Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 1954

Improving prognostication and treatment choices for patients with AML

ALBIN ÖSTERROOS

ACTA UNIVERSITATIS
UPSALIENSIS
2023

ISSN 1651-6206
ISBN 978-91-513-1829-5
urn:nbn:se:uu:diva-501512

UPPSALA
UNIVERSITET

Dissertation presented at Uppsala University to be publicly examined in H:son Holmdahlsalen, Uppsala University Hospital, Entrance 100, Uppsala, Friday, 1 September 2023 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Professor Marc H G P Raaijmakers (Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands).

Abstract

Österroos, A. 2023. Improving prognostication and treatment choices for patients with AML. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1954. 69 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1829-5.

The treatment landscape of the aggressive haematological malignancy acute myeloid leukaemia (AML) has expanded but the prognosis is still unsatisfactory poor. Here, we aimed at improving prognostication and treatment choices in AML by addressing current clinical obstacles to successful AML treatment.

Acute promyelocytic leukaemia (APL) is an AML subset characterised by a high rate of early death (ED). In **Paper I**, we developed a novel risk score for ED in APL. We identified three risk groups for ED based on regression analyses on first a training cohort from the population-based Swedish AML Registry (n=301) and later an external validation cohort from a hospital-based registry (n=129). The presented risk score included age, platelets and white blood cell (WBC) count. Importantly, already sub-normal to normal WBC counts conferred higher risks of ED.

Molecular studies of elderly AML patients are sparse. In **Paper II**, we focused on patients ≥65 years to investigate the prognostic effect of molecular markers and to propose an algorithm for response to intensive chemotherapy (IC) in this patient group. We combined clinical data with targeted DNA- and RNA-sequencing of 182 patients. Notably, we identified and externally validated three risk categories for complete remission achievement after IC based on mutational status of *TP53* and gene expression levels of *ZBTB7A* and *EEPD1*.

Hypomethylating agents (HMAs) constitute a backbone for AML patients ineligible for IC. There are limited studies on their effectiveness in the real-world setting. In **Paper III**, we compared the utility of HMAs against IC and palliative care in all AML patients ≥60 years in Sweden (n=3135) during 2008-2018. Propensity score matching in this population-based cohort showed that HMAs are as effective as IC upfront when patient characteristics were balanced. Additionally, predictive factors for overall survival in HMA treated patients were different to IC treated patients.

The HMA azacitidine combined with venetoclax is the current frontline option to AML patients unfit for IC. Few studies have addressed how this synergism arises. In **Paper IV**, we characterised the epigenetic and transcriptomic effects of azacitidine-venetoclax *in vitro* and elucidated potential survival/resistance mechanisms in AML blasts including the serine synthesis pathway and NTRK signaling. Furthermore, we utilised obtained RNA-seq data and *in silico* predictions to propose add-ons to azacitidine-venetoclax to further strengthen the synergy.

In summary, the research presented herein contributes to improved personalised medicine in AML via real-world data, risk stratification algorithms and insights into potential novel therapeutic approaches.

Keywords: Acute myeloid leukaemia, Acute promyelocytic leukaemia, Prognostication, Personalised medicine, Risk stratification, Real-world data, Next-generation sequencing, Azacitidine, Venetoclax

Albin Österroos, Department of Medical Sciences, Haematology, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-513-1829-5
URN urn:nbn:se:uu:diva-501512 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-501512)

Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.

Marie Curie

List of Papers

The thesis is based on the following papers and referred to in the text by their Roman numerals.

I. **Österroos, A.**, Maia, T., Eriksson, A., Jädersten, M., Lazarevic, V., Wennström, L., Antunovic, P., Cammenga, J., Deneberg, S., Lorenz, F., Möllgård, L., Uggla, B., Ölander, E., Aguiar, E., Trigo, F., Höglund, M., Juliusson, G., Lehmann, S. *A risk score based on real-world data to predict early death in acute promyelocytic leukemia.* Haematologica. 2022, 107, 1528–1537.

II. **Österroos, A.**, Björklund, M., Eriksson, A., Lindberg, J., Nilsson, C., Mareschal, S., Rantalainen, M., Grönberg, H., Lehmann, S. *Integrated transcriptomic and genomic analysis improves prediction of complete remission and survival in elderly patients with acute myeloid leukemia.* Blood Cancer Journal. 2020, 10, 67.

III. **Österroos, A.**, Eriksson, A., Antunovic, P., Cammenga, J., Deneberg, S., Lazarevic, V., Lorenz, F., Möllgård, L., Derolf, Å.R., Uggla, B., Wennström, L., Ölander, E., Höglund, M., Juliusson, G., Lehmann, S. *Real-world data on treatment patterns and outcomes of hypomethylating therapy in patients with newly diagnosed acute myeloid leukaemia aged ≥ 60 years.* British Journal of Haematology. 2020, 189.

IV. **Österroos, A.**, Zhong, X., Gamboa Cedeno, A., Junkunlo, K., Bengtzén, S., Eriksson, A., Lehmann, S. *Integrated multi-omic profiling of azacitidine-venetoclax in AML reveals additional targetable pathways to improve the treatment.* Manuscript.

Other scientific publications by the PhD candidate

Eriksson, A., Engvall, M., Mathot, L., **Österroos, A.**, Rippin, M., Cavelier, L., Ladenvall, C., Baliakas, P. *Somatic exonic deletions in RUNX1 constitutes a novel recurrent genomic abnormality in acute myeloid leukemia.* Clin Cancer Res. 2023, CCR-23-0122.

Trac, QT., Pawitan, Y., Mou, T., Erkers, T., Östling, P., Bohlin, A., **Österroos, A.**, Vesterlund, M., Jafari, R., Siavelis, I., Bäckvall, H., Kiviluoto, S., Orre, LM., Rantalainen, M., Lehtiö, J., Lehmann, S., Kallioniemi, O., Vu, TN. *Prediction model for drug response of acute myeloid leukemia patients.* NPJ Precis Oncol. 2023, 7(1):32.

Mou, T., Pawitan, Y., Stahl, M., Vesterlund, M., Deng, W., Jafari, R., Bohlin, A., **Österroos, A.**, Siavelis, L., Bäckvall, H., Erkers, T., Kiviluoto, S., Seashore-Ludlow, B., Östling, P., Orre, LM., Kallioniemi, O., Lehmann, S., Lehtiö, J., Vu, TN. *The transcriptome-wide landscape of molecular subtype-specific mRNA expression profiles in acute myeloid leukemia.* American J Hematol. 2021, 96(5):580-588.

Österroos, A., Kashif, M., Haglund, C., Blom, K., Höglund, M., Andersson, C., Gustafsson, MG., Eriksson, A., Larsson, R. *Combination screening in vitro identifies synergistically acting KP372-1 and cytarabine against acute myeloid leukemia.* Biochemical Pharmacology. 2016, 118:40-49.

Eriksson, A., **Österroos, A.**, Hassan, S., Gullbo, J., Rickardson, L., Jarvius, M., Nygren, P., Fryknäs, M., Höglund, M., Larsson, R. *Drug screen in patient cells suggests quinacrine to be repositioned for treatment of acute myeloid leukemia.* Blood Cancer J. 2015, 5(4):e307.

Contents

Abbreviations

Allo-HSCT	Allogeneic haematopoietic stem cell transplantation
AML	Acute myeloid leukaemia
AML-MRC	Acute myeloid leukaemia with myelodysplasia-related changes
APL	Acute promyelocytic leukaemia
ATAC-seq	Assay for Transposase-Accessible Chromatin using sequencing
ATO	Arsenic trioxide
ATRA	All-*trans*-retinoic acid
BCL-2	B-cell lymphoma 2
CBF	Core-binding factor
cDNA	Complementary DNA
CEBPA	CCAAT/enhancer-binding protein alpha
CIT	Conditional inference trees
CK-AML	Acute myeloid leukaemia with a complex karyotype
CMML	Chronic myelomonocytic leukaemia
CR	Complete remission
DAP	Differentially accessible peak
DEG	Differentially expressed gene
ECOG PS	Eastern Cooperative Oncology Group performance status
ED	Early death
ELN	European LeukemiaNet
FDA	Food and Drug Administration
FDR	False discovery rate
FISH	Fluorescence *in situ* hybridisation
FLT3	FMS-related tyrosine kinase 3
GO	Gemtuzumab ozogamicin
GRCh	Genome Reference Consortium Human
Hb	Haemoglobin
HL	Hyperleukocytosis
HMA	Hypomethylating agent
HOMER	Hypergeometric Optimization of Motif EnRichment
IC	Intensive chemotherapy
ICC	International Consensus Classification
IDH	Isocitrate dehydrogenase

ITD	Internal tandem duplication
LAIP	Leukaemia-associated immunophenotype
LDH	Lactate dehydrogenase
LSC	Leukaemic stem cells
MDS	Myelodysplastic syndrome
MPN	Myeloproliferative neoplasm
MRD	Measurable residual disease
ncRNA	Non-coding RNA
NGS	Next-generation sequencing
OS	Overall survival
PC	Palliative care
PML	Promyelocytic leukaemia
PSM	Propensity-score matching
RARα	Retinoic acid receptor alpha
RNA-seq	RNA-sequencing
RT-qPCR	Real-time quantitative polymerase chain reaction
RUNX1	Runt-related transcription factor 1
s-AML	Secondary acute myeloid leukaemia
SAALR	Swedish Adult Acute Leukaemia Registry
SEER	Surveillance, Epidemiology, and End Results
TKD	Tyrosine kinase domain
WBC	White blood cell
WHO	World Health Organization

Acute myeloid leukaemia

The severe and highly heterogenous disease entity acute myeloid leukaemia (AML) constitutes the most common form of acute leukaemia among adults. Major advances have been made with regard to biological and therapeutic aspects since the discovery of the 'globuli alicantes', the white cells, in the early 1700s and the identification of leukaemia as a distinct blood disorder in the mid-19th century, made independently by Bennett and Virchow[1]. These advances include among other a detailed delineation of the genetic aberrations recurrently observed in AML as well as steady improvements in clinical outcomes. Nonetheless, major unmet clinical needs persist as far from every AML patient is cured. In fact, only approximately 25-35% survive for more than 5 years after a diagnosis of AML[2] and the median overall survival (OS) is 6-7 months when including all adult AML cases[3].

Despite its genetically and clinically diverse manifestations, overt AML emanates from the clonal expansion of immature myeloid cells and the subsequent impaired synthesis of normal blood. These myeloblasts occur as a result of numerous genetic aberrations accumulated in a haematopoietic precursor cell[4,5]. In comparison to normal haematopoiesis where multipotent haematopoietic stem cells are capable of differentiation and under tight control with regard to proliferation, the genetic alterations in AML cause blocked differentiation and rampant expansion of the myeloblasts.

The pathogenesis behind AML is multifaceted and not fully clarified. However, the leukaemogenic process includes among other the acquisition of one or more driver mutations, Darwinian selective pressure by the bone marrow microenvironment and/or immune system as well as epigenetic aberrations with the eventual formation of leukaemic stem cells (LSCs)[6,7]. This stepwise evolution also results in the presence of genetically and epigenetically distinct subclones at time of AML diagnosis[8,9]. LSCs fuel the bulk of the leukaemia as they harbour unlimited self-renewal capacity alongside a capability of reinitiating AML as shown upon transplantation into immunocompromised mice[10,11]. Moreover, the LSCs are inherently therapy-resistant to conventional chemotherapy as they, in parallel with normal haematopoietic stem cells, may enter a transiently dormant state and hence form a reservoir for relapse after remission[12,13,14]. In fact, relapsing disease in addition to primary refractory AML currently constitute two major challenges for successful AML

treatment[15] whereby improved prognostic tools and powerful treatment options upfront are necessary to avoid the selection of difficult-to-treat AML clones.

Epidemiology

AML accounts for approximately 80% of all adult acute leukaemias. The disease onset of AML is highly age-dependent with a median age at diagnosis of 72 years in Sweden[3]. Overall age-adjusted incidence rates of 4.2-5.4 per 100.000 person-years have been reported in the Western world[16,17,18] with an age-adjusted incidence of 20.1 per 100.000 person-years in individuals ≥ 65 years[19]. This translates into around 350-400 annual AML cases in Sweden with a minor male preponderance (2.9 per 100.000 men, 2.6 per 100.000 women[20]).

This strong age-dependency has led to an absolute increase over time in the incidence as the population ages. In 2022, 20% of all individuals in Sweden were ≥65 years as compared to 17% in 2000 and 16% in 1980[21]. Projected estimates have in fact pointed towards a growth in incident AML cases of approximately 30% globally until 2040[22] with Swedish data showing an annual increase of 0.7% for *de novo* AML and 4.5% for therapy-related AML[23]. The latter is mainly due to the improved survival rates of other malignancies[24].

This is likely to cause shifts in the therapeutic demands of AML in the layout of providing optimal AML care in addition to the fact that different genetic subgroups show different age distributions. This may be exemplified by core-binding factor (CBF) AML, i.e. AML with t(8;21)(q22;q22.1), *RUNX1-RUNX1T1* or inv(16)(p13.1q22), *CBFB-MYH11,* that presents at a younger age (median age 43 years) whereas AML with myelodysplasia-related aberrations such as monosomy 7, a complex karyotype and/or myelodysplasia-related mutations often appears at higher ages[25].

Clinical presentation

The expansion of leukaemic blast cells eventually results in bone marrow failure and its pertaining clinical manifestations. In addition to the malignancy-related general loss of sense of well-being, pyrexia and/or anorexia, the symptoms mainly emanate from the development of anaemia, thrombocytopenia, leukopenia or leukostasis[26]. Typical signs and symptoms of anaemia include fatigue, pallor, dyspnoea on exertion and palpitations. Thrombocytopenia causes epistaxis, easy bruising, prolonged bleeding times and petechiae whereas leukopenia mainly results in frequent infections. Leukostasis is caused by an increase in blood viscosity and its cardinal features include neurologic perturbations and respiratory distress. Circulating leukemic blasts may

also infiltrate most tissues and such extramedullary lesions may occasionally be the first disease manifestation.

As mentioned, AML is a heterogenous disease. This holds true also with regard to the clinical manifestations as the spectrum covers cases discovered *en passant* upon routine blood sampling to cases with disabling organ dysfunction requiring intensive care upon presentation.

Diagnosis and classification

The diagnostic criteria for AML are an ever-evolving subject. Before 2022 a diagnosis of AML was set in the following cases i) with >20% blast cells in the bone marrow or peripheral blood, ii) irrespective of blast count whether CBF related abnormalities, e.g. t(8;21) or inv(16), or the acute promyelocytic leukaemia (APL) defining t(15;17) were present or iii) the presence of extramedullary myeloblasts (myeloid sarcoma) as defined by the World Health Organization (WHO) in 2016[27].

The current diagnostic landscape of AML now consists of an updated version of the previous WHO edition[28] as well as a new system named the International Consensus Classification (ICC)[29]. The growing knowledge of molecular markers in AML as well as the mounting possibilities of next-generation sequencing (NGS) in clinical routine are reflected in both diagnostic classifications (Table 1).

The updated WHO criteria advocate the blast cut-off 20% apart from AML with defining genetic aberrations where no blast threshold needs to be surpassed (apart from AML with *BCR::ABL1* fusion or mutated *CEBPA* where 20% remains the limit). In cases without a defining genetic abnormality but with ≥20% blasts, the diagnosis is determined upon degree of differentiation.

