survival was similar to the rest of the cohort (12 vs 15 months, $p = 0.529$), as shown in Supplementary Fig. S2A. Complex aberrations (≥2 additional cytogenetic abnormalities) were associated with shorter OS, with median OS of 9 vs 18 months for the rest of the cohort ($p = 0.046$, Supplementary Fig. S2B). Otherwise, we did not find significant differences in OS between patients with trisomy 8 when compared to the remainder of patients ($p = 0.920$, Supplementary Fig. S2C). Patients with isolated trisomy 8 as the only additional cytogenetic aberration also presented with similar OS compared to the rest of the cohort ($p = 0.402$, Supplementary Fig. S2D).

Given that NGS information on mutational landscape was available for the vast majority of the patients of the cohort (90%, $n = 185$), the impact of gene mutations on OS was further analyzed. *KRAS* mutations were present in 36 patients and were associated with shorter OS, with a median OS of 8 vs 19 months for *KRAS*-mut vs *KRAS*-wt patients ($p = 0.042$, Fig. 3A). *NRAS*-mut was the most frequent mutation (39 patients), but it was not associated with shorter OS ($p = 0.883$, Supplementary Fig. S3A) and only patients presenting both mutations (*NRAS* and *KRAS*, 7 patients) had reduced survival when compared to the rest of the cohort ($p = 0.041$, Supplementary Fig. S3B). Patients with *DNMT3A*-mut also presented with shorter OS with a median OS of 8 vs 18 months for *DNMT3A*-mut vs *DNMT3A*-wt patients ($p = 0.011$, Fig. 3B), and there was a trend for poor outcome in *TP53*-mut patients ($p = 0.053$, Fig. 3C).

Patients harboring a mutation of any of the above-mentioned high-risk genes (either *KRAS*, $n = 36$; *DNMT3A*, $n = 12$ or *TP53*, $n = 16$) represented 32% of the cohort and had a median OS of 7 months, while the rest of the patients had a median OS of 20 months ($p < 0.001$, Fig. 3D). A multivariate Cox regression model for intensively treated patients identified the following independent pretreatment variables regarding OS: age > 60 years (HR 2.1, $p = 0.001$), secondary AML after MDS (HR 2.2, $p = 0.043$), *DNMT3A*-mut (HR 2.1, $p = 0.047$), *KRAS*-mut (HR 2.0, $p = 0.005$), as shown in Fig. 4. Of note, therapy-related AML, complex cytogenetic aberrations, t(9;11) or *TP53*-mut were not significant in this model.

Effect of allo-HSCT in *KMT2Ar* AML

Allo-HSCT was performed in 88 patients (42.9%), at CR1 in 48 (23.4%) and CR2 or later in 15 (7.3%), while the response at allo-HSCT was not known in 25 (12.2%). The estimated survival

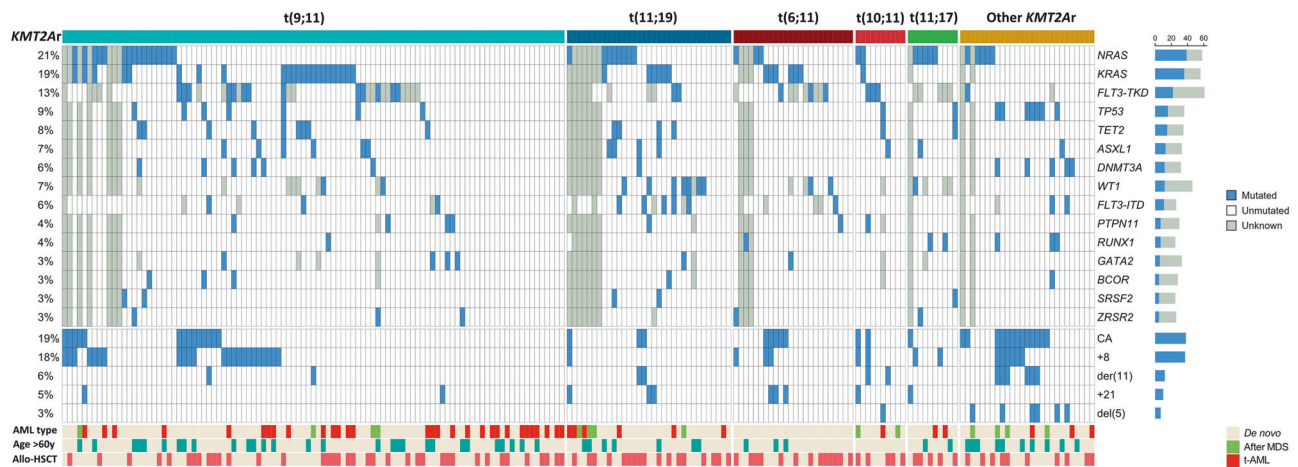


Fig. 1 Mutational landscape and additional cytogenetic aberrations in *KMT2Ar* adult AML by fusion partner. Only mutations present in at least five patients and most frequent cytogenetic aberrations are shown. Allo-HSCT allogeneic hematopoietic stem cell transplantation; CA complex cytogenetic aberrations (≥ 2 apart from *KMT2Ar*); MDS myelodysplastic syndrome; t-AML therapy-related acute myeloid leukemia.

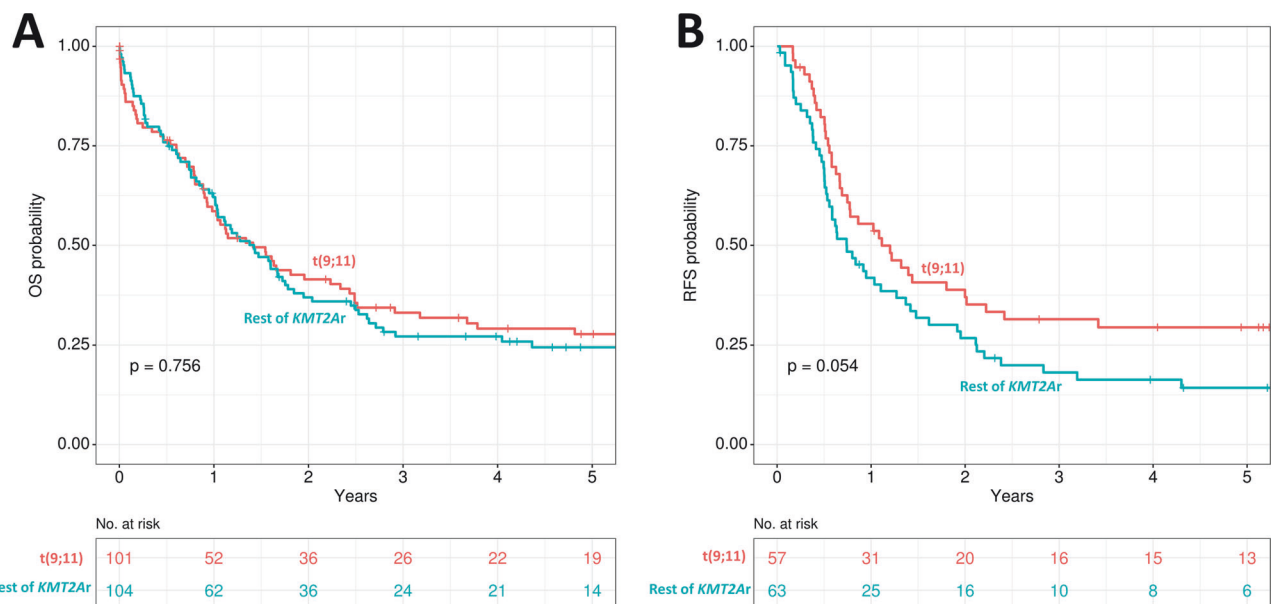


Fig. 2 Comparison of survival outcomes between *t(9;11)* and the rest of the *KMT2Ar* cohort. **A** Overall survival. **B** Relapse-free survival.

probabilities using Simon-Makuch method with clock-back correction revealed a significant OS benefit of allo-HSCT in CR1 ($p = 0.003$, Supplementary Fig. S4A). Moreover, patients with *KRAS*-mut, *TP53*-mut or *DNMT3A*-mut AML also benefited from allo-HSCT in CR1 ($p = 0.026$, Supplementary Fig. S4B). Remarkably, from all patients who underwent allo-HSCT in CR1, OS for *KRAS*-mut, *TP53*-mut or *DNMT3A*-mut patients was similar to those patients lacking these mutations ($p = 0.530$, Supplementary Fig. S4C), as well as RFS ($p = 0.884$, Supplementary Fig. S4D). Allo-HSCT in CR1 was an independent prognostic variable for increased OS in a multivariate Cox regression model that included the same variables stated previously ($p < 0.001$, Supplementary Fig. S5).

Outcome in intensively treated younger (<60 years) *KMT2Ar* de novo AML patients

As the general cohort of 205 patients was heterogeneous in terms of age, origin of AML and treatment, an analysis in a subgroup of 115 patients aged <60 years at diagnosis, who received intensive

chemotherapy regimens and who presented with de novo *KMT2Ar* AML (excluding both therapy-related and AML after MDS) was carried out. Median age of this subgroup was 42 years (range 19–59) and it included 51% females. Median follow-up for those patients alive was 5.1 year, CRc rate was 75.7%, allo-HSCT was performed in 53.9% and median OS was 1.7 years.

Notably, OS was still similar across the different translocation partners, without significant differences in OS and similar RFS between *t(9;11)* and the remainder of the patients ($p = 0.916$, Supplementary Fig. S6A, and $p = 0.193$, Supplementary Fig. S6B, respectively). Interestingly, there was a trend for longer OS for patients who harbored isolated trisomy 8 ($n = 10$) as the only additional cytogenetic abnormality ($p = 0.072$, Supplementary Fig. S7A). In fact, 80% of the patients achieving a CRc had long-lasting responses with a 2-year RFS of 75% vs 31% for the rest of cases ($p = 0.016$, Supplementary Fig. S7B).

Regarding gene mutations, patients with *KRAS*-mut ($n = 22$) had a significantly shorter OS ($p = 0.001$, Fig. 5A) when compared to *KRAS*-wt ($n = 84$). Patients showing *TP53*-mut ($n = 9$) were also

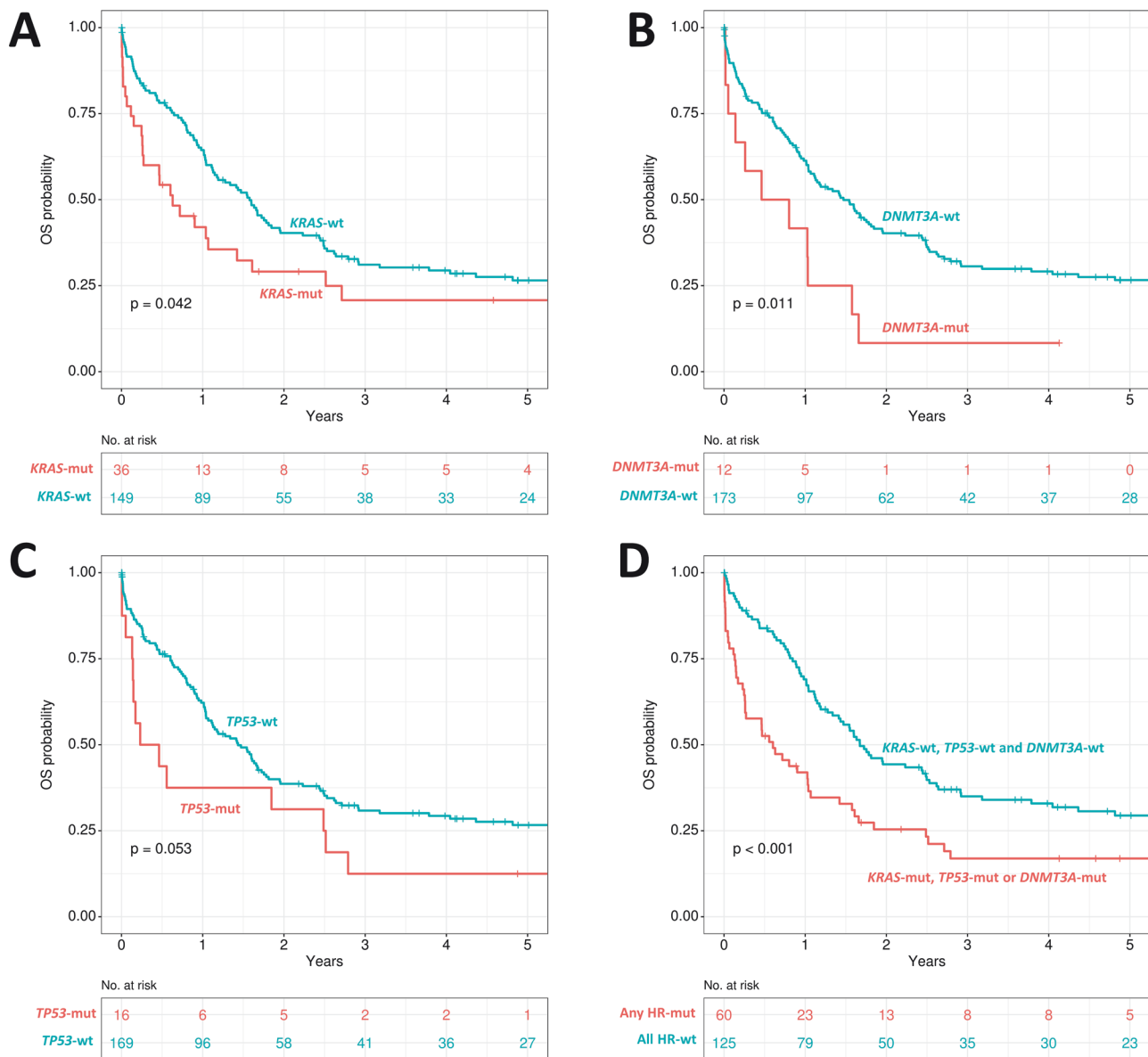


Fig. 3 Impact of different gene mutations on overall survival in *KMT2Ar* AML. **A** *KRAS*, **B** *DNMT3A*, **C** *TP53*, **D** comparison of patients with either *KRAS*-mut, *DNMT3A*-mut or *TP53*-mut and the rest of the *KMT2Ar* AML cohort; OS overall survival; mut mutated; wt wildtype.

associated with shorter OS ($p = 0.015$, Fig. 5B), while *DNMT3A*-mut was not significant ($p = 0.242$), although there was also a low number of patients who presented with *DNMT3A*-mut ($n = 5$) in this subset of younger AML patients (<60 years of age).

Therefore, *KRAS* and *TP53* were the most relevant genes linked to OS in this subgroup of patients. Grouped together (either *KRAS*-mut or *TP53*-mut) these mutations defined 28% ($n = 30$) of the cohort to have an adverse prognosis. These patients showed a significantly lower rate of CRc (50% vs 85.5%, $p < 0.001$). Median OS was 7 months and 5-year OS only 15%, while for the rest of patients median OS was 30 months and 5-year OS 42% ($p < 0.001$, Fig. 5C). Moreover, median RFS was 6 months for patients with *KRAS*-mut or *TP53*-mut, while median RFS was 13 months in all other younger *KMT2Ar* cases ($p = 0.039$, Fig. 5D).

A multivariate Cox regression in this subset of patients identified the following independent variables regarding OS: *KRAS*-mut (HR 2.6, $p = 0.002$), complex cytogenetic aberrations (HR 2.3, $p = 0.019$), and allo-HSCT in CR1 (HR 0.7, $p = 0.003$), while there was a trend for *TP53*-mut (HR 2.2, $p = 0.077$), as shown in Supplementary Fig. S8.

Historical comparison of outcomes in *KMT2Ar* AML

As the cohort was accrued over several decades, the impact of the year of AML diagnosis on OS was also analyzed. As we have reported improved patient outcomes for patients treated in the decade between 2007 and 2016 when compared to the decade between 1997 and 2006 in the HARMONY AML database [32] the same cut-off was used for this analysis. However, we could not find differences in OS when stratifying by the year of diagnosis ($p = 0.458$, Supplementary Fig. S9A). In the subset of de novo AML patients aged <60 years, early-death rates were similar with 10% for the 1996–2006 period and 6.2% for the 2007–2020 period ($p = 0.508$) and again without statistical differences in OS (median OS 19 vs 24 months respectively, $p = 0.396$, Supplementary Fig. S9B).

DISCUSSION

KMT2Ar are recognized as recurrent genetic abnormalities in AML, although they are present only in a minority of adult AML patients [4, 5, 23]. In the HARMONY AML database, we found an incidence

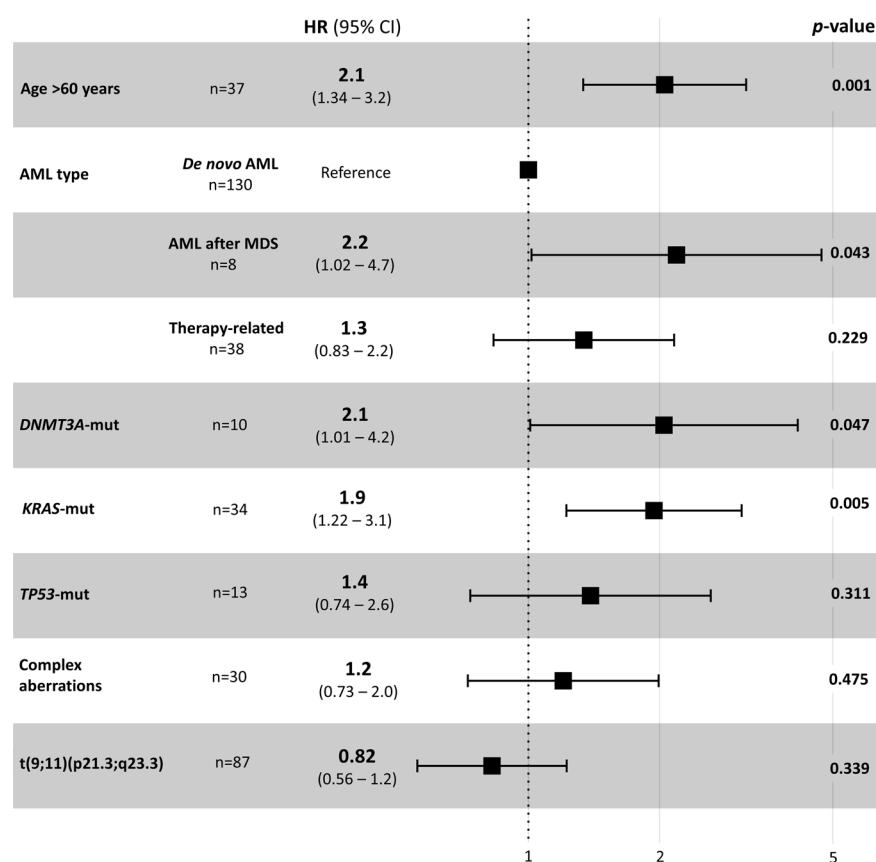


Fig. 4 Multivariate Cox regression model for prediction of overall survival in intensively treated patients. HR hazard ratio; CI confidence interval; AML acute myeloid leukemia; MDS myelodysplastic syndrome; mut mutated.

of 3.4% (205 out of 6005) for *KMT2Ar* AML, which is in line with previous studies [6, 7, 11, 15, 19]. This highlights the importance of large databases in order to obtain a representative cohort of *KMT2Ar* adult AML. However, based on conventional diagnostics the incidence of *KMT2Ar* AML might be slightly underestimated, as the development of molecular techniques has allowed the identification of cryptic *KMT2A* fusions, and thus the true prevalence might be higher [12]. While the incidence of *KMT2Ar* AML was recently shown to be significantly higher in patients <60 years, the median age of 48 years in our cohort was well within the range of previous reports [6, 7, 14, 15, 17, 19].

The prognostic impact of different translocation partners in *KMT2Ar* AML has been studied for several decades, with quite heterogeneous results [6, 7, 14–19]. Recently, Bill et al. [14] observed that the prognostic significance of t(9;11) was dependent on patient age, as it was associated with better outcomes only for patients aged <60 years with de novo AML. However, we could not corroborate that finding when we analyzed the same subset of patients. Interestingly, Bill et al. reported an uneven distribution of *KRAS* mutations among the different *KMT2Ar*, as 47% of their t(6;11) patients presented with *KRAS*-mut while only 3% of the t(9;11) had *KRAS*-mut. By contrast, in our cohort, *KRAS*-mut distribution was similar among the different translocations, with a prevalence of 19.5% for the global cohort and 24.2% for t(9;11) patients. Given the negative impact of *KRAS*-mut, this could explain the more favorable outcome of the t(9;11) cases reported in the Bill et al. study. In line with our observations, Grossman et al. [17] report a prevalence of 22.9% *KRAS*-mut in t(9;11) patients and they were also not able to find differences in OS for the distinct fusion partners. Therefore, the heterogeneous results reported in the literature with regard to a more favorable outcome of t(9;11) might be due to a potential bias in the distribution of *KRAS*

mutations (rather than the fusion partner per se), although further studies will be required in order to confirm our hypothesis.

As genomic features have been described as the most powerful predictors of OS in adult AML and the interaction of genomic aberrations has been linked to outcome [3], it is reasonable to think that additional cytogenetic aberrations or concomitant gene mutations might also play a role in *KMT2Ar* AML. In our study, 40% of *KMT2Ar* patients presented with additional cytogenetic abnormalities resulting in higher early-death rates, although poorer long-term outcome was only associated with cases with more complex genomic alterations. While both observations need to be confirmed in independent cohort, they definitely point to additional heterogeneity within *KMT2Ar*-driven leukemia biology that needs to be further understood.

In accordance, comprehensive molecular analysis of the HARMONY *KMT2Ar* adult AML cohort, which to the best of our knowledge is the largest cohort studied by panel sequencing to date, revealed additional heterogeneity on the molecular level. In line with previous studies [10, 14, 17, 19], we observed a relatively low incidence of gene mutations, with a median of only one mutation per patient. This is in contrast to the average amount of additional driver mutations seen in other AML subtypes [3], most likely due to the fact that *KMT2A* is one of the strongest oncogenic drivers and is sufficient to transform healthy hematopoietic stem cells in murine models as a single molecular lesion [33].

With regard to additional oncogenic drivers, *NRAS* was the most frequently mutated gene (21% of the patients). Overall, we found a high incidence (42.1%) of gene mutations involving the *RAS* signaling pathway (*NRAS*, *KRAS*, *PTPN11* and *BRAF*), which is consistent with previous reports [14, 17, 34]. Furthermore, we found constitutive activation of *FLT3* by mutations in 19.1% of cases, with TKD mutations being more frequent than ITD

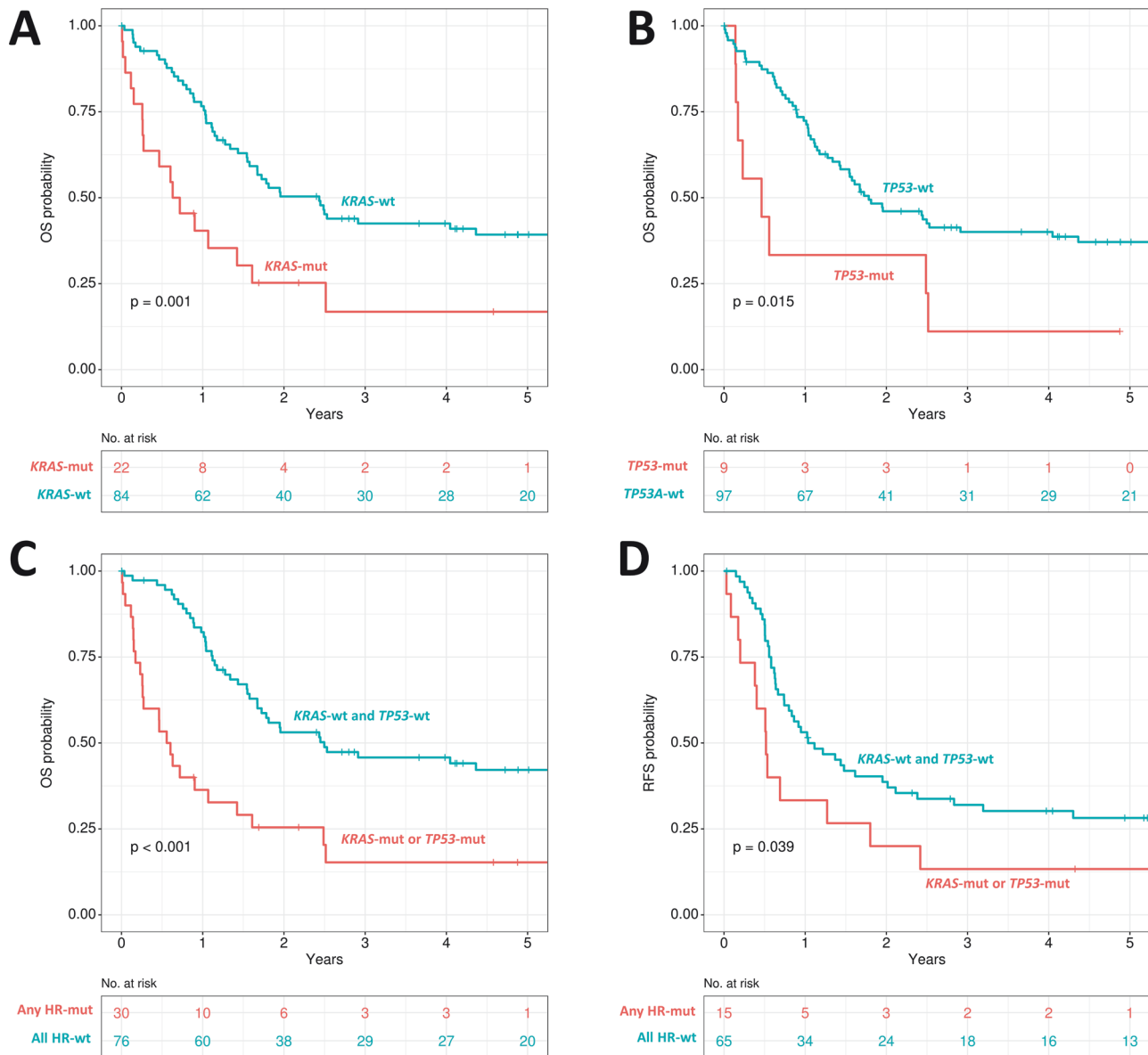


Fig. 5 Impact of different gene mutations on overall survival in patients with de novo *KMT2Ar* AML and aged ≤ 60 years. **A** *KRAS*, **B** *TP53*, **C** comparison of overall survival of patients with any high-risk genes mutated (either *KRAS*-mut, or *TP53*-mut) and the rest of patients aged ≤ 60 years with de novo AML, **D** comparison of relapse-free survival of patients with any high-risk mutated genes (either *KRAS*-mut or *TP53*-mut) and the rest of patients aged ≤ 60 years with de novo AML. OS overall survival; RFS relapse-free survival; mut mutated; wt wildtype; HR high-risk genes (*KRAS*, *TP53*).

aberrations. This is in line with a previous report in childhood *KMT2Ar* leukemia that also reported more TKD than ITD mutations [35]. Of note, *NPM1* mutations were detected only in 1% of the cohort. While this constitutes a different mutational landscape in *KMT2Ar* adult AML when compared to that described for the rest of adult AML [1–3], additional drivers do contribute to *KMT2Ar* leukemia pathogenesis and their impact has to be further unraveled.

With regard to outcome, we were able to demonstrate that adult patients with *KMT2Ar* AML have shorter OS when they present with a *KRAS* mutation. *KRAS* mutations have recently been described as an independent adverse prognostic factor in pediatric *KMT2Ar* AML by the Japanese Pediatric Leukemia/Lymphoma Study Group, which was validated using a smaller cohort of adult *KMT2Ar* patients [36]. However, several studies of *KMT2Ar* adult AML were not able to find differences in OS for *KRAS*-mut patients, which is most likely due to the relatively small

sample size and a potential bias in the distribution of *KRAS*-mut [14, 17, 19]. While *TP53*-mut have been described as an adverse prognostic marker in a univariate OS analysis in one previous study [17], we also observed an unfavorable impact of this tumor suppressor gene mutation.

The importance of gene mutations was confirmed in a subset of patients aged < 60 years at diagnosis receiving intensive chemotherapy regimens, with *KRAS* and *TP53* being the most relevant genes for patient outcomes. Only 50% of the patients with either *KRAS*-mut or *TP53*-mut achieved CRc, and for those who did, median RFS was only 6 months, which resulted in a significantly shorter OS when compared to the rest of the patients of the subset. Previous studies have shown that *KRAS* mutations are likely subclonal and therefore relatively late events in *KMT2Ar* AML [9, 14, 17, 34]. However, they might promote disease progression and clonal expansion of *KMT2Ar* cells, as reported in a recent study using a retroviral mouse AML model [37], which

could explain the low rate of treatment responses and early relapses that we observed in our patients.

On the other hand, allo-HSCT in CR might help overcome the poor prognostic impact of these additional driver mutations. The effect of allo-HSCT for *KMT2A*r AML patients achieving CR has been explored in several studies, favoring the performance of allo-HSCT whenever feasible to improve patient outcomes [19, 20, 22]. We were able to confirm these findings in our cohort, showing the benefit of allo-HSCT in CR1. In fact, our results suggest that allo-HSCT could mitigate the deleterious effect of *KRAS*-mut, *TP53*-mut and *DNMT3A*-mut, as mutated patients that underwent allo-HSCT had similar OS and RFS when compared to unmutated patients.

However, the prognosis of adult *KMT2A*r AML still remains poor, especially in patients who are not able to receive an allo-HSCT. While the development of menin inhibitors shows encouraging results in early-phase clinical trials and could change the treatment landscape of *KMT2A*r AML in the future [38, 39], *KRAS* inhibitors that have been recently approved for advanced solid tumors [40] might also open novel possibilities of targeted AML therapy and might change the prognostic impact of the markers presented in this study.

In conclusion, our study reveals a different mutational landscape of *KMT2A*r adult AML, which has important prognostic and therapeutic implications. Future studies will have to continue to better understand the disease biology underlying *KMT2A*r leukemia, especially to better guide not only conventional but also novel targeted treatment approaches and further improve patient outcome.

DATA AVAILABILITY

After the publication of this article, data collected for this analysis and related documents will be made available to others upon reasonably justified request, which needs to be written and addressed to the attention of the corresponding author LB at the following e-mail address: lars.bullinger@charite.de. The HARMONY Alliance, via the corresponding author LB, is responsible to evaluate and eventually accept or refuse every request to disclose data and their related documents, in compliance with the ethical approval conditions, in compliance with applicable laws and regulations, and in conformance with the agreements in place with the involved subjects, the participating institutions, and all the other parties directly or indirectly involved in the participation, conduct, development, management and evaluation of this analysis.

REFERENCES

- Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373:1136–52.
- Bullinger L, Döhner K, Döhner H. Genomics of acute myeloid leukemia diagnosis and pathways. *J Clin Oncol*. 2017;35:934–46.
- Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374:2209–21.
- Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. 2022;140:1345–77.
- Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia*. 2022;36:1703–19.
- Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102:2395–402.
- Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116:354–65.
- Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368:2059–74.
- Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Görlich D, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128:686–98.
- Eisfeld AK, Mrózek K, Kohlschmidt J, Nicolet D, Orwick S, Walker CJ, et al. The mutational oncoprint of recurrent cytogenetic abnormalities in adult patients with de novo acute myeloid leukemia. *Leukemia*. 2017;31:2211–8.
- Vetro C, Haferlach T, Meggendorfer M, Stengel A, Jeromin S, Kern W, et al. Cytogenetic and molecular genetic characterization of *KMT2A*-PTD positive acute myeloid leukemia in comparison to *KMT2A*-rearranged acute myeloid leukemia. *Cancer Genet*. 2020;240:15–22.
- Meyer C, Larghero P, Almeida Lopes B, Burmeister T, Gröger D, Sutton R, et al. The *KMT2A* recombinome of acute leukemias in 2023. *Leukemia*. 2023;37:988–1005.
- Bhatnagar B, Blachly JS, Kohlschmidt J, Eisfeld AK, Volinia S, Nicolet D, et al. Clinical features and gene-and microRNA-expressions patterns in adult acute leukemia patients with t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). *Leukemia*. 2016;30:1586–9.
- Bill M, Mrózek K, Kohlschmidt J, Eisfeld AK, Walker CJ, Nicolet D, et al. Mutational landscape and clinical outcome of patients with de novo acute myeloid leukemia and rearrangements involving 11q23/*KMT2A*. *Proc Natl Acad Sci USA*. 2020;117:26340–6.
- Mrózek K, Heinonen K, Lawrence D, Carroll AJ, Koduru PRK, Rao KW, et al. Adult patients with de novo acute myeloid leukemia and t(9; 11)(p22; q23) have a superior outcome to patients with other translocations involving band 11q23: a Cancer and Leukemia Group B Study. *Blood*. 1997;90:4532–8.
- Krauter J, Wagner K, Schäfer I, Marschalek R, Meyer C, Heil G, et al. Prognostic factors in adult patients up to 60 years old with acute myeloid leukemia and translocations of chromosome band 11q23: individual patient data-based meta-analysis of the German acute myeloid leukemia intergroup. *J Clin Oncol*. 2009;27:3000–6.
- Grossmann V, Schnittger S, Poetzinger F, Kohlmann A, Stiel A, Eder C, et al. High incidence of RAS signalling pathway mutations in MLL-rearranged acute myeloid leukemia. *Leukemia*. 2013;27:1933–6.
- Balgobind BV, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*. 2009;114:2489–96.
- Issa GC, Zarka J, Sasaki K, Qiao W, Pak D, Ning J, et al. Predictors of outcomes in adults with acute myeloid leukemia and *KMT2A* rearrangements. *Blood Cancer J*. 2021;11:1–10.
- Pigneux A, Labopin M, Maertens J, Cordonnier C, Volin L, Socié G, et al. Outcome of allogeneic hematopoietic stem-cell transplantation for adult patients with AML and 11q23/MLL rearrangement (MLL-r AML). *Leukemia*. 2015;29:2375–81.
- Menghrajani K, Gomez-arteaga A, Madero-Marroquin R, Zhang M, Bo-subait K, Sanchez J, et al. Risk classification at diagnosis predicts post-HCT outcomes in intermediate-, adverse-risk, and *KMT2A*-rearranged AML. *Blood Adv*. 2022;6:828–47.
- Chen Y, Kantarjian H, Pierce S, Faderl S, O'Brien S, Qiao W, et al. Prognostic significance of 11q23 aberrations in adult acute myeloid leukemia and the role of allogeneic stem cell transplantation. *Leukemia*. 2013;27:836–42.
- Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140:1200–28.
- Jongen-Lavrencic M, Grob T, Hanekamp D, Kavelaars FG, al Hinai A, Zeilemaker A, et al. Molecular minimal residual disease in acute myeloid leukemia. *N Engl J Med*. 2018;378:1189–99.
- Terwijn M, Kelder A, Huijgens PC, Dräger AM, Oussoren YJM, Scholten WJ, et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: Data from the HOVON/SAKK AML 42A study. *J Clin Oncol*. 2013;31:3889–97.
- Turki AT, Tsachakis-Muck N, Leserer S, Crivello P, Liebrechts T, Betke L, et al. Impact of CMV reactivation on relapse of acute myeloid leukemia after HCT is dependent on disease stage and ATG. *Blood Adv*. 2022;6:28–36.
- Belenkaya R, Gurley MJ, Golozar A, Dymshyts D, Miller RT, Williams AE, et al. Extending the OMOP common data model and standardized vocabularies to support observational cancer research. *JCO Clin Cancer Inform*. 2021;5:12–20.
- McGowan-Jordan J, Hastings RJ, Moore S, editors. ISCN 2020: an international system for human cytogenomic nomenclature. S. Karger; 2020.
- Gabert J, Beillard E, van der Velden VHJ, Bi W, Grimwade D, Pallisaard N, et al. Standardization and quality control studies of 'real time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – a Europe Against Cancer Program. *Leukemia*. 2003;17:2318–57.
- Bernasconi DP, Rebora P, Iacobelli S, Valsecchi MG, Antolini L. Survival probabilities with time-dependent treatment indicator: quantities and non-parametric estimators. *Stat Med*. 2016;35:1032–48.
- Döhner K, Thiede C, Jahn N, Panina E, Gambietz A, Larson RA, et al. Impact of NPM1/FLT3-ITD genotypes defined by the 2017 European LeukemiaNet in patients with acute myeloid leukemia. *Blood*. 2020;135:371–80.
- Sobas MA, Villaverde Ramiro A, Hernández Sánchez A, Martínez Elicegui J, González T, Azibeiro Melchor R, et al. Long-term follow-up of AML patients treated intensively before the era of targeted agents. A big data analysis from the Harmony Collaboration. *Blood*. 2022;140:6053–5.

33. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer*. 2007;7:823–33.
34. Lavallée VP, Baccelli I, Kros J, Wilhelm B, Barabé F, Gendron P, et al. The transcriptomic landscape and directed chemical interrogation of MLL-rearranged acute myeloid leukemias. *Nat Genet*. 2015;47:1030–7.
35. Fedders H, Alsaadeq A, Schmäh J, Vogiatzi F, Zimmermann M, Möricke A, et al. The role of constitutive activation of FMS-related tyrosine kinase-3 and NRas/KRas mutational status in infants with KMT2A-rearranged acute lymphoblastic leukemia. *Haematologica*. 2017;102:e438–42.
36. Matsuo H, Yoshida K, Nakatani K, Harata Y, Higashitani M, Ito Y, et al. Fusion partner-specific mutation profiles and KRAS mutations as adverse prognostic factors in MLL-rearranged AML. *Blood Adv*. 2020;4:4623–31.
37. Hyrenius-Wittsten A, Pilheden M, Stureson H, Hansson J, Walsh MP, Song G, et al. De novo activating mutations drive clonal evolution and enhance clonal fitness in KMT2A-rearranged leukemia. *Nat Commun*. 2018;9:1770.
38. Issa GC, Aldoss I, Diersio J, Cuglievan B, Stone R, Arellano M, et al. The Menin inhibitor revumenib in KMT2A-rearranged or NPM1-mutant leukaemia. *Nature*. 2023;615:920–4.
39. Fiskus W, Boettcher S, Daver N, Mill CP, Sasaki K, Birdwell CE, et al. Effective Menin inhibitor-based combinations against AML with MLL rearrangement or NPM1 mutation (NPM1c). *Blood Cancer J*. 2022;12:1–11.
40. Hong DS, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, et al. KRAS^{G12C} inhibition with sotorasib in advanced solid tumors. *N Engl J Med*. 2020;383:1207–17.

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AUTHOR CONTRIBUTIONS

AHS, TG, JMHR, BH, GO, HD and LB designed the study. AHS, MS, TG, MA, PJMV, AVR, KHM, RA, JMT, JML, MP, JME, KIM, CT, GS, KD, MH, TH, ATT, DR and JMHR performed data collection and assembly of the data. AHS, TG, RS, GC, AVR, AB, JME, KD, ATT, JMHR, BH, GO, HD and LB performed data analysis and interpretation. AHS, JMHR and LB wrote the manuscript. BH, GO and HD critically reviewed the manuscript. LB supervised research and coordinated the HARMONY AML group. All authors had access to primary data, read and approved the final manuscript.

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COMPETING INTERESTS

MS: honoraria from Novartis, Celgene, AOP Orphan, AbbVie. KHM: honoraria from AbbVie, Bristol Myers Squibb, Celgene, Janssen, Novartis, Pfizer, Otsuka; research funding from AbbVie. RA: honoraria from Astellas, Bristol Myers Squibb, Incyte, and Novartis. MP: honoraria from Novartis. CT: co-owner and CEO of AgenDix GmbH and has received lecture fees and/or participated in Ad-Boards from Novartis, Jazz Pharmaceuticals, Astellas, Janssen, Illumina; research funding from Novartis, Bayer.

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ADDITIONAL INFORMATION

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Unravelling Co-Mutational Patterns with Prognostic Implications in *NPM1* Mutated Adult Acute Myeloid Leukemia – A HARMONY Study

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ABSTRACT

PURPOSE: *NPM1*-mutated (*NPM1*-mut) acute myeloid leukemia (AML) is generally associated with a more favorable outcome, although the presence of additional gene mutations, such as a *FLT3*-internal tandem duplication (ITD), can influence patient prognosis. Today, only *FLT3*-ITD is considered for risk stratification, while the significance of other co-mutations remains to be determined.

PATIENTS AND METHODS: We analyzed intensively-treated adult *NPM1*-mut AML patients included in the HARMONY Alliance database. A newly developed risk stratification classification, which included combinations of co-mutations in *FLT3*-ITD, *DNMT3A*, *IDH1/IDH2*, and *TET2* genes, was applied to a training cohort of *NPM1*-mut AML patients included in clinical trials (n=1,001), an internal validation cohort more representative of real-world setting cases (n=762), and an external validation cohort enrolled in UK-NCRI trials (n=585).

RESULTS: The HARMONY classification considered 51.8% of the *NPM1*-mut AML training cohort patients as favorable, 24.8% as intermediate and 23.4% as adverse risk, with median overall survival (OS) of 14.4, 2.2, and 0.9 years, respectively; p<0.001), thereby reclassifying 42.7% of *NPM1*-mut patients into a different European LeukemiaNet (ELN) 2022 risk category. These results were confirmed both in an internal (median OS 8.2, 2.8, and 0.8 years for favorable, intermediate, and adverse *NPM1*-mut subgroups, respectively; p<0.001), and external validation cohort (median OS not reached, 3.8, and 1.2 years for favorable, intermediate, and adverse *NPM1*-mut subgroups, respectively; p<0.001). Allogeneic hematopoietic stem cell transplantation (allo-HSCT) in first complete remission (CR1) showed the highest benefit in the *NPM1*-mut adverse-risk subgroup.

CONCLUSIONS: The HARMONY classification provides the basis for a refined genetic risk stratification for adult *NPM1*-mut AML with potential clinical impact on allo-HSCT decision-making.

INTRODUCTION

Acute myeloid leukemia (AML) is a clinically heterogeneous disease, where genomic alterations provide crucial prognostic insights that inform clinical decision-making¹. *NPM1* mutations (*NPM1*-mut) have been described in approximately 30% of adult AML and define the largest disease subtype in younger adults, with distinct biologic and clinical features²⁻⁴. While the prognosis is generally considered favorable, a significant variability in outcomes has been reported. In fact, the vast majority of patients present several co-mutations that could influence the prognosis, such as *FLT3* internal tandem duplication (ITD) that has been recognized as a deleterious mutation. However, for almost two decades *FLT3*-ITD is the only co-mutation that is considered for risk stratification in *NPM1*-mut AML in current guidelines^{5,6}. Nevertheless, there are several other genes frequently co-mutated with *NPM1* that might

influence the prognosis of this AML subtype. For example, *DNMT3A*-mut is present in around 50% of *NPM1*-mut AML and it has been associated with adverse outcomes in several studies⁷⁻¹¹, although others found contradictory results¹². In fact, the prognostic impact of *DNMT3A*-mut seems to be modulated by the frequent co-mutation of *FLT3*-ITD^{13, 14}. This “triple-mutated” AML (*NPM1*-mut, *FLT3*-ITD and *DNMT3A*-mut) is a significant group as it represents approximately 25% of *NPM1*-mut AML. On the other hand, the relatively poor outcomes attributed to *FLT3*-ITD might be influenced by the frequent co-mutation with *DNMT3A*¹⁵. Additional gene mutations have been reported in *NPM1*-mut AML, although their prognostic impact remains unclear¹⁶.

Cytogenetic aberrations are uncommon in this AML subtype, where a normal karyotype has been reported in 80-88% of the patients¹⁷⁻²¹. Most of them do not seem to affect the risk stratification,

with the exception of infrequent (<3%) adverse cytogenetic abnormalities. Moreover, an aberrant karyotype does not seem to influence the immunophenotype nor gene expression profile in *NPM1*-mut AML, whereas they could be related to concomitant gene mutations^{15, 19, 22, 23}.

Hence, this AML subtype is an ideal setting for analyzing complex gene-gene interactions and co-mutational patterns with potential prognostic implications.

In order to address these uncertainties, we analyzed a large cohort of patients with *NPM1*-mut AML included in the Healthcare Alliance for Resourceful Medicine Offensive against Neoplasms in Hematology (HARMONY) AML international database and validated the findings using both HARMONY real-world data as well as publicly available data.

METHODS

Patients

The HARMONY Alliance AML database was used for this study, where only patients fulfilling the following criteria were selected: age >18 years at AML diagnosis, presence of *NPM1*-mut, treatment with intensive chemotherapy regimens, availability of cytogenetic study and next-generation sequencing (NGS) myeloid panel data. Patients who received targeted therapies (e.g. *FLT3* or *IDH1/IDH2* inhibitors, anti-CD33 antibodies) or non-intensive treatment approaches were not included. Patients with class-defining cytogenetic abnormalities concomitant to *NPM1*-mut were excluded.

A total of 1,763 *NPM1*-mut AML adult patients were selected for this analysis, contributed by eight European centers or cooperative groups. The training cohort comprised 1,001 *NPM1*-mut patients from three prospective multicenter clinical trials of the German–Austrian AML Study Group (AMLHD98A, AML-HD98B and AMLSG-07-04) and from three prospective multicenter clinical trials of HOVON-SAKK (HO102, HO103, HO132), representing a clinical trial setting^{1, 24–26}. An internal validation cohort was formed with the remainder of patients, contributed by the Study Alliance Leukemia AML registry (Germany), the Munich Leukemia Laboratory (Germany), the AML Cooperative Group registry (Germany), the Swedish AML registry (Sweden), the Hospital Universitario 12 de Octubre (Spain), and the Queen’s University of Belfast (North Ireland), with a total of 762 *NPM1*-

mut patients that were more representative of the “real-world” setting in Europe²⁷.

Patient data uploaded to the HARMONY Big Data Platform underwent a rigorous double brokerage pseudonymization process adhering to the General Data Protection Regulation (GDPR). Subsequently, the data were harmonized and converted using the Observational Medical Outcomes Partnership (OMOP) Common Data Model²⁸.

The study was performed in accordance with the Declaration of Helsinki and received approval from the HARMONY steering committee and AML working group. The HARMONY research project underwent review and approval by the Medicinal Research Ethics Committee of the University of Salamanca (PI 2018 10 128). HARMONY has established an ethical and data-protection framework for the secondary use of data, including *de facto* anonymization. Prior written informed consent had been obtained from all patients at respective HARMONY partner institutions.

NPM1-mut risk stratification

A multi-step analysis of clinically significant gene co-mutations associated to *NPM1*-mut was performed. At each step, combinations of up to two additional genes (either mutated or wildtype) were explored. The 2-year overall survival (OS) for each combination was estimated using 100-fold bootstrap sampling and compared to the 2-year OS of *NPM1* wildtype (-wt) patients in the same dataset (German–Austrian AML Study Group and HOVON-SAKK clinical trials, n=2473). Gene mutation combinations that allowed patient reclassification into different risk categories were selected. The classification included only genes that were analyzed in both NGS panels of the training cohort (**Supplementary Table S1**), provided that each gene mutation was found in at least 10 patients (≥1% of the cohort). While *IDH2*-R172K mutation has proven to be associated with distinct outcomes when compared to other *IDH2*-mut and *IDH1*-mut, it is also mutually exclusive to *NPM1*-mut and therefore rarely found in this AML subtype^{11, 29}. Moreover, exploratory analyses demonstrated similar findings with *IDH1*-mut and *IDH2*-mut in *NPM1*-mut, so they were combined as *IDH*-mut (any mutated) or *IDH*-wt (both wildtype) in the final risk classification. The HARMONY *NPM1*-mut classification was tested in the aforementioned internal validation cohort.