The classification scheme of ICC sets the blast threshold at 10% for a diagnosis of AML with recurrent genetic abnormalities (again with the exception of AML with *BCR::ABL1* where 20% blasts are required). Blast counts of 10-19% result in a diagnosis of myelodysplastic syndrome (MDS)/AML whereas those with ≥20% blasts qualify as AML. Moreover, ICC proposes a hierarchical approach for diagnostic labelling where recurrent genetic abnormalities have supremacy over *TP53* followed by AML with myelodysplasia related gene mutations or cytogenetic aberrations and eventually AML not otherwise specified.

Both working groups suggest additional labels to the diagnosis when applicable. These include AML with germline predisposition and AML following cytotoxic therapy in the WHO update whereas ICC have supplemental qualifiers for instances with germline predisposition, a previous diagnosis of MDS or myelodysplastic/myeloproliferative neoplasm (MDS/MPN) and therapy-related AML.

Hence, an accurate diagnosis of AML currently requires the cooperation of numerous clinical and laboratory specialists including haematologists, haematopathologists, and geneticists as recommended by the European LeukemiaNet (ELN)[30]. In addition to the sampling of peripheral blood and bone marrow at diagnosis, further characterisation is typically done by immunophenotyping by flow cytometry, mutational screening by NGS based gene panels and cytogenetic analyses. The latter includes fluorescence *in situ* hybridisation (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR) to rapidly find certain clinically important chromosomal aberrations such as APL- or CBF-defining lesions whereas conventional karyotyping may be used to find other aberrations.

Table 1. Adapted compilation of diagnostic classifications as presented by WHO 2016 (ref. 27), WHO 2022 (ref. 28) and ICC 2022 (ref. 29). Footnotes omitted.

WHO 2016	WHO 2022	ICC 2022
APL with *PML-RARA*	APL with *PML::RARA* fusion	APL with t(15;17)(q24.1;q21.2)/*PML::RARA* or APL with other *RARA* rearrangements
AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*	AML with *RUNX1::RUNX1T1* fusion	AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*	AML with *CBFB::MYH11* fusion	AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
AML with t(9;11)(p21.3;q23.3);*KMT2A-MLLT3*	AML with *KMT2A* rearrangement	AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A* or AML with other *KMT2A* rearrangements
AML with t(6;9)(p23;q34.1);*DEK-NUP214*	AML with *DEK::NUP214* fusion	AML with t(6;9)(p22.3;q34.1)/*DEK::NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*	AML with *MECOM* rearrangement	AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2; MECOM(EVI1) or other *MECOM* rearrangements
-	AML with *NUP98* rearrangement	-
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*	-	-
Provisional entity: AML with BCR-ABL1	AML with *BCR::ABL1* fusion	AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1*
AML with mutated *NPM1*	AML with *NPM1* mutation	AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*	AML with *CEBPA* mutation	AML with in-frame bZIP *CEBPA* mutations
-	-	AML with mutated *TP53*
Provisional entity: AML with mutated RUNX1	-	-
AML with myelodysplasia-related changes	AML, myelodysplasia-related	AML with myelodysplasia-related gene mutations or cytogenetic abnormalities
-	AML with other defined genetic alterations	AML with other rare recurring translocations
AML, Not Otherwise Specified	AML, defined by differentiation	AML, not otherwise specified
Myeloid sarcoma	Myeloid sarcoma	Myeloid sarcoma
Therapy-related myeloid neoplasms	-	-

Prognostic factors and risk stratification

Despite advances in the clinicobiological understanding of the disease, the prognosis of AML is unsatisfactorily poor. Nonetheless, progress has been made which can be illustrated by an increase in the relative 5-year survival from 2.7-3.6% in the early 1970s to 34.3-35.3% in the late 2010s in Sweden[31] with similar numbers reported from the Surveillance, Epidemiology, and End Results (SEER) program[32].

Numerous factors affect the prognosis and a holistic assessment is required to properly judge a patient's trajectory. These factors can to a large extent be divided into patient-specific, leukaemia-intrinsic and treatment-related variables (Figure 1).

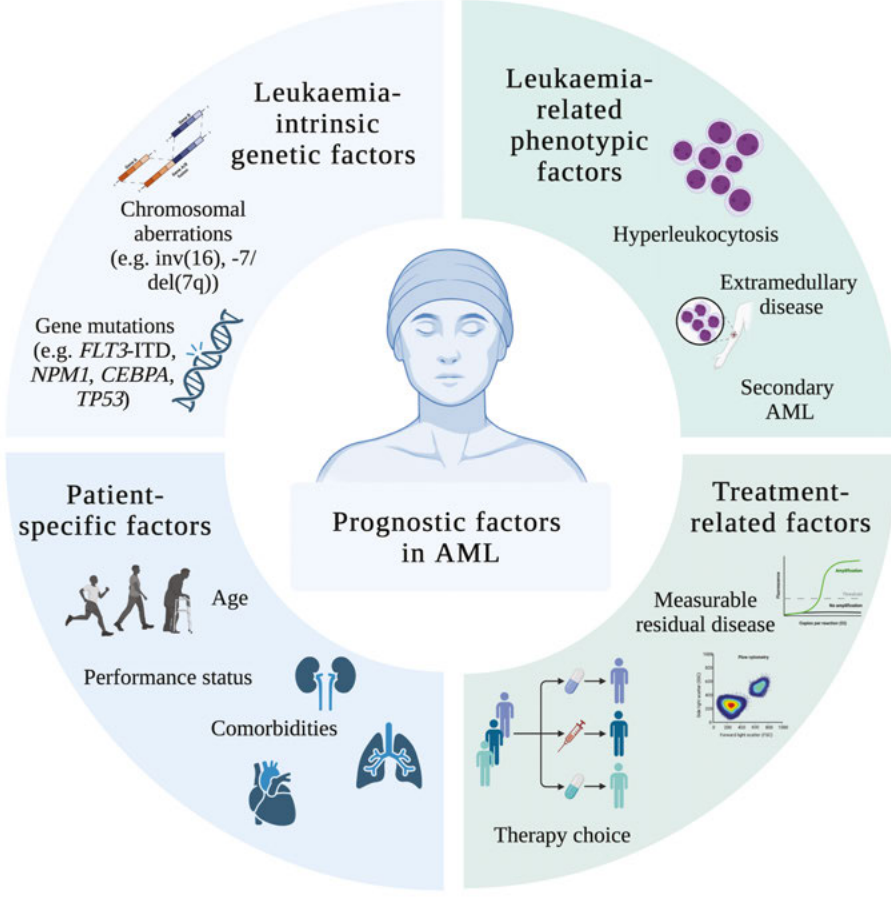

Figure 1. Prognostic factors in AML.

Patient-specific factors

Age

The patient's age at diagnosis remains one of the strongest patient-related factors with regard to prognostication. Adult patients <50 years have estimated overall 1- and 5-year survival rates of approximately 70% and 55%, respectively. In comparison, patients 60-75 years have 1- and 5-year OS of approximately 40% and 20%, respectively, and patients >75 years have a chance at 1-year OS of 10%[33,34]. A diagnosis of AML when ≥65 years conveys a median OS of 2.7 months according to SEER data which in fact is the shortest of numerous malignancies including other aggressive cancer types such as pancreatic and lung cancer[19].

The grim prognosis in elderly AML patients is mainly caused by the higher prevalence of comorbid conditions, worse performance status at diagnosis, higher presence of a preceding phase of MDS or MPN as well as the frequent finding of adverse-risk molecular markers.

Performance status

Prognosis becomes worse as the Eastern Cooperative Oncology Group performance status (ECOG PS) increases, in particular in elderly patients and in particular if >2[3,35]. Moreover, higher ECOG PS values have been associated with treatment toxicity and early mortality, especially in combination with age[36,37].

Comorbidities

The prevalence of comorbidity increases with age. Approximately 40% of all AML patients have a comorbid disease at diagnosis but the prevalence ranges from 15% in patients 18-49 years to 59% in patients ≥80 years[38]. Validated comorbidity indices may be used to predict outcomes in AML. These include the Charlson Comorbidity Index[39] and the Hematopoietic Cell Transplantation–Specific Comorbidity Index[40]. These tools have been shown to independently predict complete remission (CR), early death (ED) and OS[41,42,43,44,45].

Leukaemia-intrinsic factors

Hyperleukocytosis

Hyperleukocytosis (HL) is usually defined as a white blood cell (WBC) count >100 x 10^9/L and is considered a haematologic emergency in the context of AML[46]. As such, HL portends a high risk of complications, ED and an overall association with poor outcomes[47,48]. The worse prognosis may in large part be attributable to HL causing leukostasis, tumor lysis syndrome and disseminated intravascular coagulation.

Extramedullary disease

The presence of extramedullary disease at AML diagnosis has been reported to be present in 2-17% of newly diagnosed patients and mainly involves the skin, the central nervous system or the pleura[49,50,51]. Extramedullary involvement has been associated with lower odds of achieving CR, higher rates of ED and shorter OS as compared to AML patients without extramedullary disease[52].

Secondary AML

The development of AML after previous cytotoxic treatment, commonly defined as therapy-related AML, or following an antecedent haematological disorder, usually MDS or MPN, may collectively be defined as secondary AML (s-AML)[53,54]. In comparison to *de novo* AML, s-AML is associated with lower CR rates and shorter OS[55,56]. These poorer outcomes are mainly attributable to numerous genetic abnormalities as described below.

Chromosomal aberrations

Cytogenetic aberrations have long been known to impact rates of CR, relapse and OS in AML[57,58,59,60]. Approximately 50-60% of all AML cases will have detectable cytogenetic abnormalities at the time of diagnosis. These are mainly categorised as balanced chromosomal rearrangements, complex karyotypes (i.e. ≥3 unrelated chromosomal abnormalities), and other unbalanced abnormalities including trisomies and deletions. Cytogenetic aberrations currently included in the latest risk stratification by the ELN are outlined below.

CBF AML constitutes a subset with balanced chromosomal rearrangements with a more favourable prognosis compared to other cytogenetic subgroups[61]. CBF AML is defined as the presence of t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) that results in the fusion genes *RUNX1/RUNX1T1* and *CBFB/MYH11*, respectively. These chimeric proteins will in turn negatively affect the transcriptional activity of RUNX1 which is required for normal haematopoiesis[62]. CBF AML has consistently been associated with good responses to intensive chemotherapy and with younger age at time of diagnosis[25,58,63].

AML with a complex karyotype (CK-AML) exists on the other end of the prognostic spectrum including an association with higher age and s-AML[64]. CK-AML accounts for approximately 10% of all cases. Moreover, relapse rates are high as more than half of all CK-AML patients who undergo an allogeneic haematopoietic stem cell transplantation (allo-HSCT) will relapse within 2 years from transplantation[65]. CK-AML mainly reflects deregulations in genomic stability and mutated *TP53* is detectable in up to two-thirds of all CK-AML patients[66]. Other adverse cytogenetic markers include AML with a monosomal karyotype, monosomy 5 or deletion 5q, 3q abnormalities (involving *MECOM*) and the most frequently recurrent high-risk marker; monosomy

7 or deletion 7q. These markers usually co-occur and are indicative of s-AML and confer low responses to conventional chemotherapy[67].

Other though more infrequently observed cytogenetic aberrations that still imply a poor prognosis include translocations resulting in the fusion genes *DEK*::*NUP214*, *BCR::ABL1* and *KAT6A::CREBBP*. Each of these accounts for around 1% of all AML cases annually and they have repeatedly been associated with low CR rates upon intensive chemotherapy[68,69,70,71]. Likewise, *KMT2A*-rearranged AML that accounts for 3-7% of all AML cases predicts a poor prognosis due to treatment resistance and relapses[72,73]. Of note, the balanced translocation t(9;11)(p21.3;q23.3) that results in the fusion gene *MLLT3*::*KMT2A* is stratified as an 'intermediate' risk AML[30]. As the most common *KMT2A* rearrangement, it is found in approximately 30-40% of all *KMT2A* rearranged AML cases. This subgroup has been associated with superior outcomes compared to other *KMT2A* rearranged subgroups, especially in younger patients[72].

Lastly, around 40% of all cases will present with a normal karyotype[57] and the diversified prognoses observed within this large subset are mainly attributable to the context of other molecular aberrations in addition to the above-mentioned patient-specific factors.

Somatic mutations

The number of acquired somatic mutations in AML is lower than for most other malignancies. Whole-genome and whole-exome sequencing has shown an average of 13 mutations per AML case[74]. Nonetheless, somatic mutations are found in 97% of all cases[75]. The prognostic impact of somatic mutations in AML is an expanding field which may be illustrated by the fact that the most recent ELN risk classification includes 13 driver genes as compared to 6 in the version from 2017 and 3 in the version published in 2010[30,76,77] with seemingly improved prognostic usefulness[78,79]. Molecular aberrations included in ELN 2022 are outlined below.

NPM1

Mutated *NPM1* is one of the most commonly found molecular aberrations in AML and detectable in approximately one-third of all cases[80,81]. Around half of all patients with normal cytogenetics harbours a mutation in *NPM1*. Other co-occurring genetic lesions are necessary for leukemic transformation and the prognosis of *NPM1* mutated AML is thereby contextual[81,82]. This is particularly evident with regard to the presence of internal tandem duplications in *FLT3* (*FLT3*-ITD) or not. ELN 2022 risk stratifies AML with mutated *NPM1* without *FLT3*-ITD as "favourable" and cases with *FLT3*-ITD as "intermediate".

Moreover, the co-occurrence of mutations in *NPM1* with either *NRAS*, *RAD21* or in the tyrosine kinase domain of *FLT3* (*FLT3*-TKD) has been associated with better prognosis whereas mutated *WT1* or adverse cytogenetics

confer worse prognosis[75,83,84]. Importantly, *NPM1* mutated AML responds well to treatment with a hypomethylating agent (HMA) and venetoclax[85], underlining the importance of molecular testing also in patients ineligible for intensive chemotherapy. It shall however be emphasised that the presence of adverse risk cytogenetics, which is more common in the elderly, nullifies the impact of a mutated *NPM1*[86].

CEBPA

CCAAT/enhancer-binding protein alpha (CEBPA) is a transcription factor with pivotal roles in myeloid differentiation. *CEBPA* mutations are found in around 10% of all AML patients and confer a good prognosis. Initial studies indicated that the favourable prognosis was restricted to patients with biallelic mutations[87]. Later studies have now confined the beneficial impacts on prognosis to in-frame mutations in the basic leucine zipper region of *CEBPA* regardless of whether these occur as single or double mutations[88,89]. *CEBPA* mutated AML is highly sensitive to intensive chemotherapy which is reflected by reported CR rates of over 90%[89,90].

FLT3

A mutated *FLT3* in the form of *FLT3*-ITD is found in 25% of all AML patients whereas *FLT3*-TKD is detected in 5-10% of all cases[74,75]. The prognostic importance of the latter has not been established, although both *FLT3*-ITD and *FLT3*-TKD result in constitutive activation of the FLT3 tyrosine kinase with the promotion of cell survival and proliferation. In fact, *FLT3*-ITD mutated AML often presents with a high leukaemic burden including leukocytosis and high blast counts[91,92].