External validation dataset

An external validation was also performed, using the publicly available dataset published by Tazi *et al.*, comprising AML adult patients enrolled in UK-NCRI trials (AML17, AML16, AML11, AML12, AML14 and AML15) who were not included in HARMONY at the time of the analysis³⁰. Patients with *NPM1*-mut, treated intensively, with cytogenetic and NGS myeloid panel information were selected for HARMONY classification validation. Of note, some of these patients received gemtuzumab ozogamicin (GO) or *FLT3* inhibitor lestaurtinib in addition to intensive chemotherapy regimens, as part of AML17 randomizations.

Statistical analysis

Clinical endpoints were defined as recommended by international guidelines⁶. Composite complete remission (CRc) was defined as either complete remission (CR) or CR with incomplete hematologic recovery (CRi). OS and relapse-free survival (RFS) were estimated the Kaplan-Meier method and differences between survival distributions were evaluated using the log-rank test. Patients who underwent allo-HSCT in first complete remission (CR1) were censored at transplant date for OS and RFS analyses in both training and internal validation cohort. Cox proportional hazards model was used for multivariable survival analysis, including clinically-significant variables as well as HARMONY *NPM1*-mut classification. Imputation was not performed for missing values. Co-occurrence and mutual exclusivity were tested for gene mutations present in at least 3% of patients, calculating q-value as previously reported³¹. All reported p-values were two-sided at the conventional 5% significance level. Analyses were performed using R statistical software (v3.6.3).

RESULTS

Patient characteristics of AML training cohort

The training cohort of 1,001 adult *NPM1*-mut AML patients included 54% females. The median age at diagnosis was 53 years, and 73% were younger than 60 years (Table 1). Normal karyotype was observed in 87% of patients and 39% had *FLT3*-ITD mutation at diagnosis. CRc after induction treatment was achieved in 87% of patients, while 4% died before response assessment (early-death). Allo-HSCT was performed in 34% patients (24% in CR1). Median

follow-up was 6 years, with a median OS of 8.3 years.

Mutational landscape of *NPM1*-mut AML training cohort

In the training cohort, the most frequently mutated genes were *FLT3* in 56% (ITD 39%, TKD 17%), *DNMT3A* (53%), *NRAS* (20%), *PTPN11* (17%), *TET2* (17%), *IDH2* (16%), and *IDH1* (14%), while other gene mutations were present in less than 10% of patients (Figure 1). Of note, 59.5% of patients with *FLT3*-ITD also had *DNMT3A*-mut, whereas 43.7% of *DNMT3A*-mut patients also presented with a *FLT3*-ITD, showing a strong co-occurrence of these two mutations in *NPM1*-mut AML (odds ratio [OR] 1.5, $q=0.014$). Other significant co-occurring gene pairs, exploring all genes mutated in at least 3% of patients, were *IDH2* and *SRSF2* (OR 7.3, $q<0.001$) as well as *IDH1* and *NRAS* (OR 1.8, $q=0.041$). *FLT3*-ITD correlated negatively with *NRAS* (OR 0.2, $q<0.001$), *KRAS* (OR 0.32, $q=0.008$), *PTPN11* (OR 0.36, $q<0.001$), and *SRSF2* (OR 0.1, $q<0.001$). *DNMT3A* showed a negative correlation with *STAG2* (OR 0.05, $q<0.001$) and *SRSF2* (OR 0.22, $q<0.001$), while *IDH* correlated negatively with *TET2* (*IDH1* OR 0.19, $q<0.001$; *IDH2* OR 0.16, $q<0.001$) and *WT1* (*IDH1* OR 0.2, $q=0.049$; *IDH2* OR 0.18, $q=0.028$).

Development of the HARMONY risk classification for *NPM1*-mut AML

In order to summarize clinically significant gene co-mutational patterns, a risk classification was developed using the *NPM1*-mut AML training cohort. Two-year OS of *NPM1*-wt intensively-treated patients in the same dataset was used as a reference and was 29.9% (95% CI 26.5-33.3%), 47.8% (42.4-53.3%) and 79% (74.3-83.7%) for European LeukemiaNet (ELN) 2022 adverse, intermediate and favorable risk, respectively (Supplementary Table S2). In the first step (all 1,001 *NPM1*-mut patients), co-mutation of *FLT3*-ITD and *DNMT3A*-mut was selected, as patients with both mutations had an estimated 2-year OS of 29.1% (21.6-36.6%), similar to ELN2022 adverse (Supplementary Figures S1-S4). In the subgroup with *FLT3*-ITD and *DNMT3A*-wt, *IDH*-mut patients had a predicted 2-year OS of 72.7% (56.4-89%), comparable to ELN2022 favorable, while *IDH*-wt had an estimated 2-year OS of 47.4% (36.1-58.8%), close to ELN2022 intermediate (Supplementary

Figures S5 and S6). In accordance, 60% of patients with *FLT3*-ITD in *NPM1*-mut AML were classified as adverse, 27% as intermediate and 13% as favorable risk (median OS 0.9 years, 1.5 years and not reached, respectively, $p < 0.001$) (**Supplementary Figure S7**). In the next step, patients with absence of *FLT3*-ITD were analyzed and the combination of *DNMT3A* and *IDH* mutations was selected. Patients with *DNMT3A*-mut and *IDH*-mut had an estimated 2-year OS of 55.1% (43.6-66.5%), comparable to ELN2022 intermediate, while patients with *DNMT3A*-mut and *IDH*-wt presented a predicted 2-year OS of 77.2% (70.6-83.8%), similar to ELN2022 favorable (**Supplementary Figures S8-S11**). As a result, 44% of patients with *DNMT3A*-mut in *NPM1*-mut AML were classified as adverse, 17% as intermediate and 39% as favorable risk (median OS 0.9, 2.3 and 9.5 years, respectively, $p < 0.001$) (**Supplementary Figure S12**). In the last step (absence of *FLT3*-ITD and *DNMT3A*-wt), patients with *TET2*-mut presented an estimated 2-year OS of 56.9% (41-72.8%), in line with ELN2022 intermediate, while patients *TET2*-wt had a predicted 2-year OS of 73.5% (67.7-79.3%), closer to ELN2022 favorable (**Supplementary Figures S13 and S14**). Of note, the OS curve of *TET2*-mut patients did not present a plateau at the 2-year mark, which resulted in significant differences in OS when compared to *TET2*-wt patients ($p = 0.022$, **Supplementary Figure S15**). In the subgroup of patients with *FLT3*-ITD absence, *DNMT3A*-wt and *TET2*-wt, further risk reclassification could not be made according to the classification requisites, resulting in those patients being categorized as *NPM1*-mut favorable (**Figure 2A**, **Supplementary Figure S16**).

The HARMONY *NPM1*-mut risk classification stratified 51.8% of *NPM1*-mut AML patients as favorable, 24.8% as intermediate and 23.4% as adverse risk (median OS 14.4, 2.2 and 0.9 years, respectively, $p < 0.001$) (**Figure 2B**). CRc rates after induction treatment were 90.7%, 83.5% and 83.7% for favorable, intermediate and adverse risk, respectively ($p < 0.001$). Median RFS was not reached for favorable risk, while it was 1.2 years for intermediate (95% CI 0.92-1.49) and 0.6 years for adverse (95% CI 0.46-0.74) (**Figure 2C**). Of note, gender distribution, median patient age, AML type, hemoglobin and platelet values were similar among these three *NPM1*-mut risk categories (**Table 2**). *FLT3*-ITD was present in 10%, 43% and 100% of

favorable, intermediate and adverse risk patients, respectively, which could explain differences in WBC at diagnosis, bone marrow blasts and allo-HSCT rates in CR1 among the subgroups.

Comparison to ELN 2022 risk classification

OS of *NPM1*-mut AML patients according to HARMONY categories was similar to reference ELN2022 subgroups in *NPM1*-wt in the training cohort: median OS 11.2 vs 14.4 years for favorable ($p = 0.396$), 1.7 vs 2.2 for intermediate ($p = 0.386$) and 1.1 vs 0.9 for adverse risk categories ($p = 0.117$) (**Supplementary Figure S17**). The HARMONY classification was able to reassign 42.7% of *NPM1*-mut patients into a different risk category: 234 shifted from intermediate to adverse, 141 from favorable to intermediate and 52 from intermediate to favorable (**Figure 2D**). Within the ELN2022 favorable subgroup, HARMONY *NPM1*-mut favorable patients had significant better outcomes than *NPM1*-mut intermediate cohort (median OS 14.4 vs 2.4 years, respectively, $p < 0.001$) (**Figure 2E**). Within the ELN2022 intermediate group, the HARMONY classification was able to discriminate three different subgroups with distinct outcomes: *NPM1*-mut favorable, intermediate and adverse (median OS not reached, 1.5 and 0.9 years, respectively, $p < 0.001$) (**Figure 2F**).

The HARMONY classification was also able to stratify older patients (i.e. >60 years at diagnosis) into three subgroups with distinct outcomes, with a median OS of 3.5, 1.1 and 0.6 years for favorable, intermediate and adverse subgroups, respectively ($p < 0.001$) (**Supplementary Figure S18**).

Multivariable analysis

A multivariable Cox regression model of OS, censoring at transplant date those patients who underwent allo-HSCT in CR1, identified the following pretreatment independent variables: age >60 years (hazard ratio [HR] 2.32, $p < 0.001$), hyperleukocytosis ($>100 \times 10^9/L$) at diagnosis (HR 1.77, $p < 0.001$), prior hematological malignancy (HR 2.51, $p = 0.01$) and HARMONY *NPM1*-mut classification (using favorable category as reference, intermediate HR 1.86 [$p < 0.001$] and adverse HR 2.98 [$p < 0.001$]). Remarkably, ELN2022 risk categories were not significant in this model ($p = 0.602$) (**Figure 3**).

Finally, a multivariable Cox regression model of OS, considering allo-HSCT in CR1 as a time-

dependent covariate, in patients aged ≤ 70 years (i.e. potential transplant candidates) was performed. In the training cohort, allo-HSCT in CR1 improved OS in all HARMONY *NPM1*-mut subgroups, although the highest benefit was seen in *NPM1*-mut adverse patients (HR 0.66, 95% CI 0.57-0.77, $p < 0.001$) (**Supplementary Table S3**).

Internal validation HARMONY *NPM1*-mut classification

A multivariable Cox regression model of OS, The internal validation cohort of 762 adult *NPM1*-mut AML patients had significant differences compared to the training cohort, as patients were older (median 57 vs 53 years, $p < 0.001$; age ≥ 60 years 42% vs 27%, $p < 0.001$), with an increased proportion of patients with history of prior hematological malignancies (7.6% vs 1.5%), higher *FLT3*-ITD prevalence (45.8% vs 39.3%, $p = 0.007$) and increased WBC at diagnosis (37 vs 24 $\times 10^9/L$, $p < 0.001$) (**Table 1**). CRc rates after induction treatment were lower (79.5% vs 87.3%, $p < 0.001$), early-death rates were higher (30-day mortality 8% vs 3.8%, $p < 0.001$) and fewer patients received allo-HSCT in CR1 (17% vs 24%, $p = 0.01$). Median follow-up was 7.2 years, with a median OS of 2.8 years (vs 8.3 years in the training cohort, $p < 0.001$). The HARMONY *NPM1*-mut classification stratified 44.8% of the internal validation cohort as favorable, 29.4% as intermediate and 25.8% as adverse risk, with significant differences in OS (median OS 8.2, 2.8 and 0.8 years, respectively, $p < 0.001$) (**Figure 4A**) and RFS (median RFS 4.8, 2.1 and 0.5 years, respectively, $p < 0.001$) (**Figure 4B**). In patients aged > 60 years at diagnosis, median OS was 3, 1.6 and 0.6 years, respectively ($p < 0.001$) (**Supplementary Figure S18**).

In the internal validation cohort, allo-HSCT in CR1 did not enhance OS of HARMONY *NPM1*-mut favorable patients (HR 0.99, 95% CI 0.94-1, $p = 0.605$), but it showed improved outcomes for the *NPM1*-mut intermediate subgroup (HR 0.84, 95% CI 0.75-0.94, $p = 0.003$) and again the highest benefit for *NPM1*-mut adverse patients (HR 0.78, 95% CI 0.69-0.88, $p < 0.001$) in the multivariable Cox regression model of OS, considering allo-HSCT in CR1 as a time-dependent covariate, in patients aged ≤ 70 years (**Supplementary Table S4**).

External validation of HARMONY *NPM1*-mut classification

The external validation cohort of 585 adult *NPM1*-mut AML patients also presented significant differences compared to the training cohort (**Supplementary Table S5**). In the external validation cohort, patients were also older (median 56 vs 53 years, $p < 0.001$; age ≥ 60 years 38% vs 27%, $p < 0.001$), with an increased proportion of patients with history of prior hematological malignancies (4.8% vs 1.5%, $p < 0.001$), higher WBC at diagnosis (33 vs 24 $\times 10^9/L$, $p < 0.001$), but similar *FLT3*-ITD prevalence (41.5% vs 39.3%, $p = 0.401$). Composite complete remission rates after induction treatment were similar (89.6% vs 87.3%), although early death rate was higher (30-day mortality 6% vs 3.8%, $p = 0.045$). Allo-HSCT rates were higher (41.7% vs 34.1% at any time, $p = 0.003$), although information regarding the transplant timing was not provided for most of the patients. Therefore, OS and RFS were analyzed without censoring at transplant date in the external validation cohort. Median follow-up was 6.4 years, with a median OS of 4.5 years (vs 8.25 years in the training cohort, $p < 0.001$). In summary, the external validation cohort exhibited features that, in general, fell between those of the training cohort and the internal validation cohort (**Supplementary Table S6**).

The HARMONY *NPM1*-mut classification stratified 47.7% of the external validation cohort as favorable, 28.5% as intermediate and 23.8% as adverse risk, with significant differences in OS (median OS not reached, 3.8 and 1.2 years, respectively, $p < 0.001$) (**Figure 4C**) and RFS (median RFS 3.5, 1.3 and 0.8 years, respectively, $p < 0.001$) (**Figure 4D**). In patients aged > 60 years at diagnosis, median OS was 3.6, 1.7 and 0.7 years, respectively ($p < 0.001$) (**Supplementary Figure S18**).

DISCUSSION

NPM1-mut AML comprises the largest adult AML subtype in younger adults, making accurate risk stratification of paramount importance to inform clinical decisions¹¹. Since the initial discovery of this entity, *FLT3*-ITD has been the only co-mutation consistently associated with inferior OS and remains as the only significant co-mutation affecting prognosis in this AML subtype in current guidelines^{2, 5, 6, 32-34}. In this study, which to the best of our knowledge is the largest *NPM1*-mut AML cohort studied by panel sequencing to date, we

conducted a comprehensive analysis of the mutational landscape, identifying additional co-mutations with prognostic implications. *DNMT3A* and *FLT3*-ITD are the most frequent co-mutations, but they tend to appear together, making it difficult to address the prognostic value of each mutation individually in smaller cohorts. In our study, *FLT3*-ITD with *DNMT3A*-wt was present in only 16% of patients, *DNMT3A*-mut in the absence of *FLT3*-ITD was found in 30%, while both mutations were present in 23% of the patients, which is in line with recent reports⁷. This “triple-mutated” AML subgroup (*NPM1*-mut, *FLT3*-ITD, *DNMT3A*-mut) was associated with dismal outcomes, with a median RFS of less than 9 months in all datasets included in our study.

The interaction between these three mutations has been reported previously, consistently associated with inferior OS^{1, 7, 13, 14, 35}. Moreover, recent studies have found that this triple-mutated AML shows distinct characteristics, such as aberrant leukemia-specific GPR56^{high} and CD34^{low} immunophenotype, high leukemia stem cell frequency and upregulation of hepatic leukemia factor¹⁵. While it remains unclear if the *NPM1*-mut, *FLT3*-ITD, *DNMT3A*-mut subgroup will be recognized as a distinct biologic entity in the future, it seems reasonable to consider this subgroup as adverse risk, at least with conventional chemotherapy approaches.

In addition to this important confirmatory aspect of our study, we identified also a subset of *FLT3*-ITD positive *NPM1*-mut patients with favorable outcomes (i.e. *NPM1*-mut, *FLT3*-ITD, *DNMT3A*-wt, and *IDH*-mut) that has not been reported in previous studies. Remarkably, *IDH*-mut were associated with a favorable outcome in patients with *FLT3*-ITD and *DNMT3A*-wt, while a deleterious effect was shown in the subgroup of patients with *DNMT3A*-mut and absence of *FLT3*-ITD, which is consistent with the results reported by Paschka *et al* in the latter subgroup³⁶. Moreover, this paradoxical prognostic effect has been documented for other gene mutations in adult AML, such as *DNMT3A* and *PTPN11*, highlighting the importance of careful evaluation of co-mutational patterns for accurate patient risk stratification^{9, 37}. While the biological mechanisms underlying the impact of *IDH*-mut on treatment outcome in *NPM1*-mut AML remain to be fully elucidated, it could be related to the epigenetic state in which the

transforming events occur, such as *NPM1*-mut and *FLT3*-ITD, as these events are generally acquired at a later stage^{7, 38}. In contrast, the acquisition of *DNMT3A*-mut and *IDH*-mut are early events both linked to clonal hematopoiesis³⁹, and deregulated epigenetic states that are distinct between *DNMT3A*-mut and *IDH*-mut⁴⁰.

The incorporation of myelodysplastic syndrome (MDS)-related gene mutations (i.e. *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2*) in the ELN2022 guidelines as adverse risk, has raised the question of whether their presence could influence outcome in *NPM1*-mut AML, with contradictory results to date^{41, 42}. We found that only three of these mutations (*SRSF2*, *STAG2*, *RUNX1*) were present in more than 1% of our cohort, which precluded the consideration of most MDS-related gene mutations into the classification. Notably, in our cohort the presence of a *RUNX1*-mut was associated with adverse outcome, *SRSF2*-mut with intermediate prognosis, while *STAG2*-mut identified a subgroup of patients with favorable outcomes in the absence of *FLT3*-ITD (**Supplementary Figures S1 and S8**). These findings suggest that MDS-related gene mutations, which are also early genetic events like clonal hematopoiesis associated gene mutations, provide a different basis for transforming events, which in the case of *NPM1*-mut can impact patient outcome in distinct ways.

In *NPM1*-mut AML, a median of up 13 gene mutations per patient when whole exome analysis is performed has been reported, so hundreds of co-mutational combinations can form in this AML subtype⁴³. In our study, we aimed to reduce this complexity in the final classification by taking only five of the most prevalent gene mutations into account: *FLT3*-ITD, *DNMT3A*-mut, *IDH1/IDH2*-mut, and *TET2*-mut. The HARMONY classification was able to stratify *NPM1*-mut patients into favorable, intermediate and adverse risk categories, with comparable outcomes to their *NPM1*-wt counterparts according to ELN2022 in the same dataset. Remarkably, this risk stratification was able to reclassify more than 40% of *NPM1*-mut patients into a different risk category when compared to ELN2022 guidelines, underscoring the importance of understanding respective co-mutational patterns that can affect AML subtype-specific outcomes⁶.

Importantly, the distribution of *NPM1*-mut risk

categories was similar in all cohorts analyzed, both the training as well as the internal and external validation cohorts, accounting for 45-52% favorable, 25-29% intermediate, and 23-26% adverse risk patients contained within the *NPM1*-mut AML subtype. Prediction of outcomes in intensively-treated older patients (i.e. >60 years) is challenging with current stratification systems, so novel approaches are warranted^{44, 45}. The HARMONY classification was also applicable to older patients and, similarly to the entire cohort, identified three different outcome subgroups in the training as well as internal validation and external validation cohorts.

However, there are also several limitations to our study that should be taken into account. First, it is a retrospective, multicenter analysis, where patients were treated in various European countries with different intensive chemotherapy regimens. Accordingly, the HARMONY *NPM1*-mut classification was first developed in a cohort of patients included in clinical trials from large cooperative groups (AMLSG and HOVON-SAKK), thereby harboring a potential selection bias. However, findings could be validated in an independent validation cohort comprising real-world data derived from European institutions. Although this cohort did not include patients treated with intensive combination therapies including targeted therapeutic such as *FLT3*-ITD inhibitors or GO, which have demonstrated to improve outcomes in *NPM1*-mut AML⁴⁶⁻⁴⁹, an additional validation of the HARMONY classification was carried out with a cohort of patients enrolled in UK-NCRI trials, where patients received GO or *FLT3* inhibitor lestaurtinib in addition to intensive chemotherapy. In accordance, further studies will be required re-evaluate the prognostic and predictive impact of the HARMONY *NPM1*-mut classification to stratify patients treated with midostaurin or non-intensive approaches. Our analyses did not include assessment of measurable residual disease (MRD). While MRD in peripheral blood after two treatment cycles has proven to be a strong prognostic factor in *NPM1*-mut AML, novel treatment strategies have reduced the MRD positivity rate to less than 20% of patients, limiting the proportion of patients identified as high-risk^{50, 51}. Moreover, a 3-year cumulative incidence of relapse of up to 40% has been reported even in MRD negative patients⁷, suggesting that

combination of genotype at diagnosis and MRD assessment after treatment would provide the most accurate estimation of patient outcomes.

In summary, this study provides novel insights regarding co-mutational patterns with prognostic implications in intensively-treated *NPM1*-mut AML adult patients. The HARMONY classification suggests that more than 40% of *NPM1*-mut patients might be re-classified into a different ELN2022 risk category, taking additional markers into account. Further evaluation is warranted prior to clinical application, especially in the light of age-dependent differences in co-mutational patterns⁵² and novel combinatorial treatments for this AML subtype.

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AUTHOR CONTRIBUTIONS

AHS, JMHR, BH, GO, HD and LB designed the study. AHS, AVR, ES, ATT, MA, JV, IT, MS, JML, MP, KM, SL, CR, CT, KHM, KD, MH, TH, PJMV, NR, HD and JMHR performed data collection and assembly of the data. AHS, AVR, ES, GC, AB, RA, JMT, RM, GS, MTV, KD, JMHR, BH, GO, HD and LB performed data analysis and interpretation. AHS and LB wrote the manuscript. BJPH, GO and HD critically reviewed the manuscript. LB supervised research and coordinated the HARMONY AML group. All authors had access to primary data, read and approved the final manuscript.

COMPETING INTERESTS

ATT: Consultancy for CSL Behring, Maat Pharma, Biomarín and Onkowissen; travel reimbursements from Neovii Biotech and Novartis. **MS:** honoraria from Novartis, Celgene, AOP Orphan, AbbVie. **KHM:** honoraria from AbbVie, Bristol Myers Squibb, Celgene, Janssen, Novartis, Pfizer, Otsuka; research funding from AbbVie.

RA: honoraria from Astellas, Bristol Myers Squibb, Incyte, and Novartis. **MP:** honoraria from Novartis. **GS:** honoraria from Takeda and has participated in Ad-Boards from Novartis, Celgene, Abbvie, Helsinn and Takeda. **CR:** honoraria from AbbVie, Astellas, Bristol-Meyer-Squibb, Daiichi Sankyo, Jazz, Janssen, Novartis, Otsuka, Pfizer, Servier; institutional research funding from AbbVie, Astellas, Novartis, Pfizer. **CT:** co-owner and CEO of AgenDix GmbH and has received lecture fees and/or participated in Ad-Boards from Novartis, Jazz Pharmaceuticals, Astellas, Janssen, Illumina; research funding from Novartis, Bayer. **KD:** honoraria from Novartis, Jazz Pharmaceuticals, Abbvie; has participated in Ad-Boards from Novartis, Bristol Myers Squibb, Jazz Pharmaceuticals, Abbvie; research funding from Novartis, Astellas, Agios, Bristol Myers Squibb, Kronos. **MH:** honoraria from Astellas, Daiichi Sankyo, Janssen, Miltenyi, Otsuka, Qiagen, Servier and has participated in Ad-Boards from Abbvie, AvenCell, Ascentage Pharma, Bristol Myers Squibb, Janssen, Jazz Pharmaceuticals, LabDelbert, Novartis, Pfizer, Servier; research funding from Abbvie, Bayer Pharma AG, Jazz Pharmaceuticals, Glycostem, Karyopharm, PinotBio, Servier, Toray. **TH:** current employment at Munich Leukemia Laboratory, with part ownership. **JMHR:** honoraria from Bristol Myers Squibb, Pfizer, Amgen, Celgene, GSK, Novartis; Advisory role for Bristol Myers Squibb, Pfizer, Amgen, Celgene, Novartis, Janssen, Roche, Abbvie, AstraZeneca, Beigene, Lilly, Gilead, Takeda, Jazz Pharmaceuticals, Rovi, Incyte; research funding from Bristol Myers Squibb, Celgene, Novartis. **BH:** honoraria from Pfizer, Bristol Myers Squibb, Novartis; research funding from AstraZeneca. **GO:** honoraria from Abbvie, Jazz Pharmaceuticals, Astellas, Gilead, Bristol Myers Squibb, Servier, Roche. **HD:** Advisory role for AbbVie, Agios, Amgen, Astellas, AstraZeneca, BERLIN-CHEMIE, Bristol Myers Squibb, Celgene, GEMoaB, Gilead Sciences, Janssen, Jazz Pharmaceuticals, Novartis, Syndax; research funding from AbbVie, Agios, Amgen, Astellas, Bristol Myers Squibb, Jazz Pharmaceuticals, Kronos-Bio, Novartis. **LB:** honoraria from AbbVie, Amgen, Astellas, Bristol Myers Squibb, Celgene, Daiichi Sankyo, Gilead, Hexal, Janssen, Jazz Pharmaceuticals, Menarini, Novartis, Otsuka, Pfizer, Roche, and Sanofi; research funding from Bayer, Jazz Pharmaceuticals.

DATA SHARING STATEMENT

After the publication of this article, data collected for this analysis and related documents will be made available to others upon reasonably justified request, which needs to be written and addressed to the attention of the corresponding author Dr Lars Bullinger at the following e-mail address: lars.bullinger@charite.de. The HARMONY Alliance, via the corresponding author Dr Lars Bullinger, is responsible to evaluate and eventually accept or refuse every request to disclose data and their

related documents, in compliance with the ethical approval conditions, in compliance with applicable laws and regulations, and in conformance with the agreements in place with the involved subjects, the participating institutions, and all the other parties directly or indirectly involved in the participation, conduct, development, management, and evaluation of this analysis.

REFERENCES

1. Papaemmanuil E, Gerstung M, Bullinger L, et al: Genomic Classification and Prognosis in Acute Myeloid Leukemia. *New England Journal of Medicine* 374:2209–2221, 2016
2. Falini B, Nicoletti I, Martelli MF, et al: Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): Biologic and clinical features. *Blood* 109:874–885, 2007
3. Arber DA, Orazi A, Hasserjian RP, et al: International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood* 140:1200–1228, 2022
4. Khoury JD, Solary E, Abla O, et al: The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* 36:1703–1719, 2022
5. Falini B, Mecucci C, Tiacci E, et al: Cytoplasmic Nucleophosmin in Acute Myelogenous Leukemia with a Normal Karyotype. *New England Journal of Medicine* 352:254–266, 2005
6. Döhner H, Wei AH, Appelbaum FR, et al: Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 140:1345–1377, 2022
7. Othman J, Potter N, Ivey A, et al: Molecular, clinical, and therapeutic determinants of outcome in NPM1 mutated AML. *Blood* 144:714–728, 2024
8. Guryanova OA, Shank K, Spitzer B, et al: DNMT3A mutations promote anthracycline resistance in acute myeloid leukemia via impaired nucleosome remodeling. *Nat Med* 22:1488–1495, 2016
9. Gale RE, Lamb K, Allen C, et al: Simpson's paradox and the impact of different DNMT3A mutations on outcome in younger adults with acute myeloid leukemia. *Journal of Clinical Oncology* 33:2072–2083, 2015
10. Ivey A, Hills RK, Simpson MA, et al: Assessment of Minimal Residual Disease in Standard-Risk AML. *New England Journal of Medicine* 374:422–433, 2016
11. Bullinger L, Döhner K, Döhner H: Genomics of acute myeloid leukemia diagnosis and pathways. *Journal of Clinical Oncology* 35:934–946, 2017

12. Oñate G, Bataller A, Garrido A, et al: Prognostic impact of DNMT3A mutation in acute myeloid leukemia with mutated NPM1. *Blood Adv* 6:882–890, 2022
13. Ley T, Miller C, Ding L, et al: Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. *New England Journal of Medicine* 368:2059–2074, 2013
14. Bezerra MF, Lima AS, Piqué-Borràs MR, et al: Co-occurrence of DNMT3A, NPM1, FLT3 mutations identifies a subset of acute myeloid leukemia with adverse prognosis. *Blood* 135:870–875, 2020
15. Garg S, Reyes-Palomares A, He L, et al: Hepatic leukemia factor is a novel leukemic stem cell regulator in DNMT3A, NPM1, and FLT3-ITD triple-mutated AML. *Blood* 134:263–276, 2019
16. Falini B, Brunetti L, Sportoletti P, et al: NPM1-mutated acute myeloid leukemia: From bench to bedside. *Blood* 136:1707–1721, 2020
17. Balsat M, Renneville A, Thomas X, et al: Postinduction minimal residual disease predicts outcome and benefit from allogeneic stem cell transplantation in acute myeloid leukemia with NPM1 mutation: A study by the acute leukemia French association group. *Journal of Clinical Oncology* 35:185–193, 2017
18. Angenendt L, Röhlig C, Montesinos P, et al: Chromosomal abnormalities and prognosis in NPM1-mutated acute myeloid leukemia: A pooled analysis of individual patient data from nine international cohorts. *Journal of Clinical Oncology* 37:2632–2642, 2019
19. Haferlach C, Mecucci C, Schnittger S, et al: AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. *Blood* 114:3024–3032, 2009
20. Moukalled N, Labopin M, Versluis J, et al: Complex karyotype but not other cytogenetic abnormalities is associated with worse posttransplant survival of patients with nucleophosmin 1-mutated acute myeloid leukemia: A study from the European Society for Blood and Marrow Transplantation Acute Leukem. *Am J Hematol* 99:360–369, 2024
21. Othman J, Meggendorfer M, Tiacci E, et al: Overlapping features of therapy-related and de novo NPM1-mutated AML. *Blood* 141:8–10, 2023
22. Mason EF, Hasserjian RP, Aggarwal N, et al: Blast phenotype and comutations in acute myeloid leukemia with mutated NPM1 influence disease biology and outcome. *Blood Adv* 3:3322–3332, 2019
23. Wang B, Yang B, Wu W, et al: The correlation of next-generation sequencing-based genotypic profiles with clinicopathologic characteristics in NPM1-mutated acute myeloid leukemia. *BMC Cancer* 21:1–11, 2021
24. Löwenberg B, Pabst T, Maertens J, et al: Therapeutic value of clofarabine in younger and middle-aged (18–65 years) adults with newly diagnosed AML. *Blood* 129:1636–1645, 2017
25. Ossenkoppele GJ, Breems DA, Stuessi G, et al: Lenalidomide added to standard intensive treatment for older patients with AML and high-risk MDS. *Leukemia* 34:1751–1759, 2020
26. Löwenberg B, Pabst T, Maertens J, et al: Addition of lenalidomide to intensive treatment in younger and middle-aged adults with newly diagnosed AML: The HOVON-SAKK-132 trial. *Blood Adv* 5:1110–1121, 2021
27. Metzeler KH, Herold T, Rothenberg-Thurley M, et al: Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood* 128:686–698, 2016
28. Belenkaya R, Gurley MJ, Golozar A, et al: Extending the OMOP Common Data Model and Standardized Vocabularies to Support Observational Cancer Research. *JCO Clin Cancer Inform* 12–20, 2021
29. Middeke JM, Metzeler KH, Rollig C, et al: Differential impact of IDH1/2 mutational subclasses on outcome in adult AML: results from a large multicenter study. *Blood Adv* 6:1394–1405, 2022
30. Tazi Y, Arango-Ossa JE, Zhou Y, et al: Unified classification and risk-stratification in Acute Myeloid Leukemia. *Nat Commun* 13:4622, 2022
31. Storey JD: A direct approach to false discovery rates. *J R Stat Soc Series B Stat Methodol* 64:479–498, 2002
32. Schnittger S, Schoch C, Kern W, et al: Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood* 106:3733–3739, 2005
33. Döhner H, Estey EH, Amadori S, et al: Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* 115:453–474, 2010
34. Döhner K, Schlenk RF, Habdank M, et al: Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: Interaction with other gene mutations. *Blood* 106:3740–3746, 2005
35. Straube J, Ling VY, Hill GR, et al: The impact of age, NPM1mut, and FLT3ITD allelic ratio in patients with acute myeloid leukemia. *Blood* 131:1148–1153, 2018
36. Paschka P, Schlenk RF, Gaidzik VI, et al: IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *Journal of Clinical Oncology*

- 28:3636–3643, 2010
37. Alfayez M, Issa GC, Patel KP, et al: The Clinical impact of PTPN11 mutations in adults with acute myeloid leukemia. *Leukemia* 35:691–700, 2021
 38. Cocciardi S, Dolnik A, Kapp-Schwoerer S, et al: Clonal evolution patterns in acute myeloid leukemia with NPM1 mutation. *Nat Commun* 10:1–11, 2019
 39. Chan SM, Majeti R: Role of DNMT3A, TET2, and IDH1/2 mutations in pre-leukemic stem cells in acute myeloid leukemia. *Int J Hematol* 98:648–657, 2013
 40. Glass JL, Hassane D, Wouters BJ, et al: Epigenetic identity in AML depends on disruption of nonpromoter regulatory elements and is affected by antagonistic effects of mutations in epigenetic modifiers. *Cancer Discov* 7:868–883, 2017
 41. Eckardt JN, Bill M, Rausch C, et al: Secondary-type mutations do not impact outcome in NPM1-mutated acute myeloid leukemia – implications for the European LeukemiaNet risk classification. *Leukemia* 37:2282–2285, 2023
 42. Chan O, Al Ali N, Tashkandi H, et al: Mutations highly specific for secondary AML are associated with poor outcomes in ELN favorable risk NPM1-mutated AML. *Blood Adv* 8:1075–1083, 2024
 43. Tyner JW, Tognon CE, Bottomly D, et al: Functional genomic landscape of acute myeloid leukaemia. *Nature* 562:526–531, 2018
 44. Park S, Kim TY, Cho BS, et al: Prognostic value of European LeukemiaNet 2022 criteria and genomic clusters using machine learning in older adults with acute myeloid leukemia. *Haematologica* 109:1095–1106, 2024
 45. Versluis J, Metzner M, Wang A, et al: Risk Stratification in Older Intensively Treated Patients With AML. *Journal of Clinical Oncology* 42:4084–4094, 2024
 46. Stone RM, Mandrekar SJ, Sanford BL, et al: Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *New England Journal of Medicine* 377:454–464, 2017
 47. Oñate G, Pratcorona M, Garrido A, et al: Survival improvement of patients with FLT3 mutated acute myeloid leukemia: results from a prospective 9 years cohort. *Blood Cancer J* 13:69, 2023
 48. Schlenk RF, Paschka P, Krzykalla J, et al: Gemtuzumab ozogamicin in NPM1-mutated acute myeloid leukemia: Early results from the prospective randomized AMLSG 09-09 Phase III study. *Journal of Clinical Oncology* 38:623–632, 2020
 49. Kapp-Schwoerer S, Weber D, Corbacioglu A, et al: Impact of gemtuzumab ozogamicin on MRD and relapse risk in patients with NPM1-mutated AML: Results from the AMLSG 09-09 trial. *Blood* 136:3041–3050, 2020
 50. Othman J, Potter N, Ivey A, et al: Postinduction molecular MRD identifies patients with NPM1 AML who benefit from allogeneic transplant in first remission. *Blood* 143:1931–1936, 2024
 51. Russell NH, Wilhelm-Benartzi C, Othman J, et al: Fludarabine, Cytarabine, Granulocyte Colony-Stimulating Factor, and Idarubicin With Gemtuzumab Ozogamicin Improves Event-Free Survival in Younger Patients With Newly Diagnosed AML and Overall Survival in Patients With NPM1 and FLT3 Mutations. *Journal of Clinical Oncology* 42:1158–1168, 2024
 52. Cocciardi S, Saadati M, Weiß N, et al: Impact of myelodysplasia-related and additional gene mutations in intensively treated patients with NPM1-mutated AML. *Hemasphere* 9:e70060, 2025

Table 1. Baseline characteristics of *NPM1*-mut adult AML patients included in HARMONY database, comparing the training cohort to the internal validation cohort.

	Training cohort (n=1001)	Internal validation cohort (n=762)	p-value
Female sex	543 (54.2%)	419 (55%)	0.7938
Median age in years (range)	52.9 (18 - 81)	57 (18 - 86)	<0.0001
Age ≥60 years	269 (26.8%)	320 (42%)	<0.0001
AML type			
De novo AML	963 (96.2%)	573 (90.5%)	<0.0001
Secondary AML	38 (3.8%)	60 (9.5%)	
Prior HM	15 (1.5%)	48 (7.6%)	<0.0001
Therapy-related	23 (2.3%)	12 (1.6%)	0.2811
Hemoglobin (g/dL)	8.9 [Q1 = 7.6, Q3 = 10.3]	9.2 [Q1 = 8.2, Q3 = 10.5]	0.0037
WBC (x10⁹/L)	23.8 [Q1 = 6.9, Q3 = 62.8]	36.8 [Q1 = 13.4, Q3 = 85.6]	<0.0001
WBC > 100 x10 ⁹ /L	136 (13.6%)	144 (20.4%)	
Platelets (x10⁹/L)	66.5 [Q1 = 38, Q3 = 116]	66.5 [Q1 = 38.2, Q3 = 111]	0.8798
BM % of blasts	75 [Q1 = 45, Q3 = 89]	72.75 [Q1 = 52, Q3 = 87]	0.9417
ELN 2022			
Favorable	603 (60.2%)	408 (53.5%)	
Intermediate	391 (39%)	347 (45.5%)	0.0185
Adverse	7 (0.7%)	7 (0.9%)	
FLT3-ITD	393 (39.3%)	349 (45.8%)	0.0068
Treatment response			
CRc	874 (87.3%)	606 (79.5%)	
Refractory	89 (8.9%)	95 (12.5%)	<0.0001
Not evaluable	38 (3.8%)	61 (8%)	
Early death			
30-day mortality	38 (3.8%)	61 (8%)	0.0002
60-day mortality	62 (6.2%)	88 (11.5%)	<0.0001
Allogeneic HSCT	341 (34.1%)	215 (29.3%)	0.0419
In CR1	242 (24.2%)	130 (17.1%)	
In other situations	99 (9.9%)	85 (11.1%)	0.0104
Median survival in years (95% CI)	8.25 (5.14-9.77)	2.84 (2.06 - 4.09)	<0.0001

Abbreviations: AML, acute myeloid leukemia; HM, hematological malignancy; WBC, white blood count; ELN, European LeukemiaNet; ITD, internal tandem duplication; CRc, composite complete remission; HSCT, hematopoietic stem cell transplantation; CR1, first complete remission; CI, confidence interval

Table 2. Comparison of patient characteristics across HARMONY *NPM1*-mut risk categories.

	<i>NPM1</i> Favorable (n=519)	<i>NPM1</i> Intermediate (n=248)	<i>NPM1</i> Adverse (n=234)	p-value
Female sex	275 (53%)	134 (54%)	134 (57.3%)	0.5500
Median age in years (range)	52.2 [18 - 81]	53.1 [18.8 - 80]	52.3 [24 - 77]	0.5290
Age ≥60 years	136 (26.2%)	72 (29%)	61 (26.1%)	0.6758
AML type				
De novo AML	497 (95.8%)	238 (96%)	228 (97.4%)	0.5249
Secondary AML	22 (4.2%)	10 (4%)	6 (2.6%)	
AML after MDS	10 (1.9%)	4 (1.6%)	1 (0.4%)	
Therapy-related	12 (2.3%)	6 (2.4%)	5 (2.6%)	
Hemoglobin (g/dL)	9.15 [Q1=7.8, Q3=10.4]	8.89 [Q1=7.5, Q3=10]	8.65 [Q1=7.5, Q3=10.4]	0.2938
WBC (x10⁹/L)	14.2 [Q1=4.7, Q3=42.5]	26.8 [Q1=8.6, Q3=71.2]	50.6 [Q1=22, Q3=93.9]	<0.0001
(> 100 x10 ⁹ /L)	37 (7.1%)	44 (17.7%)	55 (23.5%)	
Platelets (x10⁹/L)	68 [Q1=38, Q3=118]	67 [Q1=39, Q3=120.5]	63 [Q1=38, Q3=104]	0.5066
Bone marrow % of blasts	66 [Q1=36, Q3=86]	79.5 [Q1=52, Q3=90]	80 [Q1=63, Q3=90]	
ELN 2022				
Favorable	461 (88.8%)	141 (56.9%)	0 (0%)	<0.0001
Intermediate	52 (10%)	106 (42.7%)	234 (100%)	
Adverse	6 (1.2%)	1 (0.4%)	0 (0%)	
<i>FLT3</i>-ITD present	52 (10%)	107 (43.1%)	234 (100%)	<0.0001
Treatment response				
CRc	471 (90.7%)	207 (83.5%)	196 (83.7%)	<0.0001
Refractory	30 (5.8%)	30 (12.1%)	29 (12.5%)	
Not evaluable	18 (3.5%)	11 (4.4%)	9 (3.8%)	
Early death				
30-day mortality	18 (3.5%)	11 (4.4%)	9 (3.8%)	0.7317
60-day mortality	28 (5.4%)	21 (8.5%)	13 (5.6%)	0.2298
Allogeneic HSCT	131 (25.2%)	94 (37.9%)	116 (49.6%)	<0.0001
In CR1	93 (17.9%)	56 (22.6%)	93 (39.8%)	0.0011
In other situations	38 (7.3%)	38 (15.3%)	23 (9.8%)	
Median survival in years (95% CI)	14.37 (9.51-NA)	2.56 (1.98-4.38)	1.13 (0.966-1.5)	<0.001

Abbreviations: AML, acute myeloid leukemia; HM, hematological malignancy; WBC, white blood count; ELN, European LeukemiaNet; ITD, internal tandem duplication; CRc, composite complete remission; HSCT, hematopoietic stem cell transplantation; CR1, first complete remission; CI, confidence interval.

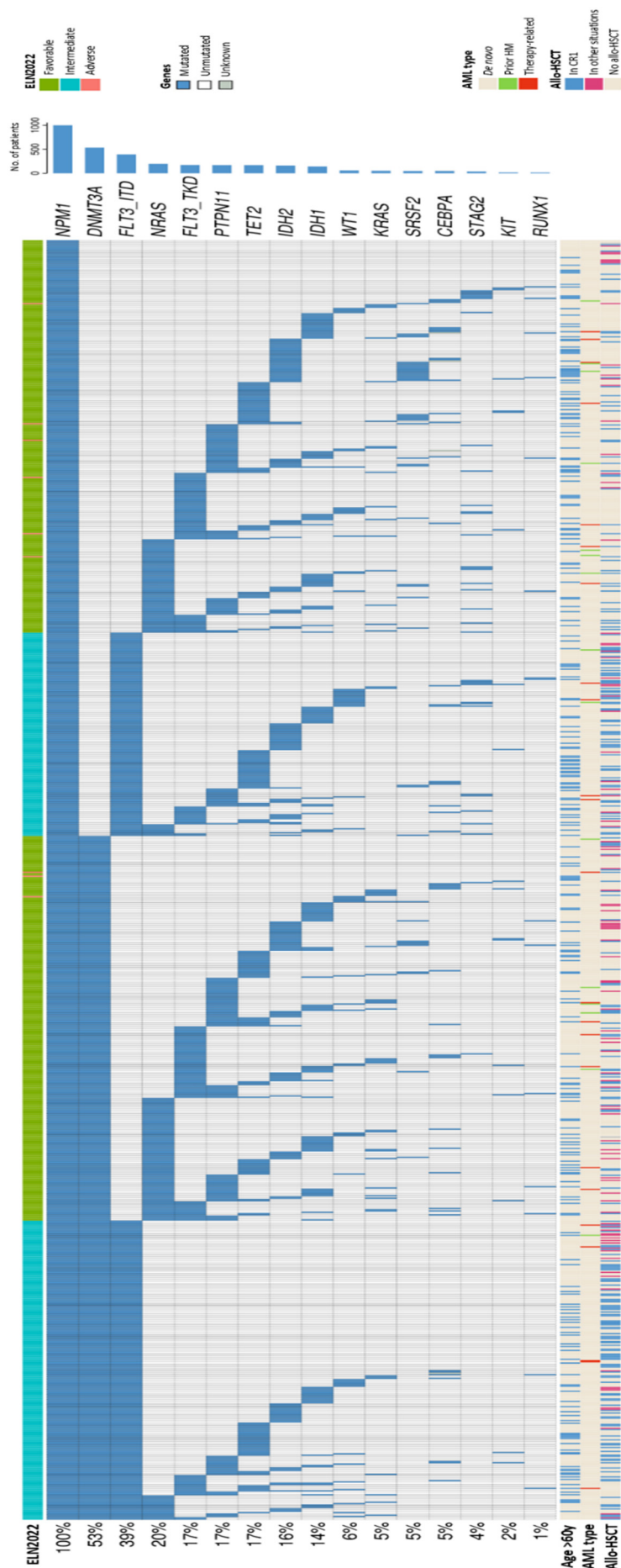
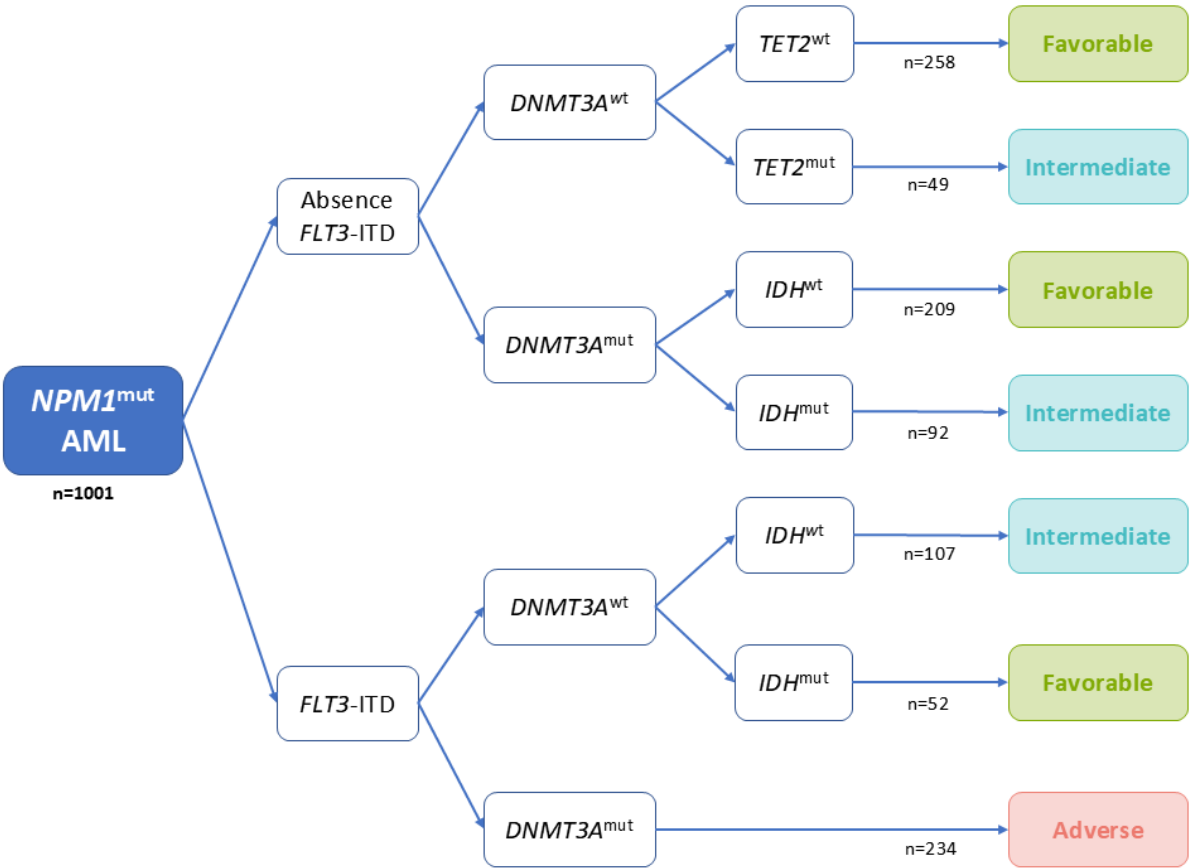
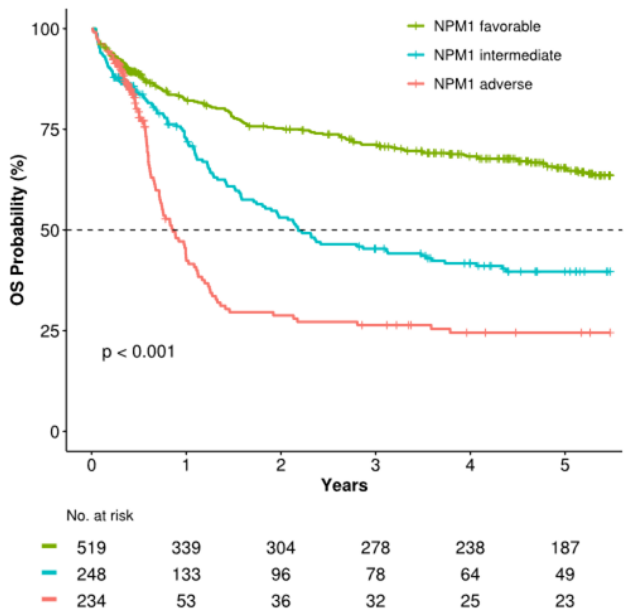


Figure 1. Mutational landscape of *NPM1*-mut adult AML. Only mutations present in both next-generation sequencing panels and in at least ten patients (1% of the training cohort) are shown. Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR1, first complete remission; ELN, European LeukemiaNet; HM, hematological malignancy.