Hence, *FLT3*-ITD is associated with worse prognosis, in particular in patients <60 years[93], and commonly persists as a driver mutation also at relapse[94]. The high proportion of AML patients with mutated *FLT3* as well as *FLT3* as a well-identified and targetable tyrosine kinase has lately led to the development of numerous *FLT3* inhibitors with evident clinical benefits[95]. Their roles in the treatment of *FLT3* mutated AML remain to be clarified.

TP53

TP53 stands as the mutation with the highest unmet clinical need. Mutated *TP53* is present in 5-15% of all AML cases[75] and aberrations in this vital tumour suppressor gene result in genomic instability and complex cytogenetic abnormalities. The median OS of *TP53* mutated AML is around 6 months irrespective of treatment with intensive chemotherapy or HMA with or without venetoclax[96]. The poor prognosis conferred by *TP53* mutated AML is mainly caused by an intrinsic resistance to DNA-damaging treatments as *TP53* is pivotal in driving the apoptosis upon chemotherapeutic exposure[63].

Myelodysplasia related gene mutations

Since the introduction of AML with myelodysplasia-related changes (AML-MRC) in 2008 by the WHO classification[97], there have been major advances in the biological understanding of this category. Hence, this entity has now been removed from both the WHO and ICC classification schemes and replaced by genetic and cytogenetic categories that define an s-AML ontogeny. These changes are mainly caused by the fact that AML-MRC defining dysplasias lack prognostic value and that the genetic aberrations override the clinical history with regard to AML biology and prognosis[98]. The list of myelodysplasia related gene mutations currently includes spliceosome genes (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*) and chromatin modifying genes (*ASXL1*, *STAG2*, *EZH2* and *BCOR*) in both the WHO and ICC classifications with the addition of *RUNX1* by the latter. All of these have been associated with a poor prognosis and thereby stratified as "adverse" risk AML by ELN 2022[30]. Importantly, these mutations confer an equally inferior prognosis in *de novo* AML as they do in s-AML[99].

Mutations in *RUNX1* are detectable in 5-10% of all AML cases[74,100]. *RUNX1* mutations more commonly co-occur with *FLT3*-ITD, mutated *NRAS* and partial tandem duplication of the *MLL* gene and is also associated with higher age[101,102]. These contextual factors likely contribute to the adverse impact of *RUNX1* mutations. In addition, co-mutations in *ASXL1*, *SRSF2* and *PHF6* confer an even worse prognosis[101].

The combined subgroup of chromatin-spliceosome mutations comprises the second largest subgroup in AML, only superseded by *NPM1* mutated AML[75]. This fits well with the previous notion of AML-MRC representing approximately 25-30% of all AML diagnoses.

Spliceosome gene mutations are detectable in up to 50% of all s-AML cases as compared to approximately 7% of *de novo* AML patients[98]. Interestingly, spliceosome mutations seem to be mutually exclusive from each other and hence synthetically lethal when co-expressed[75,103]. These mutations have in addition to the linkage with s-AML shown age-dependency[104] and an association with other adverse genetic risk factors such as mutated *RUNX1*[105].

ASXL1 is the most frequently mutated chromatin modifying gene in AML and mutated in 5-15% and 20-30% of all patients with *de novo* AML and s-AML, respectively[106,107]. Mutations in *STAG2*, *EZH2* and *BCOR* are genewise detectable in approximately 2-5% of *de novo* AML cases and 5-15% of s-AML cases[108].

The factors driving the seemingly chemoresistant phenotype caused by the above-mentioned myelodysplasia related gene mutations are multifaceted. These include among other a different mutational burden in s-AML as compared to *de novo* AML, in particular with higher rates of *TP53* mutations as well as subclonal heterogeneity[98]; a higher degree of DNA hypermethylation and thereby the silencing of tumour suppressor genes[109]; as well as a higher expression of antiapoptotic proteins such as BCL-2 and BCL-XL[110].

Other leukaemia-intrinsic factors

Currently, only recurrent and validated genetic and cytogenetic aberrations are implemented in clinical practice. Nonetheless, numerous attempts have been made to further refine the prognostic stratification. These endeavours have mainly focused on epigenetic markers although predictions based on gene expression, non-coding RNAs (ncRNAs) and proteomics also have been put forward[111,112,113].

DNA methylation patterns have been shown to be altered in AML with subsequent transcriptional dysregulation. Moreover, the potential reversibility of these DNA methylation changes has resulted in the development of epigenetically active drugs including HMAs such as azacitidine and decitabine, in particular with the now evident knowledge that disrupted epigenetic mechanisms are important for AML initiation and perpetuation[114].

DNA methylation signatures have been utilised to define distinct subgroups of AML within the already defined genetic subgroups. This may be exemplified by *NPM1* mutated AML and AML with normal karyotype that based on DNA methylation profiling can be subdivided into 4 and 5 clusters, respectively, with disparate outcomes[115]. Moreover, methylation scores have been presented with suggested utility in prognostication[116,117]. However, the implementation of these biomarkers in clinical routine would require further validation and/or replication in uniformly treated and bigger cohorts as well as the development of accredited laboratory methods.

Likewise, a plethora of ncRNA molecules has been suggested to contribute to AML heterogeneity and thereby potential utility as biomarkers[113,118]. These ncRNAs have been shown to harbour both oncogenic and tumour suppressive roles. Prognostic studies to date have mainly focused on microRNAs and long non-coding RNAs with shown potential as diagnostic and prognostic markers, indicative of treatment resistance and as possible therapeutic targets[118,119]. Nonetheless, the implementation of these markers is currently hindered by the same factors as for DNA methylation signatures; a lack of well-designed, prospective cohort studies of adequate sizes.

Treatment-related factors

The presence of measurable residual disease (MRD) is highly associated with worse prognosis[120], in particular MRD positivity after two courses of chemotherapy[121,122,123]. Moreover, conversion from MRD negativity to MRD positivity during follow-up after completed treatment is also highly indicative of a morphologic relapse. MRD positivity after non-intensive induction has also been shown to confer worse prognosis[124]. The current ELN 2022 recommendation emphasises the fact that MRD status and response to upfront treatment are pivotal in assessing a patient's risk in addition to the genetic classification.

In fact, these treatment-related factors may clinically be used to re-stratify patients dependent on treatment response.

MRD is nowadays assessed in clinical routine by multiparameter flow cytometry or molecular methods, mainly real-time quantitative PCR (RT-qPCR). The diagnostic work-up of AML includes immunophenotyping with flow cytometry and by that also a leukaemia-associated immunophenotype (LAIP) and/or an aberrant immunophenotype different from normal. Flow cytometry is limited by the fact that not all patients have an LAIP and a relatively low sensitivity where it identifies one leukaemic cell per 10^3-10^4 healthy cells. RT-qPCR is more sensitive and recommended by national guidelines for MRD assessment in patients with mutated *NPM1*, CBF AML or APL.

Risk stratification

For clinical routine, the ELN presented their latest genetic risk classification in 2022 (Table 2). It integrates recurrent chromosomal abnormalities and somatic mutations with regard to prognosis. The prognostic impact of cytogenetic aberrations has been known for decades whereas the list of risk stratifying gene mutations keeps on growing. The risk classification is mainly used as a tool to identify patients in the "intermediate" or "adverse" groups where allo-HSCT should be considered given a high risk of relapse if only treated with chemotherapy.

Table 2. Genetic risk stratification by ELN 2022 (ref. 30). Footnotes omitted.

Risk	Genetic abnormality
Favourable	▸ t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* ▸ inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/ *CBFB*::*MYH11* ▸ Mutated *NPM1* without *FLT3*-ITD ▸ bZIP in-frame mutated *CEBPA*
Intermediate	▸ Mutated *NPM1* with *FLT3*-ITD ▸ Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) ▸ t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A* ▸ Cytogenetic and/or molecular abnormalities not classified as favourable or adverse
Adverse	▸ t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* ▸ t(v;11q23.3)/*KMT2A*-rearranged ▸ t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* ▸ t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* ▸ inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/ *GATA2*, *MECOM(EVI1)* ▸ t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged ▸ −5 or del(5q); −7; −17/abn(17p) ▸ Complex karyotype, monosomal karyotype ▸ Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* and/or *ZRSR2* ▸ Mutated *TP53*

Treatment regimens

The treatment armamentarium of non-APL AML has for decades mainly been limited to conventional chemotherapy. A detailed molecular understanding and the delineation of distinct AML subsets have recently been translated into the approval of numerous novel agents and targeted drugs as depicted in Figure 2. The expanded treatment arsenal now requires tactful clinical judgements, especially with regard to treatment intentions.

Treatment goals should be tailored to and in dialogue with each individual patient. A holistic assessment of the leukaemia-intrinsic features in combination with the patient's health state and preferences is necessary to appropriately identify the goals of care. Long-term survival and cure should be the primary aim in patients who are medically fit and this is mainly accomplished by achieving CR by the use of intensive chemotherapy. On the contrary, the goals in patients who are frail or medically unfit should be focused on life prolongation, quality of life improvements and symptom alleviation.

The definition of unfitness to intensive therapies is multifaceted although numerous attempts have been made to find stringent criteria including age, ECOG PS and/or comorbidities. Age alone cannot be used as a biomarker as older patients also benefit from more intensive treatments and may achieve long-standing remissions[125,126]. Nonetheless, it shall be emphasised that the risks of intensive chemotherapy may outweigh the benefits in patients with poor ECOG PS and/or adverse risk genetic aberrations as exemplified by higher rates of induction-related mortality and lower chances of CR achievement[127,128].

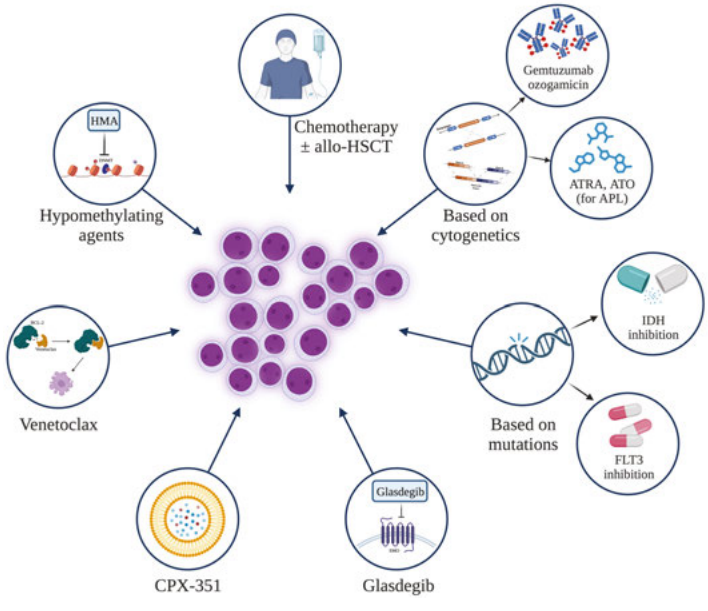

Figure 2. Overview of currently approved AML therapies.

Medically fit patients

The vast majority of medically fit AML patients undergo induction treatment with intensive chemotherapy, mainly cytarabine for 7 days (or 5 days in Sweden) combined with an anthracycline for 3 days, hence nicknamed "7 + 3". This cytotoxic strategy reaches CR rates of up to 80% in patients <60-65 years of age[129] and have constituted the backbone of AML treatment since its presentation in a landmark publication in 1973[130].

The achievement of CR is followed by courses of consolidation therapy. For patients not achieving CR by induction treatment, other chemotherapeutic drug combinations will be utilised for reinduction. Allo-HSCT should be considered for patients in the "intermediate" and "adverse" risk categories as defined by ELN due to an otherwise high risk of relapse. Allo-HSCT confers the possibilities of maximal anti-leukaemic activity by the means of intensive conditioning regimens containing chemotherapeutic and immunosuppressive agents and graft-versus-leukaemia effects induced by the new immune system emanating from the donor. However, the procedure of allo-HSCT is lined with a high rate of complications including excessive nonrelapse mortality including the risk of immune-mediated graft-versus-host disease[26,131]. Reduced-intensity conditioning regimens have thus been developed as an alternative to traditional myeloablative conditioning regimens to abrogate the risk of transplant related mortality in older patients.

Moreover, the notion of *FLT3* as a targetable and common AML mutation has led to the development of numerous more or less specific FLT3 inhibitors mainly to mitigate the increased relapse risk noted in *FLT3*-ITD AML. Currently, midostaurin is recommended as an adjunct to "7 + 3" with shown benefits with regard to OS and event-free survival in patients with *FLT3* mutated AML[132,133].

The recommended treatment for patients with *de novo* CBF AML and CD33-positivity now includes the immunoconjugate gemtuzumab ozogamicin (GO) in addition to "7 + 3". The targeted actions of GO are based on the linkage between the cytotoxic substance calicheamin and an anti-CD33 monoclonal antibody. In spite of conflicting results in randomised trials, a more recent meta-analysis has shown improved OS and lower rates of relapse with the addition of GO to conventional chemotherapy in untreated patients with CBF AML[134].

Secondary AML constitutes a persisting clinical challenge and treatment successes have been scarce. The synergistic effects of cytarabine and daunorubicin are maximised *in vitro* in a 5:1 molar ratio given that the drugs reach the myeloblasts[135]. These findings have led to the development of CPX-351, a liposomal encapsulation of cytarabine and daunorubicin that maintain the synergistic ratio. CPX-351 has shown OS benefits compared to conventional induction therapy and consolidation in patients with therapy-related AML, a

history of MDS or chronic myelomonocytic leukaemia (CMML), or AML-MRC[136,137].

Non-intensive alternatives

Hypomethylating agents

The HMA azacitidine and its deoxy derivative 5-aza-2'deoxycytidine (decitabine) were initially developed in the 1960-1970s as cytotoxic agents[138]. However, clinical usage was abandoned due to dose-dependent and extended myelosuppression and neurotoxicity. Further studies pointed to a differentiating effect via the inhibition of DNA methylation upon exposure to lower doses of HMAs[139,140] whereas later studies have elucidated pleiotropic effects of HMAs. The latter includes the induction of tumour suppressor genes as well as immune response activation[141,142,143]. Importantly, clinical trials utilising lower dose strategies eventually showed clinical benefits which led to the approval of azacitidine and decitabine in the early 2000s for the treatment of higher-risk MDS and CMML[144].

Ultimately, phase 3 randomised trials demonstrated superior results with the use of an HMA versus best supportive care (including low-dose cytarabine)[145,146,147], the latter being the main prevailing option to patients ineligible for intensive chemotherapy before the introduction of HMAs. Nonetheless, monotherapeutic usage of HMAs has resulted in CR rates of 20-30% with little to no definite improvements in OS and hence the necessity of developing other alternatives to HMAs as well as combination regimens with an HMA as a backbone given the clinical effectiveness.

The United States Food and Drug Administration (FDA) has also approved oral azacitidine (CC-486) as maintenance treatment after achieving a first remission. This strategy has resulted in OS benefits in a phase 3 trial in non-transplantable patients[148].