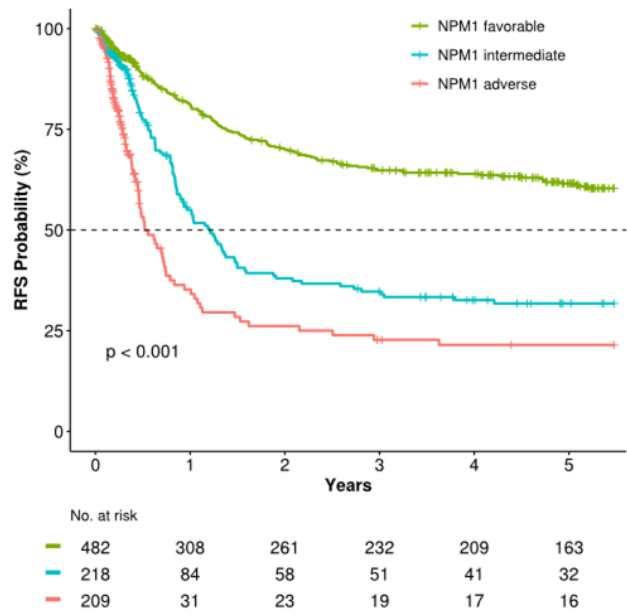
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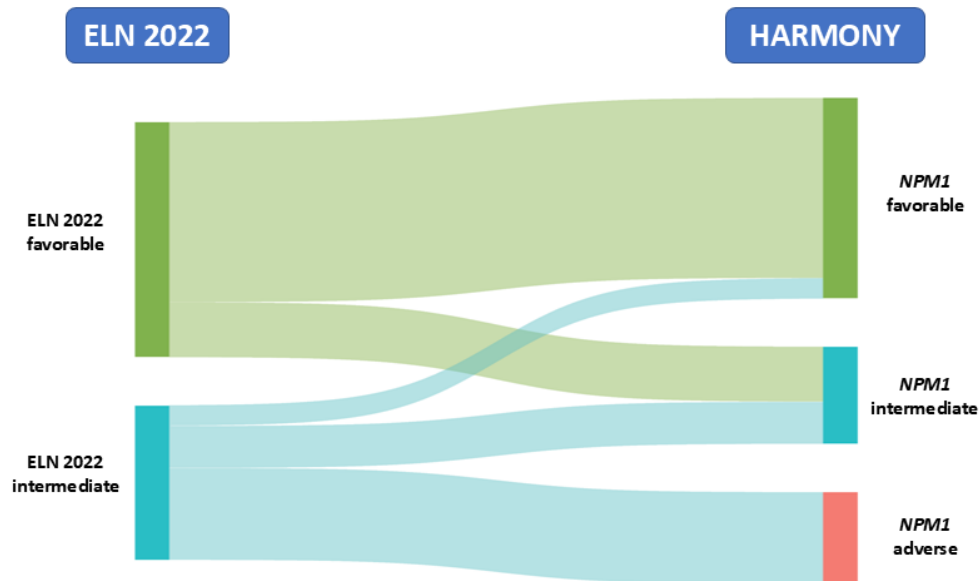
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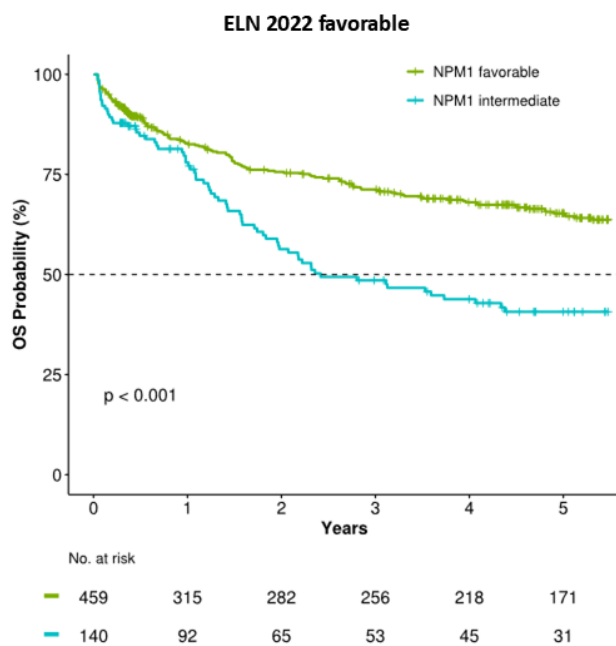
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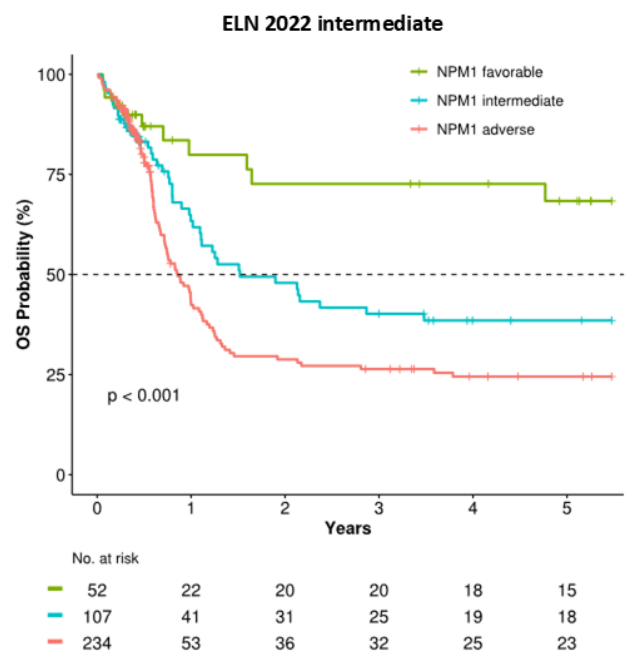


Figure 2. HARMONY NPM1-mut risk classification (A) Classification of patients according to the presence or absence of mutations in *FLT3* (ITD), *DNMT3A*, *IDH* and *TET2*. (B) Overall survival according to HARMONY NPM1-mut risk categories. (C) Relapse-free survival according to HARMONY NPM1-mut risk categories. (D) Sankey plot of patient reclassification from ELN2022 to HARMONY NPM1-mut categories. (E) Overall survival of ELN2022 favorable patients, stratified by HARMONY NPM1-mut classification. (F) Overall survival of ELN2022 intermediate patients, stratified by HARMONY NPM1-mut classification.

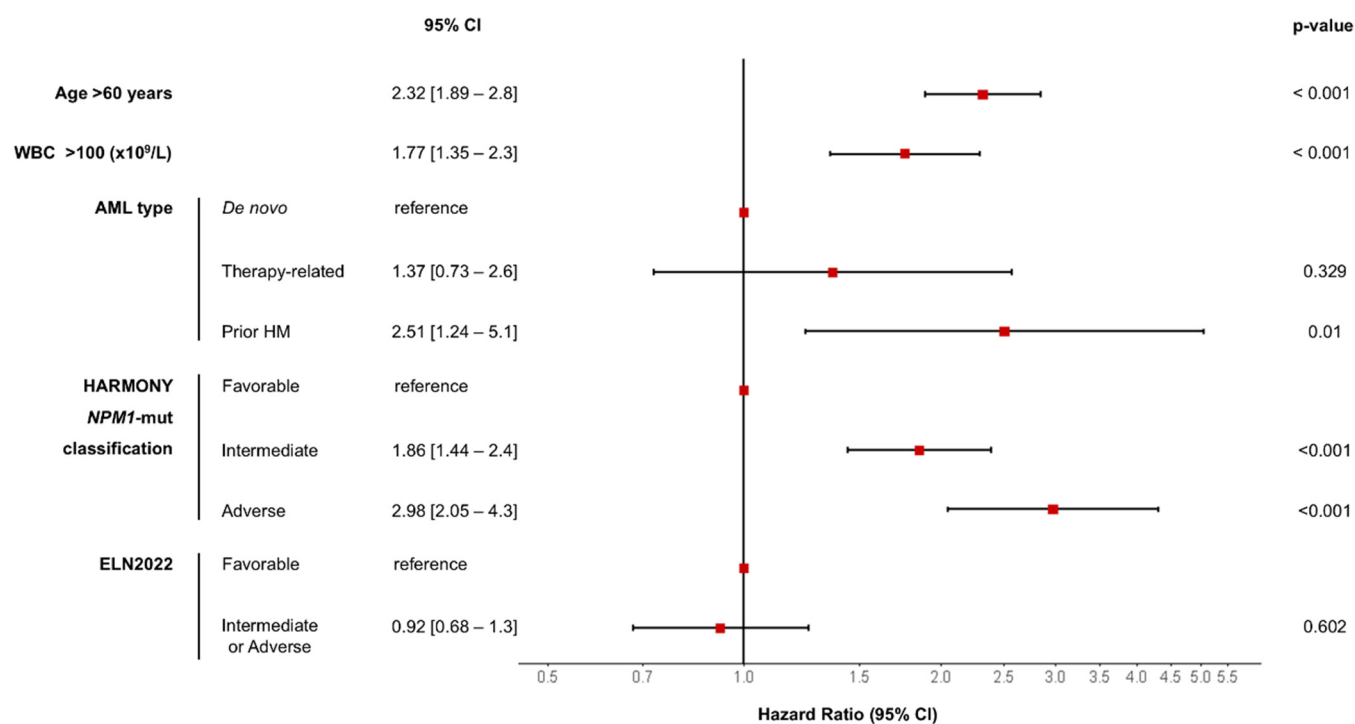


Figure 3. Multivariable Cox regression model of factors associated with OS. Patients that underwent allo-HSCT in CR1 were censored at the transplant date. Abbreviations: HR, hazard ratio; CI, confidence interval; WBC, white blood count; AML, acute myeloid leukemia; HR, hematological malignancy; mut, mutated.

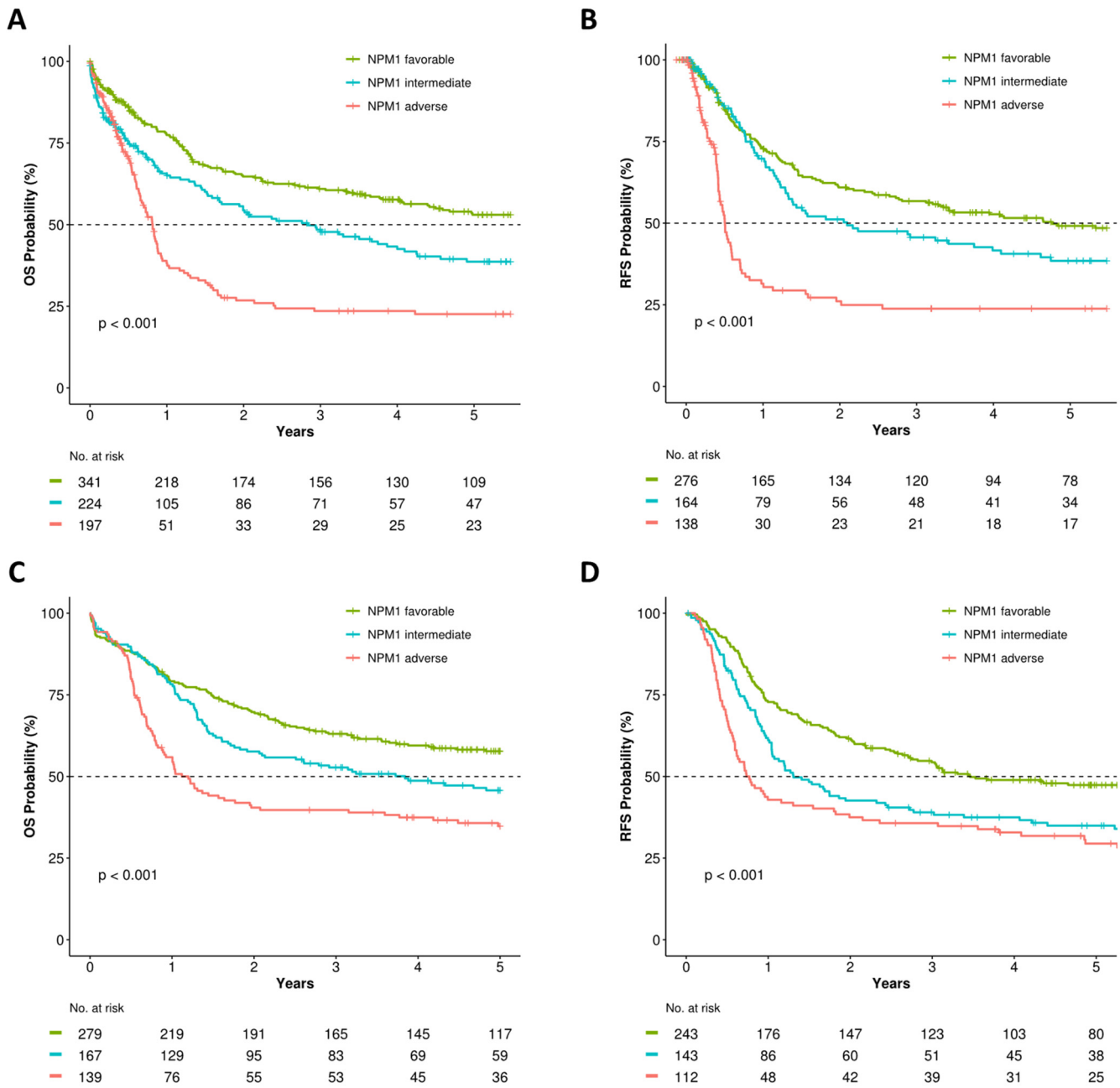


Figure 4. Stratification of outcomes in the validation cohorts according to HARMONY *NPM1*-mut risk categories. (A) OS in internal validation cohort (B) RFS in internal validation cohort (C) OS in external validation cohort (D) RFS in external validation cohort.

Machine Learning Provides Individualized Prediction of Outcomes after First Complete Remission in Adult Acute Myeloid Leukemia – A HARMONY Study

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ABSTRACT

PURPOSE: Allogeneic stem cell transplantation (allo-HSCT) remains the only curative treatment option for a significant proportion of patients with acute myeloid leukemia (AML), and it is generally recommended when the estimated relapse risk without allo-HSCT is >40%. While current recommendations for allo-HSCT are based on risk groups, there is considerable heterogeneity within the individual risk groups.

PATIENTS AND METHODS: We analyzed 2,550 intensively-treated AML patients aged 18–70 with cytogenetic and next-generation sequencing data from the HARMONY Alliance database, who did not receive allo-HSCT in first complete remission (CR1). A non-parametric machine learning (ML) model based on Bayesian Additive Regression Trees (BART) was developed, integrating clinical variables and genomic aberrations to provide individualized outcome estimations. Predictive performance for overall survival (OS), relapse-free survival (RFS), and cumulative incidence of relapse (CIR) was compared to European LeukemiaNet (ELN) 2022 risk classification. External validation was performed in a cohort of 714 patients enrolled in UK-NCRI trials.

RESULTS: The predictive performance of the HARMONY ML model, measured by the area under the receiver operating curve (AUC), was superior to ELN2022 classification in estimating 5-year OS (AUC 0.745 vs 0.702), 5-year RFS (AUC 0.748 vs 0.705) and 5-year CIR (0.739 vs 0.706), which was confirmed in the external validation cohort. Notably, the model revealed substantial heterogeneity within ELN2022 risk groups, identifying a significant proportion of favorable-risk patients with a predicted 5-year CIR >40%, who could potentially benefit from allo-HSCT in CR1.

CONCLUSIONS: The HARMONY ML model provides individualized risk prediction in intensively-treated adult AML patients and supports more tailored therapeutic decisions regarding allo-HSCT in CR1. Integrating measurable residual disease (MRD) could further enhance its predictive performance.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease in terms of underlying genomic aberrations that have a large impact on clinical outcomes¹. The prognostic significance of recurrent cytogenetic abnormalities has been well-established for decades, whereas gene mutations have increasingly been acknowledged for their role in predicting patient outcomes. Thus, the European LeukemiaNet (ELN) risk classification system initially considered three gene mutations in its 2010 edition². This number expanded to six in the 2017 edition and further increased to 13 genes in the most recent 2022 update^{3, 4}. This classification divides intensively treated adult AML patients into three risk categories (i.e. favorable, intermediate and adverse) with distinct outcomes, even when allogeneic hematopoietic stem cell transplantation (allo-HSCT) is performed⁵. While the ELN2022 stratification system has been validated in

numerous studies, further refinement can be achieved^{5–7}. Moreover, the vast majority of AML patients present several gene mutations and certain co-mutational patterns have been associated with distinct outcomes, which underscores the importance of taking the whole mutational landscape into account^{1, 8, 9}.

Allo-HSCT is the pillar of treatment with curative potential for most fit adult AML patients, although a subset of patients might achieve long-term remission without transplantation. Besides, allo-HSCT entails a considerable non-relapse mortality (NRM) due to complications related to the procedure. Therefore, a careful evaluation between risk of disease relapse and NRM is needed for the challenging decision of whom to transplant in first complete remission (CR1). Accordingly, an AML relapse risk of >40% has been suggested for recommending an allo-HSCT in potential candidates^{10–12}. Overall, ELN intermediate-risk and

adverse-risk patients usually benefit from allo-HSCT, while favorable-risk patients are considered to have a cumulative incidence of relapse (CIR) of <40% and therefore allo-HSCT is not routinely performed^{11, 12}. However, a subset of favorable-risk patients might have a higher relapse risk and could also potentially benefit from allo-HSCT, which highlights the importance of more tailored strategies¹³.

The advancement of machine learning (ML) techniques has allowed a novel approach to risk stratification in adult AML. Some models have demonstrated increased accuracy in predicting the probability of early-death following intensive chemotherapy treatment or chance of obtaining complete remission (CR)^{14–16}. In fact, failure to achieve CR has been recognized as the most powerful predictor of overall survival (OS) in AML, highlighting the challenge of predicting outcomes for patients who have already reached CR1¹⁷. However, this prediction plays a crucial role in guiding which patients should undergo allo-HSCT in CR1^{10, 11}.

In order to address this gap, we analyzed a large cohort of patients included in the Healthcare Alliance for Resourceful Medicine Offensive against Neoplasms in Hematology (HARMONY) AML international database. All patients had comprehensive genetic data available and achieved CR1, but were not consolidated with allo-HSCT. We applied a non-parametric machine learning approach based on Bayesian Additive Regression Trees to develop a model that provides individualized estimations of outcomes, with increased predictive performance than current risk classifications, which was validated in a large external dataset that is publicly available.

METHODS

Patients

The HARMONY Alliance AML database was used for this study, which included 8,232 patients at the time of the analysis. Inclusion criteria were patients aged 18-70 years, who received intensive chemotherapy regimens, with available cytogenetic study and next-generation sequencing (NGS) myeloid panel at AML diagnosis. Exclusion criteria were missing information regarding CR, relapse or allo-HSCT dates, administration of targeted therapies (e.g. anti-CD33 antibodies, *FLT3* or *IDH1/2* inhibitors) or failure to achieve CR1. Finally,

patients that underwent allo-HSCT in CR1 as consolidation treatment were also excluded (**Figure 1**).

A total of 2,550 adult AML patients were selected for this analysis, contributed by eight European centers or cooperative groups. The training cohort comprised patients from prospective multicenter clinical trials of the German–Austrian AML Study Group (AMLHD98A, AML-HD98B and AMLSG-07-04) and HOVON-SAKK (HO102, HO103 and HO132), as well as patients contributed by the Study Alliance Leukemia AML registry (Germany), the Munich Leukemia Laboratory (Germany), the AML Cooperative Group registry (Germany), the Swedish AML registry (Sweden) and the University Hospital October 12 (Spain)^{1, 9, 18–20}. Finally, a multicenter cohort of patients with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1* AML was also included¹³.

Patient data uploaded to the HARMONY Big Data Platform underwent a thorough double-broker pseudonymization process in compliance to the General Data Protection Regulation (GDPR). The data were then harmonized and transformed using the Observational Medical Outcomes Partnership (OMOP) Common Data Model²¹.

The study was performed in accordance with the Declaration of Helsinki and was approved by the HARMONY steering committee and the AML working group. The HARMONY research project was reviewed and authorized by the Medicinal Research Ethics Committee of the University of Salamanca (PI 2018 10 128). HARMONY has implemented a robust ethical and data-protection framework for the secondary use of data. Prior written informed consent had been obtained from all patients at the respective HARMONY partner institutions.

Model development and statistical analysis

Bayesian Additive Regression Trees (BART), a non-parametric ML model that is able to handle complex non-linear associations and incorporate uncertainty in predictions, was used to provide individualized outcome estimations. The analysis was performed R statistical software (v3.6.3) and BART package^{22–24}. Clinical variables (age, gender, history of prior hematological malignancy or previous cytotoxic and/or radiation therapy) as well as cytogenetic abnormalities and gene mutations that were found in at least 10 patients in the training cohort were included in the model. Overall survival, relapse-free survival (RFS) and CIR were defined as

recommended by international guidelines⁴, with the exception that OS was also calculated from CR date, and therefore analytical variables at diagnosis were not included in the model.

The predictive performance of the model was evaluated using 10-fold cross-validation, calculating the area under the receiver operating curve (AUC) at pre-defined time points (1, 2, 3, 4 and 5 years) using timeROC package²⁵, which was subsequently compared to ELN2022 risk classification system⁴.

OS and RFS were estimated using the Kaplan-Meier method, with differences in survival distributions assessed through the log-rank test. CIR was calculated using the Aalen-Johansen method for competing risks and differences between distributions were analyzed using Gray's test. All reported p-values were two-sided at the conventional 5% significance level. Further details are available in the **Supplementary Appendix**.

External validation dataset

The model was externally validated using a publicly available dataset published by Tazi *et al.*, which was not part of the HARMONY AML database at the time of the analysis²⁶. This cohort was comprised by AML adult patients enrolled in UK-NCRI trials (AML17, AML16, AML11, AML12, AML14 and AML15). Of note, some of these patients received gemtuzumab ozogamicin (GO) or *FLT3* inhibitor lestaurtinib in addition to intensive chemotherapy regimens, as part of AML17 randomizations. The inclusion and exclusion criteria for the external validation dataset matched those of the HARMONY cohort, with the previously noted exception regarding certain targeted therapies.

RESULTS

Patient characteristics of HARMONY cohort

The training cohort of 2,550 adult AML patients in CR1 included 52% males. The median age at diagnosis was 52 years (range 18-70) and 72% were younger than 60 years (**Table 1**). Five percent of patients had a clinical history of prior hematological malignancy and 3% presented with therapy-related AML. The majority of patients (53%) were classified as favorable risk according to the ELN2022 classification, while 25% and 22% were considered intermediate and adverse-risk, respectively. Although none of the patients received allo-HSCT in

CR1 (exclusion criteria for this study), 15.5% underwent allo-HSCT following salvage therapy. Median follow-up was 6.4 years, with a 5-year CIR of 52% and median OS of 5 years.

Genomic landscape of HARMONY cohort

Most frequent cytogenetic abnormalities were t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1* (14.3%), inv(16)(p13.1q22)/t(16;16)(p13.1;q22)/*CBFB::MYH11* (7.9%) and loss of Y chromosome (6.9%), while 52% of patients had a normal karyotype. Most frequently mutated genes were *NPM1* (36.1%), *FLT3* in 29.9% (ITD 19%, TKD 10.9%), *TET2* (13.5%), *IDH2* (11.8%), *KIT* (8.4%), *PTPN11* (8.4%) and *RUNX1* (8%). Overall, a total of 76 genomic aberrations were present in at least 10 patients and were therefore included in the ML model (**Supplementary Table S1**).

HARMONY ML model

A BART ML model was developed in order to estimate individualized prediction of outcomes, incorporating clinical variables and the aforementioned genomic features. The performance of the HARMONY ML model for OS discrimination, as measured by mean AUC after 10-fold cross-validation, was 0.755, 0.753 and 0.745 for 1-year, 3-year and 5-year OS, respectively. Conversely, ELN2022 risk classification resulted in AUC values of 0.696, 0.706 and 0.702 for OS at the same time points. The predictive performance of HARMONY ML model was also higher than ELN2022 for 1-year RFS (AUC 0.746 vs 0.706), 3-year RFS (AUC 0.751 vs 0.709), 5-year RFS (AUC 0.748 vs 0.705), 1-year CIR (AUC 0.75 vs 0.71), 3-year CIR (0.741 vs 0.707) and 5-year CIR (0.739 vs 0.706) (**Figure 2, Supplementary Table S2**).

ELN2022 classification divided the cohort into three subgroups with distinct cumulative incidence of relapse: 5-year CIR was 38% (CI 35-40%) for favorable-risk, 63% (CI 59-67%) for intermediate-risk and 75% (CI 71-79%) for adverse risk patients (**Figure 3A**). Similarly, HARMONY ML model predicted a median 5-year CIR of 38% for ELN2022 favorable-risk patients, which was 66% and 74% for intermediate and adverse-risk patients, respectively. However, most ELN2022 favorable subgroup (95% CI) included patients with an individual 5-year CIR estimation by HARMONY ML model that ranged from 18% to 60%, ELN2022 intermediate subset had patients with predicted 5-

year CIR from 46% to 86%, and ELN2022 adverse-risk patients ranged from 52% to 91% (**Supplementary Figure S1**). In consequence, 139 favorable-risk, 345 intermediate-risk and 203 adverse-risk patients according to ELN2022 classification had a predicted 5-year between 50% and 70% according to HARMONY ML model. Remarkably, the actual CIR of these subgroups, estimated by Aalen-Johansen method, was similar: 5-year CIR of 66%, 59% and 59% for ELN2022 favorable, intermediate and adverse-risk, respectively ($p=0.815$) (**Figure 3B**).

Overall, the 5-year CIR of ELN2022 favorable-risk subset was 38%. However, the HARMONY model predicted an individual 5-year CIR >40% in 593 patients. Accordingly, 5-year CIR of this subgroup, estimated by Aalen-Johansen method, was higher than ELN2022 favorable-risk with predicted 5-year CIR <40% (48% vs 30% respectively, $p<0.001$) (**Figure 3C**). In ELN2022 favorable with *NPM1* mutation (i.e. absence of *FLT3*-ITD), the HARMONY model predicted an individual 5-year CIR >40% in 50.7% of the patients, with significantly higher CIR when compared to the subset with predicted CIR <40% (48% vs 29%, $p<0.001$) (**Figure 3D**). Of note, the *NPM1*-mutated (-mut) cohort with predicted 5-year CIR >40% by HARMONY ML model was enriched in *DNMT3A*-mut (85% vs 9%, $p<0.001$) and clinical history of prior HM (6.7% vs 0.3%, $p<0.001$), while *STAG2*-mut was less frequent (0.3% vs 5.6%, $p<0.001$) than in the subset with predicted 5-year CIR <40%. Similarly, HARMONY ML model predicted an individual 5-year CIR >40% in 18.4% patients with *t(8;21)(q22;q22.1)/RUNX1::RUNX1T1* and that subgroup presented a significantly higher CIR when compared to the subset with predicted CIR <40% (56% vs 27%, $p<0.001$) (**Figure 3E**). The former subgroup had a higher proportion of *RUNX1*-mut (9% vs 1.3%, $p=0.004$), *DNMT3A*-mut (9% vs 1%, $p=0.002$) and *TP53*-mut (4.5% vs 0%, $p=0.006$). Finally, heterogeneity was also observed within ELN2022 intermediate subgroup, as patients with predicted 5-year CIR >65% by HARMONY model presented a significantly higher CIR than those with individual prediction <65% (5-year CIR 71% vs 52%, respectively, $p<0.001$).

External validation of HARMONY ML model

The external validation cohort of 714 adult AML patients in CR1, who had not received allo-HSCT as consolidation treatment, showed significant

differences compared to the training cohort, as patients were younger (median 50 vs 52 years, $p=0.004$; age ≥ 60 years 20% vs 28%, $p<0.001$), with a decreased proportion of bone marrow blasts at diagnosis (61.5% vs 64%, $p<0.001$), lower proportion of patients classified as ELN2022 favorable-risk (44.7% vs 53.2%, $p<0.001$), higher percentage of adverse-risk patients (27.5% vs 22.2%, $p=0.004$) and increased allo-HSCT rates as salvage treatment (25.5% vs 15.5%, $p<0.001$) (**Table 1**). The distribution of genomic aberrations included in the HARMONY ML model in the validation cohort is indicated in **Supplementary Table S3**.

The predictive performance of the HARMONY ML model, as measured by AUC, was also higher than ELN2022 in the external validation cohort for 1-year OS (0.775 vs 0.699), 3-year OS (0.763 vs 0.717), 5-year OS (0.755 vs 0.714), 1-year RFS (0.697 vs 0.647), 3-year RFS (0.71 vs 0.673), 5-year RFS (0.715 vs 0.679), 1-year CIR (0.696 vs 0.636), 3-year CIR (0.698 vs 0.662) and 5-year CIR (0.716 vs 0.672) (**Figure 2, Supplementary Table S4**).

ELN2022 classification also divided the validation cohort into three subgroups with distinct cumulative incidence of relapse: 5-year CIR was 42% (CI 37-48%) for favorable-risk, 59% (CI 52-66%) for intermediate-risk and 68% (CI 62-75%) for adverse risk patients (**Figure 4A**). Individual 5-year CIR, predicted by HARMONY ML model, showed substantial heterogeneity within each ELN2022 risk category: it ranged (95% CI) from 20% to 56% for favorable-risk patients, ELN2022 intermediate subset had patients with predicted 5-year CIR from 49% to 82% and ELN2022 adverse-risk patients ranged from 49% to 91% (**Supplementary Figure S2**). Accordingly, 10.6% of favorable-risk, 60.8% of intermediate-risk and 31.1% of adverse-risk patients in the validation cohort had a predicted 5-year CIR between 50% and 70% according to HARMONY ML model and the actual CIR of these subgroups, estimated by Aalen-Johansen method, was similar ($p=0.351$) (**Figure 4B**).

Overall, the 5-year CIR of ELN2022 favorable-risk subgroup was 42%, although the HARMONY ML model predicted an individual 5-year CIR >40% in 47.6% patients. Remarkably, 5-year CIR of this subset, estimated by Aalen-Johansen method, was higher (54% vs 32% for ELN2022 favorable-risk with predicted 5-year CIR <40%, $p<0.001$) (**Figure 4C**). HARMONY ML model was able to identify that

50.6% ELN2022 favorable-risk patients with *NPM1*-mut and 33.3% with *t*(8;21)(q22;q22.1)/*RUNX1::RUNX1T1* had an individual 5-year CIR >40% (**Figures 4D-E**). Heterogeneity was also confirmed within ELN2022 intermediate subgroup, as patients with predicted 5-year CIR >65% by HARMONY ML model presented a significantly higher CIR than those with individual prediction <65% (5-year CIR 67% vs 50%, respectively, $p<0.006$) (**Figure 4F**).

Online tool for individual risk estimation

A web-based interface was developed to calculate individualized estimation of outcomes according to HARMONY ML model. Using five input variables (age, gender, AML type, cytogenetic abnormalities and gene mutations), a 1000-fold patient simulation is performed to provide estimation of each outcome measure (OS, RFS, CIR) with confidence interval for each prediction (**Figure 5**). The interface also includes a 5-year CIR sensitivity analysis, reflecting potential change on outcome prediction if the patient had different genomic alterations.

DISCUSSION

Allo-HSCT plays a crucial role as part of the curative treatment for the majority of eligible adult AML patients. Whether to proceed with an allogeneic transplant in CR1 depends on multiple factors, including disease biology, as it is generally recommended for patients with intermediate or adverse-risk features. However, it has been suggested that a predicted relapse risk >40% with chemotherapy alone should also be considered as an indication for allo-HSCT¹⁰⁻¹². In our study, we analyzed the outcomes of a large dataset of potential allo-HSCT candidates (i.e. 18-70 years, who achieved CR1 following intensive chemotherapy regimens), confirming distinct relapse risks for ELN2022 favorable, intermediate and adverse-risk groups. While the relapse risk without allo-HSCT was approximately 40% in the ELN2022 favorable-risk group, we demonstrated considerable heterogeneity within this subset of patients, as a significant proportion of favorable-risk patients presented a 5-year CIR >40% in both training and external validation datasets. This observation is relevant from the clinical perspective, as those patients could have potentially benefited from allo-HSCT as

consolidation treatment. Moreover, a substantial variability was also observed within intermediate group, highlighting the importance of individualized outcome estimations in AML patients.

The HARMONY ML model was developed combining data from randomized clinical trials as well as national registries and was validated with data from UK-NCRI clinical trials²⁶. The predictive performance of the HARMONY ML model, measured by AUC, was superior to ELN2022 risk classification in all outcome measures (i.e. OS, RFS and CIR) and timepoints (1-5 years), in both the training cohort and the external independent validation dataset. While previous studies have also reported improved performance compared to ELN classifications, some models rely on analyses that are not routinely performed in clinical practice, such as gene expression profiles or epigenetic features^{27, 28}. On the other hand, the HARMONY ML model was developed using genetic tests that are recommended at AML diagnosis in current guidelines (i.e. cytogenetics and NGS myeloid panel diagnostics), facilitating its implementation in clinical practice⁴. In this regard, estimation of outcomes is calculated individually, with a confidence interval provided for each prediction, and the process is performed promptly.

The decision of whom to perform allo-HSCT in CR1 is one of the most challenging in the clinical management of adult AML, as both disease relapse risk and NRM related to the procedure need to be taken into account. While the former could be estimated with the HARMONY ML model, the latter depends on diverse factors such as patient comorbidities, donor features and management of early complications²⁹⁻³¹. Advances in donor selection, conditioning regimens and post-transplant care, have led to a significant reduction of transplant-related mortality over the last decades, with a 1-year NRM of 24.4% in the 1990s decreasing to 9.5% in the 2013-2016 period³². In this context, an increasing number of patients could potentially benefit from allo-HSCT in CR1, highlighting the evolving assessment of allo-HSCT risk/benefit balance.

However, there are some aspects of our study that have to be taken into account. Although it includes multiple patient cohorts from various European institutions and has been validated by an independent UK cohort, it remains a retrospective analysis that warrants prospective evaluation. The

inclusion and exclusion criteria of the study contributed to a relatively homogeneous cohort of intensively-treated adult AML patients who achieved CR1 and were not consolidated with allo-HSCT, where genomic characteristics have been described as the major determinants of patient outcomes³³.

AML treatment has evolved over the last decade, with an increasing number of targeted therapies becoming available, including the frontline setting³⁴. In our training cohort, only patients treated with intensive-chemotherapy regimens were included. However, the model was validated using an external cohort, which included a proportion of patients who were also treated with GO or *FLT3* inhibitors. The HARMONY ML algorithm will require further refinement as optimal treatment evolves, as the inclusion of patients treated with additional targeted therapies or non-intensive approaches could enhance its ability to provide even more accurate risk estimations.

In addition, our study did not incorporate assessment of measurable residual disease (MRD), which has been established as a significant prognostic factor in AML^{35, 36}, and could add valuable information with regard to the relapse risk of an individual patient. While novel treatment strategies have reduced the MRD positivity rate to less than 20% in certain subtypes, such as *NPM1*-mut AML³⁷, a 3-year CIR of up to 40% has been observed even in certain MRD negative genomic subgroups. This suggests that the combination of genomic features at diagnosis and evaluation of treatment response by MRD measurement can provide an even more precise estimation of patient outcomes. While we are currently performing a respective MRD study within the HARMONY consortium, our current model can already provide valuable insights into which patients would benefit most from MRD evaluation.

In summary, the HARMONY ML model provides individualized outcome estimation for intensively treated adult AML patients that achieve CR1 and are consolidated without allo-HSCT. Clinicians could balance the estimated relapse risk calculated by the model against NRM evaluated by specific scores, in order to assess the relative benefit of allo-HSCT for their patients. The HARMONY ML model can be accessed as an online tool (https://taxonomy.harmony-platform.eu/AML_outcome_predictor/).

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AUTHOR CONTRIBUTIONS

AHS, JMHR, BH, GO, HD and LB designed the study. AHS, JME, AVR, ES, ATT, MA, JV, IT, MS, JML, MP, KM, SL, CR, KHM, KD, MH, TH, PJMV, NR, HD and JMHR performed data collection and assembly of the data. AHS, JME, AVR, ES, GC, AB, JMT, RM, GS, MTV, KD, JMHR, BH, GO, HD and LB performed data analysis and interpretation. AHS and LB wrote the manuscript. BJPH, GO and HD critically reviewed the manuscript. LB supervised research and coordinated the HARMONY AML group. All authors had access to primary data, read and approved the final manuscript.

COMPETING INTERESTS

MS: honoraria from Novartis, Celgene, AOP Orphan, AbbVie. **KHM:** honoraria from AbbVie, Bristol Myers Squibb, Celgene, Janssen, Novartis, Pfizer, Otsuka; research funding from AbbVie. **ATT:** Consultancy for CSL Behring, Maat Pharma, Biomarin and Onkowissen; travel reimbursements from Neovii Biotech and Novartis. **MP:** honoraria from Novartis. **GS:** honoraria from Takeda and has participated in Ad-Boards from Novartis, Celgene, Abbvie, Helsinn and Takeda. **CR:** honoraria from AbbVie, Astellas, Bristol-Meyer-Squibb, Daiichi Sankyo, Jazz, Janssen, Novartis, Otsuka, Pfizer, Servier; institutional research funding from AbbVie, Astellas, Novartis, Pfizer. **KD:** honoraria from Novartis, Jazz Pharmaceuticals, Abbvie; has participated in Ad-Boards from Novartis, Bristol Myers Squibb, Jazz Pharmaceuticals, Abbvie; research funding from Novartis, Astellas, Agios, Bristol Myers Squibb, Kronos. **MH:** honoraria from Astellas, Daiichi Sankyo, Janssen, Miltenyi, Otsuka, Qiagen, Servier and has participated in Ad-Boards from Abbvie, AvenCell, Ascentage Pharma, Bristol Myers Squibb, Janssen, Jazz Pharmaceuticals, LabDelbert, Novartis, Pfizer, Servier; research funding from Abbvie, Bayer

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DATA SHARING STATEMENT

After the publication of this article, data collected for this analysis and related documents will be made available to others upon reasonably justified request, which needs to be written and addressed to the attention of the corresponding author Dr Lars Bullinger at the following e-mail address: lars.bullinger@charite.de. The HARMONY Alliance, via the corresponding author Dr Lars Bullinger, is responsible to evaluate and eventually accept or refuse every request to disclose data and their related documents, in compliance with the ethical approval conditions, in compliance with applicable laws and regulations, and in conformance with the agreements in place with the involved subjects, the participating institutions, and all the other parties directly or indirectly involved in the participation, conduct, development, management, and evaluation of this analysis.

REFERENCES

1. Papaemmanuil E, Gerstung M, Bullinger L, et al: Genomic Classification and Prognosis in Acute Myeloid Leukemia. *New England Journal of Medicine* 374:2209–2221, 2016
2. Döhner H, Estey EH, Amadori S, et al: Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* 115:453–474, 2010
3. Döhner H, Estey E, Grimwade D, et al: Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 129:424–447, 2017
4. Döhner H, Wei AH, Appelbaum FR, et al: Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 140:1345–1377, 2022
5. Jentzsch M, Bischof L, Ussmann J, et al: Prognostic impact of the AML ELN2022 risk classification in patients undergoing allogeneic stem cell transplantation. *Blood Cancer J* 12, 2022
6. Rausch C, Rothenberg-thurley M, Dufour A, et al: Validation and refinement of the 2022 European LeukemiaNet genetic risk stratification of acute myeloid leukemia. *Leukemia* 37:1234–1244, 2023
7. Sargas C, Ayala R, Larráyo MJ, et al: Comparison of the 2022 and 2017 European LeukemiaNet risk classifications in a real-life cohort of the PETHEMA group. *Blood Cancer J* 13, 2023
8. Alfayez M, Issa GC, Patel KP, et al: The Clinical impact of PTPN11 mutations in adults with acute myeloid leukemia. *Leukemia* 35:691–700, 2021
9. Metzeler KH, Herold T, Rothenberg-Thurley M, et al: Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood* 128:686–698, 2016
10. Cornelissen JJ, Blaise D: Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood* 127:62–70, 2016
11. Dewolf S, Tallman MS, Rowe JM, et al: What Influences the Decision to Proceed to Transplant for Patients With AML in First Remission? *Journal of Clinical Oncology* 41:4693–4703, 2023
12. Loke J, Buka R, Craddock C: Allogeneic Stem Cell Transplantation for Acute Myeloid Leukemia: Who, When, and How? *Front Immunol* 12:1–18, 2021
13. Christen F, Hoyer K, Yoshida K, et al: Genomic landscape and clonal evolution of acute myeloid leukemia with t(8;21): An international study on 331 patients. *Blood* 133:1140–1151, 2019
14. Walter RB, Othus M, Paietta EM, et al: Effect of genetic profiling on prediction of therapeutic resistance and survival in adult acute myeloid leukemia. *Leukemia* 29:2104–2107, 2015
15. Eckardt JN, Röllig C, Metzeler K, et al: Prediction of complete remission and survival in acute myeloid leukemia using supervised machine learning. *Haematologica* 108:690–704, 2023
16. Siddiqui NS, Klein A, Godara A, et al: Predicting In-Hospital Mortality After Acute Myeloid Leukemia Therapy: Through Supervised Machine Learning Algorithms. *JCO Clin Cancer Inform* 1–11, 2022
17. Tefferi A, Gangat N, Al-Kali A, et al: A dynamic 3-factor survival model for acute myeloid leukemia that accounts for response to induction chemotherapy. *Am J Hematol* 97:1127–1134, 2022
18. Löwenberg B, Pabst T, Maertens J, et al: Therapeutic value of clofarabine in younger and middle-aged (18-65 years) adults with newly diagnosed AML.

- Blood 129:1636–1645, 2017
19. Ossenkoppele GJ, Breems DA, Stuessi G, et al: Lenalidomide added to standard intensive treatment for older patients with AML and high-risk MDS. *Leukemia* 34:1751–1759, 2020
 20. Löwenberg B, Pabst T, Maertens J, et al: Addition of lenalidomide to intensive treatment in younger and middle-aged adults with newly diagnosed AML: The HOVON-SAKK-132 trial. *Blood Adv* 5:1110–1121, 2021
 21. Belenkaya R, Gurley MJ, Golozar A, et al: Extending the OMOP Common Data Model and Standardized Vocabularies to Support Observational Cancer Research. *JCO Clin Cancer Inform* 12–20, 2021
 22. Sparapani RA, Logan BR, McCulloch RE, et al: Nonparametric survival analysis using Bayesian Additive Regression Trees (BART). *Stat Med* 35:2741–2753, 2016
 23. Sparapani R, Logan BR, McCulloch RE, et al: Nonparametric competing risks analysis using Bayesian Additive Regression Trees. *Stat Methods Med Res* 29:57–77, 2020
 24. Sparapani R, Spanbauer C, McCulloch R: Nonparametric Machine Learning and Efficient Computation with Bayesian Additive Regression Trees: The BART R Package. *J Stat Softw* 97:1–66, 2021
 25. Blanche P, Dartigues JF, Jacqmin-Gadda H: Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat Med* 32:5381–5397, 2013
 26. Tazi Y, Arango-Ossa JE, Zhou Y, et al: Unified classification and risk-stratification in Acute Myeloid Leukemia. *Nat Commun* 13, 2022
 27. Ng SWK, Mitchell A, Kennedy JA, et al: A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature* 540:433–437, 2016
 28. Kosvira, Karadimitris, Papaioannou, et al: Machine learning and integrative multi-omics network analysis for survival prediction in acute myeloid leukemia. *Comput Biol Med* 178:108735, 2024
 29. Shouval R, Labopin M, Bondi O, et al: Prediction of allogeneic hematopoietic stem-cell transplantation mortality 100 days after transplantation using a machine learning algorithm: a European Group for Blood and Marrow Transplantation Acute Leukemia Working Party Retrospective Data Mining Stud. *Journal of Clinical Oncology* 33:3144–3151, 2015
 30. Potdar R, Varadi G, Fein J, et al: Prognostic Scoring Systems in Allogeneic Hematopoietic Stem Cell Transplantation: Where Do We Stand? *Biology of Blood and Marrow Transplantation* 23:1839–1846, 2017
 31. Shouval R, Fein JA, Shouval A, et al: External validation and comparison of multiple prognostic scores in allogeneic hematopoietic stem cell transplantation. *Blood Adv* 3:1881–1890, 2019
 32. Penack O, Peczynski C, Mohty M, et al: How much has allogeneic stem cell transplant-related mortality improved since the 1980s? A retrospective analysis from the EBMT. *Blood Adv* 4:6283–6290, 2020
 33. Gerstung M, Papaemmanuil E, Martincorena I, et al: Precision oncology for acute myeloid leukemia using a knowledge bank approach. *Nat Genet* 49:332–340, 2017
 34. DiNardo CD, Erba HP, Freeman SD, et al: Acute myeloid leukaemia. *The Lancet* 401:2073–2086, 2023
 35. Jongen-Lavrencic M, Grob T, Hanekamp D, et al: Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N Engl J Med* 378:1189–1199, 2018
 36. Heuser M, Freeman SD, Ossenkoppele GJ, et al: 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. *Blood* 138:2753–2767, 2021
 37. Othman J, Potter N, Ivey A, et al: Molecular, clinical, and therapeutic determinants of outcome in NPM1 mutated AML. *Blood* 144:714–728, 2024

Table 1. Baseline characteristics of adult AML patients included in HARMONY database that were selected for this study (training cohort) and comparison with external validation cohort.