Venetoclax

The current recommended frontline therapy to older patients and patients ineligible for intensive chemotherapy consists of an HMA in combination with venetoclax. The latter was developed to overcome one of the hallmarks of malignant cells, namely their inherent resistance to apoptosis. Venetoclax acts as a BH3 mimetic with highly selective binding to the anti-apoptotic protein BCL-2[149]. This binding prevents the interaction of BCL-2 with pro-apoptotic molecules, mainly BAX and BIM, and therefore the induction of apoptosis[150].

Clinical gains of BCL-2 inhibition were first accomplished in chronic lymphocytic leukaemia[151,152] and later also as monotherapy in AML[153], largely based on the frequent observation of BCL-2 overexpression in AML cell lines[154] in addition to their dependency on BCL-2 for survival[155]. However, when firstly evaluated in a clinical trial (then mainly in relapsed/refractory

AML patients), the response rates were modest with venetoclax as single-agent therapy, namely 6% and 13% reaching CR or incomplete CR, respectively, and a median CR duration of only 1.6 months[153]. Consequently, combinatorial regimens including venetoclax were actively investigated and preclinical studies were able to show positive synergistic activities with both low-dose cytarabine[156] and HMAs[157,158]. These results were ultimately translated into clinical benefits as a large phase 3 placebo-controlled trial was able to show a distinct survival benefit of azacitidine and venetoclax as compared to azacitidine alone, notably in a usually hard-to-treat aged study population (median age 76 years)[159,].

The efficacy of azacitidine-venetoclax is now supported by follow-up[160] and real-world data[161,162] but questions remain, in particular with regard to venetoclax resistance (both intrinsic and acquired), cycle lengths, potential biomarkers for response and the role for other potent venetoclax-based combinations.

IDH

One-fifth of all AML patients harbours a mutation in the isocitrate dehydrogenase (IDH) genes *IDH1* or *IDH2*[163]. These mutations cause an accumulation of the oncometabolite 2-hydroxyglutarate and thereby alterations in cell differentiation and methylation[164,165]. Targeted inhibition of mutated *IDH1* with ivosidenib and of *IDH2* with enasidenib was approved by the FDA in 2017 for patients with relapsed/refractory AML based on shown benefits in clinical studies where approximately one-fifth achieved CR[166,167]. Olutasidenib, an *IDH1* inhibitor, has also been FDA approved for the relapsed/refractory setting[168].

Moreover, ivosidenib has been approved by the FDA as frontline treatment, either as monotherapy or in combination with azacitidine, to AML patients with an *IDH1* mutation and ineligible for intensive chemotherapy[169]. However, it is important to emphasise that mutations in *IDH1/2* seem to predict durable responses also to azacitidine-venetoclax[85,170]. Hence, the role of IDH inhibitors in the AML armamentarium is currently actively investigated including clinical studies on their efficacy in combination with induction chemotherapy and/or as maintenance drugs.

FLT3

Likewise, the position of *FLT3* inhibition is and has been duly examined also in *FLT3* positive patients unfit for intensive chemotherapy. Gilteritinib is currently approved as monotherapy in relapsed/refractory AML patients based on OS gains when compared to salvage chemotherapy, especially in patients with co-occurring mutations in both *DNMT3A* and *NPM1*[171].

Nonetheless, remissions are short if not followed by allo-HSCT as emergence of resistant clones is frequent[172]. Ongoing clinical trials thereby aim at synergistic combinations with prolonged remissions and avoidance of

resistance. Moreover, *FLT3* inhibition in the posttransplant setting is a tempting maintenance strategy to avoid relapse, as shown in clinical trials with the multikinase inhibitor sorafenib[173,174].

Glasdegib

Aberrant Hedgehog (Hh) signalling is common in AML and includes the transcription of anti-apoptotic proteins as well as LSC maintenance and expansion[175,176]. This fact has ultimately led to the development of the Hh signalling inhibitor glasdegib which currently is approved for use in combination with low-dose cytarabine in patients not eligible for intensive chemotherapy[177]. However, in the current era of venetoclax-azacitidine as recommended frontline therapy to this patient category it shall be emphasised that glasdegib-cytarabine has shown far lower response rates and shorter OS than venetoclax-azacitidine. Future studies will in other words need to aim at decipher what AML subgroups benefit most from glasdegib and especially how to optimally combine glasdegib with other treatment options.

Acute promyelocytic leukaemia

Acute promyelocytic leukaemia (APL) stands as a role model for successful precision medicine in the field of AML although it once was described as the "most malignant form of acute leukaemia"[178,179]. This distinct subtype accounts for 5-10% of all AML cases annually[180] and harbours specific clinical, molecular and prognostic characteristics.

Clinically, the median age at diagnosis is approximately 58 years[181] which is clearly lower than for most other AML subtypes and thrombo-haemorrhagic manifestations are usually prominent at time of diagnosis[182,183]. The characteristic consumptive coagulopathy typically presents as skin and mucous membrane haemorrhages but pulmonary or intracranial bleedings are not infrequent. Consequently, APL is a haematologic emergency and requires a high degree of suspicion, especially due to long-term cure rates >90% with current treatment regimens[184].

The cytogenetic lesion defining APL involves the rearrangement of the retinoic acid receptor alpha (*RARα*) gene on chromosome 17(q21). The most commonly found aberration is the balanced reciprocal translocation t(15;17)(q21;q22) that is seen in >95% of all APL cases[185], a translocation also involving the promyelocytic leukaemia (*PML*) gene on chromosome 15. The resulting fusion protein PML-RARα causes blockage in myeloid differentiation by acting as a transcriptional repressor on retinoic acid response elements including genes such as *CDKN1A, C/EBPE* and *HOXA1*[186]. In addition, PML-RARα has been shown to cause epigenetic aberrations by recruiting methylating enzymes to critical domains[187]. Treatment with all-*trans*-retinoic acid (ATRA) induces differentiation as ATRA nullifies the transcriptional gene repression by PML-RARα[188].

Numerous factors contribute to the phenotypic hyperfibrinolytic and procoagulant state seen in APL. These include an activation of the tissue factor promoter by PML-RARα[189], the surface expression of annexin II on leukaemic promyelocytes[190] as well as the release of chromatin and phosphatidylserine as APL cells undergo ETosis[191].

Early death

The above-mentioned thrombo-haemorrhagic state will, if left untreated, result in ED from bleeding diathesis. ED is usually defined as death within 30 days of diagnosis. Rates of ED range from 5-10% within clinical trials whereas real-world data have reported ED rates of up to 20-30%[184]. Moreover, more than half of all EDs occur within the first week of presentation and most of these already within the first 24 h. Hence, a high degree of suspicion, a rapid initiation of ATRA and aggressive means to correct the coagulopathy are all necessary to abrogate the risk of ED.

Different variables associated with a higher risk of ED include higher age, worse ECOG PS, high-risk disease (as defined below), concurrent infection as well as delays in the administration of ATRA[181,192,193,194]. In addition, lethal bleedings are more frequently seen in APL patients with laboratory perturbations including high levels of WBCs, lactate dehydrogenase and/or creatinine, low fibrinogen levels as well as a prolonged prothrombin or activated partial thromboplastin time[192].

Risk stratification and induction treatment

The current widely used risk stratification of APL, known as the Sanz score, dates back to 2000 and is based on a total of 217 patients included in two clinical trials[195]. The model was originally developed to predict three-year relapse-free survival after treatment with ATRA and idarubicin. It has henceforth been shown useful in distinguishing non-high-risk patients that show sustained responses to ATRA and arsenic trioxide (ATO) as compared to ATRA and chemotherapy. The model includes three categories:

- Low risk; WBC $<10x10^9$/L and platelets $>40x10^9$/L
- Intermediate risk; WBC $<10x10^9$/L and platelets $<40x10^9$/L
- High risk; WBC $>10x10^9$/L

The recommended induction therapy to APL patients with WBC $<10x10^9$/L, i.e. the "low" and "intermediate" risk groups, consists of ATRA combined with ATO[196]. This approach results in haematologic CR rates and estimated 4-year event free-survival rates above 95% and 4-year OS rates of up to 99%[197,198]. ATRA in combination with chemotherapy is the current preferred treatment for patients with "high" risk disease, mainly due to lack of data supporting ATRA plus ATO for these patients[196].

Aims of the thesis

The overall aim of this thesis was to improve the outcomes for patients with AML by better risk stratification and by improving antileukaemic drug treatment. The specific aims of included papers were:

Paper I

To develop and validate a prediction model for ED in newly diagnosed APL patients in the real-world setting.

Paper II

To investigate the prognostic effect of molecular markers (including somatic mutations and transcriptome data) in AML patients ≥ 65 years.

To identify clinical and molecular features that may be utilised to predict the achievement of CR in AML patients ≥ 65 years treated with intensive chemotherapy.

Paper III

To investigate the usage and effectiveness of HMAs upfront during 2008-2018 in AML patients ≥ 60 years in Sweden.

To characterise clinical factors predictive of response to HMAs.

Paper IV

To characterise the epigenetic and transcriptomic effects of azacitidine and venetoclax in AML to better understand the synergy between the two drugs.

To elucidate potential survival/resistance mechanisms upon azacitidine-venetoclax exposure.

To suggest triplet combinations via *in silico* drug predictions based on obtained molecular data to further enhance the synergy.

Patient cohorts, materials and methods

Patient cohorts

Swedish Adult Acute Leukaemia Registry

Clinical data for **Paper I, Paper II** and **Paper III** were retrieved from the Swedish Adult Acute Leukaemia Registry (SAALR). This nationwide registry was founded in 1997 and comprises information such as demographics, disease characteristics and treatment intention on patients diagnosed with acute leukaemia. The coverage is approximately 98% as compared to the legislative registration in the Swedish Cancer Registry[3].

In **Paper I** we extracted basic demographic and diagnostic laboratory values for all adult APL patients (n = 301) diagnosed between January 1997 and December 2020 from the SAALR. Additional data were collected from the patients' health records. The Swedish patients constituted a training cohort for the development of a prediction model for ED in APL.

The given therapy as well as outcomes are reported to SAALR by the treating clinician or hospital. For **Paper III** we included all reported AML patients ≥60 years (n = 3 135) with a diagnosis of AML between January 2008 and December 2018.

ClinSeq

Paper II is based on the research group's own well-characterised AML cohort named ClinSeq which contains Swedish AML cases diagnosed between 1997 and 2014[199,200]. ClinSeq includes clinical data obtained from SAALR and patient records as well as molecular data including gene panel sequencing, RNA-sequencing (RNA-seq), whole-genome sequencing and methylome characterisation. For the scope of **Paper II**, we extracted data from ClinSeq on 182 AML patients ≥65 years of which 130 patients were treated upfront with intensive chemotherapy, 45 patients with palliative treatment and 7 patients with HMAs.

Validation cohorts

In **Paper I**, the proposed model for ED prediction was externally validated in a single-centre APL cohort (n = 129) diagnosed and treated at the University

Hospital Centre of São João, Porto, Portugal, between January 2005 and April 2019.

We developed a risk-stratifying algorithm with regard to CR achievement in intensively treated patients in **Paper II**. The obtained algorithm was externally validated by using the beatAML cohort which contains clinical and molecular information on 562 primary AML patients by the joint venture of 11 academic medical centres in the United States[201]. Ninety-four intensively treated AML patients ≥50 years with available RNA-seq data constituted a validation cohort in **Paper II**.

Primary patient samples and cell lines

Paper II includes sequencing analyses on primary patient samples which were collected from newly diagnosed AML patients by bone marrow or peripheral blood sampling before treatment initiation. Mononuclear cells were isolated via Ficoll-Plaque density-gradient centrifugation and were then vitally frozen until use. Samples were collected after informed consent.

Paper IV includes the two myeloid leukaemia cell lines Kasumi-1 and KG-1. These were used for *in vitro* exposure to azacitidine and/or venetoclax and downstream sequencing analyses.

Genetic profiling

The diagnosis of APL was confirmed in all included cases in **Paper I** per clinical routine with the use of cytogenetics, FISH and/or RT-PCR.

ClinSeq data on the mutational status of 25 different genes was utilised in **Paper II**. These somatic variants had previously been manually curated after DNA isolation, library construction and sequencing of vitally frozen diagnostic mononuclear cells from the 182 AML patients included in **Paper II**.

RNA-sequencing

Transcriptome profiling by bulk RNA-seq allows for the quantification and examination of the RNA sequences present in a given sample at a given time point. In short, the steps of RNA-seq include RNA isolation, selection and reverse transcription of RNA into complementary DNA (cDNA) to which ends sequencing adaptors are ligated. The cDNA is amplified and sequenced on an NGS platform. The obtained reads are then aligned to a reference genome in order to get relative gene expression values as quantification of each gene can be obtained by counting the number of reads mapped per gene[202].

RNA-seq has been performed and analysed in **Paper II** and **Paper IV** with a focus on differential expression analyses between samples.

Epigenetic analyses

ATAC-sequencing

Genome-wide profiling of chromatin accessibility by the Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) has become a widely used method to investigate the chromatin landscape both at steady state and upon perturbations such as genetic alterations, substance exposure and/or disease[203]. Importantly, ATAC-seq does not require any presumptive hypotheses on the underlying epigenetic modifications or involved transcription factors as compared to other methods.

ATAC-seq is based on a Tn5 transposase made hyperactive via genetic engineering that then identifies and cuts open chromatin regions and simultaneously ligates high-throughput sequencing adapters to these regions. These tagged and cut regions can then undergo paired-end sequencing on an NGS platform to identify euchromatic regions as well as transcriptional factor binding sites[204].

Paper IV includes ATAC-seq analyses on the AML cell line Kasumi-1 at baseline and after 24 h exposure to azacitidine, venetoclax or the combination of azacitidine and venetoclax.

Methylation profiling

Paper IV also includes methylation profiling of the AML cell lines Kasumi-1 and KG-1 at baseline and after 24 h exposure to azacitidine and/or venetoclax. The array-based Infinium MethylationEPIC BeadChip (Illumina) allows for the investigation of the methylation state of approximately 850 000 CpG sites across the genome[205,206]. Here, isolated genomic DNA undergoes sodium bisulphite conversion in which unmethylated cytosines are converted to uracils whereas methylated cytosines stay unchanged.

After DNA amplification, this polymorphism is then investigated by the hybridisation of the DNA products to pre-specified array probes and hapten-labeled dideoxynucleotides. The colour intensities at each CpG site can eventually after antibody-staining be measured. The methylation states are commonly reported as Beta values, usually defined as the methylated probe intensity divided by the overall intensity. Beta values range from 0 to 1 with a value of 1 representing a fully methylated locus.

Statistics and bioinformatic processing

Descriptive statistics and regression analyses

All statistical analyses were performed using the computing environment R[207]. Detailed information on the statistical analyses are found in the corresponding paper and/or supplementary material included in the thesis.

Descriptive statistics for discrete variables included the utilisation of Fisher's exact test for categorical and the Mann–Whitney U test for continuous variables in **Papers I-III**. Kaplan-Meier analyses and the log-rank test were used for estimations of OS and to assess survival differences, respectively, in **Paper II** and **Paper III**.

Univariate and multivariate logistic regression analyses were applied to model the relationship between binary dependent variables and one (univariate) or more (multivariate) independent variables in **Paper I** and **Paper II**. Final covariates for the multivariate models were obtained via backward selection. **Paper I** also included a multivariate penalised logistic regression analysis with ridge penalisation in order to avoid model overfitting. Ridge regression works by the addition of a penalty term to the likelihood function so that the sum of the squared regression coefficients is minimised. Hence, ridge regression results in reduced coefficient estimates compared to standard regression models[208]. This commonly leads to more accurate predictions, in particular if the ratio of events in the dataset divided by the number of included variables in the risk model is low[209] as in **Paper I**.