	Training cohort (n=2550)	External validation cohort (n=714)	p-value
Female sex	1215 (47.6%)	347 (48.6%)	0.67
Median age in years (range)	52 [18 - 70]	50 [20 - 70]	0.004
Age ≥60 years	714 (28%)	143 (20%)	<0.001
AML type			0.7
De novo AML	2353 (92.3%)	662 (93.7%)	
Secondary AML	197 (7.7%)	52 (7.3%)	
Prior HM	122 (4.8%)	30 (4.2%)	
Therapy-related AML	75 (2.9%)	22 (3.1%)	
Hemoglobin (g/dL)	8.9 [Q1=7.7 Q3=10.3]	9.4 [Q1=8.1 Q1=11.1]	0.33
WBC (x10⁹/L)	14.3 [Q1=4.5 Q1=43.4]	15 [Q1=4.4 Q1=42]	0.86
(WBC > 100 x10 ⁹ /L)	224 (8.8%)	63 (8.8%)	1
Platelets (x10⁹/L)	53 [Q1=29 Q1=98.5]	57.5 [Q1=31.2 Q1=101.8]	0.63
Bone marrow % of blasts	64 [Q1=40 Q1=81.5]	61.5 [Q1=37 Q1=81]	<0.001
ELN 2022			<0.001
Favorable	1357 (53.2%)	319 (44.7%)	<0.001
<i>NPM1</i> -mut without <i>FLT3</i> -ITD	611 (24%)	170 (23.8%)	0.97
t(8;21)(q22;q22.1)/ <i>RUNX1::RUNX1T1</i>	365 (14.3%)	66 (9.2%)	<0.001
inv(16)(p13.1;q22) or t(16;16)(p13.1;q22)/ <i>CBFB::MYH11</i>	202 (7.9%)	58 (8.1%)	0.87
bZIP in-frame mutated <i>CEBPA</i>	181 (7.1%)	27 (3.8%)	<0.001
Intermediate	626 (24.5%)	199 (27.9%)	0.072
Adverse	567 (22.2%)	196 (27.5%)	0.004
Most frequently mutated genes			
<i>NPM1</i>	921 (36.1%)	269 (37.7%)	0.45
<i>DNMT3A</i>	671 (26.3%)	204 (28.6%)	0.23
<i>FLT3</i> -ITD	502 (19.7%)	133 (18.6%)	0.56
<i>NRAS</i>	484 (19%)	121 (16.9%)	0.23
<i>TET2</i>	345 (13.5%)	82 (11.5%)	0.17
Salvage allo-HSCT (not in CR1)	396 (15.5%)	182 (25.5%)	<0.001
5-year cumulative incidence of relapse (%)	52% [50% - 54%]	54% [50% - 58%]	0.71
Median overall survival in years (95% CI)	4.97 [4.14 - 6.37]	8.38 [4.76 - NA]	0.07
Median overall follow-up in years (95% CI)	6.38 [6.14 - 6.58]	6.45 [6.25 - 6.66]	0.02

Abbreviations: AML, acute myeloid leukemia; HM, hematological malignancy; WBC, white blood count; ELN, European LeukemiaNet; ITD, internal tandem duplication; allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR1, first complete remission; CI, confidence interval.

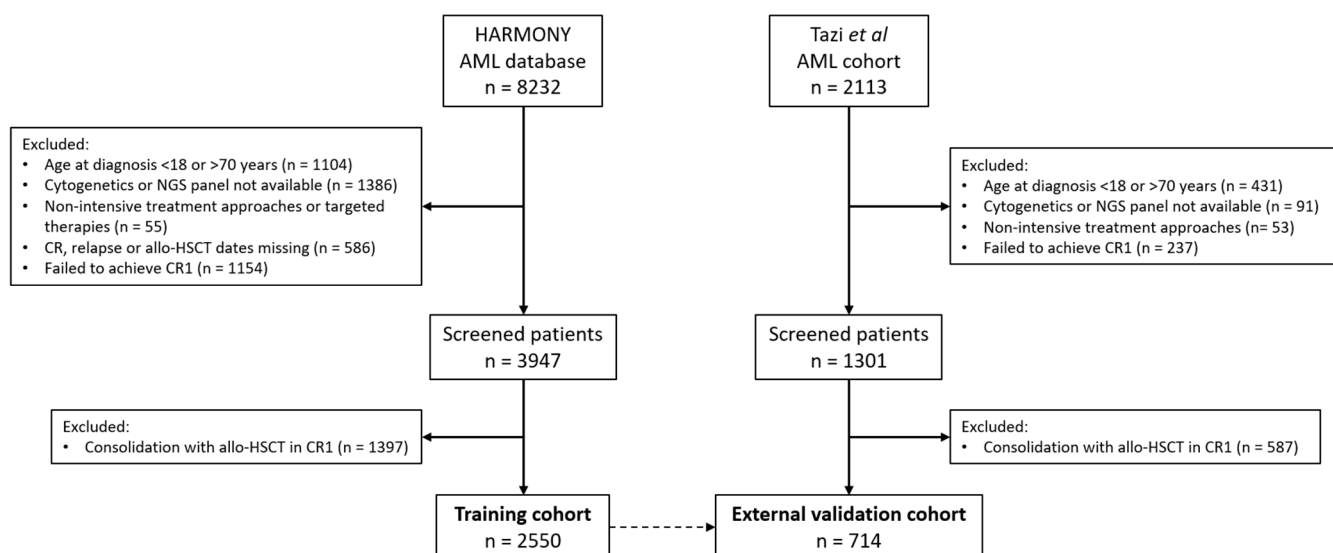


Figure 1. Study flow diagram. Abbreviations: AML, acute myeloid leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR1, first complete remission; NGS, next generation sequencing.

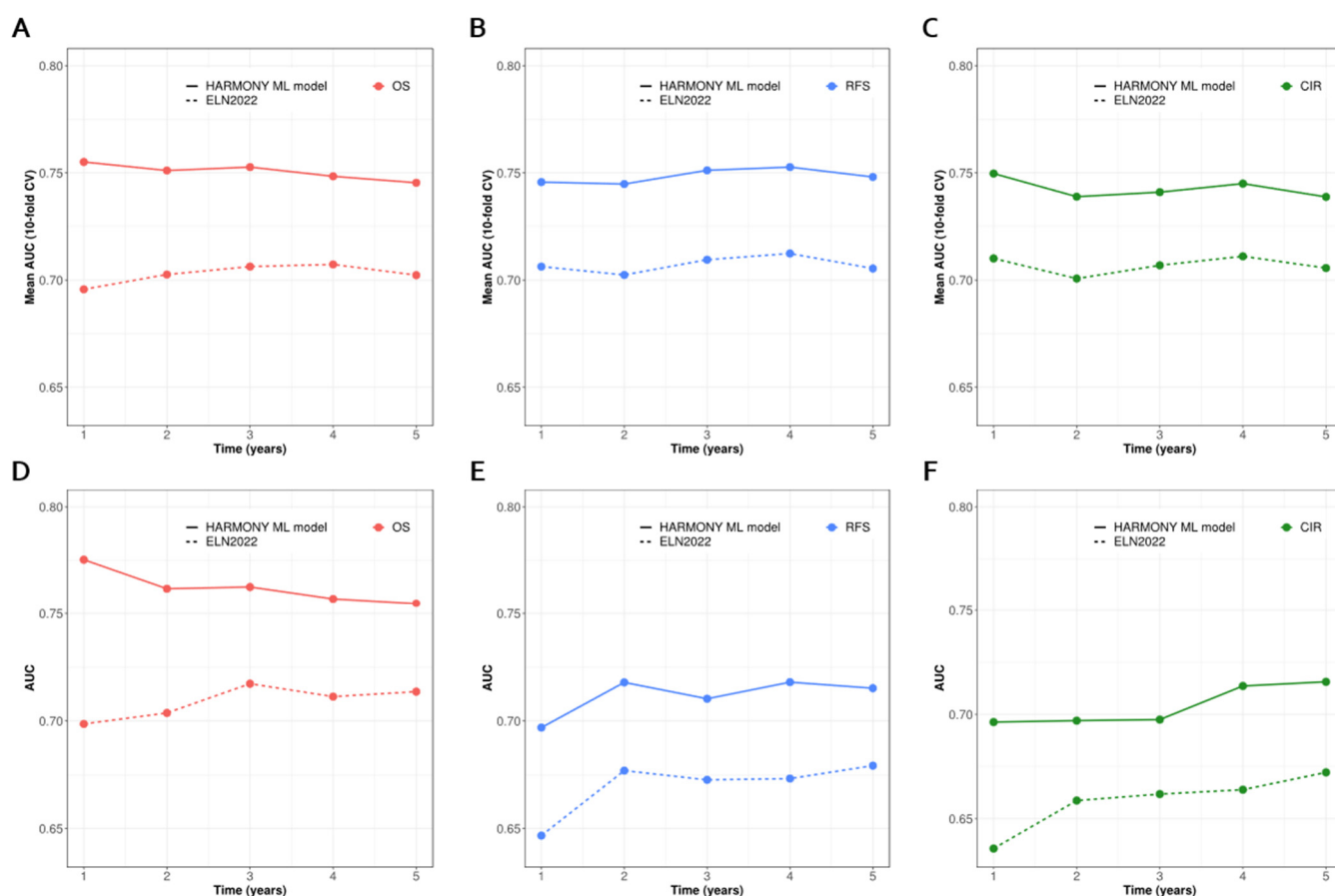


Figure 2. Predictive performance of HARMONY ML model compared to ELN2022 risk classification, measured by AUC at 1, 2, 3, 4 and 5 years (A) Overall survival in training cohort. (B) Relapse-free survival in training cohort. (C) Cumulative incidence of relapse in training cohort. (D) Overall survival in external validation cohort. (E) Relapse-free survival in external validation cohort. (F) Cumulative incidence of relapse in external validation cohort.

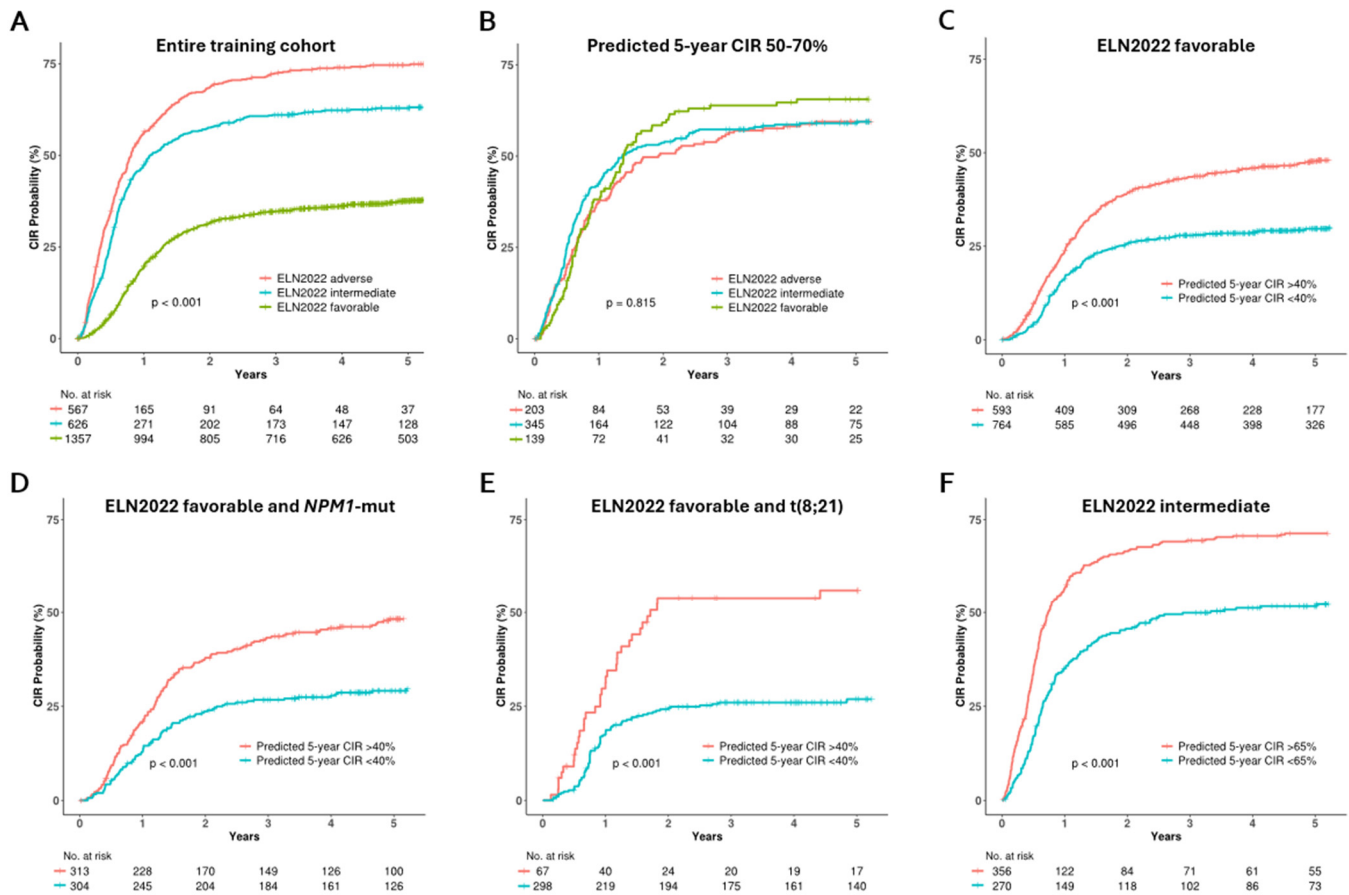


Figure 3. Cumulative incidence of relapse in training cohort (A) Entire training cohort, stratified by ELN2022 risk classification. **(B)** Only patients with predicted 5-year CIR of 50-70% according to HARMONY ML model, stratified by ELN2022 risk classification. **(C)** ELN2022 favorable-risk patients, stratified by predicted 5-year CIR according to HARMONY ML model. **(D)** ELN2022 favorable-risk patients with *NPM1* mutation, stratified by predicted 5-year CIR according to HARMONY ML model. **(E)** ELN2022 favorable-risk patients with *t(8;21)(q22;q22.1)/RUNX1::RUNX1T1*, stratified by predicted 5-year CIR according to HARMONY ML model. **(F)** ELN2022 intermediate-risk patients, stratified by predicted 5-year CIR according to HARMONY ML model.

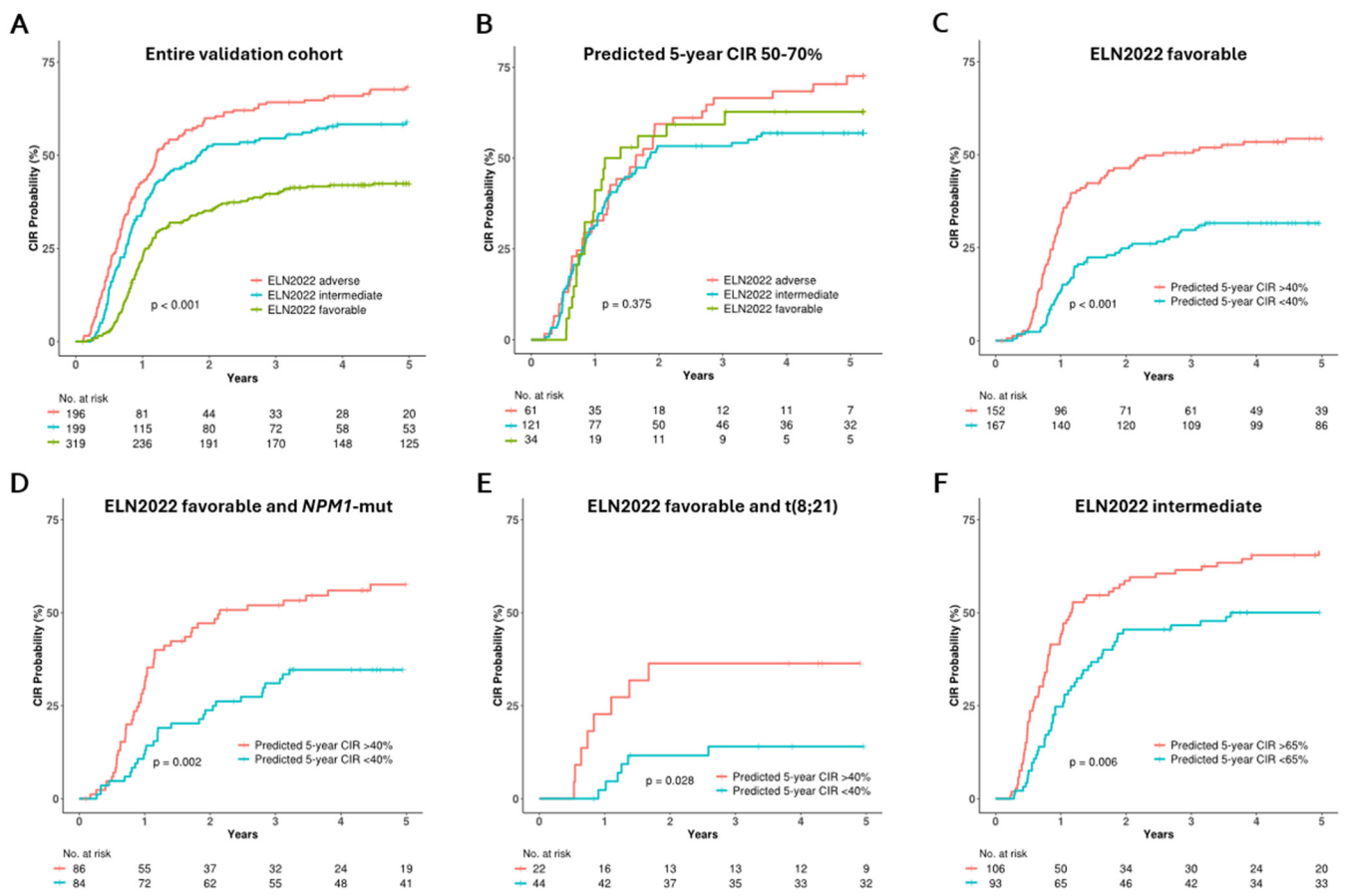


Figure 4. Cumulative incidence of relapse in external validation cohort (A) Entire external validation cohort, stratified by ELN2022 risk classification. **(B)** Only patients with predicted 5-year CIR of 50-70% according to HARMONY ML model, stratified by ELN2022 risk classification. **(C)** ELN2022 favorable-risk patients, stratified by predicted 5-year CIR according to HARMONY ML model. **(D)** ELN2022 favorable-risk patients with *NPM1* mutation, stratified by predicted 5-year CIR according to HARMONY ML model. **(E)** ELN2022 favorable-risk patients with *t(8;21)(q22;q22.1)/RUNX1::RUNX1T1*, stratified by predicted 5-year CIR according to HARMONY ML model. **(F)** ELN2022 intermediate-risk patients, stratified by predicted 5-year CIR according to HARMONY ML model.

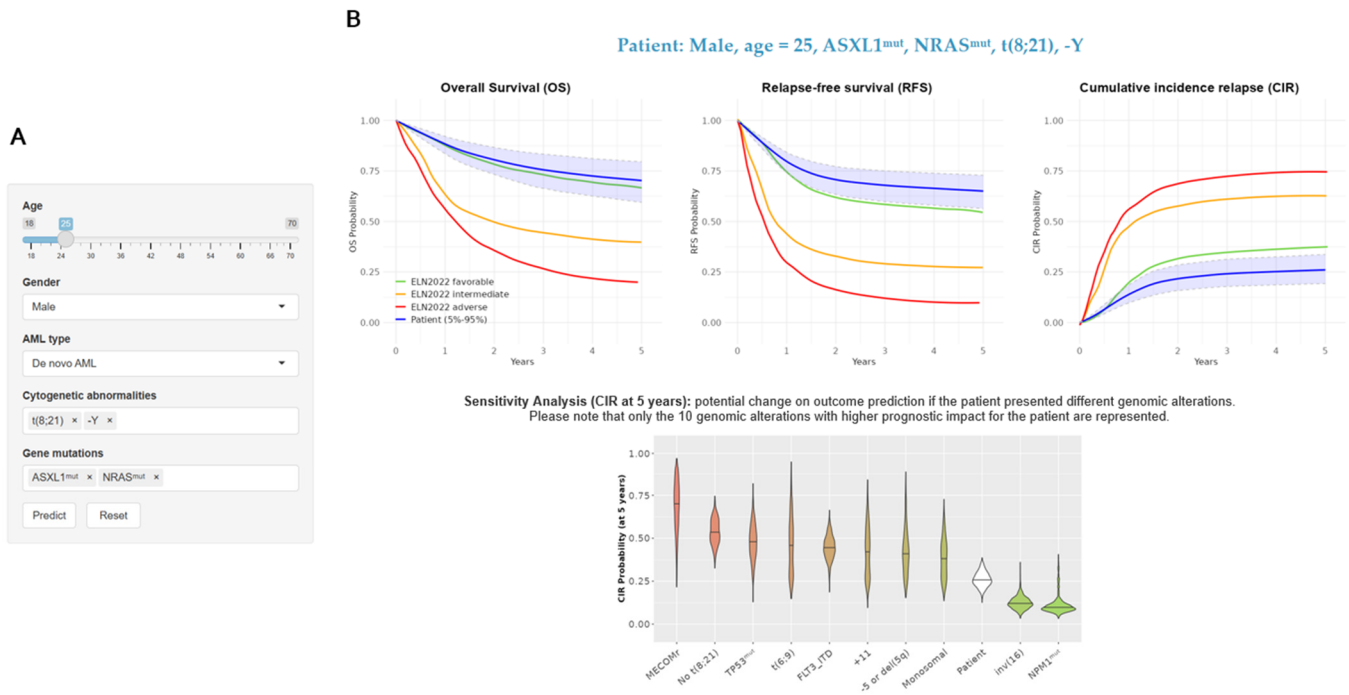


Figure 5. Web-based interface integrating HARMONY ML model. (A) Data input **(B)** Data output, including individualized patient outcome estimation (blue line), reference of ELN2022 favorable-risk patients (green line), ELN2022 intermediate-risk patients (yellow line) and ELN2022 adverse-risk patients (red line). All outcome estimations are calculated from CR date. **(C)** Sensitivity analysis of 5-year CIR.

DISCUSIÓN GENERAL

La leucemia mieloblástica aguda (LMA) es una enfermedad heterogénea, en la que el pronóstico del paciente está condicionado por las alteraciones genómicas^{84,86,196}. Inicialmente las clasificaciones de riesgo estaban basadas fundamentalmente en alteraciones citogenéticas¹⁵⁰, pero la implementación de técnicas como la secuenciación masiva de nueva generación (NGS) ha permitido descubrir un perfil mutacional muy diverso, con más de 20 genes mutados de forma recurrente en esta patología⁸¹. Muchos de ellos se han asociado con la supervivencia global (SG) de los pacientes, por lo que se han incorporado en las actualizaciones de las clasificaciones de riesgo, como la de la *European LeukemiaNet* de 2022 (ELN2022)³⁹.

Los estudios genéticos han puesto de manifiesto que la inmensa mayoría de los pacientes con LMA presenta varias alteraciones citogenéticas y/o mutaciones de forma concomitante, dando lugar a un perfil genómico más complejo^{81,84}. Además, la concurrencia de dichas alteraciones no es aleatoria y hay ciertas anomalías que están íntimamente relacionadas. Por ejemplo, el 70% de los pacientes con t(6;9)(p22.3;q34.1)/*DEK::NUP214* tiene mutación en *FLT3*-ITD, mientras que el 80% de las LMA con mutación (-mut) en *DNMT3A* presenta también *NPM1*-mut^{86,157}. Si se evalúa el valor pronóstico de cada alteración de forma independiente, es posible que una anomalía de riesgo favorable enmascare el efecto adverso de una segunda alteración, como sucede precisamente con la mutación de *DNMT3A*. En cambio, el análisis estratificado por subgrupos (en este caso, evaluando el impacto de *DNMT3A*-mut por una parte en pacientes con *NPM1*-mut y por otra en aquellos con *NPM1* *wildtype*) desvela el efecto adverso de la mutación de *DNMT3A*¹⁵⁷. Por otra parte, ciertas mutaciones podrían tener implicaciones pronósticas únicamente en determinados subtipos, algunos de los cuales representan menos del 5% de la LMA del adulto⁴⁶. Por ello, la colaboración multicéntrica internacional resulta crucial para reunir la cantidad de datos suficientes para realizar estos análisis detallados. Las principales barreras para dichos estudios colaborativos son la armonización y la privacidad de los datos.

El proyecto HARMONY (*Healthcare Alliance for Resourceful Medicine Offensive against Neoplasms in Hematology*) resuelve ambos problemas mediante la “traducción” de los datos enviados por los diferentes centros al modelo de datos común de OMOP (*Observational Medical Outcomes Partnership*)¹⁹⁷ y la doble pseudonimización. Es un

proceso laborioso, que requiere constante comunicación con el centro que envía los datos para asegurar una correcta transferencia de la información. Posteriormente un equipo formado por hematólogos, informáticos y estadísticos realiza una depuración de los datos, para reducir las inconsistencias, corregir posibles errores del mapeo y preparar las herramientas de análisis para cada proyecto.

En el primer trabajo de la presente tesis doctoral (**capítulo 1** de la sección de resultados: “Rearrangements involving 11q23.3/*KMT2A* in adult AML: mutational landscape and prognostic implications – a HARMONY study”) analizamos las LMA con reordenamiento de *KMT2A* (*KMT2Ar*). A pesar de que contábamos con 6.342 pacientes adultos con LMA de 11 centros o grupos cooperativos y 6.005 tenían un cariotipo valorable, solo se pudo confirmar el reordenamiento de *KMT2A* en 205 (3,4%). Sin embargo, 185 de ellos disponían de información sobre panel de NGS mieloide, lo que la convierte en la mayor serie publicada hasta la fecha. Los pacientes con t(9;11)(p21.3;q23.3)/*KMT2A::MLL3* presentaron una SG similar a aquellos con otros reordenamientos de *KMT2A*, lo cual contradice la clasificación de la *European LeukemiaNet*, que considera la t(9;11) de riesgo intermedio y el resto de *KMT2Ar* de riesgo adverso, tanto en la versión de 2017 como en la de 2022^{39,136}. Las implicaciones pronósticas de los diferentes reordenamientos de *KMT2A* ha sido un tema de controversia durante décadas, ya que algunos estudios reportaron una mejor supervivencia en pacientes adultos^{198–200} o pediátricos²⁰¹ con t(9;11), mientras que otros no encontraron diferencias^{202–204}. Cabe destacar que en el estudio publicado por Bill *et al.*²⁰⁵, la t(9;11) únicamente se asoció a un mejor pronóstico un grupo específico de pacientes jóvenes (<60 años) con LMA *de novo*. Por ello, realizamos un subanálisis en la base de HARMONY y, aunque dicho grupo representaba el 56% de la cohorte con *KMT2Ar*, tampoco observamos diferencias en la SG entre las diferentes traslocaciones. Sin embargo, la mutación de *KRAS* fue un factor pronóstico independiente de la SG, lo cual es concordante con los resultados publicados por Matsuo *et al.* en población pediátrica²⁰⁶. Conviene señalar que Bill *et al.*²⁰⁵ reportaron una distribución heterogénea de la mutación de *KRAS*, con la mayor prevalencia en los pacientes con t(6;11) (47%), que fue el subgrupo con el peor pronóstico en su serie. Sin embargo, el subgrupo con la menor prevalencia de *KRAS*-mut fue precisamente la t(9;11) (3%). En la cohorte de

HARMONY, la distribución de la mutación de *KRAS* fue más homogénea, con un 28% de *KRAS*-mut en pacientes con t(6;11) y un 20% en aquellos con t(9;11) (capítulo 1 de la sección de resultados, **Figura 1**). Por otra parte, Grossman *et al.*²⁰⁷ tampoco encontraron diferencias en la SG en pacientes con t(9;11) y la proporción de pacientes con *KRAS*-mut fue similar a la de HARMONY (23%). Estos resultados sugieren que la mutación de *KRAS* sería la que otorgaría un peor pronóstico a los pacientes con LMA y *KMT2Ar* y no los diversos reordenamientos del gen, lo cual estaría respaldado por el estudio de Hyrenius-Wittsten *et al.*²⁰⁸ en modelo murino. Esta explicación podría poner fin a la controversia en la LMA con *KMT2Ar* y contribuir a perfeccionar la siguiente edición de la clasificación pronóstica de esta patología. Además, constituye un ejemplo representativo de la importancia de las grandes bases de datos para el estudio de subtipos poco frecuentes y de la relevancia del perfil mutacional en determinados subtipos de LMA definidos por alteraciones citogenéticas. No obstante, deberá ser confirmado en cohortes independientes de pacientes con LMA con *KMT2Ar* caracterizados mediante panel de NGS.

En el segundo trabajo de la tesis doctoral (**capítulo 2** de la sección de resultados: “Unravelling co-mutational patterns with prognostic implications in *NPM1* mutated adult acute myeloid leukemia – a HARMONY study”) estudiamos la LMA con mutación en *NPM1*, que constituye el subtipo más frecuente en pacientes <60 años^{84,86}. La inmensa mayoría de los pacientes no presenta alteraciones citogenéticas, pero sí otras mutaciones concomitantes, por lo que representa el subtipo ideal para el estudio de las interacciones gen-gen. Dado que el tipo de tratamiento puede influir en gran medida en los factores pronósticos de la LMA^{39,209–211}, seleccionamos de la base de HARMONY únicamente pacientes que hubieran recibido tratamiento con quimioterapia intensiva y tuvieran información de NGS mediante un panel de genes implicados en patología mieloide. Con el fin de obtener grupos más homogéneos, dividimos los 1.763 pacientes con LMA y *NPM1*-mut en una cohorte de entrenamiento (n=1.001), con datos provenientes de ensayos clínicos de grandes grupos cooperativos^{84,212–214} y una cohorte de validación interna (n=762) con pacientes incluidos en registros de nacionales, de grupos cooperativos o centros individuales, más representativa de la población en “vida real”. Cabe destacar que las dos cohortes presentaban diferencias significativas en la

edad mediana de los pacientes, la tasa de neoplasias hematológicas previas, la cifra de leucocitos totales o incluso algunas mutaciones como *FLT3*-ITD, lo que se traducía en diferencias significativas en la supervivencia global de los pacientes (capítulo 2 de la sección de resultados, **Tabla 1**), incluso teniendo en cuenta que en ambas cohortes se habían aplicado los mismos criterios de inclusión y exclusión. Esto pone de manifiesto la necesidad de validar los resultados obtenidos en los ensayos clínicos en poblaciones no seleccionadas, que presentan características clínicas y biológicas diferentes. Para ello, resulta fundamental la colaboración entre centros e instituciones a nivel nacional e internacional.

Las co-mutaciones más frecuentes en la LMA con *NPM1*-mut fueron *DNMT3A* (53%) y *FLT3*-ITD (39%). El 60% de los pacientes con mutación *FLT3*-ITD también presentaba *DNMT3A*-mut, mientras que el 44% de los pacientes con *DNMT3A*-mut tenía asimismo *FLT3*-ITD, mostrando una gran correlación entre ambas mutaciones. Estos datos sugieren que no es posible evaluar el impacto pronóstico de una mutación de forma aislada, sin tener en cuenta el resto del perfil mutacional. Las guías actuales (ELN2022) consideran que la LMA con *NPM1*-mut y ausencia de *FLT3*-ITD es de riesgo favorable, mientras que la presencia concomitante de *FLT3*-ITD modifica el riesgo a intermedio, sin otras distinciones en relación al perfil genético al diagnóstico³⁹. Con los datos incluidos en HARMONY, pudimos demostrar que los pacientes con la triple mutación (*NPM1*-mut, *FLT3*-ITD y *DNMT3A*-mut) presentan una supervivencia similar a la de otros pacientes clasificados como riesgo adverso según la ELN2022, con una SG a 2 años de tan solo el 29%. Estos resultados son concordantes con estudios previos, que también asocian este subgrupo a un pronóstico adverso^{81,84,134,135,215}. De hecho, la triple mutación podría identificar un subtipo de LMA con características biológicas propias, como un inmunofenotipo característico (*GPR56*^{high} y *CD34*^{low}), una alta frecuencia de células madre leucémicas y aumento del factor leucémico hepático²¹⁶. Cabe destacar que esta triple combinación de mutaciones representa el 23% de la serie de HARMONY de LMA con mutación en *NPM1*. Teniendo en cuenta que este subtipo representa el 25-30% de la LMA del adulto^{84,86}, la triple mutación estaría presente en aproximadamente el 6% de los pacientes con LMA, lo cual supondría una frecuencia mucho mayor que otros subtipos reconocidos en esta patología, como la *t(6;9)(p22.3;q34.1)/DEK::NUP214*, los

reordenamientos de *MECOM* o incluso *KMT2Ar*. Aunque no está claro si la LMA con *NPM1*-mut, *FLT3*-ITD y *DNMT3A*-mut será reconocida como una entidad biológica en el futuro, sería razonable considerar a este subgrupo como de riesgo adverso, al menos en pacientes que reciben esquemas de tratamiento intensivo con quimioterapia convencional. Por otra parte, demostramos que los pacientes con *FLT3*-ITD podían clasificarse como de riesgo favorable (13%), intermedio (27%) o adverso (60%) en la LMA con *NPM1*-mut, en función de las co-mutaciones concomitantes. Asimismo, aquellos con *DNMT3A*-mut también se podían estratificar en las tres categorías, siendo el 39%, 17% y 44% de riesgo favorable, intermedio y adverso, respectivamente. Estos datos sugieren que la clasificación de la ELN refleja la tendencia general de estas alteraciones a nivel pronóstico, pero podría no ser precisa en la evaluación del riesgo de determinadas combinaciones de co-mutaciones en la LMA con *NPM1*-mut. No obstante, hay que tener en cuenta que la ELN2022 asume que los pacientes con mutación en *FLT3* reciben midostaurina como parte del tratamiento, ya que es un fármaco que ha demostrado mejorar la supervivencia de los pacientes con dicha alteración y está aprobado en primera línea²¹⁷. Por ello, futuros estudios deberán confirmar si este subgrupo con la triple mutación mantiene su pronóstico adverso o si la incorporación de la midostaurina también permitiría incrementar la supervivencia en este perfil de pacientes. En cualquier caso, nuestro estudio sobre la LMA con mutación en *NPM1* sería una demostración conceptual de la importancia de las co-mutaciones para una adecuada valoración pronóstica en esta patología.

En la misma línea, descubrimos que las mutaciones en *IDH1* o *IDH2* (*IDH*-mut) tienen implicaciones pronósticas opuestas en la LMA con *NPM1*-mut, en función del perfil de co-mutaciones. En pacientes con *FLT3*-ITD sin mutación en *DNMT3A* (*DNMT3A*-wt), *IDH*-mut se asoció con un pronóstico favorable (capítulo 2 del apéndice suplementario, **Figuras S5 y S21**). Por el contrario, en el subgrupo con *DNMT3A*-mut y ausencia de *FLT3*-ITD, *IDH*-mut identificó pacientes con una menor SG (capítulo 2 del apéndice suplementario, **Figuras S10 y S20**). Los mecanismos biológicos implicados en este fenómeno aún se desconocen, pero podrían estar relacionados con el estado epigenético en el que ocurren las mutaciones de *NPM1*-mut o *FLT3*-ITD, ya que ambas suelen ser eventos tardíos en la LMA^{135,218}. Sin embargo, *DNMT3A*-mut e *IDH*-mut

habitualmente se adquieren de forma precoz y están relacionados con la hematopoyesis clonal y estados epigenéticos alterados, con características distintivas entre *DNMT3A*-mut e *IDH*-mut^{219,220}.

La clasificación pronóstica establecida en la LMA con mutación en *NPM1* permitiría reclasificar más del 40% de los pacientes a una categoría de riesgo diferente con respecto a la propuesta de la ELN2022, teniendo en cuenta el perfil de co-mutaciones de cinco de los genes más frecuentemente mutados en este subtipo de LMA (*DNMT3A*, *FLT3*-ITD, *IDH1/2* y *TET2*). Sin embargo, es probable que otras alteraciones minoritarias también puedan tener implicaciones pronósticas. Desde la publicación de la clasificación ELN2022, ha habido un gran debate sobre el papel de las mutaciones en los genes relacionados con la mielodisplasia (MRG) en la LMA con *NPM1*-mut. Algunos estudios encontraron una asociación con una menor SG^{221,222}, mientras que otros no encontraron diferencias significativas^{223–225}. Por otra parte, es posible que la mutación de alguno de los nueve MRG tenga implicaciones pronósticas diferentes a las del resto, como sugiere el estudio de Cocciardi *et al.*²²⁶, en el que las mutaciones en genes del complejo cohesina (representados mayoritariamente por *STAG2*) se asociaron a una supervivencia libre de evento más prolongada. En la cohorte de entrenamiento de HARMONY de LMA con *NPM1*-mut, las mutaciones en MRG fueron muy poco prevalentes, probablemente condicionado por los criterios de inclusión del estudio, ya que se seleccionaron pacientes que recibieron quimioterapia intensiva dentro de ensayos clínicos. Por ello, solo tres MRG (*SRSF2*, *STAG2* y *RUNX1*) presentaban mutación en más del 1% de los pacientes, lo que impidió la consideración de la mayoría de las MRG en el estudio. No obstante, los datos obtenidos en la elaboración de la clasificación sugieren que *RUNX1*-mut y *SRSF2*-mut podrían asociarse a riesgo adverso e intermedio, respectivamente, mientras que *STAG2*-mut podría ser un marcador de riesgo favorable en los pacientes sin *FLT3*-ITD (capítulo 2 del apéndice suplementario, **Figuras S1 y S8**). Estos resultados indican que cada mutación en los genes relacionados con la mielodisplasia podría tener un impacto pronóstico distinto, al menos en la LMA con mutación en *NPM1*.

De forma global, los dos primeros trabajos de la presente tesis doctoral ponen de manifiesto la complejidad de las interacciones entre las alteraciones citogenéticas y las

combinaciones de mutaciones en la LMA del adulto, así como el valor pronóstico de las mismas. Sin embargo, la edad o el género del paciente también pueden tener un papel relevante, tanto en el perfil mutacional como en la respuesta al tratamiento o la supervivencia^{86,227-230}, lo cual incrementa aún más la complejidad de los análisis. Por ello, en el tercer trabajo de la tesis doctoral (**capítulo 3** de la sección de resultados: “Machine learning provides individualized prediction of outcomes after first complete remission in adult acute myeloid leukemia – a HARMONY study”) empleamos modelos de aprendizaje automático para predecir el pronóstico de pacientes con LMA que alcanzan la primera respuesta completa (RC1) tras recibir tratamiento de inducción con quimioterapia intensiva. La respuesta al tratamiento es el factor más determinante de la supervivencia global en la LMA del adulto¹⁹², por lo que predecir el pronóstico en los pacientes que ya han alcanzado RC1 representa un desafío. Sin embargo, puede contribuir a determinar quién debe someterse a un trasplante alogénico de progenitores hematopoyéticos (alo-TPH). De forma general, se considera que cuando el riesgo de recaída sin alo-TPH es superior al 40%, se debería considerar realizar dicho procedimiento^{152,231}. La clasificación pronóstica de la ELN resulta de gran utilidad en este contexto, ya que se estima que los pacientes de riesgo favorable tienen una probabilidad de recaída inferior al 40% sin alo-TPH. Sin embargo, en los dos trabajos previos demostramos que los grupos de riesgo de ELN pueden albergar una heterogeneidad significativa, al menos en los subtipos de *KMT2A* y *NPM1*-mut. En este tercer estudio, demostramos que el 40-50% de los pacientes clasificados por la ELN2022 como riesgo favorable podrían tener una incidencia acumulada de recaída (CIR) a los 5 años superior al 40% (capítulo 3 de la sección de resultados, **Figuras 3C y 4C**), por lo que se podría haber considerado la realización del trasplante en RC1.

Este abordaje personalizado en la predicción del riesgo de recaída ha sido validado en una cohorte externa independiente y tendría el potencial de modificar las indicaciones de alo-TPH en los pacientes con LMA en el futuro, aunque presenta una serie de limitaciones. La valoración de la enfermedad medible residual (EMR) es un factor pronóstico establecido en la LMA del adulto, por lo que podría aportar información de gran valor con respecto al riesgo de recaída de cada paciente^{39,70}. No obstante, en algunos subtipos como la LMA con mutación en *NPM1*, la tasa de EMR positiva con

nuevas estrategias de tratamiento podría llegar a ser inferior al 20%, mientras que la CIR a 3 años en determinados subgrupos genéticos (*NPM1*-mut, *FLT3*-ITD y *DNMT3A*-mut) podría alcanzar el 40% en pacientes con EMR negativa¹³⁵. Todo ello sugiere que el perfil genómico y la EMR podrían aportar información complementaria, por lo que la combinación de ambas permitiría predecir el pronóstico de los pacientes de forma más precisa. Por otra parte, la ausencia de pacientes tratados con terapias dirigidas en nuestro estudio podría dificultar la generalización de los resultados, ya que fármacos como midostaurina o gemtuzumab ozogamicina modifican el pronóstico de forma diferencial en determinados subgrupos de pacientes^{61,153,229,230}. En cualquier caso, el modelo de aprendizaje automático desarrollado con los datos incluidos en HARMONY podría ser de utilidad en regiones donde las terapias dirigidas o las técnicas de EMR no estén disponibles, aportando una predicción individualizada del riesgo de cada paciente. Asimismo, en los centros donde los recursos sean limitados, permitiría priorizar los pacientes que más se beneficiarían de la evaluación de EMR para la toma de decisiones clínicas.

En resumen, el trabajo realizado en la presente tesis doctoral destaca la relevancia de varios aspectos vinculados a la LMA del adulto. En primer lugar, es preciso disponer de grandes bases de datos, como la del proyecto HARMONY, para identificar subgrupos poco frecuentes, pero de gran importancia clínica. Esto podría contribuir a perfeccionar las clasificaciones de riesgo actuales, que tienen un impacto cada vez mayor en las decisiones terapéuticas para los pacientes con LMA. El estudio del perfil mutacional de cada uno de los subtipos de LMA definidos por alteraciones citogenéticas recurrentes podría desvelar nuevos factores pronósticos en estas entidades, como hemos demostrado en la LMA con *KMT2A*r. Además, algunas alteraciones, como *KRAS*-mut, podrían constituir dianas terapéuticas en esta patología en el futuro, ya que actualmente hay varios inhibidores en fase de desarrollo en el campo de las neoplasias sólidas^{232,233}. Esto permitiría transformar un factor pronóstico adverso en una potencial vulnerabilidad de la célula tumoral, modificando la evolución natural de la enfermedad. Por otra parte, realizamos una demostración conceptual de la relevancia de las combinaciones de co-mutaciones y las interacciones gen-gen en la LMA con *NPM1*-mut. Aunque es posible que los factores pronósticos identificados se vean modificados en pacientes que reciben

terapias dirigidas como parte del tratamiento, este trabajo sienta las bases para realizar una evaluación sistemática del perfil genómico de la LMA en futuros ensayos clínicos. Por último, confirmamos que las técnicas de inteligencia artificial son útiles para el desarrollo de modelos predictivos más precisos en esta patología, lo que supone un paso hacia adelante en la medicina de precisión para los pacientes con LMA.

PERSPECTIVAS FUTURAS

La LMA del adulto es una enfermedad compleja desde el punto de vista biológico, en la que múltiples alteraciones citogenéticas y mutaciones están presentes de forma concomitante y pueden modificar el pronóstico de los pacientes. En el segundo capítulo de la tesis doctoral demostramos que un porcentaje significativo de pacientes con *NPM1*-mut presentaban una supervivencia similar a aquellos considerados de riesgo adverso, según la clasificación ELN2022³⁹. Es decir, es probable que el riesgo de este subtipo de LMA se esté infraestimando con las recomendaciones actuales. Por otra parte, en el tercer capítulo observamos un subgrupo de pacientes con *t(8;21)(q22;q22.1)/RUNX1::RUNX1T1* cuya predicción de CIR a 5 años, según el modelo de aprendizaje automático, era superior al 40%, tanto en la cohorte de entrenamiento como en la de validación externa (capítulo 2 de la sección de resultados, **Figuras 3E y 4E**). Esto sugiere que el riesgo también se podría estar infraestimando en otros subtipos de LMA considerados de pronóstico favorable. Cabe destacar que la CIR fue mayor en la cohorte de HARMONY que en la de validación, dado que esta última incluía pacientes que recibieron tratamiento con gemtuzumab ozogamicina (GO)²³⁴. Sin embargo, en ambas la diferencia de la CIR entre los dos subgrupos identificados por inteligencia artificial era estadísticamente significativa, lo que sugiere que el tratamiento con GO podría modificar, pero no eliminar, factores pronósticos adicionales en este subtipo de LMA. Por ello, planteamos el análisis detallado de estos pacientes en la plataforma de HARMONY y los resultados preliminares fueron presentados en el 29.º Congreso de la Asociación Europea de Hematología (*Hernandez-Sanchez A, et al. Genomic landscape of adult AML with t(8;21)(q22;q22.1) and prognostic implications – results from the harmony platform. Abstract S148. Hemasphere. 2024*). En este análisis, pudimos demostrar que tanto el género como las alteraciones citogenéticas concomitantes y las mutaciones son factores pronósticos independientes de la CIR en este subtipo de LMA. Además, el valor pronóstico de las mutaciones de *KIT* es dependiente de la frecuencia alélica de la variante (VAF), asociándose un VAF>25% a una mayor incidencia de recaídas. No obstante, será preciso determinar si el tratamiento con GO modifica la CIR en algún subconjunto de pacientes de forma diferencial. La generalización de este tipo de análisis permitiría abordar el estudio sistemático de grupos considerados de buen pronóstico para definir alteraciones que puedan asociarse con una menor supervivencia

o, por el contrario, detectar subgrupos de pacientes considerados de riesgo adverso que presenten una buena respuesta al tratamiento y obtengan supervivencias más prolongadas.

Los subgrupos pronósticos establecidos en esta tesis doctoral podrían tener consecuencias a nivel biológico que expliquen las diferencias en la respuesta al tratamiento o en la incidencia de recaídas de la enfermedad, como la LMA con *NPM1*-mut, *FLT3*-ITD y *DNMT3A*-mut²¹⁶. La correlación entre la clínica y la biología de esta patología resulta fundamental para el desarrollo de nuevos fármacos y estrategias terapéuticas que permitan modificar la historia natural de los casos de riesgo adverso. En ese sentido, el grupo del Dr. Barabé (Université Laval, Québec, Canada) está investigando los mecanismos de resistencia y evolución en la LMA con *KMT2A*²³⁵, por lo que hemos iniciado un estudio colaborativo para confirmar si sus resultados experimentales se podrían traducir en consecuencias pronósticas a nivel clínico.

Los modelos de aprendizaje automático permiten una predicción pronóstica personalizada de los pacientes con LMA, como se ha demostrado en el tercer trabajo de la tesis doctoral. En ese estudio solo se evaluaron pacientes que habían recibido tratamiento con esquemas de quimioterapia intensiva y que serían potenciales candidatos a someterse a un trasplante de progenitores hematopoyéticos en primera respuesta completa, ya que representa la población mayoritaria en la base de datos de HARMONY. Sin embargo, el modelo desarrollado podría ser aplicable a otros perfiles de pacientes, como aquellos que reciben tratamientos menos intensivos con la combinación de venetoclax y agentes hipometilantes. En este contexto, la clasificación de la ELN2022 pierde su valor pronóstico, por lo que se ha publicado recientemente la propuesta ELN2024^{209,210}. Sin embargo, otros grupos han realizado también sus propuestas o modificaciones sobre la ELN2024^{211,236,237}, lo que pone de manifiesto que en el momento actual no existe consenso con respecto al sistema que permita predecir el pronóstico de estos pacientes. Por ello, la aplicación del modelo de aprendizaje automático desarrollado en esta tesis, modificado para incluir otras variables relevantes como la enfermedad medible residual, podría resultar de utilidad para predecir los pacientes que tienen más probabilidades de responder a esta combinación de fármacos o de obtener un mayor beneficio a largo plazo en términos de supervivencia global.

La posibilidad de realizar estudios colaborativos a escala internacional, donde se puedan incluir grandes cohortes de pacientes con patologías poco frecuentes, bien caracterizados a nivel clínico y genómico, abrirá nuevas perspectivas de clasificación y de tratamiento en el futuro próximo. Las clasificaciones actuales de las hemopatías malignas traducen un mayor conocimiento de las alteraciones moleculares presentes en los enfermos con LMA, pero suponen una gran segmentación de las series, por lo que es preciso incrementar el número de casos para obtener resultados valorables en cada subgrupo. Por ello, la plataforma HARMONY está abierta a cualquier propuesta de estudio que pueda mejorar el tratamiento o el pronóstico de los pacientes con LMA.

En resumen, los estudios incluidos en la presente tesis doctoral permitirán abrir nuevas vías de investigación, tanto a nivel biológico como clínico, para continuar avanzando hacia la medicina de precisión en los pacientes con leucemia mieloblástica aguda.

CONCLUSIONES

1. Los pacientes con LMA y $t(9;11)(p21.3;q23.3)/KMT2A::MLLT3$ presentan una supervivencia global comparable a la de aquellos con otros reordenamientos de *KMT2A*, en discordancia con lo establecido por la clasificación de riesgo de la ELN2022.
2. El perfil mutacional de la LMA del adulto con reordenamiento de *KMT2A* tiene implicaciones pronósticas, ya que las mutaciones de *KRAS*, *TP53* y *DNMT3A* se asocian a una menor supervivencia global.
3. La LMA con mutación en *NPM1* presenta varias combinaciones de co-mutaciones clínicamente relevantes que afectan a *FLT3*-ITD, *DNMT3A*, *IDH1/2* y *TET2*.
4. Los pacientes con LMA con mutación en *NPM1* que reciben esquemas de quimioterapia intensiva se podrían estratificar en tres categorías de riesgo establecidas según el perfil de co-mutaciones, con diferencias significativas en la supervivencia global y la supervivencia libre de recaída.
5. La estratificación pronóstica basada en los patrones de co-mutaciones en la LMA con mutación en *NPM1* podría reclasificar más del 40% de los pacientes a una categoría de riesgo diferente a la propuesta por la ELN2022.
6. El modelo de aprendizaje automático desarrollado, basado en árboles de regresión aditiva bayesiana, proporciona estimaciones pronósticas personalizadas en pacientes de 18-70 años con LMA que alcanzan la primera respuesta completa tras la administración de esquemas de quimioterapia intensiva.
7. El modelo presenta una capacidad predictiva superior a la clasificación de riesgo de la ELN2022 y se ha validado en una cohorte independiente de pacientes con LMA, por lo que permite identificar pacientes en los que se podría valorar la realización de un trasplante alogénico de progenitores hematopoyéticos.
8. El análisis de la base de datos de HARMONY, que contiene información citogenética y de secuenciación masiva de amplias cohortes de pacientes con LMA, facilita la identificación de nuevos factores pronósticos y permite desarrollar modelos predictivos aplicables a contextos clínicamente relevantes.

BIBLIOGRAFÍA

1. DiNardo CD, Erba HP, Freeman SD, Wei AH. Acute myeloid leukaemia. *Lancet*. 2023;401(10393):2073–86.
2. Khwaja A, Bjorkholm M, Gale RE, Levine RL, Jordan CT, Ehninger G, et al. Acute myeloid leukaemia. *Nat Rev Dis Primers*. 2016;2:16010.
3. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016–40 for 195 countries and territories. *Lancet*. 2018;392(10159):2052–90.
4. Acute Myeloid Leukemia - Cancer Stat Facts [Internet]. 2025 [cited 2025 Mar 25]. Available from: <https://seer.cancer.gov/statfacts/html/amyl.html>.
5. Juliusson G, Lehmann S, Lazarevic V. Epidemiology and etiology of AML. In: Röllig C, Ossenkoppele GJ, editors. *Acute Myeloid Leukemia*. Cham: Springer International Publishing; 2021. p. 1–22. Cham: Springer International Publishing; .
6. Maynadié M, Girodon F, Manivet-Janoray I, Mounier M, Mugneret F, Bailly F, et al. Twenty-five years of epidemiological recording on myeloid malignancies: data from the specialized registry of hematologic malignancies of Côte d'Or (Burgundy, France). *Haematologica*. 2011;96(1):55–61.
7. Ohnishi H, Imataki O, Kawachi Y, Ide M, Kawakami K, Waki M, et al. Age is an independent adverse prognostic factor for overall survival in acute myeloid leukemia in Japan. *World Journal of Hematology*. 2014;3(3):105–14.
8. Roman E, Smith A, Appleton S, Crouch S, Kelly R, Kinsey S, et al. Myeloid malignancies in the real-world: Occurrence, progression and survival in the UK's population-based Haematological Malignancy Research Network 2004-15. *Cancer Epidemiol*. 2016;42:186–98.
9. Juliusson G, Antunovic P, Derolf Å, Lehmann S, Möllgård L, Stockelberg D, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*. 2009;113(18):4179–87.
10. Nordcan 2.0 [Internet]. 2025 [cited 2025 Feb 17]. Available from: <https://nordcan.iarc.fr/en/database>.

11. Juliusson G, Registry for the SA, Jädersten M, Registry for the SA, Deneberg S, Registry for the SA, et al. The prognostic impact of FLT3-ITD and NPM1 mutation in adult AML is age-dependent in the population-based setting. *Blood Adv.* 2020;4(6):1094–101.
12. Brunner AM, Graubert TA. Pathobiology of acute myeloid leukemia. In: Weitz J, editor. *Hematology*. Elsevier; 2018. p. 913–23.
13. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. *Science*. 2019;366(6465):eaan4673.
14. Blokzijl F, De Ligt J, Jager M, Sasselli V, Roerink S, Sasaki N, et al. Tissue-specific mutation accumulation in human adult stem cells during life. *Nature*. 2016;538(7624):260–4.
15. Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, Quake SR, et al. Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med*. 2012;4(149):149ra118.
16. Hartmann L, Metzeler KH. Clonal hematopoiesis and preleukemia—Genetics, biology, and clinical implications. *Genes Chromosomes Cancer*. 2019;58(12):828–38.
17. Young AL, Spencer Tong R, Birmann BM, Druley TE. Clonal hematopoiesis and risk of acute myeloid leukemia. *Haematologica*. 2019;104(12):2410–7.
18. Warren JT, Link DC. Clonal hematopoiesis and risk for hematologic malignancy. *Blood*. 2020;136(14):1599–605.
19. Fabre MA, de Almeida JG, Fiorillo E, Mitchell E, Damaskou A, Rak J, et al. The longitudinal dynamics and natural history of clonal haematopoiesis. *Nature*. 2022;606(7913):335–42.
20. Bocker MT, Hellwig I, Breiling A, Eckstein V, Ho AD, Lyko F. Genome-wide promoter DNA methylation dynamics of human hematopoietic progenitor cells during differentiation and aging. *Blood*. 2011;117(19):e182-9.
21. Smith MT, Zhang L, Mchale CM, Skibola CF, Rappaport SM. Benzene, the Exposome and Future Investigations of Leukemia Etiology. 2011;192(1–2):155–9.
22. Shallis RM, Weiss JJ, Deziel NC, Gore SD. A clandestine culprit with critical consequences: Benzene and acute myeloid leukemia. *Blood Rev*. 2021;47:100736.
23. Snyder R. Leukemia and benzene. *Int J Environ Res Public Health*. 2012;9(8):2875–93.
24. Van Maele-Fabry G, Gamet-Payraastre L, Lison D. Household exposure to pesticides and risk of leukemia in children and adolescents: Updated systematic review and meta-analysis. *Int J Hyg Environ Health*. 2019;222(1):49–67.

25. Stone RM, Neuberg D, Soiffer R, Takvorian T, Whelan M, Rabinowe SN, et al. Myelodysplastic syndrome as a late complication following autologous bone marrow transplantation for non-Hodgkin's lymphoma. *J Clin Oncol*. 1994;12(12):2535–42.
26. Le Beau MM, Albain KS, Larson RA, Vardiman JW, Davis EM, Blough RR, et al. Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromosomes no. 5 and 7. *J Clin Oncol*. 1986;4(3):325–45.
27. Schanz J, Cevik N, Fonatsch C, Bräulke F, Shirneshan K, Bacher U, et al. Detailed analysis of clonal evolution and cytogenetic evolution patterns in patients with myelodysplastic syndromes (MDS) and related myeloid disorders. *Blood Cancer J*. 2018;8(3):28.
28. Jonas BA, Johnson C, Gratzinger D, Majeti R. Alkylator-Induced and Patient-Derived Xenograft Mouse Models of Therapy-Related Myeloid Neoplasms Model Clinical Disease and Suggest the Presence of Multiple Cell Subpopulations with Leukemia Stem Cell Activity. *PLoS One*. 2016;11(7):e0159189.
29. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature*. 2015;518(7540):552–5.
30. Nilsson C, Linde F, Hulegårdh E, Garelus H, Lazarevic V, Antunovic P, et al. Characterization of therapy-related acute myeloid leukemia: increasing incidence and prognostic implications. *Haematologica*. 2023;108(4):1015–25.
31. Young AL, Spencer Tong R, Birman BM, Druley TE. Clonal hematopoiesis and risk of acute myeloid leukemia. *Haematologica*. 2019;104(12):2410–7.
32. Takahashi K, Wang F, Kantarjian H, Doss D, Khanna K, Thompson E, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *Lancet Oncol*. 2017;18(1):100–11.
33. Gillis NK, Ball M, Zhang Q, Ma Z, Zhao YL, Yoder SJ, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *Lancet Oncol*. 2017;18(1):112–21.
34. Tawana K, Wang J, Renneville A, Bödör C, Hills R, Loveday C, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. *Blood*. 2015;126(10):1214–23.
35. Tawana K, Rio-Machin A, Preudhomme C, Fitzgibbon J. Familial CEBPA-mutated acute myeloid leukemia. *Semin Hematol*. 2017;54(2):87–93.

36. Li P, Brown S, Williams M, White T, Xie W, Cui W, et al. The genetic landscape of germline DDX41 variants predisposing to myeloid neoplasms. *Blood*. 2022;140(7):716–55.
37. Sébert M, Passet M, Raimbault A, Rahmé R, Raffoux E, De Fontbrune FS, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. *Blood*. 2019;134(17):1441–4.
38. Ernst MPT, Versluis J, Valk PJM, Bierings M, Tamminga RYJ, Hooimeijer LH, et al. Disease characteristics and outcomes of acute myeloid leukemia in germline RUNX1 deficiency (Familial Platelet Disorder with associated Myeloid Malignancy). *Hemasphere*. 2025;9(1):e70057.
39. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. 2022;140(12):1345–77.
40. Abdulkarim K, Girodon F, Johansson P, Maynadié M, Kutti J, Carli PM, et al. AML transformation in 56 patients with Ph– MPD in two well defined populations. *Eur J Haematol*. 2009;82(2):106–11.
41. Jain P, Kantarjian HM, Ghorab A, Sasaki K, Jabbour EJ, Nogueras Gonzalez G, et al. Prognostic factors and survival outcomes in patients with chronic myeloid leukemia in blast phase in the tyrosine kinase inhibitor era: Cohort study of 477 patients. *Cancer*. 2017;123(22):4391–402.
42. Metzeler K. Clinical manifestations and diagnosis. In: Hiddemann W, editor. *Handbook of Acute Leukemia*. Cham: Springer International Publishing; 2016. p. 15–23.
43. Wierzbowska A, Czemerska M. Clinical Manifestation and Diagnostic Workup. In: Röllig C, Ossenkoppele GJ, editors. *Acute Myeloid Leukemia*. Cham: Springer International Publishing; 2021. p. 119–26.
44. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100(7):2292–302.
45. Khoury JD, Solary E, Abela O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022;36(7):1703–19.
46. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200–28.

47. Bennett JM, Catovsky D, Daniel M -T, Flandrin G, Galton DAG, Gralnick HR, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. *Br J Haematol*. 1976;33(4):451–8.
48. Platzbecker U, Avisati G, Cicconi L, Thiede C, Paoloni F, Vignetti M, et al. Improved Outcomes With Retinoic Acid and Arsenic Trioxide Compared With Retinoic Acid and Chemotherapy in Non-High-Risk Acute Promyelocytic Leukemia: Final Results of the Randomized Italian-German APL0406 Trial. *J Clin Oncol*. 2017;35(6):605–12.
49. Ellison RR, Holland JF, Weil M, Jacquillat C, Boiron M, Bernard J, et al. Arabinosyl Cytosine: A Useful Agent in the Treatment of Acute Leukemia in Adults. *Blood*. 1968;32(4):507–23.
50. Boiron M, Weil M, Jacquillat C, Tanzer J, Levy D, Sultan C, et al. Daunorubicin in the treatment of acute myelocytic leukaemia. *Lancet*. 1969;1(7590):330–3.
51. Yates JW, Wallace HJ, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep*. 1973;57(4):485–8.
52. Rai K, Holland J, Glidewell O, Weinberg V, Brunner K, Obrecht J, et al. Treatment of Acute Myelocytic Leukemia: A Study by Cancer and Leukemia Group B. *Blood*. 1981;58(6):1203–12.
53. Burnett AK, Group on behalf of the UNAS, Russell NH, Group on behalf of the UNAS, Hills RK, Group on behalf of the UNAS, et al. A randomized comparison of daunorubicin 90 mg/m² vs 60 mg/m² in AML induction: results from the UK NCRI AML17 trial in 1206 patients. *Blood*. 2015;125(25):3878–85.
54. Röhlig C, Steffen B, Schliemann C, Mikesch JH, Alakel N, Herbst R, et al. Single or Double Induction With 7 + 3 Containing Standard or High-Dose Daunorubicin for Newly Diagnosed AML: The Randomized DaunoDouble Trial by the Study Alliance Leukemia. *J Clin Oncol*. 2025;43(1):65–74.
55. Lee JH, Kim H, Joo YD, Lee WS, Bae SH, Zang DY, et al. Prospective Randomized Comparison of Idarubicin and High-Dose Daunorubicin in Induction Chemotherapy for Newly Diagnosed Acute Myeloid Leukemia. *J Clin Oncol*. 2017;35(24):2754–63.
56. Teuffel O, Leibundgut K, Lehrnbecher T, Alonzo TA, Beyene J, Sung L. Anthracyclines during induction therapy in acute myeloid leukaemia: a systematic review and meta-analysis. *Br J Haematol*. 2013;161(2):192–203.

57. Krug U, Berdel WE, Gale RP, Haferlach C, Schnittger S, Müller-Tidow C, et al. Increasing intensity of therapies assigned at diagnosis does not improve survival of adults with acute myeloid leukemia. *Leukemia*. 2016;30(6):1230–6.
58. Cornelissen JJ, Versluis J, Passweg JR, Van Putten WLJ, Manz MG, Maertens J, et al. Comparative therapeutic value of post-remission approaches in patients with acute myeloid leukemia aged 40-60 years. *Leukemia*. 2015;29(5):1041–50.
59. Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood*. 2016;127(1):62–70.
60. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *N Engl J Med*. 2017;377(5):454–64.
61. Lambert J, Pautas C, Terré C, Raffoux E, Turlure P, Caillot D, et al. Gemtuzumab ozogamicin for de novo acute myeloid leukemia: final efficacy and safety updates from the open-label, phase III ALFA-0701 trial. *Haematologica*. 2019;104(1):113–9.
62. Hills RK, Castaigne S, Appelbaum FR, Delaunay J, Petersdorf S, Othus M, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol*. 2014;15(9):986–96.
63. Lancet JE, Uy GL, Cortes JE, Newell LF, Lin TL, Ritchie EK, et al. Cpx-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. *J Clin Oncol*. 2018;36(26):2684–92.
64. Döhner H, Wei AH, Löwenberg B. Towards precision medicine for AML. *Nat Rev Clin Oncol*. 2021;18(9):577–90.
65. Stone A, Zukerman T, Flaishon L, Yakar R Ben, Rowe JM. Efficacy outcomes in the treatment of older or medically unfit patients with acute myeloid leukaemia: A systematic review and meta-analysis. *Leuk Res*. 2019;82:36–42.
66. Burnett AK, Hills RK, Hunter AE, Milligan D, Kell WJ, Wheatley K, et al. The addition of gemtuzumab ozogamicin to low-dose Ara-C improves remission rate but does not significantly prolong survival in older patients with acute myeloid leukaemia: results from the LRF AML14 and NCRI AML16 pick-a-winner comparison. *Leukemia*. 2012;27(1):75–81.

67. Burnett AK, Hills RK, Hunter A, Milligan D, Kell J, Wheatley K, et al. The addition of arsenic trioxide to low-dose Ara-C in older patients with AML does not improve outcome. *Leukemia*. 2011;25(7):1122–7.
68. Burnett AK, Russell NH, Culligan D, Cavanagh J, Kell J, Wheatley K, et al. The addition of the farnesyl transferase inhibitor, tipifarnib, to low dose cytarabine does not improve outcome for older patients with AML. *Br J Haematol*. 2012;158(4):519–22.
69. Dennis M, Russell N, Hills RK, Hemmaway C, Panoskaltsis N, McMullin MF, et al. Vosaroxin and vosaroxin plus low-dose Ara-C (LDAC) vs low-dose Ara-C alone in older patients with acute myeloid leukemia. *Blood*. 2015;125(19):2923–32.
70. Heuser M, Freeman SD, Ossenkoppele GJ, Buccisano F, Hourigan CS, Ngai LL, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*. 2021;138(26):2753–67.
71. Hernández-Sánchez A, Bullinger L. Recent advances in precision medicine for acute myeloid leukemia. *Curr Opin Oncol*. 2023;35(6):581–8.
72. Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood*. 2015;126(3):291–9.
73. Kantarjian HM, Thomas XG, Dmoszynska A, Wierzbowska A, Mazur G, Mayer J, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol*. 2012;30(21):2670–7.
74. DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. *N Engl J Med*. 2020;383(7):617–29.
75. Kadia TM, Reville PK, Wang X, Rausch CR, Borthakur G, Pemmaraju N, et al. Phase II Study of Venetoclax Added to Cladribine Plus Low-Dose Cytarabine Alternating With 5-Azacitidine in Older Patients With Newly Diagnosed Acute Myeloid Leukemia. *J Clin Oncol*. 2022;40(33):3848–57.
76. Short NJ, Daver N, Dinardo CD, Kadia T, Nasr LF, MacAron W, et al. Azacitidine, Venetoclax, and Gilteritinib in Newly Diagnosed and Relapsed or Refractory FLT3 -Mutated AML. *Journal of Clinical Oncology*. 2024;42(13):1499–508.

77. Montesinos P, Recher C, Vives S, Zarzycka E, Wang J, Bertani G, et al. Ivosidenib and Azacitidine in IDH1 -Mutated Acute Myeloid Leukemia. *N Engl J Med*. 2022;386(16):1519–31.
78. Marvin-Peek J, Garcia JS, Borthakur G, Garcia-Manero G, Short NJ, Kadia TM, et al. A Phase Ib/II Study of Ivosidenib with Venetoclax ± Azacitidine in IDH1-Mutated Hematologic Malignancies: A 2024 Update. *Blood*. 2024;144(Supplement 1):219.
79. Döhner H, Pratz KW, DiNardo CD, Wei AH, Jonas BA, Pullarkat VA, et al. Genetic risk stratification and outcomes among treatment-naïve patients with AML treated with venetoclax and azacitidine. *Blood*. 2024;144(21):2211–22.
80. Lazarevic V, Hörstedt AS, Johansson B, Antunovic P, Billström R, Derolf Å, et al. Incidence and prognostic significance of karyotypic subgroups in older patients with acute myeloid leukemia: the Swedish population-based experience. *Blood Cancer J*. 2014;4(2):e188.
81. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059–74.
82. Itzykson R, Cerrano M, Esteve J. Prognostic Factors in AML. In: Röllig C, Ossenkoppele GJ, editors. *Acute Myeloid Leukemia*. Cham: Springer International Publishing; 2021. p. 127–75.
83. Lindsley RC, Mar BG, Mazzola E, Grauman P V., Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–76.
84. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209–21.
85. Grimwade D, Ivey A, Huntly BJP. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood*. 2016;127(1):29–41.
86. Bullinger L, Döhner K, Döhner H. Genomics of Acute Myeloid Leukemia Diagnosis and Pathways. *J Clin Oncol*. 2017;35(9):934–46.
87. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022;36(7):1703–19.

88. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200–28.
89. Xiao W, Nardi V, Stein E, Hasserjian RP. A practical approach on the classifications of myeloid neoplasms and acute leukemia: WHO and ICC. *J Hematol Oncol*. 2024;17(1):56.
90. Falini B, Martelli MP. Comparison of the International Consensus and 5th WHO edition classifications of adult myelodysplastic syndromes and acute myeloid leukemia. *Am J Hematol*. 2023;98(3):481–92.
91. Huber S, Baer C, Hutter S, Dicker F, Meggendorfer M, Pohlkamp C, et al. AML classification in the year 2023: How to avoid a Babylonian confusion of languages. *Leukemia*. 2023;37(7):1413–20.
92. Darwish C, Farina K, Tremblay D. The core concepts of core binding factor acute myeloid leukemia: Current considerations for prognosis and treatment. *Blood Rev*. 2023;62:101117.
93. Christen F, Hoyer K, Yoshida K, Hou HA, Waldhueter N, Heuser M, et al. Genomic landscape and clonal evolution of acute myeloid leukemia with t(8;21): an international study on 331 patients. *Blood*. 2019;133(10):1140–51.
94. Paschka P, Du J, Schlenk RF, Gaidzik VI, Bullinger L, Corbacioglu A, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML Study Group (AML SG). *Blood*. 2013;121(1):170–7.
95. Jahn N, Terzer T, Sträng E, Dolnik A, Cocciardi S, Panina E, et al. Genomic heterogeneity in core-binding factor acute myeloid leukemia and its clinical implication. *Blood Adv*. 2020;4(24):6342–52.
96. Ishikawa Y, Kawashima N, Atsuta Y, Sugiura I, Sawa M, Dobashi N, et al. Prospective evaluation of prognostic impact of KIT mutations on acute myeloid leukemia with RUNX1-RUNX1T1 and CBFB-MYH11. *Blood Adv*. 2020;4(1):66–75.
97. Duployez N, Marceau-Renaut A, Boissel N, Petit A, Bucci M, Geffroy S, et al. Comprehensive mutational profiling of core binding factor acute myeloid leukemia. *Blood*. 2016;127(20):2451–9.
98. Kayser S, Kramer M, Martínez-Cuadrón D, Grenet J, Metzeler KH, Sustkova Z, et al. Characteristics and outcome of patients with core-binding factor acute myeloid leukemia

- and FLT3-ITD: results from an international collaborative study. *Haematologica*. 2022;107(4):836–43.
99. Eisfeld AK, Mrózek K, Kohlschmidt J, Nicolet D, Orwick S, Walker CJ, et al. The mutational oncoprint of recurrent cytogenetic abnormalities in adult patients with de novo acute myeloid leukemia. *Leukemia*. 2017;31(10):2211–8.
 100. Grimwade D, Hills RK, Moorman A V., Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116(3):354–65.
 101. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102(7):2395–402.
 102. Meyer C, Larghero P, Almeida Lopes B, Burmeister T, Gröger D, Sutton R, et al. The KMT2A recombinoome of acute leukemias in 2023. *Leukemia*. 2023;37(5):988–1005.
 103. Krauter J, Wagner K, Schäfer I, Marschalek R, Meyer C, Heil G, et al. Prognostic factors in adult patients up to 60 years old with acute myeloid leukemia and translocations of chromosome band 11q23: individual patient data-based meta-analysis of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol*. 2009;27(18):3000–6.
 104. Chen Y, Kantarjian H, Pierce S, Faderl S, O'Brien S, Qiao W, et al. Prognostic significance of 11q23 aberrations in adult acute myeloid leukemia and the role of allogeneic stem cell transplantation. *Leukemia*. 2013;27(4):836–42.
 105. Mrózek K, Heinonen K, Lawrence D, Carroll AJ, Koduru PRK, Rao KW, et al. Adult Patients With De Novo Acute Myeloid Leukemia and t(9; 11)(p22; q23) Have a Superior Outcome to Patients With Other Translocations Involving Band 11q23: A Cancer and Leukemia Group B Study. *Blood*. 1997;90(11):4532–8.
 106. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102(7):2395–402.

107. Harrison CJ, Hills RK, Moorman A V., Grimwade DJ, Hann I, Webb DKH, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. *J Clin Oncol*. 2010;28(16):2674–81.
108. Issa GC, Zarka J, Sasaki K, Qiao W, Pak D, Ning J, et al. Predictors of outcomes in adults with acute myeloid leukemia and KMT2A rearrangements. *Blood Cancer J*. 2021;11(9):1–10.
109. Díaz-Beyá M, Labopin M, Maertens J, Alijurf M, Passweg J, Dietrich B, et al. Allogeneic stem cell transplantation in AML with t(6;9)(p23;q34);DEK-NUP214 shows a favourable outcome when performed in first complete remission. *Br J Haematol*. 2020;189(5):920–5.
110. Sitges M, Boluda B, Garrido A, Morgades M, Granada I, Barragan E, et al. Acute myeloid leukemia with inv(3)(q21.3q26.2)/t(3;3)(q21.3;q26.2): Study of 61 patients treated with intensive protocols. *Eur J Haematol*. 2020;105(2):138–47.
111. Huber S, Haferlach T, Meggendorfer M, Hutter S, Hoermann G, Baer C, et al. SF3B1 mutations in AML are strongly associated with MECOM rearrangements and may be indicative of an MDS pre-phase. *Leukemia*. 2022;36(12):2927–30.
112. Jiménez-Vicente C, Esteve J, Baile-González M, Pérez-López E, Calvo CM, Aparicio C, et al. Allo-HCT refined ELN 2022 risk classification: validation of the Adverse-Plus risk group in AML patients undergoing allogeneic hematopoietic cell transplantation within the Spanish Group for Hematopoietic Cell Transplantation (GETH-TC). *Blood Cancer J*. 2025;15(1):1–9.
113. Merz LE, Perissinotti AJ, Marini BL, Burke PW, Crouch A, Erba HP, et al. Lenalidomide Plus Hypomethylating Agent as a Treatment Option in Acute Myeloid Leukemia With Recurrent Genetic Abnormalities-AML With inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM. *Clin Lymphoma Myeloma Leuk*. 2020;20(1):24–30.
114. Paredes R, Doleschall N, Connors K, Geary B, Meyer S. EVI1 protein interaction dynamics: Targetable for therapeutic intervention? *Exp Hematol*. 2022;107:1–8.
115. Ottema S, Mulet-Lazaro R, Beverloo HB, Erpelinck C, van Herk S, van der Helm R, et al. Atypical 3q26/MECOM rearrangements genocopy inv(3)/t(3;3) in acute myeloid leukemia. *Blood*. 2020;136(2):224–34.
116. Ochi Y, Yoshida K, Huang YJ, Kuo MC, Nannya Y, Sasaki K, et al. Clonal evolution and clinical implications of genetic abnormalities in blastic transformation of chronic myeloid leukaemia. *Nat Commun*. 2021;12(1):1–13.

117. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352(3):254–66.
118. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. *Blood*. 2007;109(3):874–85.
119. Othman J, Meggendorfer M, Tiacci E, Thiede C, Schlenk R, Dillon R, et al. Overlapping features of therapy-related and de novo NPM1-mutated AML. *Blood*. 2023;141(15):1846–57.
120. Moukalled N, Labopin M, Versluis J, Socié G, Blaise D, Salmenniemi U, et al. Complex karyotype but not other cytogenetic abnormalities is associated with worse posttransplant survival of patients with nucleophosmin 1-mutated acute myeloid leukemia: A study from the European Society for Blood and Marrow Transplantation Acute Leukemia Working Party. *Am J Hematol*. 2024;99(3):360–9.
121. Haferlach C, Mecucci C, Schnittger S, Kohlmann A, Mancini M, Cuneo A, et al. AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. *Blood*. 2009;114(14):3024–32.
122. Angenendt L, Röllig C, Montesinos P, Martínez-Cuadrón D, Barragan E, García R, et al. Chromosomal Abnormalities and Prognosis in NPM1-Mutated Acute Myeloid Leukemia: A Pooled Analysis of Individual Patient Data From Nine International Cohorts. *J Clin Oncol*. 2019;37(29):2632–42.
123. Balsat M, Renneville A, Thomas X, De Botton S, Caillot D, Marceau A, et al. Postinduction Minimal Residual Disease Predicts Outcome and Benefit From Allogeneic Stem Cell Transplantation in Acute Myeloid Leukemia With NPM1 Mutation: A Study by the Acute Leukemia French Association Group. *J Clin Oncol*. 2017;35(2):185–93.
124. Herudkova Z, Culen M, Foltá A, Jeziskova I, Cerna J, Loja T, et al. Clonal hierarchy of main molecular lesions in acute myeloid leukaemia. *Br J Haematol*. 2020;190(4):562–72.
125. Loberg MA, Bell RK, Goodwin LO, Eudy E, Miles LA, SanMiguel JM, et al. Sequentially inducible mouse models reveal that Npm1 mutation causes malignant transformation of Dnmt3a-mutant clonal hematopoiesis. *Leukemia*. 2019;33(7):1635–49.

126. Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci U S A*. 2014;111(7):2548–53.
127. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506(7488):328–33.
128. Falini B, Brunetti L, Sportoletti P, Paola Martelli M. NPM1-mutated acute myeloid leukemia: from bench to bedside. *Blood*. 2020;136(15):1707–21.
129. Gaidzik VI, Weber D, Paschka P, Kaumanns A, Krieger S, Corbacioglu A, et al. DNMT3A mutant transcript levels persist in remission and do not predict outcome in patients with acute myeloid leukemia. *Leukemia*. 2018;32(1):30–7.
130. Bhatnagar B, Eisfeld AK, Nicolet D, Mrózek K, Blachly JS, Orwick S, et al. Persistence of DNMT3A R882 mutations during remission does not adversely affect outcomes of patients with acute myeloid leukaemia. *Br J Haematol*. 2016;175(2):226–36.
131. Desai P, Mencia-Trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nat Med*. 2018;24(7):1015–23.
132. Krönke J, Bullinger L, Temeanu V, Tschürtz F, Gaidzik VI, Kühn MWM, et al. Clonal evolution in relapsed NPM1-mutated acute myeloid leukemia. *Blood*. 2013;122(1):100–8.
133. Boddu P, Kantarjian H, Borthakur G, Kadia T, Daver N, Pierce S, et al. Co-occurrence of FLT3-TKD and NPM1 mutations defines a highly favorable prognostic AML group. *Blood Adv*. 2017;1(19):1546–50.
134. Bezerra MF, Lima AS, Piqué-Borràs MR, Silveira DR, Coelho-Silva JL, Pereira-Martins DA, et al. Co-occurrence of DNMT3A, NPM1, FLT3 mutations identifies a subset of acute myeloid leukemia with adverse prognosis. *Blood*. 2020;135(11):870–5.
135. Othman J, Potter N, Ivey A, Tazi Y, Papaemmanuil E, Jovanovic J, et al. Molecular, clinical, and therapeutic determinants of outcome in NPM1-mutated AML. *Blood*. 2024;144(7):714–28.
136. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424–47.

137. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–405.
138. Wakita S, Sakaguchi M, Oh I, Kako S, Toya T, Najima Y, et al. Prognostic impact of CEBPA bZIP domain mutation in acute myeloid leukemia. *Blood Adv*. 2022;6(1):238–47.
139. Taube F, Georgi JA, Kramer M, Stasik S, Middeke JM, Röllig C, et al. CEBPA mutations in 4708 patients with acute myeloid leukemia: differential impact of bZIP and TAD mutations on outcome. *Blood*. 2022;139(1):87–103.
140. Short NJ, Montalban-Bravo G, Hwang H, Ning J, Franquiz MJ, Kanagal-Shamanna R, et al. Prognostic and therapeutic impacts of mutant TP53 variant allelic frequency in newly diagnosed acute myeloid leukemia. *Blood Adv*. 2020;4(22):5681–9.
141. Grob T, Al Hinai ASA, Sanders MA, Kavelaars FG, Rijken M, Gradowska PL, et al. Molecular characterization of mutant TP53 acute myeloid leukemia and high-risk myelodysplastic syndrome. *Blood*. 2022;139(15):2347–54.
142. Weinberg OK, Siddon A, Madanat YF, Gagan J, Arber DA, Cin PD, et al. TP53 mutation defines a unique subgroup within complex karyotype de novo and therapy-related MDS/AML. *Blood Adv*. 2022;6(9):2847–53.
143. Rücker FG, Schlenk RF, Bullinger L, Kayser S, Teleanu V, Kett H, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood*. 2012;119(9):2114–21.
144. Van Der Werf I, Wojtuszkiewicz A, Meggendorfer M, Hutter S, Baer C, Heymans M, et al. Splicing factor gene mutations in acute myeloid leukemia offer additive value if incorporated in current risk classification. *Blood Adv*. 2021;5(17):3254–65.
145. Gardin C, Pautas C, Fournier E, Itzykson R, Lemasle E, Bourhis JH, et al. Added prognostic value of secondary AML-like gene mutations in ELN intermediate-risk older AML: ALFA-1200 study results. *Blood Adv*. 2020;4(9):1942–9.
146. Lindsley RC, Mar BG, Mazzola E, Grauman P V., Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–76.
147. Gao Y, Jia M, Mao Y, Cai H, Jiang X, Cao X, et al. Distinct Mutation Landscapes Between Acute Myeloid Leukemia With Myelodysplasia-Related Changes and De Novo Acute Myeloid Leukemia. *Am J Clin Pathol*. 2022;157(5):691–700.

148. Sargas C, Ayala R, Larráyoz MJ, Chillón MC, Rodríguez-Arboli E, Bilbao C, et al. Comparison of the 2022 and 2017 European LeukemiaNet risk classifications in a real-life cohort of the PETHEMA group. *Blood Cancer J.* 2023;13(1):77.
149. Fuhrmann I, Lenk M, Haferlach T, Stengel A, Hutter S, Baer C, et al. AML, NOS and AML-MRC as defined by multilineage dysplasia share a common mutation pattern which is distinct from AML-MRC as defined by MDS-related cytogenetics. *Leukemia.* 2022;36(7):1939–42.
150. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood.* 2010;115(3):453–74.
151. Ruhnke L, Bill M, Zukunft S, Eckardt J-N, Schäfer S, Stasik S, et al. Validation of the revised 2022 European LeukemiaNet risk stratification in adult patients with acute myeloid leukemia. *Blood Adv.* 2025;9(6):1392–404.
152. Dewolf S, Tallman MS, Rowe JM, Salman MY. What Influences the Decision to Proceed to Transplant for Patients With AML in First Remission? *J Clin Oncol.* 2023;41(29):4693–703.
153. Voso MT, Larson RA, Jones D, Marcucci G, Prior T, Krauter J, et al. Midostaurin in patients with acute myeloid leukemia and FLT3-TKD mutations: a subanalysis from the RATIFY trial. *Blood Adv.* 2020;4(19):4945–54.
154. Perry M, Bertoli S, Rocher C, Hayette S, Ducastelle S, Barraco F, et al. FLT3-TKD Mutations Associated With NPM1 Mutations Define a Favorable-risk Group in Patients With Acute Myeloid Leukemia. *Clin Lymphoma Myeloma Leuk.* 2018;18(12):e545–50.
155. Li S, Li N, Chen Y, Zheng Z, Guo Y. FLT3-TKD in the prognosis of patients with acute myeloid leukemia: A meta-analysis. *Front Oncol.* 2023;13:1086846.
156. Boddu P, Kantarjian H, Borthakur G, Kadia T, Daver N, Pierce S, et al. Co-occurrence of FLT3-TKD and NPM1 mutations defines a highly favorable prognostic AML group. *Blood Adv.* 2017;1(19):1546–50.
157. Gale RE, Lamb K, Allen C, El-Sharkawi D, Stowe C, Jenkinson S, et al. Simpson's Paradox and the Impact of Different DNMT3A Mutations on Outcome in Younger Adults With Acute Myeloid Leukemia. *J Clin Oncol.* 2015;33(18):2072–83.
158. Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype

- and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. *Blood*. 2010;116(25):5486–96.
159. Green CL, Evans CM, Hills RK, Burnett AK, Linch DC, Gale RE. The prognostic significance of IDH1 mutations in younger adult patients with acute myeloid leukemia is dependent on FLT3/ITD status. *Blood*. 2010;116(15):2779–82.
 160. Dinardo CD, Ravandi F, Agresta S, Konopleva M, Takahashi K, Kadia T, et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. *Am J Hematol*. 2015;90(8):732–6.
 161. Green CL, Evans CM, Zhao L, Hills RK, Burnett AK, Linch DC, et al. The prognostic significance of IDH2 mutations in AML depends on the location of the mutation. *Blood*. 2011;118(2):409–12.
 162. Chou WC, Lei WC, Ko BS, Hou HA, Chen CY, Tang JL, et al. The prognostic impact and stability of Isocitrate dehydrogenase 2 mutation in adult patients with acute myeloid leukemia. *Leukemia*. 2010;25(2):246–53.
 163. Middeke JM, Metzeler KH, Rollig C, Kramer M, Eckardt JN, Stasik S, et al. Differential impact of IDH1/2 mutational subclasses on outcome in adult AML: results from a large multicenter study. *Blood Adv*. 2022;6(5):1394–405.
 164. Cirillo D, Valencia A. Big data analytics for personalized medicine. *Curr Opin Biotechnol*. 2019;58:161–7.
 165. Shouval R, Fein JA, Savani B, Mohty M, Nagler A. Machine learning and artificial intelligence in haematology. *Br J Haematol*. 2021;192(2):239–50.
 166. Gomes B, Ashley EA. Artificial Intelligence in Molecular Medicine. *N Engl J Med*. 2023;388(26):2456–65.
 167. Beam AL, Kohane IS. Big Data and Machine Learning in Health Care. *JAMA*. 2018;319(13):1317–8.
 168. Rajkomar A, Dean J, Kohane I. Machine Learning in Medicine. *N Engl J Med*. 2019;380(14):1347–58.
 169. Esteva A, Robicquet A, Ramsundar B, Kuleshov V, DePristo M, Chou K, et al. A guide to deep learning in healthcare. *Nat Med*. 2019;25(1):24–9.
 170. Liu Y, Chen PHC, Krause J, Peng L. How to Read Articles That Use Machine Learning: Users' Guides to the Medical Literature. *JAMA*. 2019;322(18):1806–16.

171. Zini G, Bain B, Bettelheim P, Cortez J, D'Onofrio G, Faber E, et al. A European consensus report on blood cell identification: terminology utilized and morphological diagnosis concordance among 28 experts from 17 countries within the European LeukemiaNet network WP10, on behalf of the ELN Morphology Faculty. *Br J Haematol*. 2010;151(4):359–64.
172. Walter W, Pohlkamp C, Meggendorfer M, Nadarajah N, Kern W, Haferlach C, et al. Artificial intelligence in hematological diagnostics: Game changer or gadget? *Blood Rev*. 2023;58:101019.
173. Cheuque C, Querales M, León R, Salas R, Torres R. An Efficient Multi-Level Convolutional Neural Network Approach for White Blood Cells Classification. *Diagnostics (Basel)*. 2022;12(2):248.
174. Tseng T-R, Huang H-M, Rd A, Dist Z. Classification of peripheral blood neutrophils using deep learning. *Cytometry Part A*. 2023;103(4):295–303.
175. Kimura K, Tabe Y, Ai T, Takehara I, Fukuda H, Takahashi H, et al. A novel automated image analysis system using deep convolutional neural networks can assist to differentiate MDS and AA. *Sci Rep*. 2019;9(1):13385.
176. Haferlach T, Pohlkamp C, Heo I, Drescher R, Hänselmann S, Lörch T, et al. Automated Peripheral Blood Cell Differentiation Using Artificial Intelligence - a Study with More Than 10,000 Routine Samples in a Specialized Leukemia Laboratory. *Blood*. 2021;138(Supplement 1):103.
177. Matek C, Krappe S, Münzenmayer C, Haferlach T, Marr C. Highly accurate differentiation of bone marrow cell morphologies using deep neural networks on a large image data set. *Blood*. 2021;138(20):1917–27.
178. Swiderska-Chadaj Z, Hebeda KM, van den Brand M, Litjens G. Artificial intelligence to detect MYC translocation in slides of diffuse large B-cell lymphoma. *Virchows Archiv*. 2021;479(3):617–21.
179. Eckardt JN, Middeke JM, Riechert S, Schmittmann T, Sulaiman AS, Kramer M, et al. Deep learning detects acute myeloid leukemia and predicts NPM1 mutation status from bone marrow smears. *Leukemia*. 2022;36(1):111–8.
180. Wei BH, Tsai XCH, Sun KJ, Lo MY, Hung SY, Chou WC, et al. Annotation-free deep learning for predicting gene mutations from whole slide images of acute myeloid leukemia. *NPJ Precis Oncol*. 2025;9(1):1–8.

181. Kockwelp J, Thiele S, Bartsch J, Haalck L, Gromoll J, Schlatt S, et al. Deep learning predicts therapy-relevant genetics in acute myeloid leukemia from Pappenheim-stained bone marrow smears. *Blood Adv.* 2024;8(1):70–9.
182. Fein JA, Patel SS. Under AI's lens: spotting mutations visually. *Blood Adv.* 2024;8(3):827–8.
183. Walter W, Haferlach C, Nadarajah N, Schmidts I, Kühn C, Kern W, et al. How artificial intelligence might disrupt diagnostics in hematology in the near future. *Oncogene.* 2021;40(25):4271–80.
184. Nazha A, Komrokji R, Meggendorfer M, Jia X, Radakovich N, Shreve J, et al. Personalized Prediction Model to Risk Stratify Patients With Myelodysplastic Syndromes. *J Clin Oncol.* 2021;39(33):3737–46.
185. Mosquera-Orgueira A, Arellano-Rodrigo E, Garrote M, Martín I, Pérez-Encinas M, Gómez-Casares MT, et al. Integrating AIPSS-MF and molecular predictors: A comparative analysis of prognostic models for myelofibrosis. *Hemasphere.* 2024;8(3):e60.
186. Mosquera-Orgueira A, Pérez-Encinas M, Hernández-Sánchez A, González-Martínez T, Arellano-Rodrigo E, Martínez-EliceGUI J, et al. Machine Learning Improves Risk Stratification in Myelofibrosis: An Analysis of the Spanish Registry of Myelofibrosis. *Hemasphere.* 2022;7(1):e818.
187. Siddiqui NS, Klein A, Godara A, Buchsbaum RJ, Hughes MC. Predicting In-Hospital Mortality After Acute Myeloid Leukemia Therapy: Through Supervised Machine Learning Algorithms. *JCO Clin Cancer Inform.* 2022;6(6):e2200044.
188. Eckardt JN, Röllig C, Metzeler K, Kramer M, Stasik S, Georgi JA, et al. Prediction of complete remission and survival in acute myeloid leukemia using supervised machine learning. *Haematologica.* 2023;108(3):690–704.
189. Walter RB, Othus M, Paietta EM, Racevskis J, Fernandez HF, Lee JW, et al. Effect of genetic profiling on prediction of therapeutic resistance and survival in adult acute myeloid leukemia. *Leukemia.* 2015;29(10):2104–7.
190. Kosvyra, Karadimitris, Papaioannou, Chouvarda I. Machine learning and integrative multi-omics network analysis for survival prediction in acute myeloid leukemia. *Comput Biol Med.* 2024;178:108735.