Cox proportional hazards regression analyses were used to assess the impact of included predictor variables on OS in **Paper II** and **Paper III** and also on relapse-free survival in **Paper II**.

Conditional inference trees

The algorithm presented in **Paper II** was created by the application of conditional inference trees (CITs)[210]. These decision trees are based on an unbiased recursive partitioning procedure to determine the optimal splitting point of included variables in each node. CITs have numerous advantages over other modelling approaches[211]. First is its foundation on permutational statistical tests to minimise both overfitting and selection bias. Secondly, obtained trees are readily graspable and transparent as compared to models based on neural

networks or support vector machines. Thirdly, CITs may include a wide variety of covariates including nominal and ordinal variables as well as missing values.

For the scope of **Paper II**, a CIT with regard to CR achievement was developed based on the mutational status of all analysed somatic genes as well as the gene expression levels of the 20 most significant differentially expressed genes (DEGs) when comparing individuals obtaining CR or not.

Propensity-score matching

Propensity-score matching (PSM) was used in **Paper III** to minimise the risk of confounding by indication. Covariates and hence potential confounding factors are usually unbalanced between treatment groups in observational data. Although PSM is a method to eliminate this imbalance, a major drawback is its assumption that there are no unmeasured confounders. PSM builds on logistic regression modelling where values of measured covariates are used to predict the likelihood of a certain treatment allocation[212]. Individuals with a similar propensity score can thenceforth be matched between groups but now with equal values on the included covariates.

For the scope of **Paper III**, we used PSM to match 275 patients given HMA upfront to intensively and palliatively treated patients. The variables used for PSM were age, gender, ECOG-PS, cytogenetic risk and whether the patient had a previous MDS or MPN.

Bioinformatic processing

Bioinformatics has been utilised for the processing of obtained sequencing data in **Paper II** and **Paper IV** with details delineated in the respective papers and their supplementary material.

DNA sequencing data in **Paper II** underwent quality control, read trimming, mapping to the reference genome Genome Reference Consortium Human (GRCh) Build 37[213] (GRCh37) and subsequent variant calling of somatic single nucleotide variants and insertions/deletions. RNA-seq data was utilised for the detection of *NPM1* mutations due to low panel coverage in exon 11 of *NPM1*. Ultimately, somatic variants were manually curated for 25 different genes with the exclusion of variants with a variant allele fraction <0.05 and/or an allele frequency >0.01 in Caucasians as defined by the ExAC database[214].

For RNA-seq, the quality of raw reads was assessed followed by the trimming of sequencing adapters and subsequent alignment of reads to the reference genome (GRCh37[213] for **Paper II** and GRCh38[215] for **Paper IV**). Transcript quantification eventually allowed for normalisation of the counts before downstream analyses. Differential expression analyses were performed with

negative binomial generalised linear modelling in DESeq2[216] with a false discovery rate (FDR) threshold of 0.05.

A similar approach was applied to the ATAC-seq dataset in **Paper IV**. This included trimming, genome alignment (to GRCh38[215]) and filtering before the calling of narrow peaks with MACS2[217]. Differentially accessible peaks (DAPs) were finally defined with DiffBind[218] with an FDR of 0.01 and with differential analyses accomplished with DESeq2[216]. The toolbox Hypergeometric Optimization of Motif EnRichment[219] (HOMER) was used for peak annotation with genomic positions as input. Moreover, we performed *de novo* motif analyses with HOMER to investigate the potential enrichment of transcription factor binding sites in DAPs. We defined promoter peaks as peaks with centre coordinates within -1 kbp and +100 bp of RefSeq defined transcription start sites[220]. We utilised our previously collected H3K27Ac chromatin immunoprecipitation sequencing data on AML patients with normal cytogenetics to define genomic regions of enhancers[221].

For DNA methylation data processing in **Paper IV**, we utilised the R package minfi[222] which includes functions for pre-processing, normalisation and differential analyses. Here, we implemented a stratified quantile normalisation procedure on obtained Intensity Data files which was followed by filtering before probe-wise differential methylation analyses were performed using the linear modeling properties of the R package limma[223]. The FDR threshold was set at 0.05. Alterations in methylation status of significant probes were quantified by using probe- and condition-wise averaged Beta values.

In addition, **Paper IV** also included Reactome pathway gene set enrichment analyses[224] to identify biological themes enriched in obtained DEGs, DAPs and significantly altered CpG probes. All adjusted P-values were obtained using the Benjamini-Hochberg procedure.

Our obtained RNA-seq data in **Paper IV** was used as input for *in silico* predictions to suggest potential add-ons to the already evident synergistic combination azacitidine-venetoclax. This pipeline was based on the R package hCoCena[225] and publicly available drug databases. Weighted co-expression networks based on Pearson's correlation were formed to identify co-expressed clusters of genes per condition, i.e. after exposure to azacitidine and/or venetoclax for 24 h. This approach allowed for the detection of condition-unique gene modules. Next, we extracted 164 344 gene signatures from the iLINCS drug prediction databases[226]. Finally, we highlighted substances with negative enrichment scores upon Gene Set Enrichment Analysis with the up-regulated, combination-unique gene clusters and all extracted signatures as inputs.

Ethical considerations

Permission was granted by the Regional Ethics Committee for all studies and these were performed according to the Declaration of Helsinki. Utilised patient data from the SAALR in **Papers I-III** were anonymised to avoid personal harm in the form of integrity breaching. The collection of diagnostic bone marrow samples (**Paper II** and **Paper IV**) or peripheral blood samples (**Paper II**) was conducted after informed consent and conferred minimal additional personal harm as parts of the diagnostic work-up.

It shall from an ethical perspective be emphasised that a deeper understanding of AML with the use of novel techniques not currently in clinical practice, for example RNA-seq or ATAC-seq, comes with the requirement of additional resources and increased costs. However, for the included studies, no treatments were altered based on the performed research, mainly due to the retrospective nature of **Papers I-III** and the cell line-based nature of **Paper IV**.

Results

Paper I

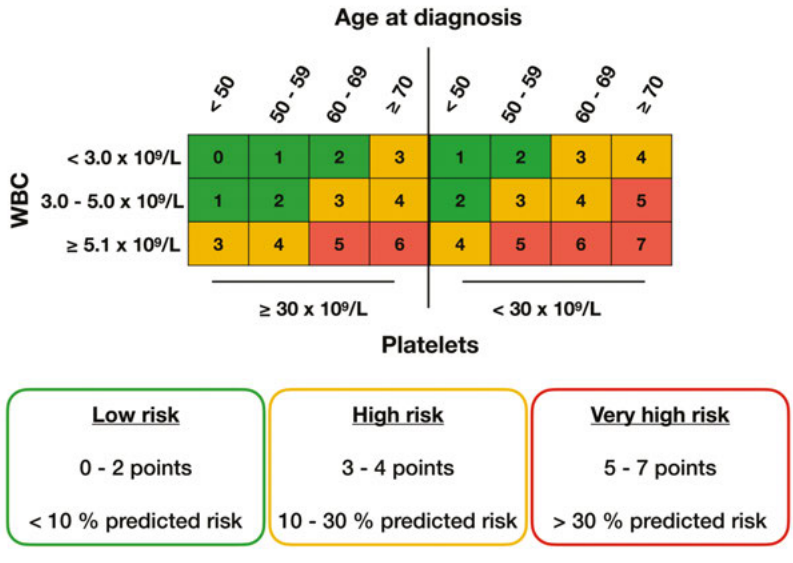

Figure 3. Risk score algorithm for ED in APL as presented in **Paper I**.

Main findings and conclusions

Paper I showed that ED still is a major obstacle to successful APL treatment as the rates of ED were 19.6% and 18.6% in the training and validation cohort, respectively.

Importantly, this was the first published study to propose a validated risk stratification tool with regard to ED risk in APL. Here, we were able to identify three risk groups based on readily-available variables at diagnosis including age, WBC count and platelets (Figure 3). These parameters were included in the final model as they showed significant prognostic power in multivariate logistic regression analysis. In order to obtain a clinically useful scoring system we assigned integer points to the risk group variables based on ridge regression coefficients after subdividing age into four categories: <50 years, 50-59 years, 60-69 years and ≥70 years; WBC into three categories: $<3.0x10^9$/L,

3.0-5.0x10^9/L and >5.0x10^9/L; and platelets into two categories: ≥30x10^9/L and <30x10^9/L.

The risk score sum ranged from 0 to 7 points where a higher cumulative score indicated a higher risk for ED. Furthermore, we defined three risk groups based on the obtained risk score: "low" (0 - 2 points), "high" (3 - 4 points) and "very high" (5 - 7 points). This stratification resulted in a clear increase in the rate of ED per risk group. ED was observed in 4.8% and 6.7% of patients in the "low" risk group in the training and validation cohort, respectively. Corresponding numbers were 20.2% and 25% for the "high" risk group and 50.9% and 36% for the "very high" risk group.

Moreover, our truly population-based study was able to strike another clinically important chord as our approach showed that the risk of ED began to increase already at sub-normal WBC levels. The risk of ED increased already from approximately 2x10^9/L and then increased steeply within the normal WBC range. Previous studies including the widely used Sanz risk score for risk stratifying APL patients have applied 10x10^9/L as a cut-off for WBCs[195,227,228]. In fact, our proposed risk score outperformed the hitherto published tools dealing with ED in APL.

The information presented in **Paper I** may guide clinicians in identifying APL patients in need of more frequent monitoring, potentially even in the intensive care unit, as well as provide clinicians with guidance on transfusion thresholds and aggressive pre-emptive measures on coagulopathy.

Limitations

The main limitation of the above-mentioned risk score is the low number of ATO/ATRA-treated patients included in the cohorts. We were thereby not able to validate the utility of the score in the era of ATO/ATRA and further validation of the proposed risk score in the current treatment milieu is warranted. Reports on ED after the introduction of ATO/ATRA as first-line treatment against APL have shown ED rates of 4.9-8.2%[229,230] which are clearly lower than hitherto observed.

In addition, ED was predicted based on retrospective data and the outcomes of the patients were known. Nonetheless, the use of real-world data without the application of exclusion criteria lends validity to the presented risk score.

Paper II

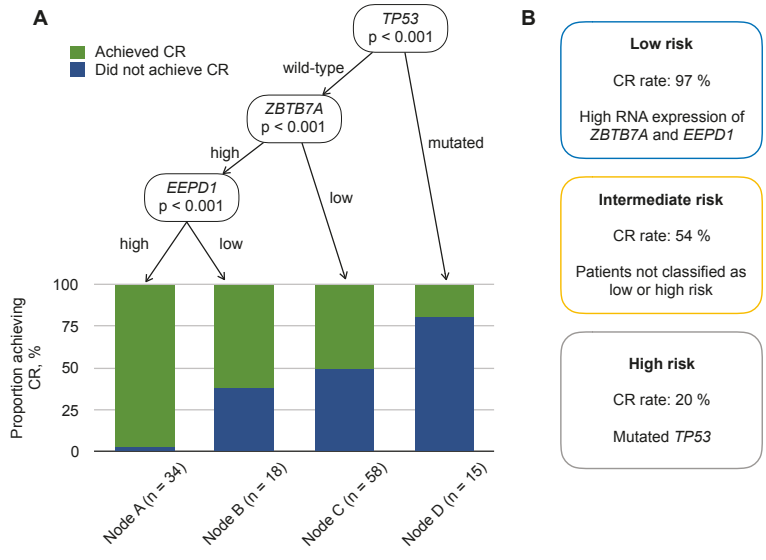

Figure 4. Risk-stratifying algorithm for CR after intensive chemotherapy in AML patients ≥65 years as presented in **Paper II**.

Main findings and conclusions

The potential clinical importance of molecular data in choosing treatment for elderly AML patients is presented in **Paper II**. Moreover, we here underlined another unmet clinical need in AML as the median OS for intensively and palliatively treated AML patients ≥65 years was only 11.4 and 1.8 months, respectively.

We applied age-adjusted multivariable Cox regression analyses to assess the impact on OS of available clinical and mutational variables. Mutations in *NPM1* and *IDH2*^{R172} were associated with better OS in patients who underwent intensive chemotherapy whereas mutated *TP53* was the only aberration associated with shorter OS and lower rates of CR. In addition, a poor ECOG PS and high levels of lactate dehydrogenase at diagnosis were indicative of shorter OS.

Patients who were deemed fit for intensive treatment and in whom CR was obtained had a median OS of 23 months as compared to 1.6 months if CR not was obtained. We applied differential gene expression analyses with regard to CR achievement using RNA-seq data from 125 intensively treated cases. Ninety-six genes showed significant expression differences between the CR groups including genes that previously have been associated with both solid and haematological malignancies.

Next, we developed a risk-stratifying algorithm with regard to CR with the application of CIT analyses. Data on all available somatic mutations as well as the 20 most significant DEGs were included. This approach resulted in three risk groups defined as "low", "intermediate" and "high" risk with CR rates of 97%, 54%, and 20%, respectively (Figure 4). The gene expression levels of *ZBTB7A* and *EEPD1* were in addition to aberrant *TP53* predictive of responsiveness to intensive chemotherapy. Interestingly, the suggested molecular risk stratification translated into significant OS differences whereas risk grouping by ELN did not.

This underscores two clinically relevant issues. Firstly, it emphasises the fact that risk stratification by ELN was developed mainly in younger patients whereby the robustness of the ELN risk groups for aged AML patients may be and have been questioned[231,232]. Secondly, we demonstrated that gene expression analysis may be utilised as a risk stratification tool for CR and/or OS by applying RNA-seq or RT-PCR for specific genes. In particular as we were able to externally validate the proposed risk-stratifying algorithm in the beatAML cohort with CR rates of 90%, 63%, and 43% for the "low", "intermediate" and "high" risk groups, respectively.

We also explored factors with a negative impact on OS in patients treated with a palliative intention (n = 34). These included aberrant *TP53*, presence of *FLT3*-ITD as well as an aberrant karyotype albeit the relatively low included number of patients. Nonetheless, studies focusing on molecular biomarkers in palliatively treated AML patients are sparse and the value of non-intensive, targeted regimens in *FLT3* and/or *TP53* mutated individuals remains to be highlighted.

Limitations

One caveat to **Paper II** includes the use of retrospective data where the risk of observer bias related to outcome(s) shall be noted. However, the use of CIT analyses, an unbiased predictor selection tool, for CR prediction based solely on multiple molecular markers reduces this risk. In order to obtain a publicly available, large enough intensively treated external validation cohort with complete data on clinical, molecular (including RNA-seq) and follow-up data, we needed to lower the age cut-off to 50 years for the beatAML cohort[201]. Nonetheless, we were able to validate our CR algorithm in this younger cohort which indicates that our findings apply also to patients <65 years although this remains to be investigated.