191. Ng SWK, Mitchell A, Kennedy JA, Chen WC, McLeod J, Ibrahimova N, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature*. 2016;540(7633):433–7.
192. Tefferi A, Gangat N, Al-Kali A, Alkhateeb H, Shah M, Patnaik MS, et al. A dynamic 3-factor survival model for acute myeloid leukemia that accounts for response to induction chemotherapy. *Am J Hematol*. 2022;97(9):1127–34.
193. Versluis J, Metzner M, Wang A, Gradowska P, Thomas A, Jakobsen NA, et al. Risk Stratification in Older Intensively Treated Patients With AML. *J Clin Oncol*. 2024;42(34):4084–94.
194. Databarometer - HARMONY Alliance [Internet]. 2025 [cited 2025 Mar 23]. Available from: <https://www.harmony-alliance.eu/bigdata-platform/databarometer>.
195. Big Data Platform - HARMONY Alliance [Internet]. 2025 [cited 2025 Mar 23]. Available from: <https://www.harmony-alliance.eu/bigdata-platform/databarometer>.
196. Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Görlich D, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128(5):686–98.
197. Belenkaya R, Gurley MJ, Golozar A, Dymshyts D, Miller RT, Williams AE, et al. Extending the OMOP Common Data Model and Standardized Vocabularies to Support Observational Cancer Research. *JCO Clin Cancer Inform*. 2021;5:12–20.
198. Krauter J, Wagner K, Schäfer I, Marschalek R, Meyer C, Heil G, et al. Prognostic factors in adult patients up to 60 years old with acute myeloid leukemia and translocations of chromosome band 11q23: individual patient data-based meta-analysis of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol*. 2009;27(18):3000–6.
199. Chen Y, Kantarjian H, Pierce S, Faderl S, O'Brien S, Qiao W, et al. Prognostic significance of 11q23 aberrations in adult acute myeloid leukemia and the role of allogeneic stem cell transplantation. *Leukemia*. 2013;27(4):836–42.
200. Mrózek K, Heinonen K, Lawrence D, Carroll AJ, Koduru PRK, Rao KW, et al. Adult Patients With De Novo Acute Myeloid Leukemia and t(9; 11)(p22; q23) Have a Superior Outcome to Patients With Other Translocations Involving Band 11q23: A Cancer and Leukemia Group B Study. *Blood*. 1997;90(11):4532–8.
201. Martinez-Climent JA, Espinosa R, Thirman MJ, Le Beau MM, Rowley JD. Abnormalities of chromosome band 11q23 and the MLL gene in pediatric myelomonocytic and monoblastic

- leukemias. Identification of the t(9;11) as an indicator of long survival. *J Pediatr Hematol Oncol*. 1995;17(4):277–83.
202. Harrison CJ, Hills RK, Moorman A V., Grimwade DJ, Hann I, Webb DKH, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. *J Clin Oncol*. 2010;28(16):2674–81.
 203. Issa GC, Zarka J, Sasaki K, Qiao W, Pak D, Ning J, et al. Predictors of outcomes in adults with acute myeloid leukemia and KMT2A rearrangements. *Blood Cancer J*. 2021;11(9):162.
 204. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102(7):2395–402.
 205. Bill M, Mrózek K, Kohlschmidt J, Eisfeld AK, Walker CJ, Nicolet D, et al. Mutational landscape and clinical outcome of patients with de novo acute myeloid leukemia and rearrangements involving 11q23/KMT2A. *Proc Natl Acad Sci U S A*. 2020;117(42):26340–6.
 206. Matsuo H, Yoshida K, Nakatani K, Harata Y, Higashitani M, Ito Y, et al. Fusion partner-specific mutation profiles and KRAS mutations as adverse prognostic factors in MLL-rearranged AML. *Blood Adv*. 2020;4(19):4623–31.
 207. Grossmann V, Schnittger S, Poetzinger F, Kohlmann A, Stiel A, Eder C, et al. High incidence of RAS signalling pathway mutations in MLL-rearranged acute myeloid leukemia. *Leukemia*. 2013;27(9):1933–6.
 208. Hyrenius-Wittsten A, Pilheden M, Stureson H, Hansson J, Walsh MP, Song G, et al. De novo activating mutations drive clonal evolution and enhance clonal fitness in KMT2A-rearranged leukemia. *Nat Commun*. 2018;9(1):1–13.
 209. Gökbüget N, Boissel N, Chiaretti S, Dombret H, Doubek M, Fielding A, et al. Diagnosis, prognostic factors, and assessment of ALL in adults: 2024 ELN recommendations from a European expert panel. *Blood*. 2024;143(19):1891–902.
 210. Döhner H, DiNardo CD, Appelbaum FR, Craddock C, Dombret H, Ebert BL, et al. Genetic risk classification for adults with AML receiving less-intensive therapies: the 2024 ELN recommendations. *Blood*. 2024;144(21):2169–73.
 211. Lachowiec CA, Ravikumar VI, Othman J, O’Nions J, Peters DT, McMahon C, et al. Refined ELN 2024 risk stratification improves survival prognostication following venetoclax-based therapy in AML. *Blood*. 2024;144(26):2788–92.

212. Löwenberg B, Pabst T, Maertens J, Gradowska P, Biemond BJ, Spertini O, et al. Addition of lenalidomide to intensive treatment in younger and middle-aged adults with newly diagnosed AML: the HOVON-SAKK-132 trial. *Blood Adv.* 2021;5(4):1110–21.
213. Ossenkoppele GJ, Breems DA, Stuessi G, van Norden Y, Bargetzi M, Biemond BJ, et al. Lenalidomide added to standard intensive treatment for older patients with AML and high-risk MDS. *Leukemia.* 2020;34(7):1751–9.
214. Löwenberg B, Pabst T, Maertens J, Van Norden Y, Biemond BJ, Schouten HC, et al. Therapeutic value of clofarabine in younger and middle-aged (18-65 years) adults with newly diagnosed AML. *Blood.* 2017;129(12):1636–45.
215. Straube J, Ling VY, Hill GR, Lane SW. The impact of age, NPM1mut, and FLT3ITD allelic ratio in patients with acute myeloid leukemia. *Blood.* 2018;131(10):1148–53.
216. Garg S, Reyes-Palomares A, He L, Bergeron A, Lavallée VP, Lemieux S, et al. Hepatic leukemia factor is a novel leukemic stem cell regulator in DNMT3A, NPM1, and FLT3-ITD triple-mutated AML. *Blood.* 2019;134(3):263–76.
217. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *N Engl J Med.* 2017;377(5):454–64.
218. Cocciardi S, Dolnik A, Kapp-Schwoerer S, Rücker FG, Lux S, Blätte TJ, et al. Clonal evolution patterns in acute myeloid leukemia with NPM1 mutation. *Nat Commun.* 2019;10(1):.
219. Chan SM, Majeti R. Role of DNMT3A, TET2, and IDH1/2 mutations in pre-leukemic stem cells in acute myeloid leukemia. *Int J Hematol.* 2013;98(6):648–57.
220. Glass JL, Hassane D, Wouters BJ, Kunimoto H, Avellino R, Garrett-Bakelman FE, et al. Epigenetic Identity in AML Depends on Disruption of Nonpromoter Regulatory Elements and Is Affected by Antagonistic Effects of Mutations in Epigenetic Modifiers. *Cancer Discov.* 2017;7(8):868–83.
221. Wang Y, Quesada AE, Zuo Z, Medeiros LJ, Yin CC, Li S, et al. The Impact of Mutation of Myelodysplasia-Related Genes in De Novo Acute Myeloid Leukemia Carrying NPM1 Mutation. *Cancers (Basel).* 2022;15(1):198.
222. Chan O, Al Ali N, Tashkandi H, Ellis A, Ball S, Grenet J, et al. Mutations highly specific for secondary AML are associated with poor outcomes in ELN favorable risk NPM1-mutated AML. *Blood Adv.* 2024;8(5):1075–83.

223. Zhou Q, Zhao D, Zarif M, Yeung YWT, Richard-Carpentier G, Chang H. Impact of secondary-type mutations in NPM1 mutated AML. *Eur J Haematol*. 2023;111(1):165–8.
224. Wright MF, Pozdnyakova O, Hasserjian RP, Aggarwal N, Shaver AC, Weinberg OK, et al. Secondary-type mutations do not impact prognosis in acute myelogenous leukemia AML with mutated NPM1. *Am J Hematol*. 2022;97(12):E462–5.
225. Eckardt JN, Bill M, Rausch C, Metzeler K, Spiekermann K, Stasik S, et al. Secondary-type mutations do not impact outcome in NPM1-mutated acute myeloid leukemia – implications for the European LeukemiaNet risk classification. *Leukemia*. 2023;37(11):2282–5.
226. Cocciardi S, Saadati M, Weiß N, Späth D, Kapp-Schwoerer S, Schneider I, et al. Impact of myelodysplasia-related and additional gene mutations in intensively treated patients with NPM1-mutated AML. *Hemasphere*. 2025;9(1):e70060.
227. Paltiel O, Ratnasingam S, Lee HP. Are we ignoring sex differences in haematological malignancies? A call for improved reporting. *Br J Haematol*. 2025;Online ahead of print.
228. Stabellini N, Tomlinson B, Cullen J, Shanahan J, Waite K, Montero AJ, et al. Sex differences in adults with acute myeloid leukemia and the impact of sex on overall survival. *Cancer Med*. 2023;12(6):6711–21.
229. Ozga M, Nicolet D, Mrózek K, Yilmaz AS, Kohlschmidt J, Larkin KT, et al. Sex-associated differences in frequencies and prognostic impact of recurrent genetic alterations in adult acute myeloid leukemia (Alliance, AMLCG). *Leukemia*. 2023;38(1):45–57.
230. Tettero JM, Cloos J, Bullinger L. Acute myeloid leukemia: does sex matter? *Leukemia*. 2024;38(11):2329–31.
231. Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood*. 2016;127(1):62–70.
232. Kim D, Herdeis L, Rudolph D, Zhao Y, Böttcher J, Vides A, et al. Pan-KRAS inhibitor disables oncogenic signalling and tumour growth. *Nature*. 2023;619(7968):160–6.
233. Hong DS, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, et al. KRASG12C Inhibition with Sotorasib in Advanced Solid Tumors. *N Engl J Med*. 2020;383(13):1207–17.
234. Tazi Y, Arango-Ossa JE, Zhou Y, Bernard E, Thomas I, Gilkes A, et al. Unified classification and risk-stratification in Acute Myeloid Leukemia. *Nat Commun*. 2022;13(1):1–16.

235. Esparza N, Bresson E, Safa-Tahar-Henni S, Defrene J, Roques É, Bonnet-Magnaval F, et al. Deciphering the Role of RAS Pathway Mutations in the Biology of Human Acute Myeloid Leukemia Using In Vivo Models. *Blood*. 2023;142(Supplement 1):4111.
236. Hoff FW, Blum W, Huang Y, Welkie RL, Swords R, Traer E, et al. Beat-AML 2024 ELN-refined risk stratification for older adults with newly diagnosed AML given lower-intensity therapy. *Blood Adv*. 2024;8(20):5297–305.
237. Gangat N, Elbeih A, Ghosoun N, McCullough K, Aperia F, Johnson IM, et al. Mayo Genetic Risk Models for Newly Diagnosed Acute Myeloid Leukemia Treated With Venetoclax + Hypomethylating Agent. *Am J Hematol*. 2025;100(2):260–71.

APÉNDICE SUPLEMENTARIO

MATERIAL SUPLEMENTARIO: CAPÍTULO 1

Leukemia

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ACUTE MYELOID LEUKEMIA

Rearrangements involving 11q23.3/*KMT2A* in adult AML: mutational landscape and prognostic implications – a HARMONY study

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SPRINGER NATURE

SUPPLEMENTARY TABLES

Table S1. List of centers and cooperative groups participating in the HARMONY Alliance AML database at the time of the analysis.

Center
AML Study Group
HOVON
University Hospital 12 de Octubre
AML Cooperative Group
Essen University Hospital
Medical University Wroclaw
CETLAM
Charité
MLL Munich Leukemia Laboratory
Queen's University of Belfast
Study Alliance Leukemia

Table S2. List of analyzed genes ordered by percentage of patients with the mutation.

Gene	Number of patients analyzed	Number of patients mutated	% patients with mutation
<i>NRAS</i>	186	39	21
<i>KRAS</i>	185	36	19.5
<i>FLT3-TKD</i>	166	22	13.3
<i>TP53</i>	185	16	8.6
<i>TET2</i>	185	15	8.1
<i>ASXL1</i>	185	13	7
<i>WT1</i>	171	12	7
<i>DNMT3A</i>	185	12	6.5
<i>FLT3-ITD</i>	190	11	5.8
<i>KDM5A</i>	51	2	3.9
<i>PTPN11</i>	182	7	3.8
<i>RUNX1</i>	187	7	3.7
<i>GATA2</i>	178	6	3.4
<i>SRSF2</i>	185	5	2.7
<i>ZRSR2</i>	184	5	2.7
<i>BCOR</i>	182	5	2.7
<i>SMC1A</i>	80	2	2.5
<i>BCORL1</i>	124	3	2.4
<i>IKZF1</i>	86	2	2.3
<i>RAD21</i>	182	4	2.2
<i>CDKN2A</i>	151	3	2
<i>ATRX</i>	51	1	2
<i>EP300</i>	51	1	2
<i>KMT2C</i>	51	1	2
<i>NF1</i>	51	1	2
<i>MYC</i>	52	1	1.9
<i>STAG2</i>	178	3	1.7
<i>BRAF</i>	177	3	1.7
<i>IDH2</i>	185	3	1.6
<i>HRAS</i>	72	1	1.4
<i>CBLB</i>	85	1	1.2
<i>CBL</i>	185	2	1.1
<i>EZH2</i>	185	2	1.1
<i>SF3B1</i>	185	2	1.1
<i>U2AF1</i>	185	2	1.1
<i>ETV6</i>	180	2	1.1
<i>KDM6A</i>	180	2	1.1
<i>KMT2A</i>	89	1	1.1
<i>NPM1</i>	202	2	1
<i>SF3A1</i>	139	1	0.7
<i>CUX1</i>	170	1	0.6
<i>IDH1</i>	185	1	0.5
<i>JAK2</i>	185	1	0.5

<i>KIT</i>	185	1	0.5
<i>MPL</i>	185	0	0
<i>PHF6</i>	181	0	0
<i>FBXW7</i>	171	0	0
<i>U2AF2</i>	136	0	0
<i>PTEN</i>	127	0	0
<i>CSF3R</i>	84	0	0
<i>SETBP1</i>	76	0	0
<i>NOTCH1</i>	73	0	0
<i>SMC3</i>	73	0	0
<i>GATA1</i>	58	0	0
<i>SH2B3</i>	55	0	0
<i>PRPF40B</i>	54	0	0
<i>SF1</i>	54	0	0
<i>ABL1</i>	51	0	0
<i>CREBBP</i>	51	0	0
<i>GNAS</i>	51	0	0
<i>KMT2D</i>	51	0	0
<i>KMT2E</i>	51	0	0
<i>RB1</i>	51	0	0
<i>CALR</i>	44	0	0
<i>ASXL2</i>	39	0	0
<i>ABCB1</i>	38	0	0
<i>ABCG2</i>	38	0	0
<i>BRINP3</i>	38	0	0
<i>DCK</i>	38	0	0
<i>DCLK1</i>	38	0	0
<i>DIS3</i>	38	0	0
<i>HNRNPK</i>	38	0	0
<i>JAK1</i>	38	0	0
<i>JAK3</i>	38	0	0
<i>MIR142</i>	38	0	0
<i>PTPRT</i>	38	0	0
<i>TERC</i>	38	0	0
<i>WAC</i>	38	0	0
<i>CBLC</i>	20	0	0
<i>EPOR</i>	3	0	0
<i>VHL</i>	3	0	0
<i>BRCC3</i>	1	0	0
<i>CCND1</i>	1	0	0
<i>CCND2</i>	1	0	0
<i>DHX15</i>	1	0	0
<i>GIGYF2</i>	1	0	0
<i>MGA</i>	1	0	0

Table S3. Induction treatments for *KMT2Ar* AML patients included in this study.

Induction treatment		% of patients
Cytarabine + anthracycline only ("7+3" or similar)		19
Cytarabine + anthracycline + additional drug	Etoposide	35
	Thioguanine	14
	Clofarabine	7
Cytarabine + mitoxantrone		15
Other treatments		10

Table S4. Comparison of patient characteristics among different *KMT2A* translocation partners. Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; WBC, white blood cell; ELN, European LeukemiaNet; HSCT, Hematopoietic Stem Cell Transplantation; CI, confidence interval

	Global cohort (n=205)	t(9;11) (n=101)	t(11;19) (n=33)	t(6;11) (n=24)	t(10;11) (n=10)	t(11;17) (n=10)	Other <i>KMT2A</i> (n=27)	p-value
Female sex	111 (54.1%)	54 (53.5%)	19 (57.6%)	14 (58.3%)	4 (40%)	6 (60%)	14 (51.9%)	0.930
Median age in years (range)	48.1 [18.6 - 86.2]	48 [18.6 - 86.2]	45 [20.4 - 77.1]	41 [20.3 - 66]	45.2 [21 - 57.5]	52.5 [38 - 68]	55.9 [27 - 83.3]	0.004
Age ≥60 years	51 (24.9%)	29 (28.7%)	7 (21.2%)	3 (12.5%)	-	2 (20%)	10 (37%)	0.127
AML type								
De novo AML	148 (72.2%)	66 (65.3%)	23 (69.7%)	24 (100%)	7 (70%)	8 (80%)	20 (74.1%)	
Secondary AML	57 (27.8%)	35 (34.7%)	10 (30.3%)	-	3 (30%)	2 (20%)	7 (25.9%)	
AML after MDS	14 (6.8%)	4 (4%)	4 (12.1%)	-	2 (20%)	-	4 (14.8%)	0.058
Therapy-related AML	43 (21%)	31 (30.7%)	6 (18.2%)	-	1 (10%)	2 (20%)	3 (11.1%)	0.012
Hemoglobin (g/dL)	9.5 [Q1 = 8.3, Q3 = 11.3]	9.8 [Q1 = 8.6, Q3 = 11.9]	9.45 [Q1 = 7.9, Q3 = 10]	10.6 [Q1 = 9, Q3 = 11.9]	10.8 [Q1 = 8.8, Q3 = 12]	11 [Q1 = 9.2, Q3 = 11.3]	8.7 [Q1 = 8, Q3 = 9.8]	0.171
WBC (x10⁹/L)	23.1 [Q1 = 4.7, Q3 = 58]	31.6 [Q1 = 5, Q3 = 49.1]	30 [Q1 = 6.4, Q3 = 69.7]	50 [Q1 = 21.7, Q3 = 82.7]	9.8 [Q1 = 3.1, Q3 = 44.8]	12.5 [Q1 = 4.2, Q3 = 17.5]	8 [Q1 = 2.3, Q3 = 24.7]	0.087
(> 100 x10 ⁹ /L)	21 (10.2%)	11 (10.9%)	3 (9.1%)	4 (16.7%)	-	-	3 (11.1%)	0.613
Platelets (x10⁹/L)	51.5 [Q1 = 29.8, Q3 = 107.5]	49.5 [Q1 = 32, Q3 = 93.8]	88 [Q1 = 36, Q3 = 140.5]	39.5 [Q1 = 19.8, Q3 = 89.2]	109 [Q1 = 60, Q3 = 155]	76.5 [Q1 = 38.2, Q3 = 207]	30 [Q1 = 19.5, Q3 = 61.5]	0.016
Bone marrow % of blasts	83 [Q1 = 70.2, Q3 = 90]	86 [Q1 = 80, Q3 = 95]	71.5 [Q1 = 48.2, Q3 = 79.8]	89 [Q1 = 80.5, Q3 = 90]	85.25 [Q1 = 74, Q3 = 91.9]	67.5 [Q1 = 47.5, Q3 = 82.5]	80 [Q1 = 52.2, Q3 = 89.5]	0.001
Treatment								
Intensive	195 (95%)	94 (93%)	33 (100%)	24 (100%)	10 (100%)	10 (100%)	24 (89%)	
Non-intensive	10 (5%)	7 (7%)	-	-	-	-	3 (11%)	
Composite complete response (information provided for n=188)	120 (63.8%)	57 (60%)	18 (69.2%)	14 (63.6%)	9 (90%)	6 (66.6%)	16 (61.5%)	
Early death								
30-day mortality	28 (13.6%)	21 (20.8%)	3 (9.1%)	3 (12.5%)	-	-	1 (3.7%)	
60-day mortality	36 (17.6%)	23 (22.8%)	4 (12.1%)	5 (20.8%)	-	-	4 (14.8%)	
Allogeneic HSCT	88 (42.9%)	38 (37.6%)	17 (51.5%)	13 (54.2%)	5 (50%)	5 (50%)	10 (37%)	0.524
Median survival in years (95% CI)	1.4 (1.1 - 1.7)	1.4 (0.98 - 2.4)	1.2 (0.95 - NA)	1.7 (1.04 - NA)	1.7 (0.85 - NA)	2.9 (1.27 - NA)	1.0 (0.6 - 1.95)	

SUPPLEMENTARY FIGURES

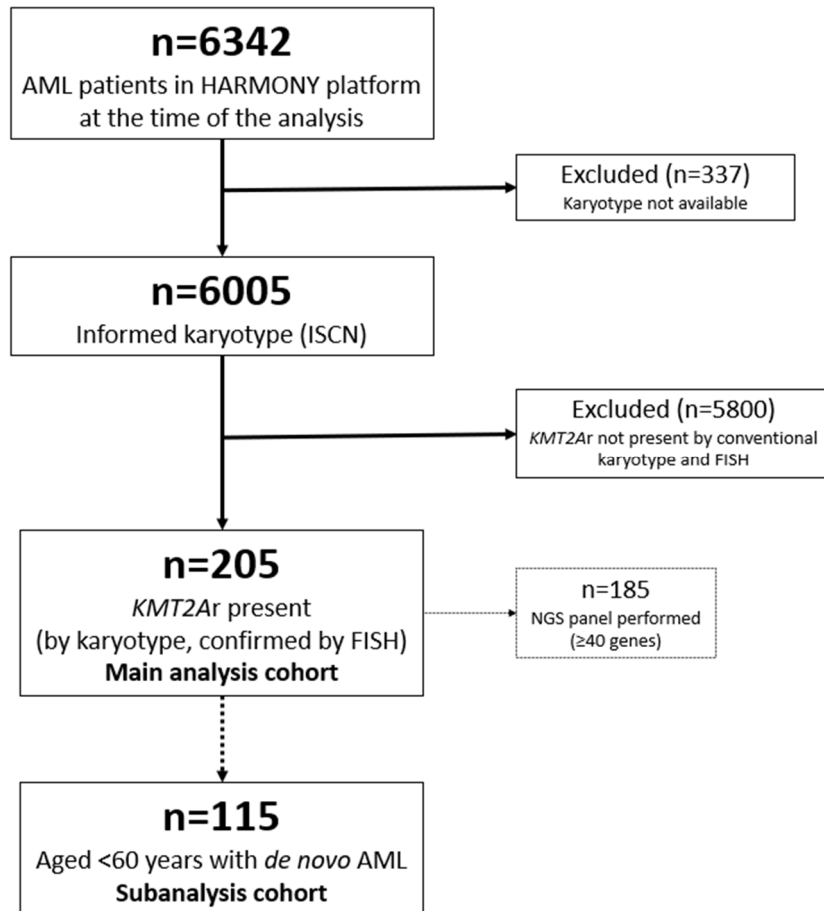


Figure S1. Selection criteria of the patients included in this retrospective analysis. AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; ISCN, International System for Human Cytogenomic Nomenclature; NGS, next-generation sequencing.

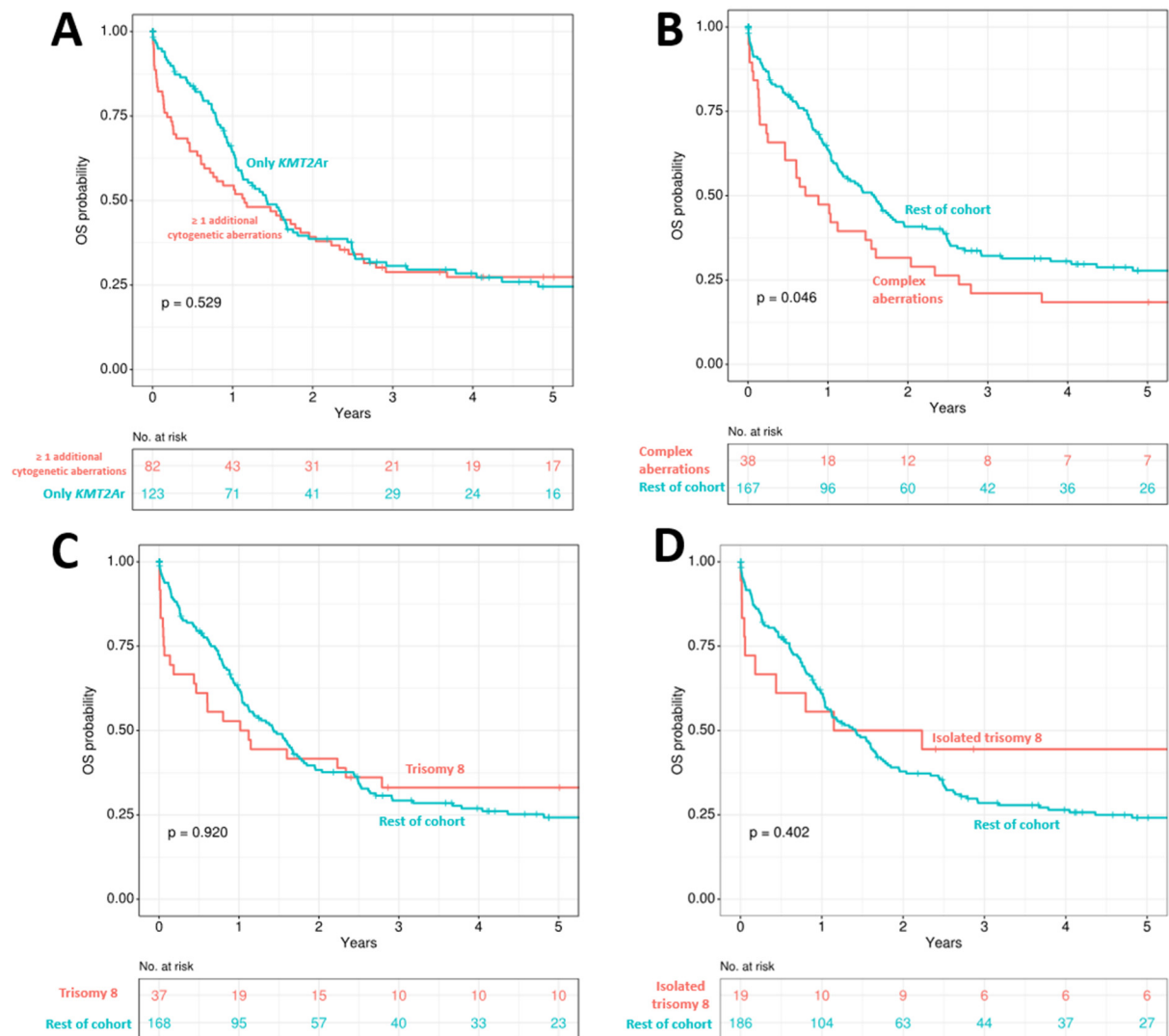


Figure S2. Impact of additional cytogenetic aberrations in overall survival (OS) of *KMT2Ar* AML patients: **(A)** Presence of any additional alteration vs no other alteration apart from *KMT2Ar*, **(B)** Complex karyotype (2 or more additional cytogenetic aberrations apart from *KMT2Ar*), **(C)** Trisomy 8 with any concomitant cytogenetic aberration, **(D)** Trisomy 8 as the sole cytogenetic abnormality apart from *KMT2Ar*.

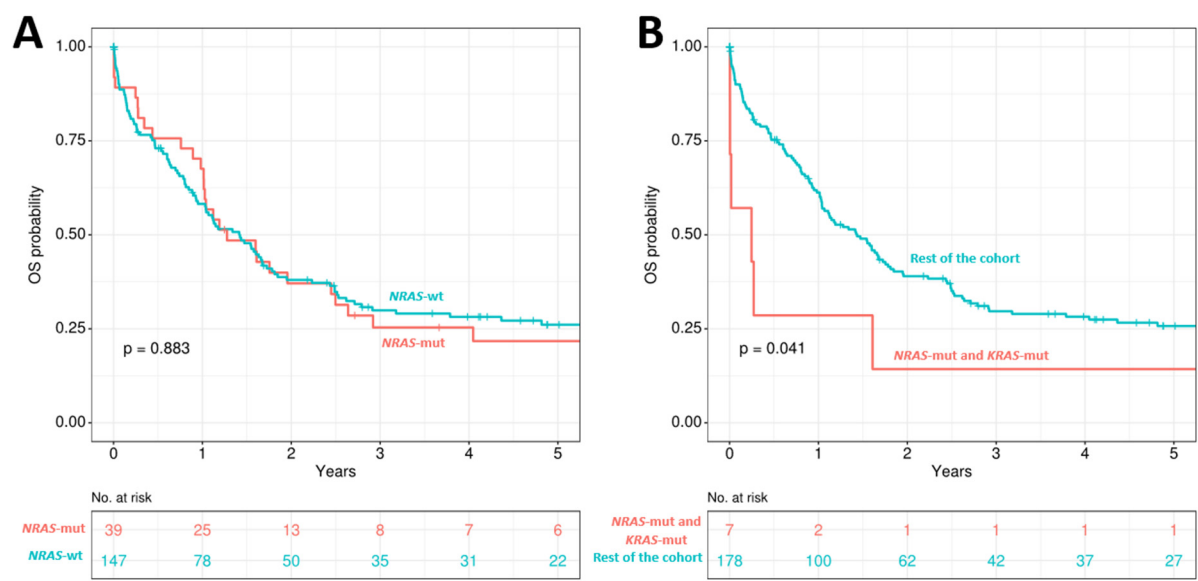


Figure S3. Comparison of overall survival regarding mutational status of *NRAS*: **(A)** *NRAS*-mut vs rest of the cohort, **(B)** Both *NRAS*-mut and *KRAS*-mut vs rest of the cohort.

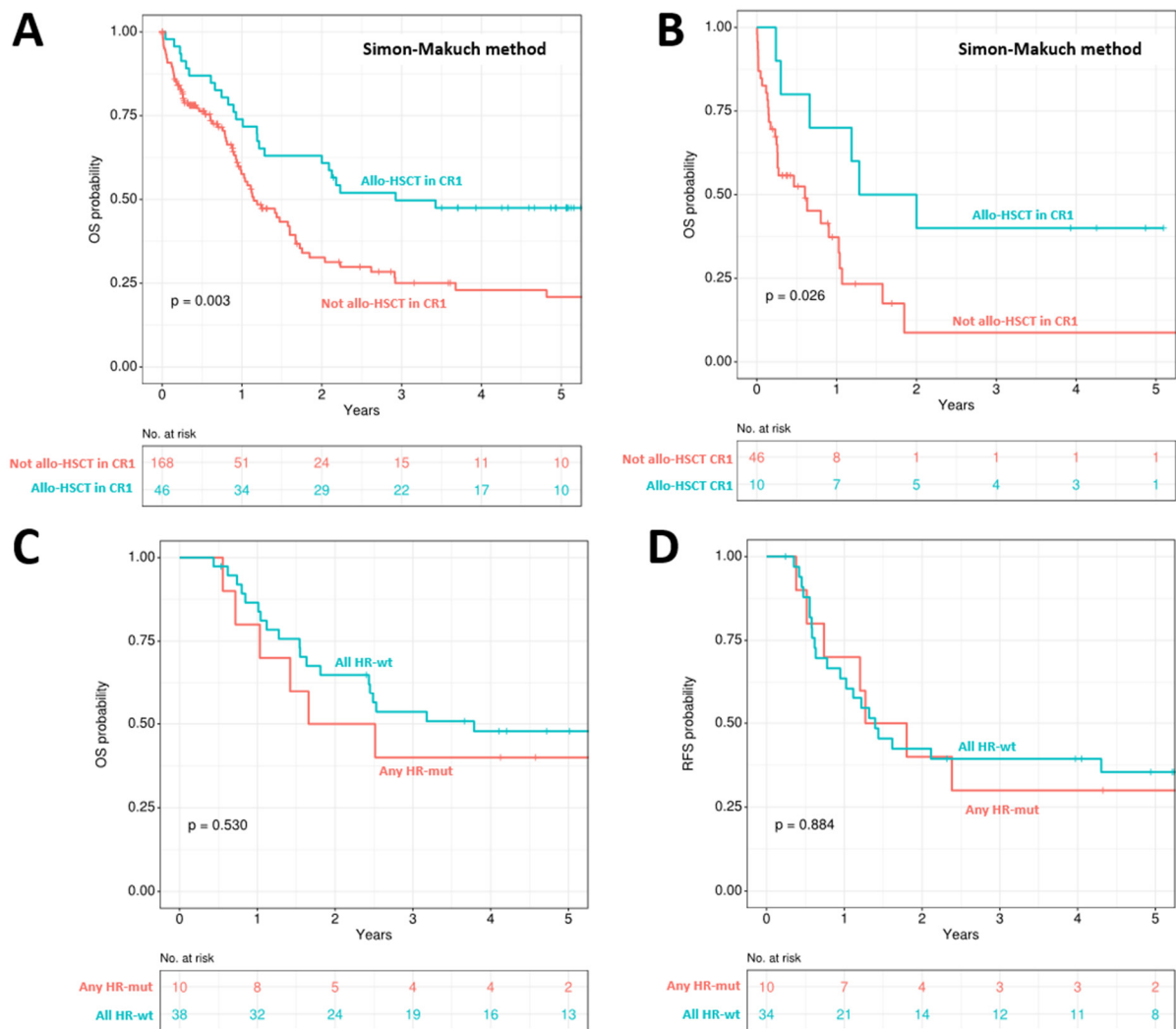


Figure S4. Impact of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in outcomes, limited to intensively-treated patients: **(A)** Survival estimations using Simon-Makuch method with clock-back correction comparing patients who underwent allo-HSCT in CR1 and those who did not **(B)** Same analysis, limited to patients with *KRAS*-mut, *TP53*-mut or *DNMT3A*-mut **(C)** Comparison of overall survival for patients that received allo-HSCT in CR1 **(D)** Comparison of relapse-free survival for patients that received allo-HSCT in CR1. HR, high-risk mutations (*KRAS*, *TP53*, *DNMT3A*).

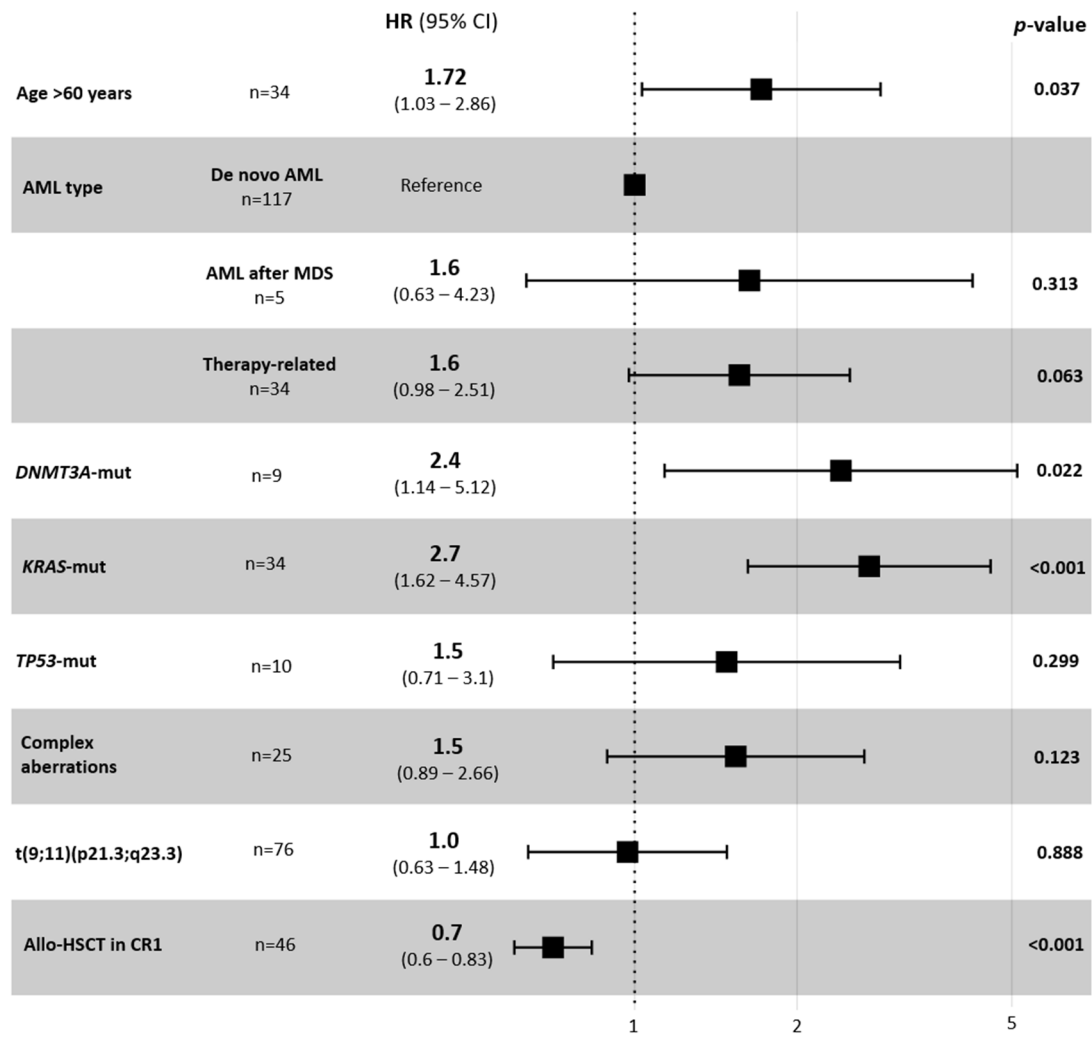


Figure S5. Multivariate Cox regression model for prediction of overall survival in intensively-treated patients with allo-HSCT in CR1 as time-depending variable. HR, hazard ratio; CI, confidence interval; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

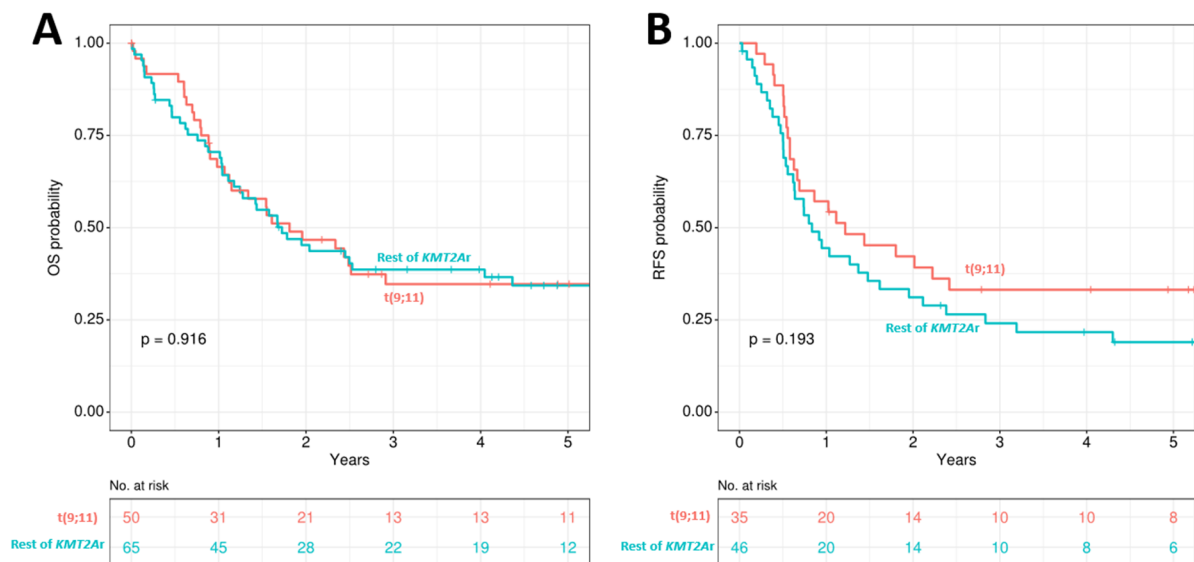


Figure S6. Comparison of survival outcomes between *t(9;11)* and the rest of the subset, limited to patients aged <60 years with de novo AML who received intensive chemotherapy: **(A)** Overall survival, **(B)** Relapse-free survival.

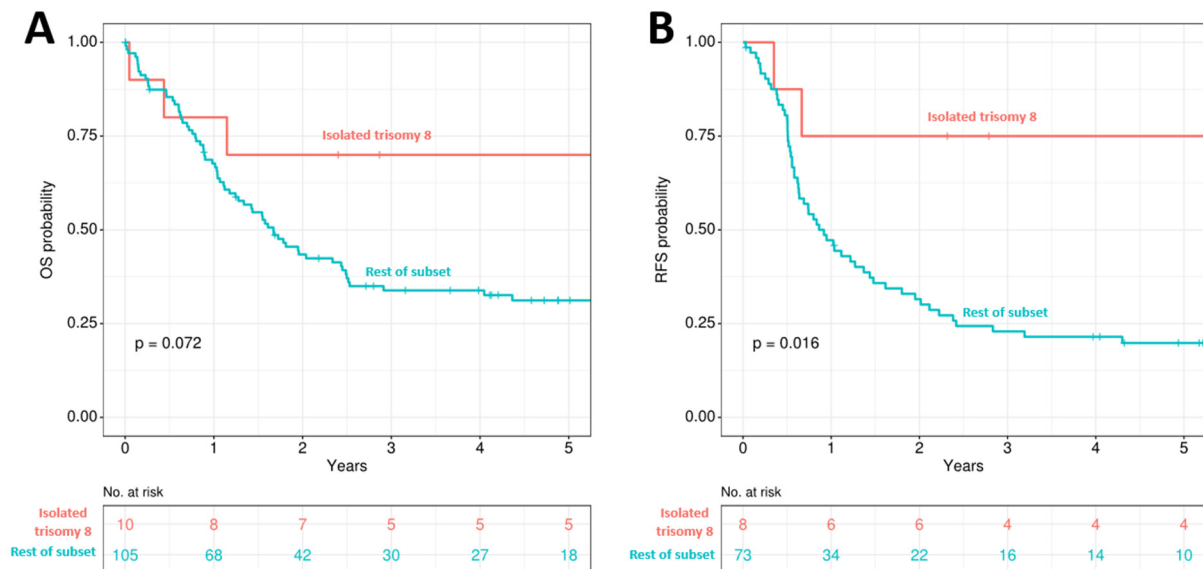


Figure S7. Comparison of survival outcomes between isolated trisomy 8 as the only additional cytogenetic aberration (apart from *KMT2Ar*) and the rest of the subset, limited to patients aged <60 years with de novo AML who received intensive chemotherapy: **(A)** Overall survival, **(B)** Relapse-free survival.

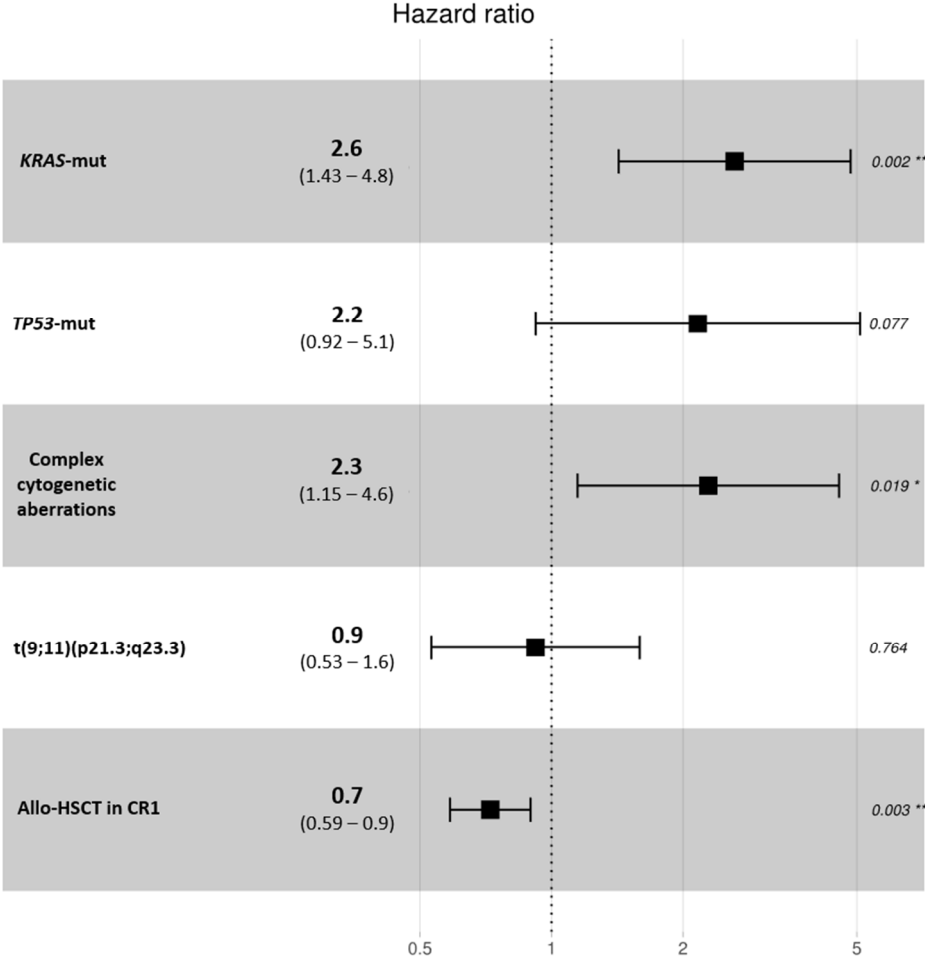


Figure S8. Multivariable Cox regression model for prediction of overall survival in patients with *de novo* AML aged <60 years at diagnosis, with allo-HSCT in CR1 as time-dependent variable. HR, hazard ratio; CI, confidence interval.

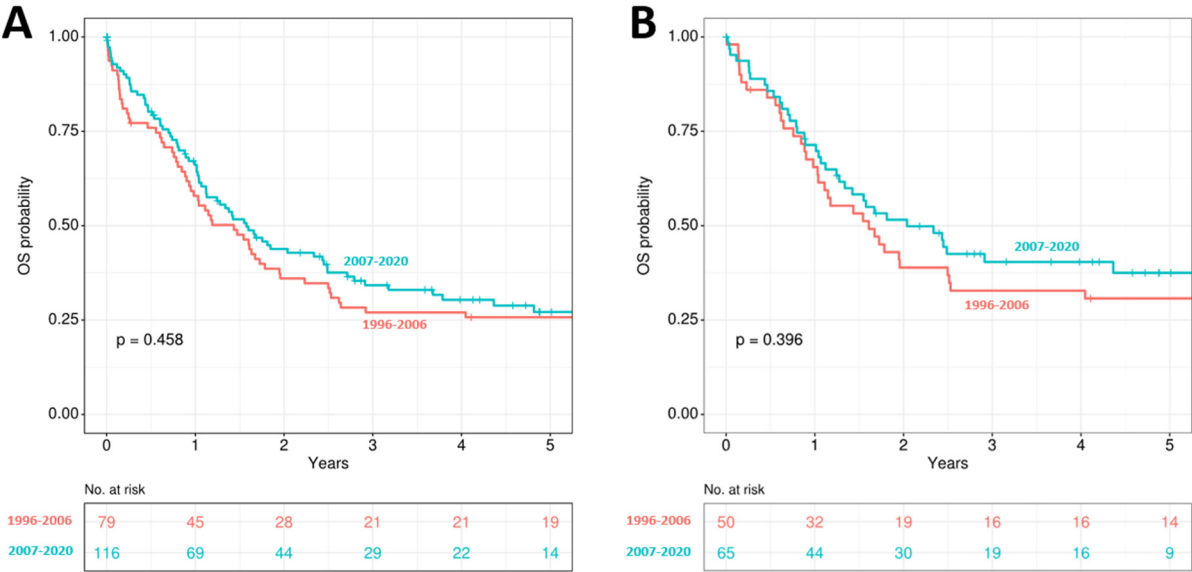


Figure S9. Comparison of survival outcomes when stratifying by year of AML diagnosis: **(A)** Intensively-treated *KMT2Ar* AML patients, **(B)** Subset of patients aged <60 years with *de novo* AML.

MATERIAL SUPLEMENTARIO: CAPÍTULO 2

Unravelling Co-Mutational Patterns with Prognostic Implications in *NPM1* Mutated Adult Acute Myeloid Leukemia – A HARMONY Study

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SUPPLEMENTARY METHODS

***NPM1*-mut risk stratification**

A multi-step analysis of clinically significant gene co-mutations associated to *NPM1*-mut was performed. At each step, combinations of up to two additional genes (either mutated or wildtype) that were present in at least 10 patients were explored. While late relapses in *NPM1*-mut AML have been reported, patient prognosis is mostly determined within the first 2 years from disease diagnosis in this AML subtype¹⁻³. In fact, in HARMONY training cohort there was <10% difference between 2-year OS (62.6%) and 5-year OS (53.5%) and an increasing number of follow-up losses between those timepoints (**Supplementary Figure S19**), so 2-year OS was selected for outcome comparison. The 2-year-OS for each combination was estimated using 100-fold bootstrap sampling and compared to the 2-year OS of *NPM1* wildtype (-wt) patients in the same dataset. Cloglog transformation of survival curves was used for testing differences at this fixed 2-year timepoint⁴. Only combinations with statistically significant differences (cloglog p-value <0.05) compared to their respective opposites, in at least 60% of bootstrap sampling comparisons, were initially selected and represented in **Supplementary Figures S1, S5, S8, S10, S13 and S16**. Finally, gene mutation combinations that allowed patient reclassification into different European LeukemiaNet (ELN) 2022 risk categories were selected⁵. When multiple combinations could lead into patient reclassification, the most representative (i.e. with higher number of patients) was selected.

In the first step, combinations that met the aforementioned criteria are depicted in **Supplementary Figure S1**. Baseline cohort (i.e. all *NPM1*-mut) presented a 2-year OS between ELN2022 favorable-risk and ELN2022 intermediate-risk for *NPM1*-wt in the same dataset. However, patients with *FLT3*-ITD and *DNMT3A* had a 2-year OS that was different to ELN2022 intermediate-risk (cloglog p-value <0.001) and closer to ELN2022 adverse-risk patients (cloglog p-value 0.834), so this combination was selected. The deleterious effect of *DNMT3A*-mut was confirmed when the subset with *NPM1*-mut and *FLT3*-ITD was analyzed (**Supplementary Figure S2**) and therefore triple-mutated patients (i.e. *NPM1*-mut, *FLT3*-ITD and *DNMT3A*-mut) were classified as “*NPM1* adverse”.

Subsequently, the subset with *NPM1*-mut, *FLT3*-ITD and *DNMT3A*-wt was evaluated (**Supplementary Figure S5**). This subset had a 2-year OS between ELN2022 favorable-risk and ELN2022 intermediate-risk, but *IDH*-mut patients had a 2-year OS that was distinct to ELN2022 intermediate-risk (cloglog p-value 0.013) and was closer to ELN2022 favorable-risk (cloglog p-value 0.409) and were therefore classified as “*NPM1* favorable”. Conversely, *IDH*-wt patients had a 2-year OS that was different from ELN2022 favorable-risk (cloglog p-value <0.001) and closer to ELN2022 intermediate-risk (cloglog p-value 0.913) and were therefore classified as “*NPM1* intermediate”. Similar methodology was applied to classify the remainder of *NPM1*-mut patients (**Supplementary Figures S8, S10 and S13**).

References

1. Bertoli S, Tavitian S, Bérard E, et al: More than ten percent of relapses occur after five years in AML patients with *NPM1* mutation. *Leuk Lymphoma* 61:1226–1229, 2020
2. Giupponi C, Bertoli D, Borlenghi E, et al: Myeloid neoplasm occurrence during stable molecular remission of *NPM1*-mutated AML: are we facing secondary disease or AML relapse? *Blood Cancer J* 13, 2023
3. Othman J, Potter N, Ivey A, et al: Molecular, clinical, and therapeutic determinants of outcome in *NPM1*-mutated AML. *Blood* 144:714–728, 2024
4. Klein JP, Logan B, Harhoff M, et al: Analyzing survival curves at a fixed point in time. *Stat Med* 26:4505–4519, 2007
5. Döhner H, Wei AH, Appelbaum FR, et al: Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 140:1345–1377, 2022

SUPPLEMENTARY TABLES

Table S1. List of genes included in both NGS panels of the training cohort.

Genes included in both NGS panels
<i>ASXL1</i>
<i>BCOR</i>
<i>DNMT3A</i>
<i>EZH2</i>
<i>FLT3</i>
<i>IDH1</i>
<i>IDH2</i>
<i>JAK2</i>
<i>KIT</i>
<i>KRAS</i>
<i>NPM1</i>
<i>NRAS</i>
<i>PTEN</i>
<i>PTPN11</i>
<i>RUNX1</i>
<i>SF3B1</i>
<i>SRSF2</i>
<i>STAG2</i>
<i>TET2</i>
<i>TP53</i>
<i>U2AF1</i>
<i>WT1</i>
<i>ZRSR2</i>
<i>CEBPA</i>

Table S2. Baseline characteristics training cohort, comparing patients with *NPM1* mutation (*NPM1*-mut) to patients with *NPM1* wildtype (*NPM1*-wt).

	<i>NPM1</i>-mut training cohort (n=1001)	<i>NPM1</i>-wt training cohort (n=2473)	p-value
Female sex	543 (54.2%)	1016 (41.1%)	<0.001
Median age in years (range)	52.9 (18 - 81)	55 [18 - 84.5]	0.025
Age ≥60 years	269 (26.8%)	763 (30.9%)	0.022
AML type			
De novo AML	963 (96.2%)	2198 (88.9%)	<0.001
Secondary AML	38 (3.8%)	275 (11.1%)	<0.001
Prior HM	15 (1.5%)	156 (6.3%)	
Therapy-related AML	23 (2.3%)	119 (4.8%)	
Hemoglobin (g/dL)	8.9 [Q1 = 7.6, Q3 = 10.3]	9.1 [Q1 = 7.8, Q3 = 10.3]	0.3062
WBC (x10⁹/L)	23.8 [Q1 = 6.9, Q3 = 62.8]	6.2 [Q1 = 2.2, Q3 = 27.2]	<0.001
(WBC > 100 x10 ⁹ /L)	136 (13.6%)	140 (5.7%)	<0.001
Platelets (x10⁹/L)	66.5 [Q1 = 38, Q3 = 116]	57 [Q1 = 31, Q3 = 108]	<0.001
Bone marrow % of blasts	75 [Q1 = 45, Q3 = 89]	55 [Q1 = 30, Q3 = 80]	<0.001
ELN 2022			<0.001
Favorable	603 (60.2%)	335 (15%)	
Intermediate	391 (39%)	578 (26%)	
Adverse	7 (0.7%)	1332 (59%)	
FLT3-ITD	393 (39.3%)	316 (13%)	<0.001
Treatment response			
CRc	874 (87.3%)	2035 (82.3%)	<0.001
Refractory	89 (8.9%)	342 (13.8%)	<0.001
Not evaluable	38 (3.8%)	96 (3.9%)	1
Early death			
30-day mortality	38 (3.8%)	96 (3.9%)	1
60-day mortality	62 (6.2%)	226 (9.1%)	0.004
Allogeneic HSCT	341 (34.1%)	1130 (45.7%)	<0.001
In CR1	242 (24.2%)	998 (40%)	<0.001
In other situations	99 (9.9%)	132 (5.7%)	<0.001
Median survival in years (95% CI)	8.25 (5.14-9.77)	1.83 (1.69-2.05)	<0.001

Table S3. Univariable and multivariable analysis of overall survival in the training cohort, including allogeneic stem cell transplantation in first complete remission (allo-HSCT in CR1) as a time-dependent covariate.

	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
All patients (n=951)				
Age >60 years	2 (1.6-2.5)	<0.001	1.74 (1.42-2.15)	<0.001
WBC >100 (x10 ⁹ /L)	2.2 (1.7-2.9)	<0.001	1.40 (1.08-1.80)	0.011
Prior HM*	2.9 (1.38-6.2)	0.005	3.42 (1.82-6.44)	<0.001
Therapy-related AML*	1.2 (0.59-2.6)	0.573	1.52 (0.86-2.71)	0.153
<i>NPM1</i> Intermediate**	1.8 (1.4-2.4)	<0.001	1.90 (1.49-2.43)	<0.001
<i>NPM1</i> Adverse**	3.2 (2.5-4.1)	<0.001	3.32 (2.35-4.68)	<0.001
ELN2022 (Intermediate/Adverse vs Favorable)	2 (1.6-2.4)	<0.001	1.01 (0.75-1.35)	0.964
Allo-HSCT in CR1	0.9 (0.86-0.94)	<0.001	0.84 (0.81-0.89)	<0.001
<i>NPM1</i> Favorable (n=496)				
Age >60 years	2.3 (1.7-3.2)	<0.001	2.39 (1.73-3.30)	<0.001
WBC >100 (x10 ⁹ /L)	1.9 (1.1-3.3)	0.025	1.80 (1.07-3.03)	0.027
ELN2022 (Intermediate/Adverse vs Favorable)	0.87 (0.48-1.6)	0.646	0.85 (0.50-1.43)	0.536
Allo-HSCT in CR1	0.88 (0.82-0.94)	<0.001	0.88 (0.82-0.94)	<0.001
<i>NPM1</i> Intermediate (n=230)				
Age >60 years	1.8 (1.2-2.7)	0.007	1.70 (1.12-2.46)	0.011
WBC >100 (x10 ⁹ /L)	1.5 (0.94-2.5)	0.084	1.40 (0.87-2.21)	0.174
ELN2022 (Intermediate/Adverse vs Favorable)	1.1 (0.72-1.6)	0.724	1.00 (0.71-1.49)	0.891
Allo-HSCT in CR1	0.9 (0.83-0.97)	0.009	0.9 (0.83-0.98)	0.017
<i>NPM1</i> Adverse (n=225)				
Age >60 years	1.8 (1.2-2.7)	0.009	1.26 (0.86-1.85)	0.23
WBC >100 (x10 ⁹ /L)	1.7 (1.1-2.7)	0.012	1.26 (0.87-1.84)	0.22
Allo-HSCT in CR1	0.66 (0.57-0.76)	<0.001	0.66 (0.57-0.77)	<0.001

* De novo AML was used as reference

** *NPM1* Favorable was used as reference

Table S4. Univariable and multivariable analysis of overall survival in the internal validation cohort, including allogeneic stem cell transplantation in first complete remission (allo-HSCT in CR1) as a time-dependent covariate.

	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
All patients (n=620)				
Age >60 years	1.7 (1.3-2.1)	<0.001	1.53 (1.22-1.92)	<0.001
WBC >100 (x10 ⁹ /L)	2.1 (1.6-2.7)	<0.001	1.61 (1.25-2.08)	<0.001
Prior HM*	1 (0.67-1.6)	0.861	1.23 (0.83-1.83)	0.302
Therapy-related AML*	1.4 (0.64-3.2)	0.38	0.99 (0.41-2.42)	0.987
<i>NPM1</i> Intermediate**	1.5 (1.1-2)	0.005	1.14 (0.85-1.53)	0.365
<i>NPM1</i> Adverse**	2.6 (2-3.3)	<0.001	1.53 (1.07-2.19)	0.021
ELN2022 (Intermediate/Adverse vs Favorable)	2.1 (1.7-2.7)	<0.001	1.49 (1.09-2.03)	0.013
Allo-HSCT in CR1	0.91 (0.87-0.95)	<0.001	0.89 (0.85-0.93)	<0.001
<i>NPM1</i> Favorable (n=267)				
Age >60 years	2.1 (1.4-3)	<0.001	1.96 (1.35-2.80)	<0.001
WBC >100 (x10 ⁹ /L)	1.8 (1.1-3.1)	0.027	1.57 (0.93-2.60)	0.094
ELN2022 (Intermediate/Adverse vs Favorable)	1.4 (0.83-2.3)	0.21	1.18 (0.72-1.90)	0.508
Allo-HSCT in CR1	0.99 (0.94-1)	0.635	0.99 (0.94-1.00)	0.605
<i>NPM1</i> Intermediate (n=176)				
Age >60 years	1.5 (0.97-2.3)	0.07	1.24 (0.81-1.90)	0.322
WBC >100 (x10 ⁹ /L)	2.3 (1.4-3.6)	<0.001	1.72 (1.09-2.72)	0.02
ELN2022 (Intermediate/Adverse vs Favorable)	1.5 (0.97-2.3)	0.066	1.56 (1.02-2.38)	0.041
Allo-HSCT in CR1	0.85 (0.76-0.94)	0.002	0.84 (0.75-0.94)	0.003
<i>NPM1</i> Adverse (n=177)				
Age >60 years	1.7 (1.1-2.5)	0.01	1.42 (0.96-2.10)	0.082
WBC >100 (x10 ⁹ /L)	1.5 (0.99-2.2)	0.055	1.57 (1.08-2.30)	0.019
Allo-HSCT in CR1	0.79 (0.71-0.88)	<0.001	0.78 (0.69-0.88)	<0.001

* De novo AML was used as reference

** *NPM1* Favorable was used as reference

Table S5. Comparison of baseline patient characteristics between training cohort and external validation cohort.

	Training cohort (n=1001)	External validation cohort (n=585)	p-value
Female sex	543 (54.2%)	322 (55%)	0.7985
Median age in years (range)	52.9 (18 - 81)	56.4 (16 - 79)	<0.0001
Age ≥60 years	269 (26.8%)	224 (38.3%)	<0.0001
AML type			
De novo AML	963 (96.2%)	551 (94.2%)	0.0628
Secondary AML	38 (3.8%)	34 (5.8%)	
Prior HM	15 (1.5%)	28 (4.8%)	0.0001
Therapy-related	23 (2.3%)	6 (1%)	0.0681
Hemoglobin (g/dL)	8.9 [Q1 = 7.6, Q3 = 10.3]	9.3 [Q1 = 7.8, Q3 = 10.9]	0.01248
WBC (x10⁹/L)	23.8 [Q1 = 6.9, Q3 = 62.8]	32.6 [Q1 = 12.2, Q3 = 74.5]	<0.0001
(WBC > 100 x10 ⁹ /L)	136 (13.6%)	103 (17.6%)	0.0308
Platelets (x10⁹/L)	66.5 [Q1 = 38, Q3 = 116]	63 [Q1 = 38, Q3 = 106]	0.2769
BM % of blasts	75 [Q1 = 45, Q3 = 89]	72 [Q1 = 50, Q3 = 89]	0.7606
ELN 2022			
Favorable	603 (60.2%)	340 (58.1%)	
Intermediate	391 (39%)	241 (41.2%)	0.4018
Adverse	7 (0.7%)	4 (0.7%)	
FLT3-ITD	393 (39.3%)	243 (41.5%)	0.401
Treatment response			
CRc	874 (87.3%)	524 (89.6%)	
Refractory	89 (8.9%)	26 (4.4%)	0.0002
Not evaluable	38 (3.8%)	35 (6%)	
Early death			
30-day mortality	38 (3.8%)	35 (6%)	0.0449
60-day mortality	62 (6.2%)	40 (6.8%)	0.6143
Allogeneic HSCT	341 (34.1%)	157 (41.7%)*	0.0028
In CR1	242 (24.2%)	NA	
In other situations	99 (9.9%)	NA	
Median survival in years (95% CI)	8.25 (5.14-9.77)	4.48 (3.2 - 9.72)	<0.0001

* Information regarding allo-HSCT was provided for 378 patients in the external validation cohort

Table S6. Comparison of baseline patient characteristics between internal validation cohort and external validation cohort.

	Internal validation cohort (n=762)	External validation cohort (n=585)	p-value
Female sex	419 (55%)	322 (55%)	1
Median age in years (range)	57 (18 - 86)	56.4 (16 - 79)	0.0933
Age ≥60 years	320 (42%)	224 (38.3%)	0.1877
AML type			
De novo AML	573 (90.5%)	551 (94.2%)	0.0166
Secondary AML	60 (9.5%)	34 (5.8%)	
Prior HM	48 (7.6%)	28 (4.8%)	0.2331
Therapy-related	12 (1.6%)	6 (1%)	0.3843
Hemoglobin (g/dL)	9.2 [Q1 = 8.2, Q3 = 10.5]	9.3 [Q1 = 7.8, Q3 = 10.9]	0.8477
WBC (x10⁹/L)	36.8 [Q1 = 13.4, Q3 = 85.6]	32.6 [Q1 = 12.2, Q3 = 74.5]	0.154
(WBC > 100 x10 ⁹ /L)	144 (20.4%)	103 (17.6%)	0.5441
Platelets (x10⁹/L)	66.5 [Q1 = 38.2, Q3 = 111]	63 [Q1 = 38, Q3 = 106]	0.373
BM % of blasts	72.75 [Q1 = 52, Q3 = 87]	72 [Q1 = 50, Q3 = 89]	0.8679
ELN 2022			
Favorable	408 (53.5%)	340 (58.1%)	
Intermediate	347 (45.5%)	241 (41.2%)	0.0886
Adverse	7 (0.9%)	4 (0.7%)	
FLT3-ITD	349 (45.8%)	243 (41.5%)	0.1319
Treatment response			
CRc	606 (79.5%)	524 (89.6%)	
Refractory	95 (12.5%)	26 (4.4%)	<0.0001
Not evaluable	61 (8%)	35 (6%)	
Early death			
30-day mortality	61 (8%)	35 (6%)	0.1528
60-day mortality	88 (11.5%)	40 (6.8%)	0.0035
Allogeneic HSCT	215 (29.3%)	157 (41.7%)	0.0151
In CR1	130 (60.5%)	NA	
In other situations	85 (39.5%)	NA	
Median survival in years (95% CI)	2.84 (2.06 - 4.09)	4.48 (3.2 - 9.72)	<0.0001

SUPPLEMENTARY FIGURES

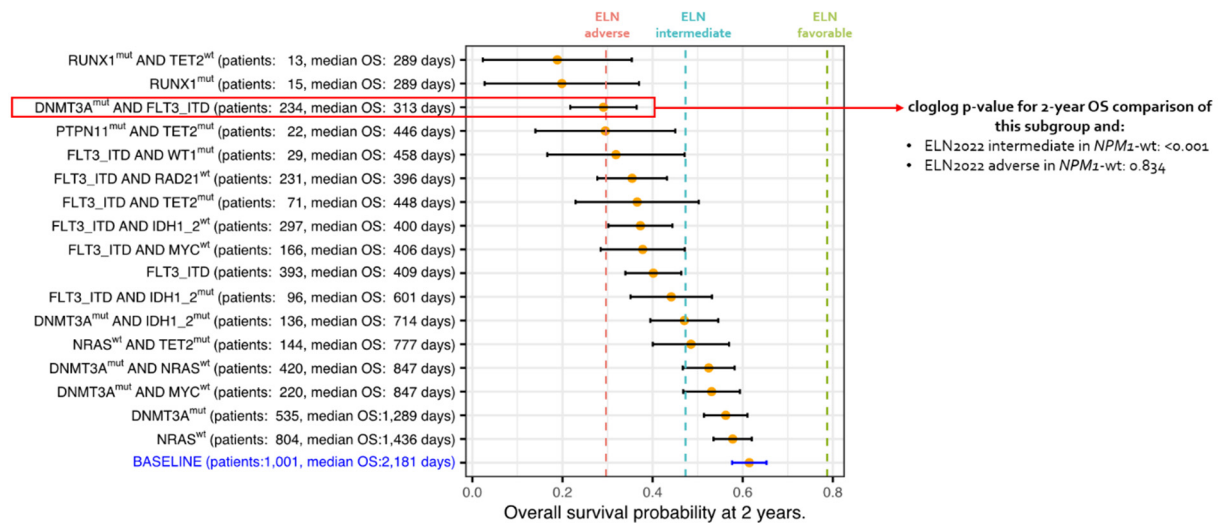


Figure S1. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut.

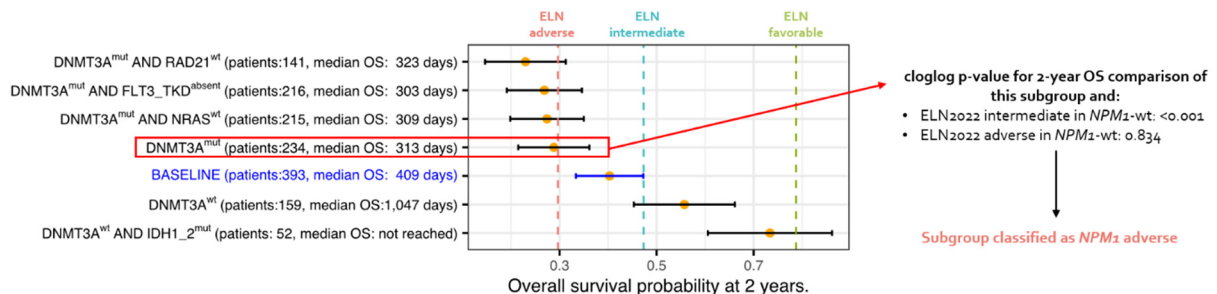


Figure S2. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut and *FLT3*-ITD.

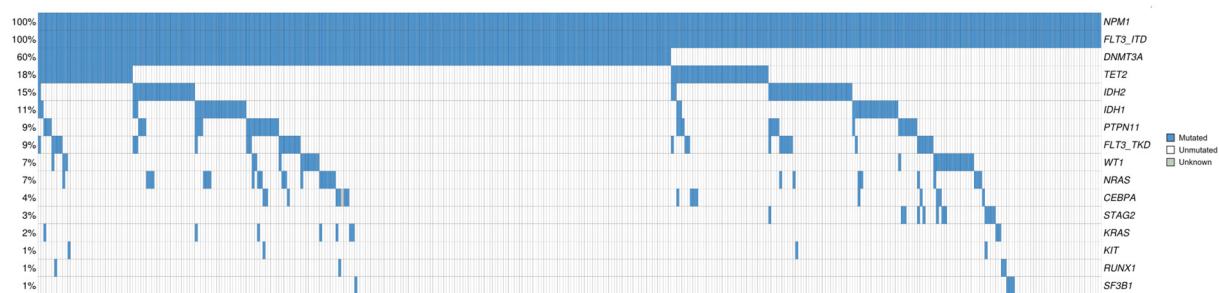


Figure S3. Co-mutational landscape of patients with *NPM1*-mut and *FLT3*-ITD.

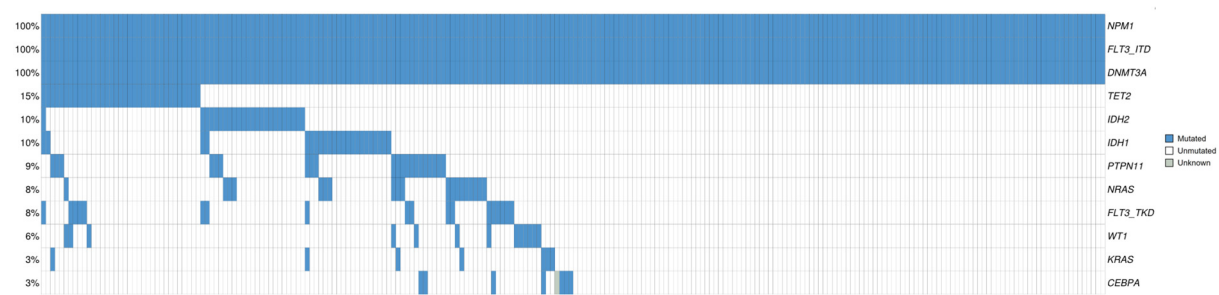


Figure S4. Co-mutational landscape of patients with *NPM1*-mut, *FLT3*-ITD and *DNMT3A*-mut.

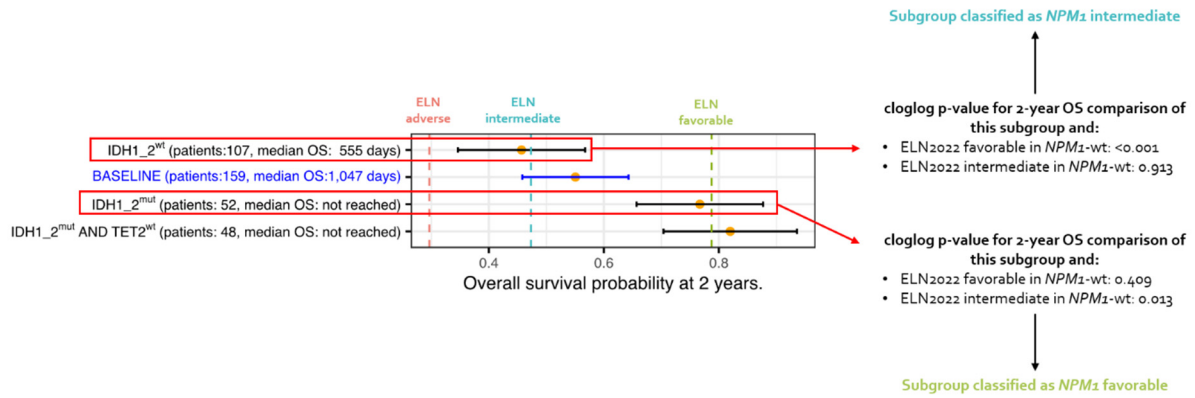


Figure S5. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut, *FLT3*-ITD and *DNMT3A*-wt.

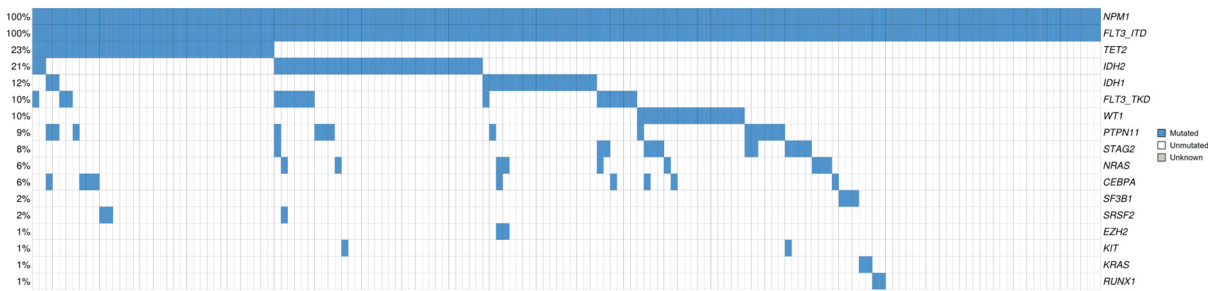


Figure S6. Co-mutational landscape of patients with *NPM1*-mut, *FLT3*-ITD and *DNMT3A*-wt.

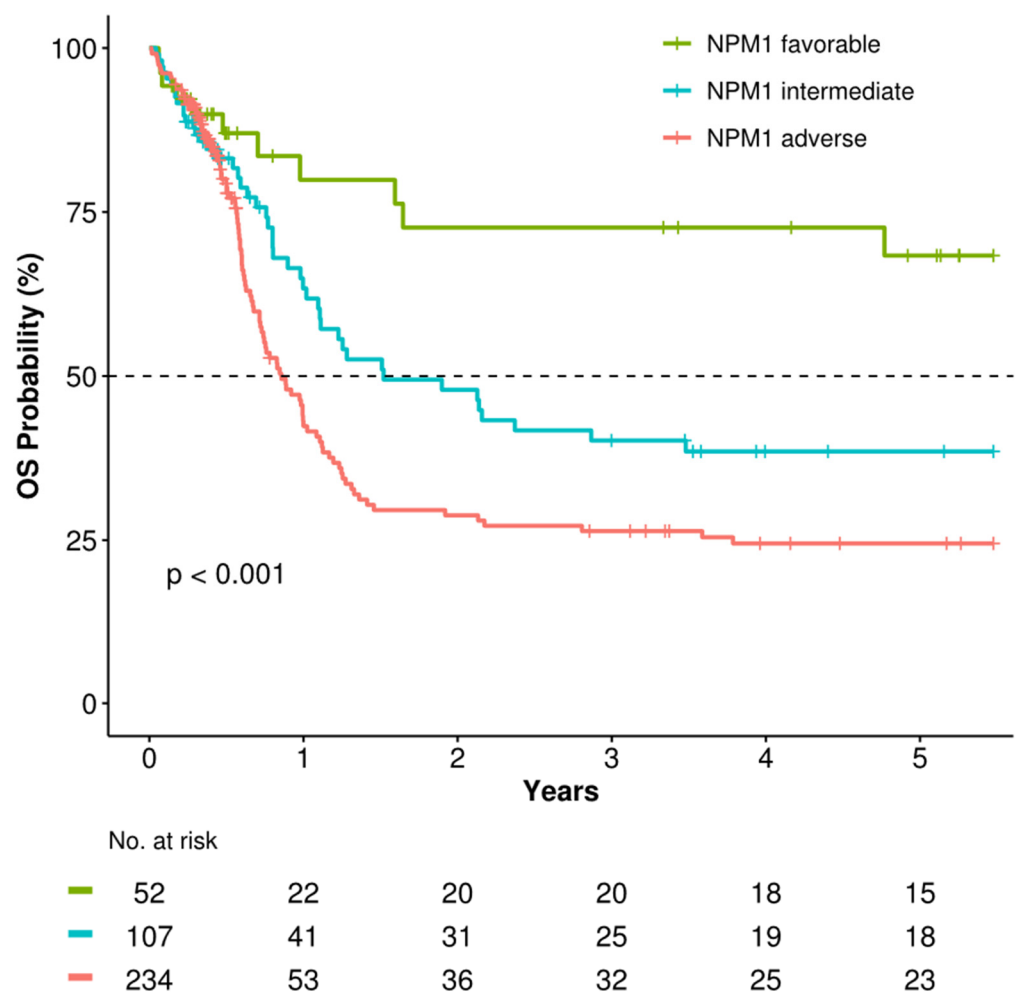


Figure S7. Overall survival of patients with *NPM1*-mut AML and *FLT3*-ITD according to HARMONY *NPM1*-mut classification.

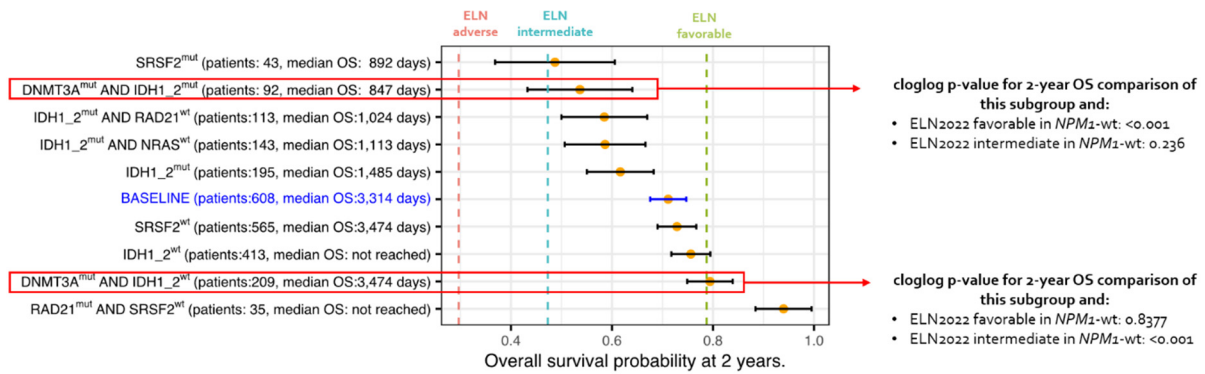


Figure S8. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut and absence of *FLT3*-ITD.

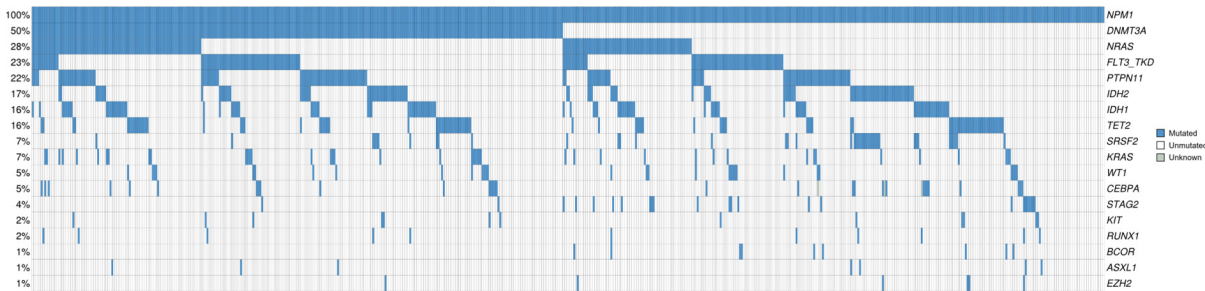


Figure S9. Co-mutational landscape of patients with *NPM1*-mut and absence of *FLT3*-ITD.

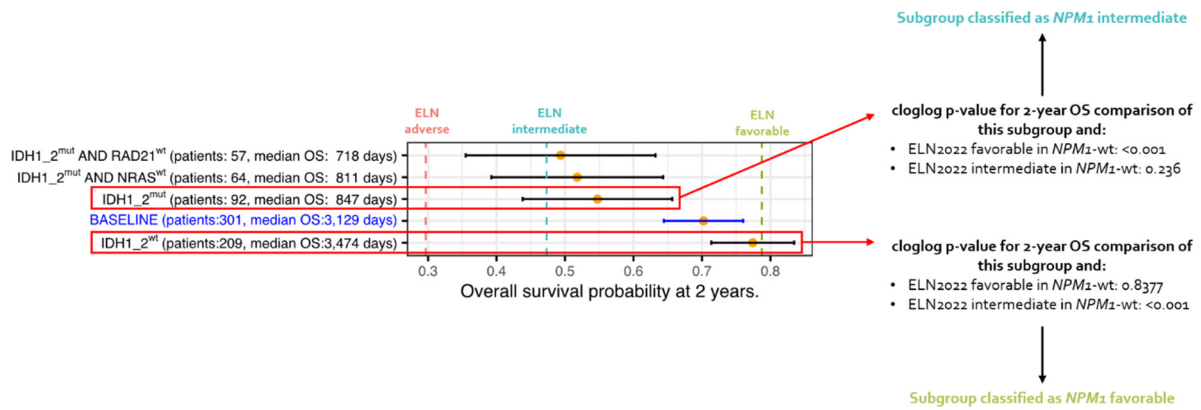


Figure S10. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut, absence of *FLT3*-ITD and *DNMT3A*-mut.

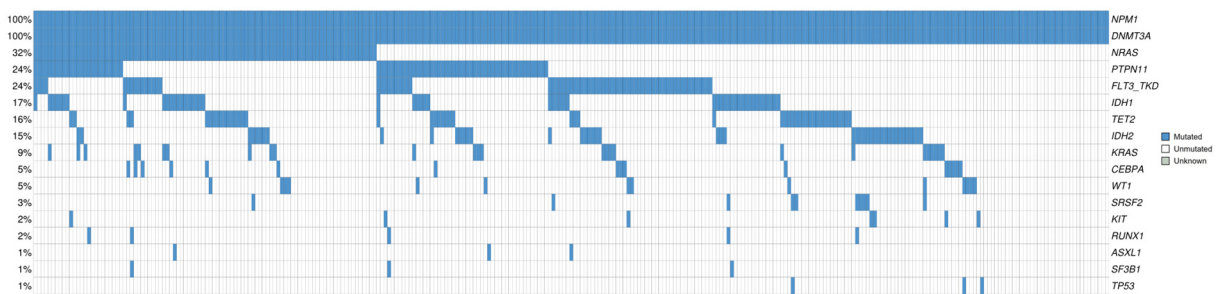


Figure S11. Co-mutational landscape of patients with *NPM1*-mut, absence of *FLT3*-ITD and *DNMT3A*-mut.

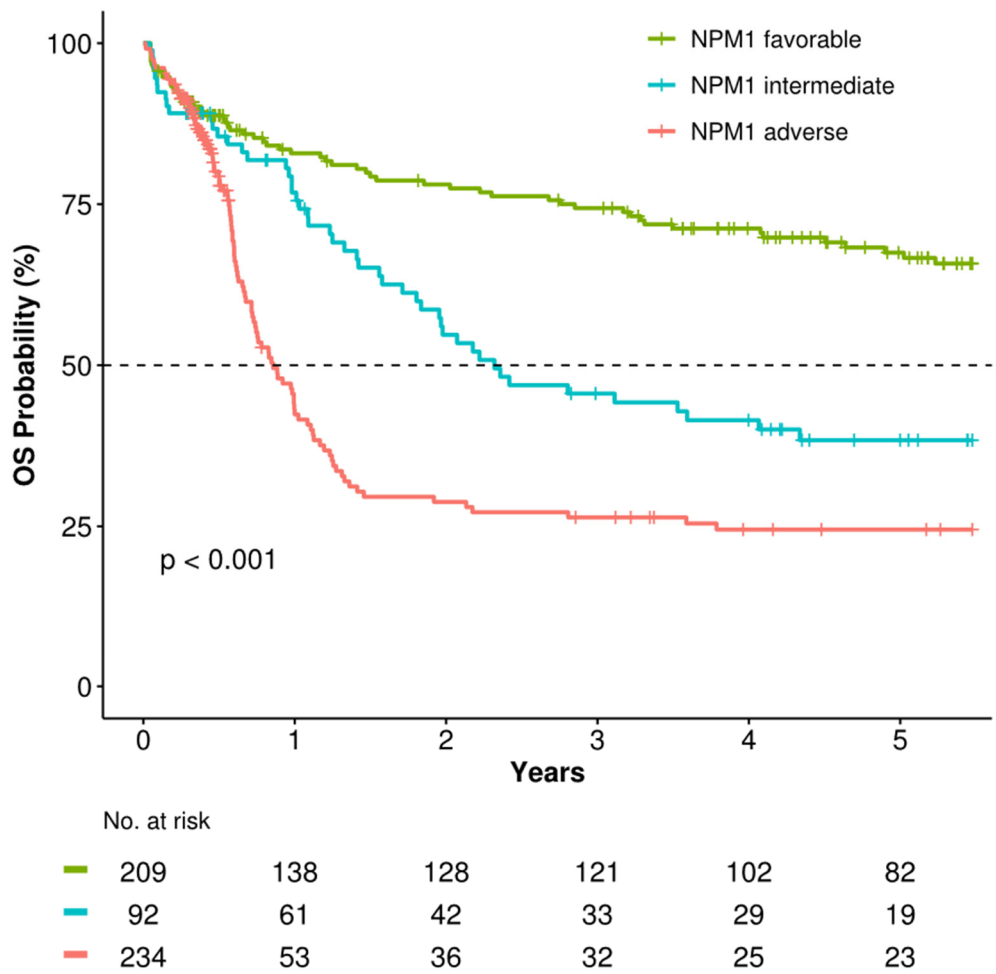


Figure S12. Overall survival of patients with *NPM1*-mut AML and *DNMT3A*-mut according to HARMONY *NPM1*-mut classification.

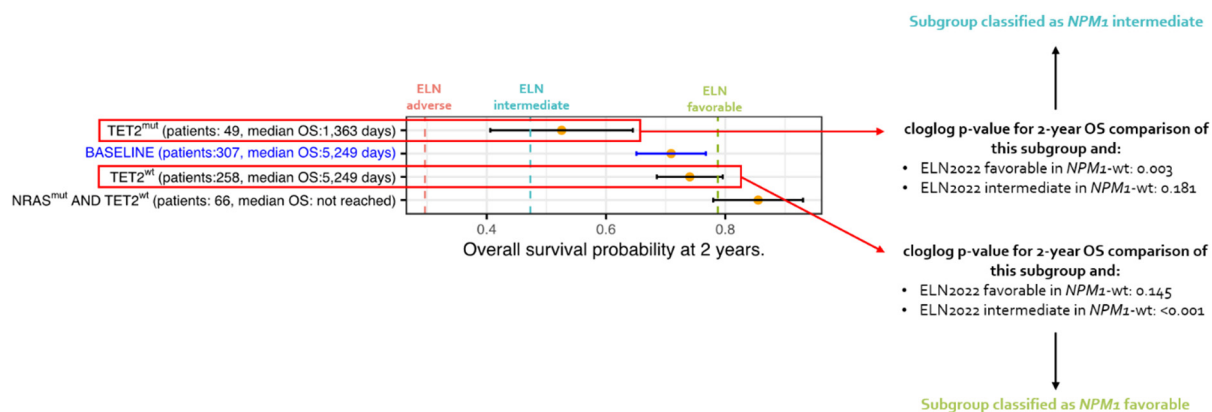


Figure S13. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut, absence of *FLT3*-ITD and *DNMT3A*-wt.

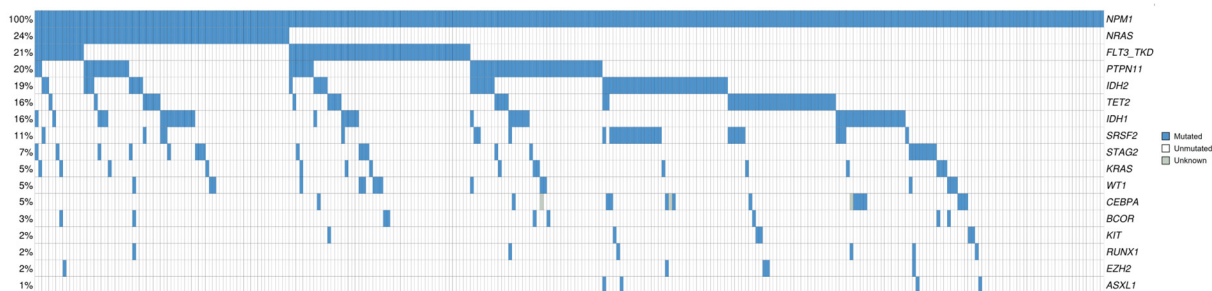


Figure S14. Co-mutational landscape of patients with *NPM1*-mut, absence of *FLT3*-ITD and *DNMT3A*-wt.

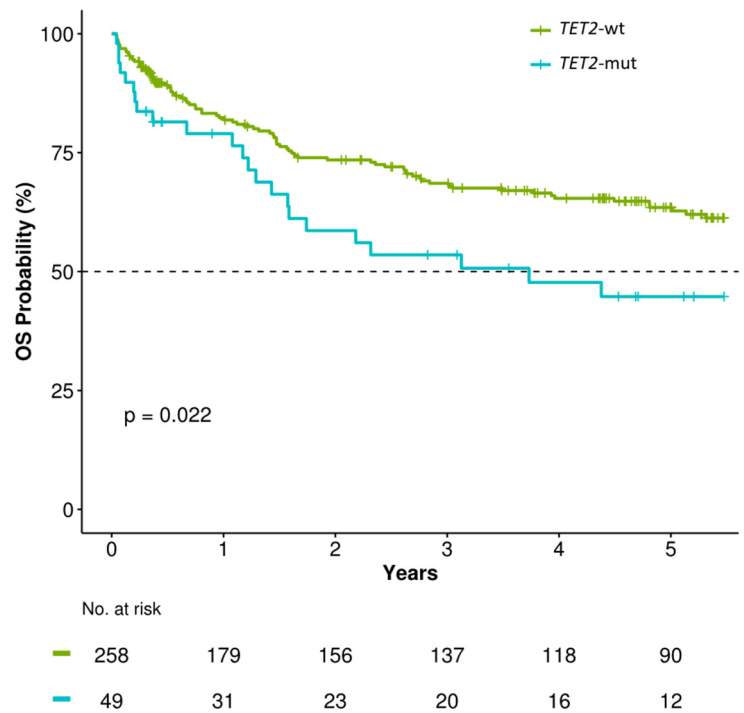


Figure S15. Overall survival of patients with *NPM1*-mut AML, absence of *FLT3*-ITD and *DNMT3A*-wt, stratified by *TET2* mutational status.

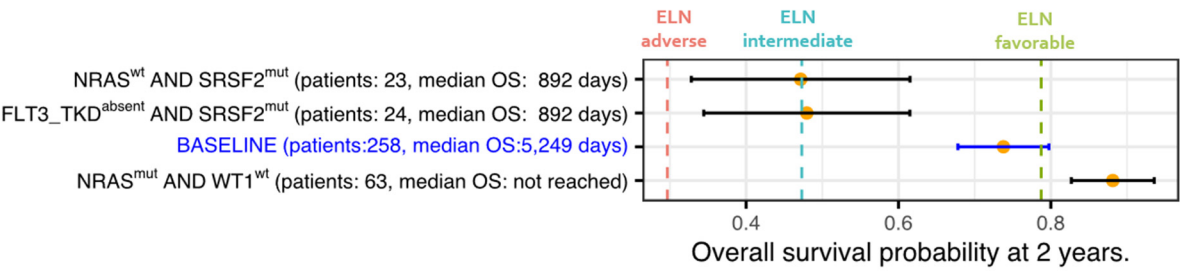


Figure S16. Two-year overall survival comparison of gene co-mutations in *NPM1*-mut AML compared to ELN2022 risk classification in *NPM1*-wt patients, in the training cohort. Baseline cohort of the figure: *NPM1*-mut, absence of *FLT3*-ITD, *DNMT3A*-wt and *TET2*-wt.

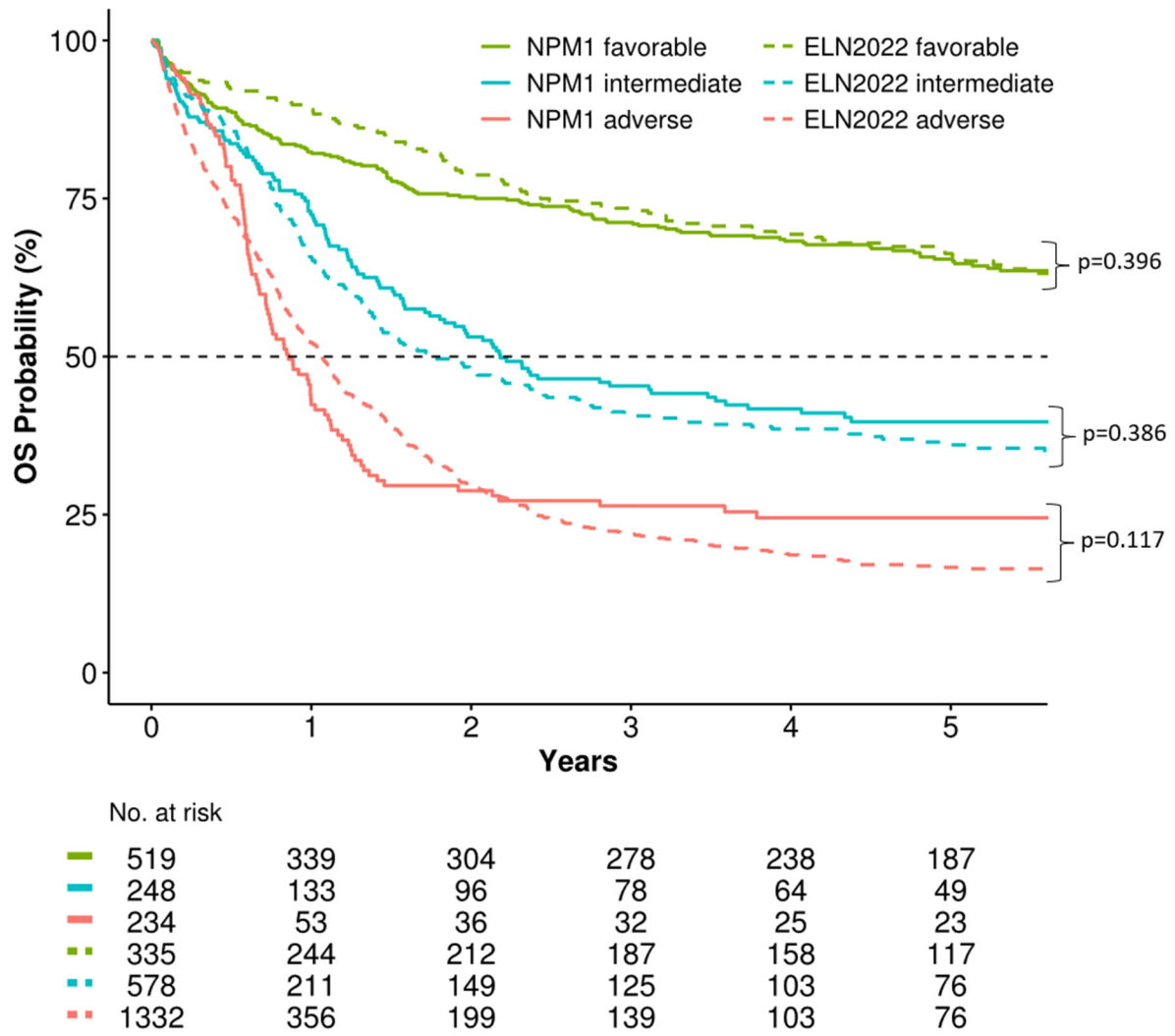


Figure S17. Comparative of overall survival according to *NPM1* classification (for *NPM1*-mut patients) and ELN2022 (for *NPM1*-wt patients), in the training cohort.