Paper III

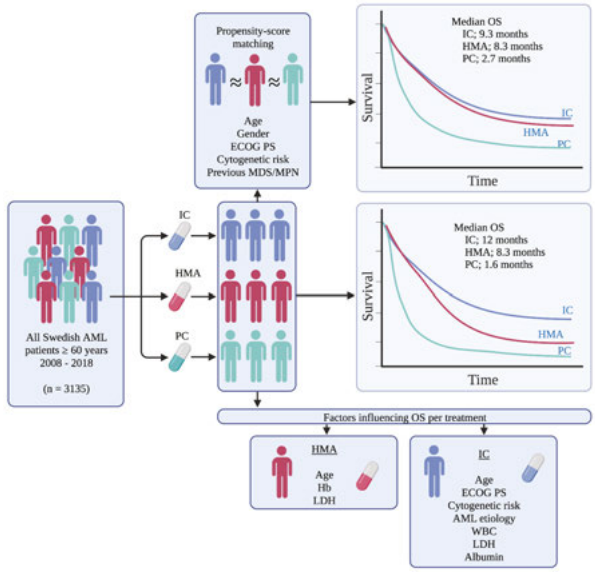

Figure 5. Graphical abstract for **Paper III**. Abbreviations: IC: Intensive chemotherapy; PC: palliative care; Hb: Haemoglobin; LDH: Lactate dehydrogenase.

Main findings and conclusions

In **Paper III** we used real-world data to show a steady increase in the use of HMAs upfront in the period 2008-2018 for AML patients ≥60 years. The proportion of intensively treated patients remained similar on an annual basis during the study period whereas the proportion of an upfront palliative approach decreased as HMAs gained grounds. HMAs were most frequently used in individuals aged 75-84 years. Importantly, crude OS estimates of HMA treated patients fell in between intensively and palliatively treated cases (Figure 5). The median OS was 8.3 months, 12 months and 1.6 months, respectively, for the mentioned treatment intentions. This highlights the longstanding unmet clinical need of an active treatment approach as well as the efficacy of HMAs on the national level in elderly patients with AML unable to undergo intensive chemotherapy.

The effectiveness of HMAs was further evaluated by PSM as well as multivariate Cox regression analyses in order to minimise treatment selection bias. Strikingly, these statistical approaches revealed no significant OS differences between patients treated with HMAs or intensive chemotherapy upfront as baseline characteristics were balanced. In addition, the effects of HMAs were observed in all cytogenetic risk groups.

Paper III also showed that predictive factors for OS were different for patients treated with intensive chemotherapy compared to HMAs. Classical prognostic markers in AML, such as cytogenetics and s-AML, were not relevant for patients treated with HMA. Instead, HMA patients with a higher haemoglobin value at diagnosis had longer OS compared to those with lower values, thus implying a patient group that would benefit from HMAs.

Limitations

Paper III illustrates, in parallel to **Paper I**, the rapidly evolving treatment field in AML during the recent 5-10 years. The current recommended frontline therapy for patients ineligible for intensive chemotherapy consists of azacitidine in combination with venetoclax. Hence, a limitation to **Paper III** is its lack of azacitidine-venetoclax treated patients.

The use of retrospective, observational data also limits our findings as the data quality is dependent on the collected data. The SAALR is a comprehensive data source but lacks for example the type of HMA used, the number of cycles completed as well as molecular markers. In particular the latter could have been informative with regard to predictive biomarkers to HMAs.

We used PSM to control for confounding by indication but it shall be emphasised that PSM cannot control for unmeasured covariates and/or unknown confounders. Nonetheless, we included available factors that are vital in treatment assignment and for the outcome (i.e. true confounders) to account for this limitation in PSM.

Paper IV

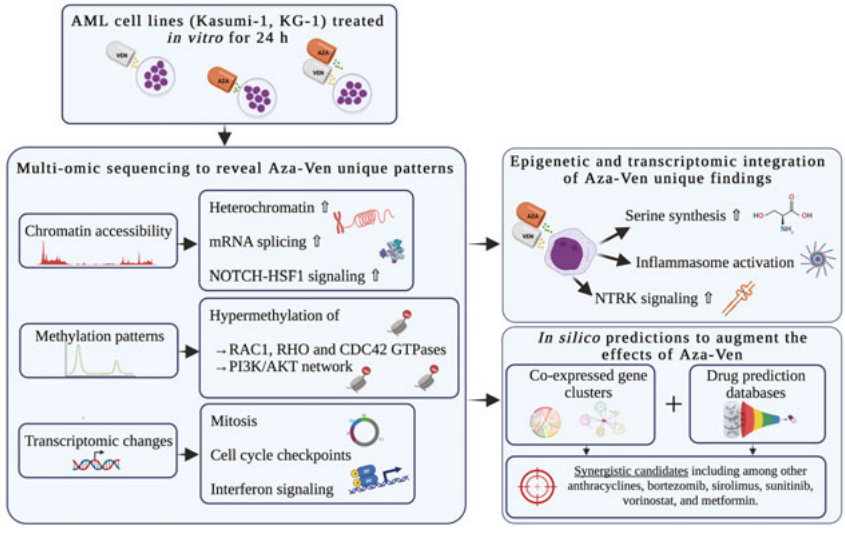

Figure 6. Graphical abstract for methods and main findings in **Paper IV**.

Main findings and conclusions

In **Paper IV** we applied an integrative multi-omic approach to examine the effects of azacitidine and venetoclax in AML (Figure 6). We showed that the two substances alone and in combination have disparate impacts with regard to chromatin accessibility, methylation patterns and gene expression changes that in part may explain the observed clinical synergy. Of interest, venetoclax single-handedly caused widespread chromatin accessibility shifts but few significant transcriptomic and methylation changes whereas the opposite was observed with azacitidine alone.

We mainly focused on combination-unique features in an attempt to define molecular changes associated with the synergism. Firstly, we applied ATAC-seq which showed that exposure to azacitidine-venetoclax resulted in euchromatic sites enriched for genes involved in mRNA splicing, HSF1 activation, TNFR1-induced proapoptotic signalling and pre-NOTCH processing. Secondly, we found that the addition of venetoclax to azacitidine enhanced the number of DNA methylation changes despite the fact that venetoclax alone did not significantly alter any CpG sites. Azacitidine-venetoclax elicited mainly hypermethylation and these combination-unique CpG alterations were enriched for genes related to the RAC1 GTPase cycle, the RHO GTPase cycle, the CDC42 GTPase cycle as well as negative regulation of the PI3K/AKT network. Thirdly, bulk RNA-seq revealed similar pathways to be affected both by azacitidine and azacitidine-venetoclax including interferon- and interleukin-related genes. Moreover, we found combination-specific DEGs to be

enriched for genes involved in mitosis, cell cycle checkpoints and RHO GTPases.

Next, we integrated the different datasets and ultimately identified 11 significantly altered genes with altered chromatin accessibility and/or methylation state. Importantly, these DEGs were observed in both cell lines upon exposure to azacitidine-venetoclax and included inflammasome-related *NLRP1*, genes involved in serine synthesis (*PHGDH*, *PSAT1*, *ATF3*), as well as the proto-oncogene *JUND*. Moreover, our integrative approach pointed towards the activation of NTRK signalling. The up-regulation of numerous of the above-mentioned pathways most likely represents potential survival mechanisms of the AML blasts upon azacitidine-venetoclax exposure and targeted treatments may thus enhance the efficacy of this combination.

Finally, we utilised our transcriptome data and developed an *in silico* prediction pipeline to suggest potent triplet therapies with azacitidine-venetoclax as a backbone. We initially defined combination-unique up-regulated gene clusters by applying weighted co-expression network analyses. Secondly, we extracted gene signatures from the iLINCS drug prediction database[226] and identified substances with negative enrichment scores for the defined upregulated clusters as we reasoned that these clusters are likely to reflect protective or surviving mechanisms. The top hits included well-known AML drugs such as daunorubicin and doxorubicin but as a further proof-of-concept also the proteasome inhibitor bortezomib with shown synergy with venetoclax[233]. Other top candidates included both approved substances such as sirolimus, sunitinib, vorinostat, cabozantinib, dasatinib and metformin as well as experimental compounds such as a dual inhibitor of BCL-2 and MCL-1 named TW 37, the Hsp90 inhibitor tanispimycin and the FLT3 inhibitor KW-2449.

The combinatorial effects of these substances with azacitidine-venetoclax remain to be investigated but our findings in **Paper IV** provide a rationale upon which the treatment of AML patients may be advanced.

Limitations

It shall be emphasised that **Paper IV** is solely based on *in vitro* and *in silico* findings in AML cell lines which constitutes a major limitation to our findings. Whether the observed molecular effects of azacitidine-venetoclax transcend into primary patient samples both *in vitro* and *in vivo* remains a question for future research. Nonetheless, the controlled environment provided by *in vitro* experiments makes it possible to isolate specific drug effects, in particular when applying exploratory sequencing techniques such as ATAC-seq.

Further limitations to our *in vitro* study include the low number of included cell lines as well as the fact that the cells only were exposed to the substances for 24 h before downstream analyses. This time frame was mainly chosen due to limitations in the different sequencing techniques as too many non-viable cells would have resulted in undesirable noise. In addition, we reasoned that

sorting (e.g. by flow cytometry) for only viable cells had the potential of distorting the sequencing results as we wanted to catch the intrinsic molecular effects of azacitidine-venetoclax in affected cells.

Concluding remarks and future perspectives

Tremendous advances have been made in the field of AML during the last decades but the similarly tremendous heterogeneity of the disease entity still poses challenges to be overcome. Nonetheless, the rapidly evolving molecular understanding of AML in combination with a growing treatment armamentarium makes the proverbial glass half full rather than half empty.

The clinical gains made with the development of targeted therapies against AML emphasise three important aspects. Firstly, this translational AML research has shown that AML can be successfully treated also without intensive chemotherapy, in particular upon the identification of AML subgroups with the greatest benefits of specific therapies and thereby a focus on further molecular characterisation of AML. Secondly, the fact that the hitherto achieved detailed biological understanding of AML has been translated into clinical utility opens up for the optimistic perspective that an even more detailed delineation may result in further benefits for AML patients. Thirdly and perhaps most importantly is the more and more refined multidisciplinary and collaborative approach behind many of the above-mentioned advances including preclinical experiments, technical developments, novel sequencing techniques, computing improvements as well as thoroughly designed clinical trials and follow-up in the form of real-world data.

A number of these approaches have been utilised in the research done within the scope of this thesis in order to contribute to improved prognostication and sharpened AML treatment. Despite all advances obtained, the prognosis for most AML patients is poor and numerous unmet clinical needs persist. A number of them have been addressed by the research of this thesis.

Firstly, ED in APL is a hurdle for successful long-term treatment in a substantial part of all APL patients (approximately 20% in our cohorts). Our proposed risk score will hopefully be clinically useful and implemented to overcome the obstacle of ED (**Paper I**). Secondly, in the era of azacitidine-venetoclax as an effective first-line option to patients ineligible for intensive chemotherapy, it is an intricate clinical question who to treat intensively and who to treat non-intensively[234]. We show that the integration of mutational data and RNA-seq may be feasible to identify elderly patients with good chances of response to intensive chemotherapy (**Paper II**). Moreover, we elucidate the mutational landscape and prognostic impact of gene mutations in both intensively and palliatively treated elderly AML patients (**Paper II**), a hitherto

largely unknown territory. Thirdly, as clinical trials rarely represent the real-world setting, the implementation of novel treatment options requires thorough follow-up including studies comparing the efficacy shown in clinical trials to the effectiveness when used clinically. We show that HMAs may be as effective as intensive chemotherapy in the real-world setting but also that there is a clear room for treatment improvements as exemplified by a median OS of 8-9 months in our matched patients regardless of treatment with an HMA or chemotherapy (**Paper III**). Fourthly, the molecular mechanisms driving the synergy of azacitidine-venetoclax are largely unknown but this understanding would potentially contribute to predictive biomarkers, the emergence of novel AML specific vulnerabilities as well as possibilities of enhancing the effect even further. Azacitidine-venetoclax causes distinct epigenetic and transcriptomic impacts of which many may be ascribed to pro-survival pathways that in turn may be targeted with small-molecule inhibitors (**Paper IV**). Similarly, we show that laboratory experiments, bioinformatic handling and large databases can be combined to advance the treatment armamentarium of AML (**Paper IV**).

Future studies will need to continue this never-ending race of improving the treatment and care of AML patients, in particular as we have just begun to get a grasp of the complexity of the disease. Moreover, the expanded treatment landscape also brings other questions including for example how to implement novel therapies timely, how to best define prognostic and predictive biomarkers for these therapies and whether sequential usage, combinatorial usage and/or maintenance therapy is feasible. It is likely that an even more comprehensive delineation of AML and its subtypes with deeper and newer sequencing techniques, e.g. chromatin profiling and single-cell sequencing, eventually will result in further clinical gains as shown to date.

Populärvetenskaplig sammanfattning

Akut myeloisk leukemi (AML) är en aggressiv blodmalignitet som drabbar omkring 400 personer per år i Sverige. Detta gör AML till den vanligaste formen av akut leukemi hos vuxna. AML drabbar i stor utsträckning äldre personer där majoriteten av patienterna är över 65 år vid insjuknande. Cancersjukdomen definieras av förekomsten av ett överskott av sjuka, omogna, vita blodceller i benmärgen vilka då tränger undan de friska blodcellerna. Prognosen vid AML är generellt mycket dålig men insikter i sjukdomens biologi och bakomliggande genförändringar har visat att riktade terapier och patientcentrerade behandlingsval kan förbättra utfallen. Idag utgör mycket intensiva cellgiftskurer standardbehandlingen för yngre patienter och för äldre patienter med gott allmäntillstånd. Dessa kurer är dock för intensiva för många äldre patienter och för patienter med samsjuklighet och för dessa behövs andra behandlingsalternativ. Det föreligger alltså flertalet förbättringsområden kring AML och dess terapier i nuläget.

Syftet med denna avhandling var att adressera en del av nuvarande hinder till förbättrad prognos och optimerad behandling av patienter med AML. Här nedan följer en sammanfattning av avhandlingens fyra studier.

Akut promyelocytleukemi (APL) utgör en specifik undergrupp av alla AML-fall och karaktäriseras av uttalad blödningsproblematik. Prognosen vid APL är mycket god förutsatt att rätt terapi sätts in tidigt i förloppet men APL måste behandlas snabbt och specifikt om inte blödningarna ska bli fatala. Olyckligtvis är det många APL-patienter som dör inom 30 dygn från diagnos just på grund av blödningskomplikationer. Syftet med **Studie I** var att utveckla ett kliniskt användbart riskskattningsverktyg för att förutsäga en APL-patients individuella risk att dö tidigt. Vi använde oss av data från det svenska populationsbaserade AML-registret för att skapa denna riskskattning baserat på ålder och nivån på blodplättar och vita blodceller vid diagnostillfället. Vi kunde på så vis identifiera tre grupper med olika risk för tidig död. Vi kunde även bekräfta dessa riskgrupper med data från en portugisisk sjukhusbaserad APL-grupp. Riskskattningsverktyget kan alltså guida kliniker i identifieringen av APL-patienter med hög risk för tidig död och där snabbt insatt behandling, nogsam övervakning och korrigering av avvikande laboratorievärden behövs för att minska denna risk.