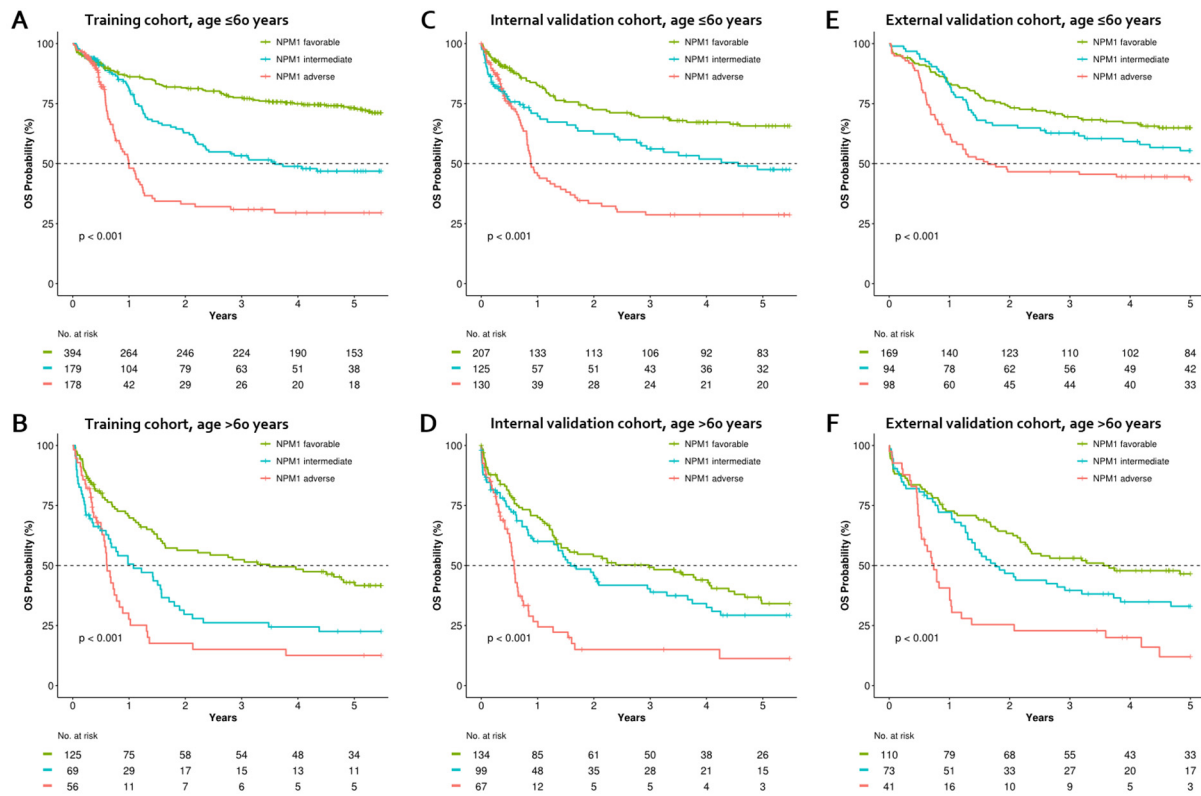


Figure S18. Overall survival according to *NPM1* classification, in training cohort (A, B), internal validation cohort (C, D) and external validation cohort (E, F), in patients aged ≤60 years (A, C, E) and >60 years at AML diagnosis (B, D, F).

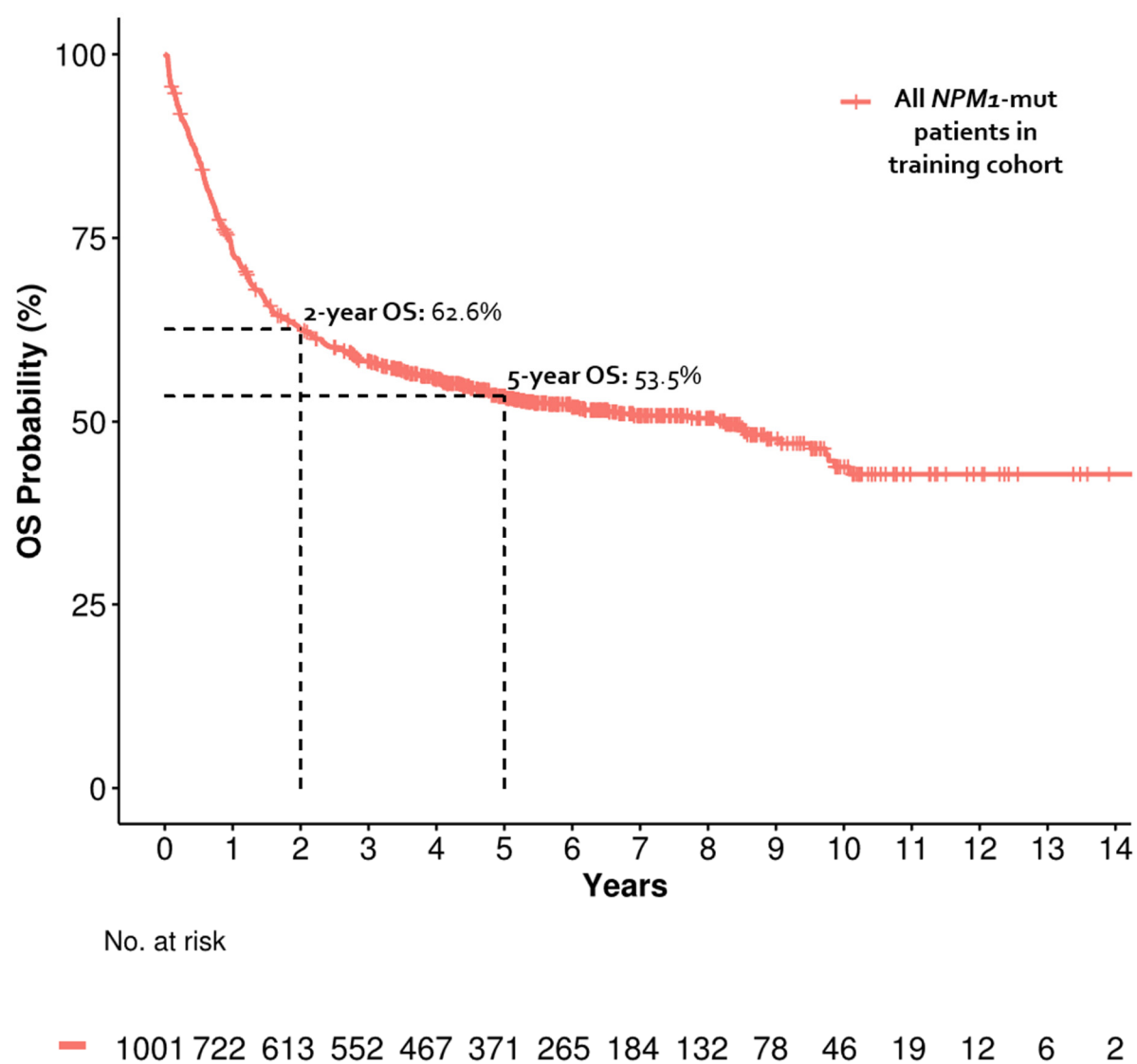


Figure S19. Overall survival of *NPM1*-mut patients in training cohort.

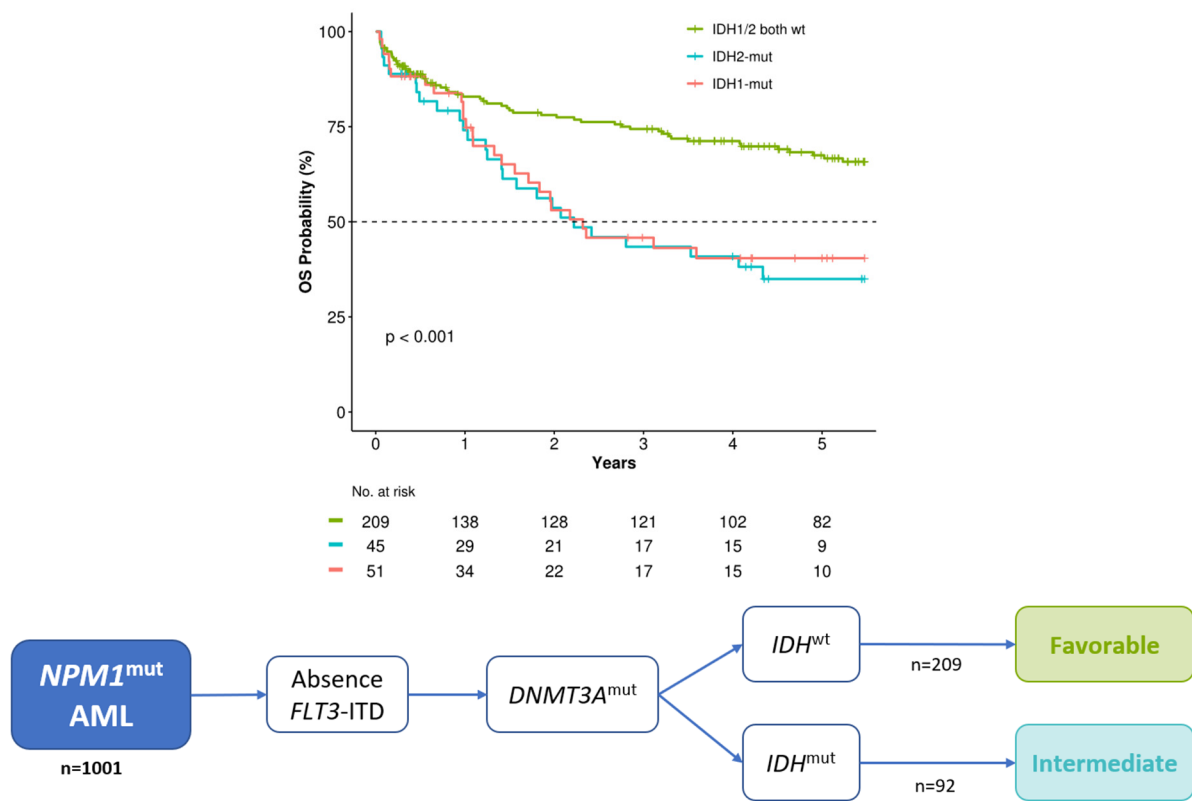


Figure S20. *IDH1/2* sub-analysis of overall survival in patients with *NPM1*-mut, absence *FLT3*-ITD and *DNMT3A*-mut.

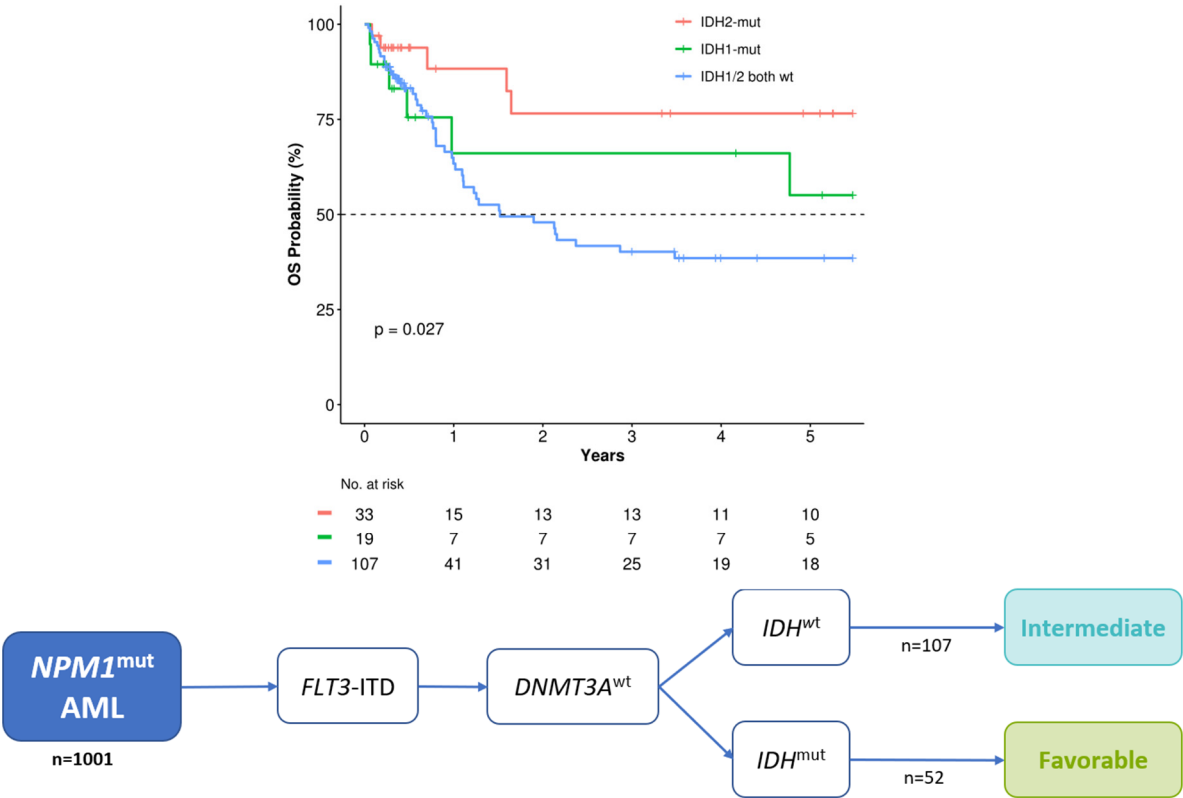


Figure S21. IDH1/2 sub-analysis of overall survival in patients with *NPM1*-mut, *FLT3*-ITD and *DNMT3A*-wt.

MATERIAL SUPLEMENTARIO: CAPÍTULO 3

Machine Learning Provides Individualized Prediction of Outcomes after First Complete Remission in Adult Acute Myeloid Leukemia – A HARMONY Study

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SUPPLEMENTARY METHODS

This section outlines the process of data extraction and statistical procedures for development of the machine learning model using Bayesian Additive Regression Trees (BART).

HARMONY database

Patient data uploaded to the HARMONY platform undergoes a thorough double-broker pseudonymization process in compliance to the General Data Protection Regulation (GDPR). The HARMONY platform harmonizes and transforms data from various sources using the Observational Medical Outcomes Partnership (OMOP) Common data model standard¹. Data is integrated into a global “data lake” containing information on patients with different diseases and separate “workspaces” are created with the required data for each research project. Access to the data is conducted through R language (version 3.6.3) via Apache Zeppelin notebooks, with a dedicated Virtual Private Network (VPN) to ensure data security.

Data pre-processing

The initial step consisted in extracting relevant data for analysis from the OMOP database. In this database, concepts are represented by numeric codes stored in various tables, accessible through Structured Query Language (SQL) queries. The extracted information was transformed into a tabular structure where rows represent patients and columns represent the different patient associated concepts, such as age at diagnosis, gender, gene mutations, International System for Human Cytogenomic Nomenclature (ISCN) karyotype formula², AML type, allogeneic hematopoietic stem cell transplantation (allo-HSCT) date, first complete remission (CR1) date, relapse date and death or last follow-up date, among others.

This initial step is time-consuming, as it requires rigorous quality controls and pre-processing activities, such as developing a parser to decode chromosomal abnormalities

from ISCN karyotype formula or calculating the European LeukemiaNet (ELN) 2022 risk classification for each patient³.

A similar process was applied to the external validation cohort with the data published by Tazi *et al.*⁴ which is publicly available from a [GitHub repository](#). As the data source was not encoded in OMOP, it required combining different files and adapting the information to a similar format to that extracted from the HARMONY database. In addition, ELN2022 risk classification had to be calculated, as the original publication and source files used the previous ELN2017 classification⁵.

Following data preparation, a cohort of 2,550 patients was selected for model training, based on the inclusion and exclusion criteria mentioned in the main manuscript (**Figure 1**). The external validation cohort of 714 patients was also selected using similar criteria, with the exception that patients who received targeted therapies in addition to intensive chemotherapy regimens were also included.

Missing data imputation

A single imputation process was applied to the HARMONY dataset to address missing data. Of note, this step was not necessary for the external validation cohort, as it did not contain missing values for the variables used in the model.

Imputation involves replacing missing values with estimated values based on the statistical relationships with other variables⁶. Multiple imputation was discarded since it requires training multiple models which are not easily handled by BART. We used *mice* (multivariate imputation by chained-equations) package and algorithm with random forest method for missing data imputation⁷. This method was chosen for its ability to capture nonlinear relationships and interactions between variables. Missing data patterns were also explored using *mice*, *nanian* and *Hmisc* packages. The main reason for missing data in HARMONY was the use of distinct next generation sequencing (NGS) myeloid panels across the different data sources. Therefore, missing variables were binary indicators of gene mutation status. Variables with over 20% of missing values (i.e. gene mutations not evaluated in a significant proportion of myeloid panels) were excluded from the imputation process. The remaining variables with missing values were

bZIP in-frame mutated *CEBPA* (19%), FLT3 tyrosine kinase domain mutation (FLT3-*TKD*), *WT1* (3%), *STAG2* (3%), *PTPN11* (3%), *BCOR* (3%) and *SRSF2* (0.2%). Logistic regression of the missingness of those variables showed significant relationships with observed variables, suggesting they were missing at random (MAR) and therefore that the aforementioned imputation process was suitable.

BART models

Once the training dataset was prepared, survival models were developed for predicting overall survival (OS), relapse-free survival (RFS) and cumulative incidence of relapse (CIR). We selected Bayesian Additive Regression Trees⁸⁻¹⁰ as our preferred modelling approach for several reasons:

- a) Bayesian approach: BART is a nonlinear model that leverages the advantages of the Bayesian paradigm. It can provide not only predictions but also measures of uncertainty, including confidence intervals for the predictions. In our opinion this feature is crucial when predicting patient outcomes.
- b) Managing numerous variables: BART can manage a large number of covariates and inherently incorporates interactions between them without requiring explicit specification in the model.
- c) Non-parametric flexibility: As a non-parametric model, BART does not require certain assumptions about the underlying data distribution of variables, enhancing its versatility and ease of use.
- d) Computational efficiency: The BART library supports parallel processing, which can significantly reduce training time when adequate computational resources are available.

The original formulation of the BART model was developed by Chipman *et al*¹¹. In simple terms, BART can be regarded as a sum-of-trees ensemble¹². The predictive function is the sum of numerous regression trees, where the first tree explains a small proportion of the variation and each subsequent tree added to the sum attempts to explain the residuals. BART consists of a set of priors for the structure and leaf parameters, along with a likelihood for the data in the terminal nodes. In order to prevent overfitting, the trees are

constrained to be small, acting as “weak” learners. A regularization prior limits the size and fit of each tree, ensuring that each one only contributes to a small proportion of the overall fit. Posterior inference in BART models is typically conducted via Markov Chain Monte Carlo (MCMC), where a Metropolis-within-Gibbs sampler is used to generate samples from the posterior distribution¹².

While BART is a “black box” machine learning model where generating functions of the covariates are difficult to interpret, other popular black-box models such as neural networks, random forests or gradient boosting have demonstrated good precision and are widely used.

Model validation

The predictive performance of BART model was evaluated through 10-fold cross-validation, calculating time-dependent areas under the receiving operator curve (AUC) at 1, 2, 3, 4 and 5 years for OS, RFS and CIR using timeROC library^{13, 14}.

Moreover, we also evaluated the predictive performance of the final model (trained with the HARMONY dataset of 2,250 patients) in the external validation cohort (n=714), comparing time-dependent AUC at 1, 2, 3, 4 and 5 years between the BART model and ELN2022 risk classification.

Online tool for individualized prediction of outcomes

A web-based interface was developed to calculate individualized estimation of outcomes according to BART model (https://taxonomy.harmony-platform.eu/AML_outcome_predictor/). We designed a responsive interface based on the Shiny library that is able to adapt to different screen sizes. Using five input variables (age, gender, AML type, cytogenetic abnormalities and gene mutations) predictions of OS, RFS and CIR can be obtained (**Figure 5**). The BART model, as we mentioned previously, is a Bayesian model that generates a probability distribution for the patient profile rather than a single prediction. In the online tool, 1,000 draws or samples from the posterior distribution are performed to obtain predictions. By averaging those samples, a more robust and stable estimate of the patient's prediction is obtained. From those

samples 10th and 90th percentiles can be obtained, which are displayed as confidence intervals in the graphical representation. The graph also includes survival curves for the ELN2022 risk categories as a reference. Finally, the interface features a 5-year CIR sensitivity analysis, illustrating how outcome predictions might vary if the patient had different genomic alterations.

References

1. Belenkaya R, Gurley MJ, Golozar A, et al: Extending the OMOP Common Data Model and Standardized Vocabularies to Support Observational Cancer Research. *JCO Clin Cancer Inform* 12–20, 2021
2. McGowan-Jordan J, Hastings RJ, Moore S (eds): *ISCN 2020: An International System for Human Cytogenomic Nomenclature* (2020). S. Karger, 2020
3. Döhner H, Wei AH, Appelbaum FR, et al: Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 140:1345–1377, 2022
4. Tazi Y, Arango-Ossa JE, Zhou Y, et al: Unified classification and risk-stratification in Acute Myeloid Leukemia. *Nat Commun* 13:4622, 2022
5. Döhner H, Estey E, Grimwade D, et al: Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 129:424–447, 2017
6. van Buuren S: *Flexible Imputation of Missing Data*, Second Edition. Chapman and Hall/CRC, 2018
7. van Buuren S, Groothuis-Oudshoorn K: mice: Multivariate Imputation by Chained Equations in R. *J Stat Softw* 45:1–67, 2011
8. Sparapani R, Spanbauer C, McCulloch R: Nonparametric Machine Learning and Efficient Computation with Bayesian Additive Regression Trees: The BART R Package. *J Stat Softw* 97:1–66, 2021
9. Sparapani R, Logan BR, McCulloch RE, et al: Nonparametric competing risks analysis using Bayesian Additive Regression Trees. *Stat Methods Med Res* 29:57–77, 2020

- 10.** Sparapani RA, Logan BR, McCulloch RE, et al: Nonparametric survival analysis using Bayesian Additive Regression Trees (BART). *Stat Med* 35:2741–2753, 2016
- 11.** Chipman HA, George EI, McCulloch RE: BART: Bayesian additive regression trees. *Annals of Applied Statistics* 6:266–298, 2012
- 12.** Hill J, Linero A, Murray J: Bayesian Additive Regression Trees: A Review and Look Forward. *Annu Rev Stat Appl* 7:251–278, 2020
- 13.** Blanche P, Dartigues JF, Jacqmin-Gadda H: Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat Med* 32:5381–5397, 2013
- 14.** Kamarudin AN, Cox T, Kolamunnage-Dona R: Time-dependent ROC curve analysis in medical research: current methods and applications. *BMC Med Res Methodol* 17:53, 2017

SUPPLEMENTARY TABLES

Table S1. Distribution of genomic aberrations included in the machine learning model, in the training cohort.

Genomic aberration / gene mutation	Number of patients	% of cohort
Normal karyotype	1327	52.04
<i>NPM1</i>	921	36.12
<i>DNMT3A</i>	671	26.31
<i>NRAS</i>	502	19.69
<i>FLT3</i> -ITD	484	18.98
t(8;21)	365	14.31
<i>TET2</i>	345	13.53
<i>IDH2</i>	302	11.84
<i>FLT3</i> -TKD	278	10.90
<i>KIT</i>	213	8.35
<i>PTPN11</i>	213	8.35
<i>RUNX1</i>	204	8.00
inv(16)/t(16;16)	202	7.92
<i>WT1</i>	193	7.57
<i>IDH1</i>	190	7.45
bZIP in-frame mutated <i>CEBPA</i>	188	7.37
<i>ASXL1</i>	177	6.94
-Y	176	6.90
+8	167	6.55
<i>KRAS</i>	149	5.84
Complex karyotype	140	5.49
<i>SRSF2</i>	138	5.41
<i>TP53</i>	117	4.59
Monosomal karyotype	111	4.35
Marker chromosome	96	3.76
<i>EZH2</i>	94	3.69
<i>STAG2</i>	94	3.69
<i>BCOR</i>	85	3.33
-5/del(5q)	85	3.33
del9q	83	3.25
-X	71	2.78
-17/abn(17p)	63	2.47
-7	62	2.43
Other <i>CEBPA</i> mutations	60	2.35
+22	55	2.16
<i>KMT2Ar</i>	53	2.08
<i>U2AF1</i>	53	2.08
-12/abn(12p)	50	1.96
+21	50	1.96

<i>JAK2</i>	49	1.92
del(7q)	48	1.88
<i>SF3B1</i>	45	1.76
-17	43	1.69
-18	41	1.61
-5	36	1.41
+13	27	1.06
-16	25	0.98
+4	24	0.94
-21	23	0.90
-13	21	0.82
-20	21	0.82
<i>ZRSR2</i>	21	0.82
-12	20	0.78
+6	19	0.75
+11	18	0.71
add(7q)	17	0.67
del(11q)	16	0.63
-3	15	0.59
+19	15	0.59
del(13q)	14	0.55
del(20q)	14	0.55
-22	14	0.55
add(21q)	13	0.51
-19	13	0.51
+10	13	0.51
add(11q)	12	0.47
add(7q)	12	0.47
-8	12	0.47
+1	12	0.47
+15	11	0.43
t(6;9)	11	0.43
add(1q)	10	0.39
del(3q)	10	0.39
<i>MECOMr</i>	10	0.39
-11	10	0.39
+9	10	0.39

Table S2. Comparison of predictive performance, measured by the area under the receiver operating curve (AUC) of HARMONY machine learning model, based on Bayesian Additive Regression Trees (BART), compared to European LeukemiaNet (ELN) 2022 risk classification in the training cohort. 10-fold cross validation was performed for each time point (1, 2, 3, 4 and 5 years) and outcome type (overall survival [OS], relapse-free survival [RFS] and cumulative incidence of relapse [CIR]).

Outcome type	Time (years)	AUC (BART)	Standard error (BART)	AUC (ELN2022)	Standard error (ELN2022)	p-value
OS	1	0.71366	0.03623	0.68061	0.03489	0.1212
OS	2	0.7354	0.03287	0.6856	0.03303	0.0108
OS	3	0.7445	0.03212	0.68456	0.03226	0.0022
OS	4	0.72291	0.03373	0.67366	0.03297	0.0201
OS	5	0.7019	0.03658	0.65566	0.03567	0.0471
OS	1	0.76339	0.0364	0.66499	0.03646	<0.0001
OS	2	0.73169	0.03377	0.66985	0.03252	0.0032
OS	3	0.74666	0.03175	0.68555	0.03106	0.0041
OS	4	0.72386	0.03366	0.66785	0.0323	0.0114
OS	5	0.72464	0.03494	0.66783	0.03461	0.0211
OS	1	0.81867	0.0325	0.73388	0.03473	0.0004
OS	2	0.75554	0.03192	0.69019	0.0326	0.0013
OS	3	0.75188	0.0315	0.71804	0.03085	0.0863
OS	4	0.72847	0.03358	0.70343	0.03158	0.2217
OS	5	0.71674	0.03634	0.70411	0.03287	0.5773
OS	1	0.75881	0.03224	0.69418	0.03393	0.0093
OS	2	0.72497	0.03224	0.70269	0.03159	0.3494
OS	3	0.73435	0.03229	0.70693	0.03141	0.2286
OS	4	0.75185	0.03206	0.72215	0.031	0.1915
OS	5	0.72885	0.03493	0.70643	0.03301	0.3523
OS	1	0.80743	0.03032	0.75155	0.03153	0.0073
OS	2	0.79136	0.03017	0.7623	0.02867	0.1079
OS	3	0.77259	0.03052	0.74638	0.02908	0.1481
OS	4	0.77034	0.0312	0.73775	0.03058	0.0846
OS	5	0.7685	0.03275	0.73643	0.03194	0.101
OS	1	0.7385	0.03749	0.68117	0.03755	0.0157
OS	2	0.75937	0.03175	0.71736	0.03163	0.0357
OS	3	0.7747	0.03107	0.7121	0.03133	0.0026
OS	4	0.78155	0.03034	0.71453	0.03124	0.0013
OS	5	0.79122	0.03041	0.71546	0.03226	0.0004
OS	1	0.75642	0.03404	0.69501	0.03643	0.0206
OS	2	0.76049	0.03181	0.68264	0.03319	0.0005
OS	3	0.77013	0.03016	0.70401	0.03102	0.0016
OS	4	0.77271	0.03087	0.72913	0.02988	0.0373
OS	5	0.76935	0.03242	0.72474	0.03059	0.0447

OS	1	0.68817	0.03801	0.61972	0.03666	0.0044
OS	2	0.72465	0.03342	0.68655	0.03214	0.0742
OS	3	0.69765	0.03388	0.6758	0.03203	0.3086
OS	4	0.69396	0.0352	0.69383	0.03238	0.9955
OS	5	0.68728	0.03675	0.68362	0.03408	0.8791
OS	1	0.80164	0.03229	0.73958	0.03414	0.0068
OS	2	0.76955	0.03234	0.72019	0.03098	0.0167
OS	3	0.7639	0.03236	0.71556	0.03054	0.0198
OS	4	0.77112	0.03189	0.72231	0.03029	0.0198
OS	5	0.76898	0.03286	0.72369	0.03104	0.0344
OS	1	0.73085	0.03679	0.67883	0.03583	0.0076
OS	2	0.75913	0.03093	0.68813	0.03249	<0.0001
OS	3	0.76139	0.0305	0.69582	0.03179	0.0001
OS	4	0.75229	0.0316	0.69172	0.03181	0.0006
OS	5	0.75384	0.03185	0.68283	0.03193	0.0001
RFS	1	0.72794	0.03264	0.69577	0.0316	0.0908
RFS	2	0.70551	0.0328	0.68237	0.03069	0.2197
RFS	3	0.70543	0.03303	0.6872	0.03075	0.3515
RFS	4	0.7106	0.03375	0.68551	0.03192	0.2214
RFS	5	0.71158	0.03486	0.68286	0.03306	0.1984
RFS	1	0.68917	0.03449	0.65783	0.03225	0.1315
RFS	2	0.70546	0.03287	0.66753	0.03095	0.0708
RFS	3	0.69725	0.03385	0.66147	0.03169	0.1112
RFS	4	0.71877	0.03357	0.67978	0.03159	0.0709
RFS	5	0.72418	0.03515	0.68401	0.03261	0.0805
RFS	1	0.78359	0.02914	0.71644	0.02993	0.0001
RFS	2	0.75334	0.03031	0.68286	0.02992	0.0001
RFS	3	0.76094	0.03062	0.68368	0.03019	0.0001
RFS	4	0.74325	0.0328	0.66648	0.0322	0.0002
RFS	5	0.74457	0.03515	0.66053	0.03508	0.0002
RFS	1	0.79223	0.02881	0.72626	0.03021	0.0001
RFS	2	0.79285	0.02773	0.72196	0.02951	<0.0001
RFS	3	0.78537	0.02884	0.72671	0.02934	0.0006
RFS	4	0.79166	0.02909	0.73568	0.02918	0.002
RFS	5	0.77502	0.03191	0.72337	0.0309	0.0069
RFS	1	0.76726	0.02977	0.73976	0.02944	0.16
RFS	2	0.76471	0.03016	0.74742	0.02775	0.3666
RFS	3	0.77028	0.03057	0.74715	0.02766	0.2318
RFS	4	0.77653	0.0314	0.74987	0.02776	0.1833
RFS	5	0.77448	0.03459	0.73964	0.03048	0.1025
RFS	1	0.71616	0.03228	0.7009	0.03034	0.3863
RFS	2	0.73801	0.03075	0.71235	0.02853	0.1579
RFS	3	0.75281	0.03053	0.72457	0.02804	0.1307
RFS	4	0.73253	0.03323	0.71141	0.02976	0.2929

RFS	5	0.7144	0.036	0.69458	0.03187	0.3659
RFS	1	0.73784	0.03182	0.67691	0.03185	0.0017
RFS	2	0.77625	0.02974	0.67761	0.02983	<0.0001
RFS	3	0.79141	0.02973	0.69566	0.02901	<0.0001
RFS	4	0.78989	0.0306	0.69999	0.02889	<0.0001
RFS	5	0.79499	0.03192	0.69687	0.0304	<0.0001
RFS	1	0.77728	0.0295	0.72823	0.03	0.0046
RFS	2	0.7961	0.02823	0.74095	0.02867	0.0016
RFS	3	0.80178	0.0283	0.74011	0.02873	0.0004
RFS	4	0.80982	0.02883	0.74282	0.02951	0.0001
RFS	5	0.80896	0.02991	0.74971	0.02956	0.0013
RFS	1	0.78708	0.02975	0.73886	0.02953	0.0069
RFS	2	0.77078	0.02904	0.71999	0.02827	0.0065
RFS	3	0.78831	0.02798	0.73104	0.02741	0.0029
RFS	4	0.78306	0.0294	0.73765	0.02732	0.0256
RFS	5	0.77918	0.03162	0.72543	0.02861	0.0166
RFS	1	0.70356	0.03411	0.68004	0.03148	0.2315
RFS	2	0.67104	0.03406	0.67111	0.03052	0.9968
RFS	3	0.70041	0.03334	0.69117	0.03005	0.6093
RFS	4	0.7126	0.03382	0.70816	0.02978	0.8069
RFS	5	0.69619	0.03666	0.69396	0.0314	0.9088
CIR	1	0.73055	0.03456	0.68074	0.03237	0.015
CIR	2	0.69113	0.03481	0.65453	0.03147	0.0762
CIR	3	0.67957	0.03579	0.65857	0.03147	0.3213
CIR	4	0.68479	0.03691	0.66604	0.0319	0.3936
CIR	5	0.6823	0.03911	0.68219	0.03211	0.9963
CIR	1	0.76202	0.03312	0.73207	0.03253	0.1549
CIR	2	0.73521	0.0329	0.72796	0.03046	0.7182
CIR	3	0.73977	0.03273	0.72796	0.03036	0.5654
CIR	4	0.73465	0.03361	0.73496	0.03029	0.9879
CIR	5	0.70226	0.03702	0.70665	0.03334	0.8547
CIR	1	0.75676	0.03349	0.72059	0.03198	0.1005
CIR	2	0.75925	0.032	0.72631	0.03044	0.1056
CIR	3	0.75284	0.03298	0.72261	0.0309	0.1531
CIR	4	0.75297	0.03422	0.72558	0.03176	0.2031
CIR	5	0.77196	0.03474	0.73771	0.03214	0.1294
CIR	1	0.73603	0.03383	0.6831	0.03372	0.02
CIR	2	0.71732	0.03437	0.67311	0.03272	0.0474
CIR	3	0.73503	0.03531	0.68763	0.03316	0.0433
CIR	4	0.73241	0.03731	0.68918	0.03417	0.0555
CIR	5	0.72293	0.03913	0.68837	0.03489	0.1327
CIR	1	0.75716	0.03452	0.73191	0.03278	0.2479
CIR	2	0.75873	0.03254	0.7136	0.03093	0.0337
CIR	3	0.77403	0.03218	0.72766	0.02992	0.0316

CIR	4	0.7831	0.03189	0.72939	0.03015	0.0134
CIR	5	0.76733	0.03451	0.71697	0.03166	0.0298
CIR	1	0.74787	0.03432	0.70176	0.03472	0.042
CIR	2	0.76588	0.03198	0.72587	0.03178	0.0421
CIR	3	0.76544	0.03229	0.7238	0.0315	0.0396
CIR	4	0.76357	0.03432	0.7382	0.03152	0.2289
CIR	5	0.75916	0.03795	0.73232	0.03308	0.2521
CIR	1	0.75731	0.03233	0.69847	0.0322	0.0047
CIR	2	0.75554	0.0321	0.67533	0.03104	0.0001
CIR	3	0.74761	0.03306	0.66965	0.03124	0.0003
CIR	4	0.76195	0.0341	0.67651	0.03164	0.0002
CIR	5	0.76207	0.03722	0.67453	0.03281	0.0002
CIR	1	0.76186	0.03349	0.72344	0.03306	0.0718
CIR	2	0.69325	0.03555	0.67434	0.03232	0.3885
CIR	3	0.71343	0.03488	0.70046	0.0311	0.5585
CIR	4	0.73027	0.0343	0.69534	0.03107	0.11
CIR	5	0.70888	0.03736	0.6733	0.03395	0.1278
CIR	1	0.76397	0.03243	0.73497	0.03081	0.1079
CIR	2	0.78369	0.02951	0.73877	0.02951	0.0127
CIR	3	0.7925	0.02913	0.75917	0.02826	0.0873
CIR	4	0.79087	0.03005	0.76938	0.02819	0.2908
CIR	5	0.78821	0.03159	0.7656	0.02987	0.2977
CIR	1	0.76498	0.03447	0.7302	0.03313	0.0787
CIR	2	0.75739	0.0325	0.72415	0.03073	0.0821
CIR	3	0.75722	0.03232	0.71624	0.03062	0.038
CIR	4	0.75379	0.03318	0.70999	0.03129	0.0313
CIR	5	0.75741	0.03412	0.70399	0.03276	0.0109

Table S3. Distribution of genomic aberrations included in the machine learning model, in the external validation cohort.

Genomic aberration / gene mutation	Number of patients	% of cohort
Normal karyotype	395	55.32
<i>NPM1</i>	269	37.68
<i>DNMT3A</i>	204	28.57
<i>FLT3</i> -ITD	133	18.63
<i>NRAS</i>	121	16.95
Complex karyotype	93	13.03
<i>IDH2</i>	89	12.46
<i>FLT3</i> -TKD	84	11.76
<i>TET2</i>	82	11.48
+8	67	9.38
<i>PTPN11</i>	66	9.24
t(8;21)	66	9.24
<i>RUNX1</i>	62	8.68
inv(16)	58	8.12
<i>IDH1</i>	57	7.98
<i>WT1</i>	54	7.56
<i>SRSF2</i>	53	7.42
<i>KIT</i>	49	6.86
Monosomal karyotype	46	6.44
<i>KRAS</i>	45	6.3
<i>STAG2</i>	34	4.76
<i>ASXL1</i>	33	4.62
bZIP in-frame mutated	30	4.2
<i>CEBPA</i>	29	4.06
<i>BCOR</i>	29	4.06
Marker chromosome	29	4.06
<i>TP53</i>	29	4.06
Other <i>CEBPA</i> mutations	28	3.92
-Y	27	3.78
del(9q)	22	3.08
-5/del(5q)	22	3.08
-7	21	2.94
<i>KMT2Ar</i>	19	2.66
<i>U2AF1</i>	18	2.52
<i>EZH2</i>	17	2.38
+22	17	2.38
-17/abn(17p)	16	2.24
del(7q)	14	1.96
+11	13	1.82
-12/abn(12p)	11	1.54
-X	11	1.54
t(9;11)	11	1.54

-17	10	1.4
+13	10	1.4
+21	10	1.4
-18	9	1.26
+4	9	1.26
<i>SF3B1</i>	9	1.26
<i>MECOMr</i>	7	0.98
<i>JAK2</i>	7	0.98
-12	6	0.84
-20	6	0.84
-21	6	0.84
-5	6	0.84
+10	6	0.84
+6	6	0.84
del(20q)	5	0.7
-13	5	0.7
-19	5	0.7
+19	5	0.7
add(7q)	4	0.56
del(11q)	4	0.56
-16	4	0.56
-22	4	0.56
-3	4	0.56
+1	4	0.56
+15	4	0.56
+9	4	0.56
-8	3	0.42
t(6;9)	3	0.42
del(13q)	2	0.28
<i>ZRSR2</i>	2	0.28
-11	1	0.14

Table S4. Comparison of predictive performance, measured by the area under the receiver operating curve (AUC) of HARMONY machine learning model, based on Bayesian Additive Regression Trees (BART), compared to European LeukemiaNet (ELN) 2022 risk classification in the external validation cohort.

Outcome type	Time (years)	AUC (BART)	Standard error (BART)	AUC (ELN2022)	Standard error (ELN2022)	p-value
CIR	1	0.6986	0.0214	0.63572	0.0214	<0.0001
CIR	2	0.69841	0.02047	0.65871	0.01978	0.0012
CIR	3	0.69965	0.02081	0.6618	0.01982	0.0021
CIR	4	0.71631	0.02116	0.66385	0.02037	<0.0001
CIR	5	0.71813	0.02222	0.6722	0.02089	0.0004
OS	1	0.77721	0.02124	0.69857	0.02207	<0.0001
OS	2	0.76304	0.01916	0.70373	0.01956	<0.0001
OS	3	0.76407	0.01845	0.71727	0.01874	0.0001
OS	4	0.75792	0.01867	0.71133	0.01892	0.0001
OS	5	0.75531	0.01932	0.7136	0.01935	0.0007
RFS	1	0.69836	0.01995	0.64669	0.01956	<0.0001
RFS	2	0.71958	0.01908	0.67687	0.0187	0.0001
RFS	3	0.71305	0.01946	0.67262	0.01891	0.0003
RFS	4	0.72045	0.01978	0.67322	0.01953	<0.0001
RFS	5	0.71742	0.02088	0.67917	0.02015	0.0014

SUPPLEMENTARY FIGURES

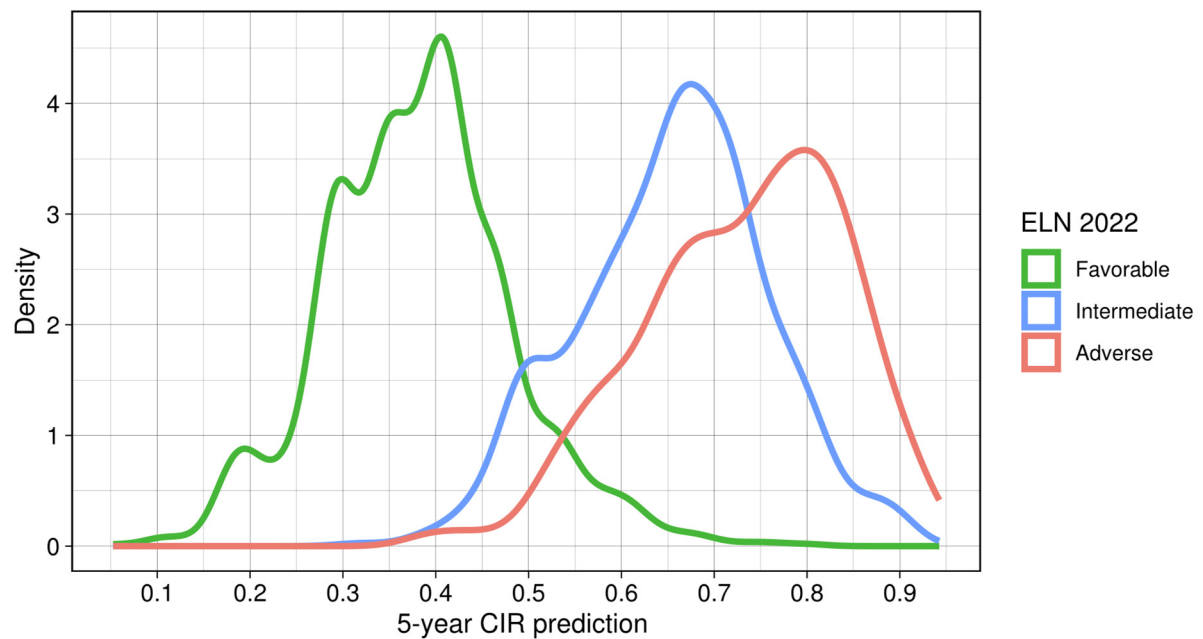


Figure S1. Density plot of predicted 5-year CIR according to HARMONY machine learning model, stratified by ELN2022 risk classification categories, in the training cohort.

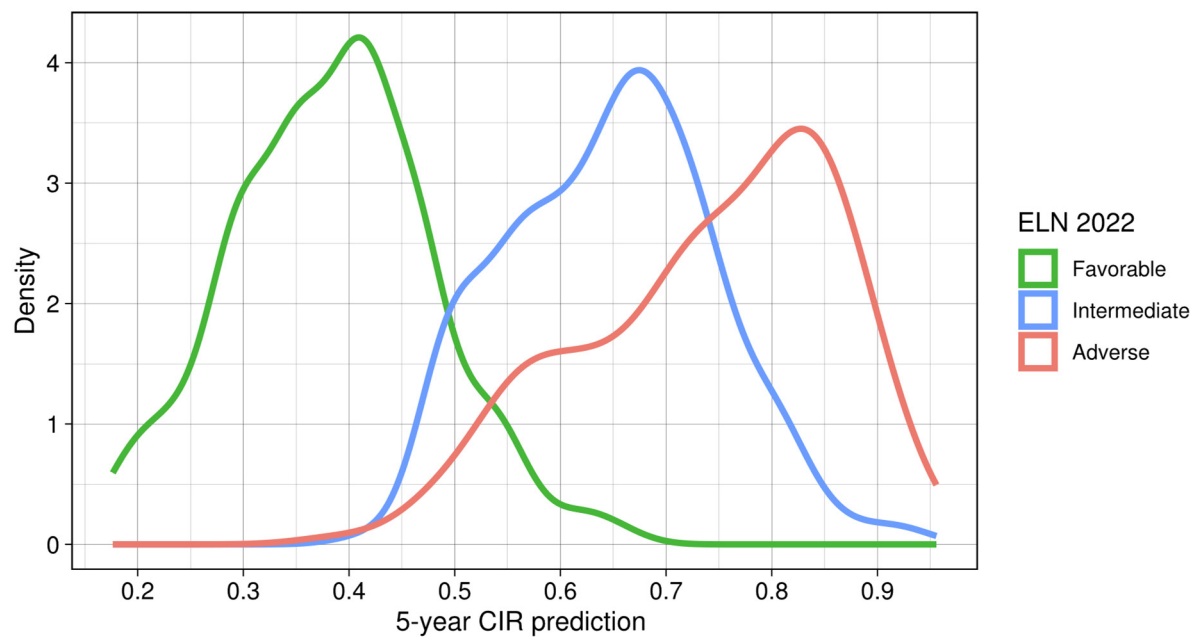


Figure S2. Density plot of predicted 5-year CIR according to HARMONY machine learning model, stratified by ELN2022 risk classification categories, in the external validation cohort.