Studie II fokuserade på AML-patienter ≥ 65 år vid diagnos. Dessa äldre patienter har ofta andra biologiska och kliniska karakteristika än yngre

patienter. Få studier har dock kartlagt förekomsten av och den prognostiska innebörden av genetiska avvikelser hos denna patientgrupp. Vi kombinerade kliniska data från drygt 180 svenska AML-patienter ≥ 65 år med molekylära markörer för att undersöka den prognostiska valören av olika mutationer. Vi kunde på så vis identifiera flera genmutationer som indikerade goda svar på cellgiftsbehandling men också ogynnsamma genförändringar. Vår karaktärisering kunde slutligen användas för utvecklandet av ytterligare ett riskskattningsverktyg för att förutsäga chansen att svara på intensiva cellgiftskurer och även detta verktyg kunde bekräftas i en annan AML-grupp. Ett av de svåraste kliniska besluten idag är huruvida en patient ska rekommenderas intensiv behandling eller inte. Detta verktyg skulle kunna användas vid ett sådant beslut förutsatt att tekniska möjligheter avseende genkaraktäriseringen föreligger.

Behandlingen av AML-patienter som inte bedöms tolerera intensiva cellgiftskurer utgörs till stor del av icke-intensiva cellgifter och/eller målinriktade terapier. De förstnämnda är vanligare varav substansen azacitidin är den allra vanligaste. Azacitidin hindrar leukemicellernas tillväxt och godkändes för behandling av AML i början av 2000-talet. Dess effektivitet i den kliniska vardagen, det vill säga utanför kliniska studier, har däremot inte undersökts i någon större utsträckning. Vi använde det svenska AML-registret för att i **Studie III** utforska hur azacitidin står sig i förhållande till intensiva cellgiftskurer och palliativt inriktad behandling hos AML-patienter ≥ 60 år vid diagnos. Vi kunde med hjälp av statistiska analyser visa att azacitidin och intensiva cellgifter resulterade i samma överlevnadsresultat när vi jämförde likvärdiga patienter och med betydligt bättre utfall än hos enbart palliativt behandlade patienter. Vi kunde också påvisa skillnader i faktorer som förutsäger ett behandlingssvar på azacitidin och intensiv cellgiftsterapi. Dessa resultat stärker användandet av azacitidin i klinisk rutin, särskilt hos utvalda patienter, och att azacitidin hos många kan utgöra ett fullgott alternativ till intensiv behandling men med färre biverkningar och därigenom bättre livskvalitet.

Azacitidin i kombination med den målinriktade tabletten venetoclax är idag den rekommenderade förstahandsbehandlingen till alla patienter som inte är kandidater för intensiva cellgifter. Goda resultat har visats i såväl studier som i den kliniska vardagen. Detta mycket tack vare att kombinationen leder till en synergistisk avdödning av leukemicellerna. Det är emellertid oklart hur denna synergi uppstår. Vi har i **Studie IV** utfört laboratorieexperiment på AML-celler där vi behandlat dessa med azacitidin och/eller venetoclax och därefter analyserat de molekylära förändringar som sker i cellerna. Dessa undersökningar har visat potentiella överlevnads- och resistensmönster hos AML-cellerna vid behandling med azacitidin-venetoclax. Denna information är viktig då dessa överlevnads-/resistenssignaler skulle kunna hämmas med tillägg av specifika terapier och på så vis stärka synergin ytterligare. Vi använde också datasimuleringar tillsammans med våra molekylära resultat för att presentera potentiella andra synergistiska läkemedelskombinationer för att ytterligare vässa behandlingen av AML.

Acknowledgements

The teamwork behind this thesis can not be emphasised enough. The works presented herein have only been possible thanks to great efforts by numerous team players and their wise guidance. Your contributions have been invaluable. The publishing costs would have become too high to name all of you in text but I know that you know who you are.

I would sincerely like to thank my main supervisor, **Sören Lehmann**, for excellent mentorship and support. Your expertise mixed with your curious mindset is inspiring. I am deeply grateful for the challenges and opportunities the research projects have brought so far. The skills learned during this journey will with certainty serve me well in my future endeavours. I would also like to express my appreciation to my co-supervisor, **Anna Eriksson**. Your timely constructive criticism has been instrumental and your energetic attitude is contagious. I look forward to continuing our collaboration in the future.

A special thank you also to **Martin Höglund** who once upon a time (2010) introduced me to the world of haematology in general and AML in particular as well as the fascinating possibilities of AML research. Who knows in what non-haematological discipline I otherwise would have ended? Similarly, a sincere acknowledgement to all members and co-authors in the **National AML Group**. Thank you for your willingness to share your expertise and knowledge and for your encouragement.

Further, I would like to express my deep appreciation to all previous and current **members of the Lehmann and Lennartsson research groups**. The obtained input and interesting discussions during and in-between all lab meetings have been indispensable and greatly instructive.

I also want to thank all **past and present colleagues**, both in Avesta and Uppsala. It has been and continues to be a privilege to have the possibility of working alongside all of you and of learning new things on a daily basis. I look forward to continuing all fruitful work and collaborations.

The financial support provided by Uppsala University, the Uppsala and Stockholm Regions, the Swedish Cancer Foundation, the Swedish Research Council, the Center for Clinical Research Dalarna, the Lions Cancer Research Foundation Uppsala-Örebro, Erik, Karin and Gösta Selander's Foundation and Gunvor Planting's Foundation made the research realisable. Sequencing analyses were performed by the National Genomics Infrastructure in Stockholm and Uppsala, platforms that in turn are funded by Science for Life

Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council. Moreover, I acknowledge the technical support and infrastructure provided via UPPMAX and the SNIC/Uppsala Multi-disciplinary Center for Advanced Computational Science.

Finally, my heartfelt appreciation to the most important individuals in my life - my family and parents.

Ulla and **Ulf**. I can not by any means thank you enough for your ever-present support, love and encouragement. I am grateful for all that you have done for me and throughout the years shaping me into the person I am today. I am particularly thankful for the way you have helped me build my confidence and self-esteem. I know that I would not have achieved half of what I have without your unwavering support.

Iris, **Arthur** and **Ellen**. You are the most perfect individuals. Being with you is the most joyful moment of my day. Your superhero abilities of intelligence, curiosity and ability of focusing on the present are and have been greatly inspirational to me. It is a privilege to be your parent and to have the possibility of following your journey as you thrive and go for your dreams.

And **Anna**, my wife, soulmate and best friend. I can not find enough words to express how much You mean to me. I am eternally grateful for all You have given me and I look forward to all beautiful adventures that lie ahead of us.

References

1. Piller GJ. Leukaemia - a brief historical review from ancient times to 1950: Historical Review. British Journal of Haematology. 2001 Feb;112(2):282–92.
2. Hemminki K, Hemminki J, Försti A, Sud A. Survival in hematological malignancies in the Nordic countries through a half century with correlation to treatment. Leukemia. 2023 Apr;37(4):854–63.
3. 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 Apr 30;113(18):4179–87.
4. 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 Aug 29;4(149).
5. 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 Feb 20;506(7488):328–33.
6. Bouligny IM, Maher KR, Grant S. Mechanisms of myeloid leukemogenesis: Current perspectives and therapeutic objectives. Blood Reviews. 2023 Jan;57:100996.
7. Trumpp A, Haas S. Cancer stem cells: The adventurous journey from hematopoietic to leukemic stem cells. Cell. 2022 Apr;185(8):1266–70.
8. Miles LA, Bowman RL, Merlinsky TR, Csete IS, Ooi AT, Durruthy-Durruthy R, et al. Single-cell mutation analysis of clonal evolution in myeloid malignancies. Nature. 2020 Nov 19;587(7834):477–82.
9. Morita K, Wang F, Jahn K, Hu T, Tanaka T, Sasaki Y, et al. Clonal evolution of acute myeloid leukemia revealed by high-throughput single-cell genomics. Nat Commun. 2020 Oct 21;11(1):5327.
10. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994 Feb;367(6464):645–8.
11. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997 Jul;3(7):730–7.
12. Stelmach P, Trumpp A. Leukemic stem cells and therapy resistance in acute myeloid leukemia. haematol. 2023 Feb 1;108(2):353–66.
13. Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, et al. Identification of Therapeutic Targets for Quiescent, Chemotherapy-Resistant Human Leukemia Stem Cells. Sci Transl Med. 2010 Feb 3;2(17).
14. Duy C, Li M, Teater M, Meydan C, Garrett-Bakelman FE, Lee TC, et al. Chemotherapy Induces Senescence-Like Resilient Cells Capable of Initiating AML Recurrence. Cancer Discovery. 2021 Jun 1;11(6):1542–61.

15. DeWolf S, Tallman MS. How I treat relapsed or refractory AML. Blood. 2020 Aug 27;136(9):1023–32.
16. Juliusson G, Lazarevic V, Hörstedt AS, Hagberg O, Höglund M. Acute myeloid leukemia in the real world: why population-based registries are needed. Blood. 2012 Apr 26;119(17):3890–9.
17. Østgård LSG, Nørgaard M, Nørgaard JM, Severinsen, Sengeløv H, Friis LS, et al. Data quality in the Danish National Acute Leukemia Registry: a hematological data resource. CLEP. 2013 Sep;335.
18. Gangatharan SA, Grove CS, P'ng S, O'Reilly J, Joske D, Leahy MF, et al. Acute myeloid leukaemia in Western Australia 1991-2005: a retrospective population-based study of 898 patients regarding epidemiology, cytogenetics, treatment and outcome: AML in Western Australia. Intern Med J. 2013 Aug;43(8):903–11.
19. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood Reviews. 2019 Jul;36:70–87.
20. NORDCAN (Association of the Nordic Cancer Registries). https://nordcan.iarc.fr/en, latest accessed 2023-05-08.
21. Statistics Sweden. https://www.scb.se, latest accessed 2023-05-08.
22. Anderson LJ, Girguis MS, Kim JH, Shewale JB, Braunlin M, Werther W, et al. A Temporal and Multinational Assessment of Acute Myeloid Leukemia (AML) Cancer Incidence, Survival, and Disease Burden. Blood. 2021 Nov 5;138(Supplement 1):4124–4124.
23. Nilsson C, Linde F, Hulegårdh E, Garelius H, Lazarevic V, Antunovic P, et al. Characterization of therapy-related acute myeloid leukemia: increasing incidence and prognostic implications. haematol. 2022 Aug 25;108(4):1015–25.
24. Morton LM, Dores GM, Tucker MA, Kim CJ, Onel K, Gilbert ES, et al. Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975-2008. Blood. 2013 Apr 11;121(15):2996–3004.
25. 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 Epidemiology. 2016 Jun;42:186–98.
26. Kaushansky K, editor. Williams hematology. Ninth edition. New York: McGraw-Hill; 2016.
27. 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 May 19;127(20):2391–405.
28. 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 Jul;36(7):1703–19.
29. 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 Sep 15;140(11):1200–28.
30. 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 Sep 22;140(12):1345–77.

31. Hemminki K, Hemminki J, Försti A, Sud A. Survival trends in hematological malignancies in the Nordic countries through 50 years. Blood Cancer J. 2022 Nov 7;12(11):150.
32. Surveillance, Epidemiology, and End Results (SEER) program. Cancer stat facts: Leukemia - acute myeloid leukemia (AML). https://seer.cancer.gov/statfacts/html/amyl.html, latest accessed 2023-05-08.
33. Juliusson G, Hagberg O, Lazarevic VL, Ölander E, Antunovic P, Cammenga J, et al. Improved survival of men 50 to 75 years old with acute myeloid leukemia over a 20-year period. Blood. 2019 Oct 31;134(18):1558–61.
34. Shah A, Andersson TML, Rachet B, Björkholm M, Lambert PC. Survival and cure of acute myeloid leukaemia in England, 1971-2006: a population-based study. Br J Haematol. 2013 Aug;162(4):509–16.
35. Østgård LSG, Nørgaard JM, Sengeløv H, Severinsen M, Friis LS, Marcher CW, et al. Comorbidity and performance status in acute myeloid leukemia patients: a nation-wide population-based cohort study. Leukemia. 2015 Mar;29(3):548–55.
36. Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood. 2006 May 1;107(9):3481–5.
37. Pollyea DA, Kohrt HE, Medeiros BC. Acute myeloid leukaemia in the elderly: a review: Review. British Journal of Haematology. 2011 Mar;152(5):524–42.
38. Mohammadi M, Cao Y, Glimelius I, Bottai M, Eloranta S, Smedby KE. The impact of comorbid disease history on all-cause and cancer-specific mortality in myeloid leukemia and myeloma – a Swedish population-based study. BMC Cancer. 2015 Dec;15(1):850.
39. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. Journal of Chronic Diseases. 1987 Jan;40(5):373–83.
40. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005 Oct 15;106(8):2912–9.
41. Dhakal P, Shostrom V, Al-Kadhimi ZS, Maness LJ, Gundabolu K, Bhatt VR. Usefulness of Charlson Comorbidity Index to Predict Early Mortality and Overall Survival in Older Patients With Acute Myeloid Leukemia. Clinical Lymphoma Myeloma and Leukemia. 2020 Dec;20(12):804-812.e8.
42. Sorror ML, Giralt S, Sandmaier BM, De Lima M, Shahjahan M, Maloney DG, et al. Hematopoietic cell transplantation–specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first remission: combined FHCRC and MDACC experiences. Blood. 2007 Dec 15;110(13):4606–13.
43. Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. Haematologica. 2012 Dec;97(12):1916–24.
44. Giles FJ, Borthakur G, Ravandi F, Faderl S, Verstovsek S, Thomas D, et al. The haematopoietic cell transplantation comorbidity index score is predictive of early death and survival in patients over 60 years of age receiving induction therapy for acute myeloid leukaemia. Br J Haematol. 2007 Feb;136(4):624–7.
45. Savic A, Kvrgic V, Rajic N, Urosevic I, Kovacevic D, Percic I, et al. The hematopoietic cell transplantation comorbidity index is a predictor of early death and survival in adult acute myeloid leukemia patients. Leuk Res. 2012 Apr;36(4):479–82.

46. Bewersdorf JP, Zeidan AM. Hyperleukocytosis and Leukostasis in Acute Myeloid Leukemia: Can a Better Understanding of the Underlying Molecular Pathophysiology Lead to Novel Treatments? Cells. 2020 Oct 17;9(10):2310.
47. Dutcher JP, Schiffer CA, Wiernik PH. Hyperleukocytosis in adult acute nonlymphocytic leukemia: impact on remission rate and duration, and survival. JCO. 1987 Sep;5(9):1364–72.
48. Röllig C, Ehninger G. How I treat hyperleukocytosis in acute myeloid leukemia. Blood. 2015 May 21;125(21):3246–52.
49. Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. Blood. 2011 Oct 6;118(14):3785–93.
50. Avni B, Rund D, Levin M, Grisariu S, Ben-Yehuda D, Bar-Cohen S, et al. Clinical implications of acute myeloid leukemia presenting as myeloid sarcoma: Acute myeloid leukemia presenting as myeloid sarcoma. Hematol Oncol. 2012 Mar;30(1):34–40.
51. Stölzel F, Lüer T, Löck S, Parmentier S, Kuithan F, Kramer M, et al. The prevalence of extramedullary acute myeloid leukemia detected by 18 FDG-PET/CT: final results from the prospective PETAML trial. Haematologica. 2020 Jun;105(6):1552–8.
52. Eckardt JN, Stölzel F, Kunadt D, Röllig C, Stasik S, Wagenführ L, et al. Molecular profiling and clinical implications of patients with acute myeloid leukemia and extramedullary manifestations. J Hematol Oncol. 2022 Dec;15(1):60.
53. Matthews A, Pratz KW. Optimizing outcomes in secondary AML. Hematology. 2022 Dec 9;2022(1):23–9.
54. McNerney ME, Godley LA, Le Beau MM. Therapy-related myeloid neoplasms: when genetics and environment collide. Nat Rev Cancer. 2017 Aug 24;17(9):513–27.
55. Kayser S, Döhner K, Krauter J, Köhne CH, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011 Feb 17;117(7):2137–45.
56. Granfeldt Østgård LS, Medeiros BC, Sengeløv H, Nørgaard M, Andersen MK, Dufva IH, et al. Epidemiology and Clinical Significance of Secondary and Therapy-Related Acute Myeloid Leukemia: A National Population-Based Cohort Study. JCO. 2015 Nov 1;33(31):3641–9.
57. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1,612 Patients Entered Into the MRC AML 10 Trial. Blood. 1998 Oct 1;92(7):2322–33.
58. 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 Jul 22;116(3):354–65.
59. Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 1998 Sep 15;58(18):4173–9.
60. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000 Dec 15;96(13):4075–83.

61. Byrd JC. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002 Dec 15;100(13):4325–36.
62. Imperato MR, Cauchy P, Obier N, Bonifer C. The RUNX1–PU.1 axis in the control of hematopoiesis. Int J Hematol. 2015 Apr;101(4):319–29.
63. Kuykendall A, Duployez N, Boissel N, Lancet JE, Welch JS. Acute Myeloid Leukemia: The Good, the Bad, and the Ugly. American Society of Clinical Oncology Educational Book. 2018 May;(38):555–73.
64. Mrózek K. Cytogenetic, Molecular Genetic, and Clinical Characteristics of Acute Myeloid Leukemia With a Complex Karyotype. Seminars in Oncology. 2008 Aug;35(4):365–77.
65. Ciurea SO, Labopin M, Socie G, Volin L, Passweg J, Chevallier P, et al. Relapse and survival after transplantation for complex karyotype acute myeloid leukemia: A report from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation and the University of Texas MD Anderson Cancer Center: AHSCT for Complex Karyotype AML. Cancer. 2018 May 15;124(10):2134–41.
66. 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 Mar 1;119(9):2114–21.
67. Rogers HJ, Vardiman JW, Anastasi J, Raca G, Savage NM, Cherry AM, et al. Complex or monosomal karyotype and not blast percentage is associated with poor survival in acute myeloid leukemia and myelodysplastic syndrome patients with inv(3)(q21q26.2)/t(3;3)(q21;q26.2): a Bone Marrow Pathology Group study. Haematologica. 2014 May;99(5):821–9.
68. Fang H, Yabe M, Zhang X, Kim Y, Wu X, Wei P, et al. Myelodysplastic syndrome with t(6;9)(p22;q34.1)/DEK-NUP214 better classified as acute myeloid leukemia? A multicenter study of 107 cases. Modern Pathology. 2021 Jun;34(6):1143–52.
69. Neuendorff NR, Burmeister T, Dörken B, Westermann J. BCR-ABL-positive acute myeloid leukemia: a new entity? Analysis of clinical and molecular features. Ann Hematol. 2016 Aug;95(8):1211–21.
70. Lamble AJ, Gerbing RB, Smith JL, Ries RE, Kolb EA, Alonzo TA, et al. *Crebbp* Alterations Are Associated with a Poor Prognosis in *De Novo* AML. Blood. 2021 Nov 5;138(Supplement 1):3451–3451.
71. Soupir CP, Vergilio JA, Dal Cin P, Muzikansky A, Kantarjian H, Jones D, et al. Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol. 2007 Apr;127(4):642–50.
72. 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 Oct 20;117(42):26340–6.
73. 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 Sep 29;11(9):162.
74. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013 May 30;368(22):2059–74.

75. 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 Jun 9;374(23):2209–21.
76. 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 Jan 26;129(4):424–47.
77. 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 Jan 21;115(3):453–74.
78. Sargas C, Ayala R, Larráyoz MJ, Chillón MC, Rodriguez-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 Journal. 2023 May 12;13(1):77.
79. Lachowiez CA, Long N, Saultz J, Gandhi A, Newell LF, Hayes-Lattin B, et al. Comparison and validation of the 2022 European LeukemiaNet guidelines in acute myeloid leukemia. Blood Advances. 2023 May 9;7(9):1899–909.
80. 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 Jan 20;352(3):254–66.
81. Falini B, Brunetti L, Sportoletti P, Martelli MP. NPM1-mutated acute myeloid leukemia: from bench to bedside. Blood. 2020 Oct 8;136(15):1707–21.
82. 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 Mar 12;135(11):870–5.
83. 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 Advances. 2017 Aug 22;1(19):1546–50.
84. Eisfeld AK, Kohlschmidt J, Mims A, Nicolet D, Walker CJ, Blachly JS, et al. Additional gene mutations may refine the 2017 European LeukemiaNet classification in adult patients with de novo acute myeloid leukemia aged <60 years. Leukemia. 2020 Dec;34(12):3215–27.
85. DiNardo CD, Tiong IS, Quaglieri A, MacRaild S, Loghavi S, Brown FC, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. Blood. 2020 Mar 12;135(11):791–803.
86. 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. JCO. 2019 Oct 10;37(29):2632–42.
87. Dufour A, Schneider F, Metzeler KH, Hoster E, Schneider S, Zellmeier E, et al. Acute Myeloid Leukemia With Biallelic *CEBPA* Gene Mutations and Normal Karyotype Represents a Distinct Genetic Entity Associated With a Favorable Clinical Outcome. JCO. 2010 Feb 1;28(4):570–7.
88. 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 Jan 6;139(1):87–103.
89. 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 Advances. 2022 Jan 11;6(1):238–47.

90. Chen X, Abuduaini D, Zhang Y, Long J, Lin X, Zhu H, et al. Characteristic immunophenotype and gene co-mutational status orchestrate to optimize the prognosis of CEBPA mutant acute myeloid leukemia. Blood Cancer J. 2023 May 5;13(1):70.
91. Yamamoto Y. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood. 2001 Apr 15;97(8):2434–9.
92. Kelly LM, Liu Q, Kutok JL, Williams IR, Boulton CL, Gilliland DG. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. Blood. 2002 Jan 1;99(1):310–8.
93. Juliusson G, Jädersten M, Deneberg S, Lehmann S, Möllgård L, Wennström L, et al. The prognostic impact of FLT3-ITD and NPM1 mutation in adult AML is age-dependent in the population-based setting. Blood Advances. 2020 Mar 24;4(6):1094–101.
94. Schmalbrock LK, Dolnik A, Cocciardi S, Sträng E, Theis F, Jahn N, et al. Clonal evolution of acute myeloid leukemia with *FLT3*-ITD mutation under treatment with midostaurin. Blood. 2021 Jun 3;137(22):3093–104.
95. Ambinder AJ, Levis M. Potential targeting of FLT3 acute myeloid leukemia. haematol. 2020 Jul 23;106(3):671–81.
96. Daver NG, Iqbal S, Renard C, Chan RJ, Hasegawa K, Hu H, et al. Treatment outcomes for newly diagnosed, treatment-naïve TP53-mutated acute myeloid leukemia: a systematic review and meta-analysis. J Hematol Oncol. 2023 Mar 6;16(1):19.
97. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009 Jul 30;114(5):937–51.
98. Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015 Feb 26;125(9):1367–76.
99. Tsai XCH, Sun KJ, Lo MY, Tien FM, Kuo YY, Tseng MH, et al. Poor prognostic implications of myelodysplasia-related mutations in both older and younger patients with de novo AML. Blood Cancer J. 2023 Jan 4;13(1):4.
100. Gaidzik VI, Bullinger L, Schlenk RF, Zimmermann AS, Röck J, Paschka P, et al. *RUNX1* Mutations in Acute Myeloid Leukemia: Results From a Comprehensive Genetic and Clinical Analysis From the AML Study Group. JCO. 2011 Apr 1;29(10):1364–72.
101. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. Leukemia. 2016 Nov;30(11):2160–8.
102. Schnittger S, Dicker F, Kern W, Wendland N, Sundermann J, Alpermann T, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood. 2011 Feb 24;117(8):2348–57.
103. 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 Advances. 2021 Sep 14;5(17):3254–65.
104. Chiara Caprioli, Federico Lussana, Silvia Salmoiraghi, Roberta Cavagna, Ksenija Buklijas, Lara Elidi, et al. Clinical significance of chromatin-spliceosome acute myeloid leukemia: a report from the Northern Italy Leukemia Group (NILG) randomized trial 02/06. haematol. 2020 Aug 27;106(10):2578–87.

105. Lachowiez CA, Loghavi S, Furudate K, Montalban-Bravo G, Maiti A, Kadia T, et al. Impact of splicing mutations in acute myeloid leukemia treated with hypomethylating agents combined with venetoclax. Blood Advances. 2021 Apr 27;5(8):2173–83.
106. Pratcorona M, Abbas S, Sanders MA, Koenders JE, Kavelaars FG, Erpelinck-Verschueren CAJ, et al. Acquired mutations in ASXL1 in acute myeloid leukemia: prevalence and prognostic value. Haematologica. 2012 Mar 1;97(3):388–92.
107. Boultwood J, Perry J, Pellagatti A, Fernandez-Mercado M, Fernandez-Santamaria C, Calasanz MJ, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. Leukemia. 2010 May;24(5):1062–5.
108. Eckardt JN, Stasik S, Röllig C, Sauer T, Scholl S, Hochhaus A, et al. Alterations of cohesin complex genes in acute myeloid leukemia: differential co-mutations, clinical presentation and impact on outcome. Blood Cancer J. 2023 Jan 24;13(1):18.
109. Jiang Y, Dunbar A, Gondek LP, Mohan S, Rataul M, O'Keefe C, et al. Aberrant DNA methylation is a dominant mechanism in MDS progression to AML. Blood. 2009 Feb 5;113(6):1315–25.
110. Parker JE, Mufti GJ, Rasool F, Mijovic A, Devereux S, Pagliuca A. The role of apoptosis, proliferation, and the Bcl-2–related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood. 2000 Dec 1;96(12):3932–8.
111. Docking TR, Parker JDK, Jädersten M, Duns G, Chang L, Jiang J, et al. A clinical transcriptome approach to patient stratification and therapy selection in acute myeloid leukemia. Nat Commun. 2021 Apr 30;12(1):2474.
112. Jayavelu AK, Wolf S, Buettner F, Alexe G, Häupl B, Comoglio F, et al. The proteogenomic subtypes of acute myeloid leukemia. Cancer Cell. 2022 Mar;40(3):301-317.e12.
113. Gourvest M, Brousset P, Bousquet M. Long Noncoding RNAs in Acute Myeloid Leukemia: Functional Characterization and Clinical Relevance. Cancers. 2019 Oct 24;11(11):1638.
114. Eriksson A, Lennartsson A, Lehmann S. Epigenetic aberrations in acute myeloid leukemia: Early key events during leukemogenesis. Experimental Hematology. 2015 Aug;43(8):609–24.
115. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA Methylation Signatures Identify Biologically Distinct Subtypes in Acute Myeloid Leukemia. Cancer Cell. 2010 Jan;17(1):13–27.
116. Bullinger L, Ehrich M, Döhner K, Schlenk RF, Döhner H, Nelson MR, et al. Quantitative DNA methylation predicts survival in adult acute myeloid leukemia. Blood. 2010 Jan 21;115(3):636–42.
117. Deneberg S, Grövdal M, Karimi M, Jansson M, Nahi H, Corbacioglu A, et al. Gene-specific and global methylation patterns predict outcome in patients with acute myeloid leukemia. Leukemia. 2010 May;24(5):932–41.
118. Trino S, Lamorte D, Caivano A, Laurenzana I, Tagliaferri D, Falco G, et al. MicroRNAs as New Biomarkers for Diagnosis and Prognosis, and as Potential Therapeutic Targets in Acute Myeloid Leukemia. Int J Mol Sci. 2018 Feb 3;19(2):460.
119. McDermott AM, Heneghan HM, Miller N, Kerin MJ. The Therapeutic Potential of MicroRNAs: Disease Modulators and Drug Targets. Pharm Res. 2011 Dec;28(12):3016–29.

120. Li Y, Solis-Ruiz J, Yang F, Long N, Tong CH, Lacbawan FL, et al. NGS-defined measurable residual disease (MRD) after initial chemotherapy as a prognostic biomarker for acute myeloid leukemia. Blood Cancer J. 2023 Apr 24;13(1):59.
121. 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 Dec 30;138(26):2753–67.
122. Yu S, Lin T, Nie D, Zhang Y, Sun Z, Zhang Q, et al. Dynamic assessment of measurable residual disease in favorable-risk acute myeloid leukemia in first remission, treatment, and outcomes. Blood Cancer J. 2021 Dec 6;11(12):195.
123. Short NJ, Zhou S, Fu C, Berry DA, Walter RB, Freeman SD, et al. Association of Measurable Residual Disease With Survival Outcomes in Patients With Acute Myeloid Leukemia: A Systematic Review and Meta-analysis. JAMA Oncol. 2020 Dec 1;6(12):1890.
124. Maiti A, DiNardo CD, Wang SA, Jorgensen J, Kadia TM, Daver NG, et al. Prognostic value of measurable residual disease after venetoclax and decitabine in acute myeloid leukemia. Blood Advances. 2021 Apr 13;5(7):1876–83.
125. Récher C, Röllig C, Bérard E, Bertoli S, Dumas PY, Tavitian S, et al. Long-term survival after intensive chemotherapy or hypomethylating agents in AML patients aged 70 years and older: a large patient data set study from European registries. Leukemia. 2022 Apr;36(4):913–22.
126. Juliusson G. Older Patients With Acute Myeloid Leukemia Benefit From Intensive Chemotherapy: An Update From the Swedish Acute Leukemia Registry. Clinical Lymphoma Myeloma and Leukemia. 2011 Jun;11:S54–9.
127. Fröhling S, Schlenk RF, Kayser S, Morhardt M, Benner A, Döhner K, et al. Cytogenetics and age are major determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: results from AMLSG trial AML HD98-B. Blood. 2006 Nov 15;108(10):3280–8.
128. Kantarjian H, O'brien S, Cortes J, Giles F, Faderl S, Jabbour E, et al. Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. Cancer. 2006 Mar 1;106(5):1090–8.
129 Dombret H, Gardin C. An update of current treatments for adult acute myeloid leukemia. Blood. 2016 Jan 7;127(1):53–61.
130. 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.
131. Loke J, Buka R, Craddock C. Allogeneic Stem Cell Transplantation for Acute Myeloid Leukemia: Who, When, and How? Front Immunol. 2021;12:659595.
132. 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 Aug 3;377(5):454–64.
133. Oñate G, Pratcorona M, Garrido A, Artigas-Baleri A, Bataller A, Tormo M, et al. Survival improvement of patients with FLT3 mutated acute myeloid leukemia: results from a prospective 9 years cohort. Blood Cancer J. 2023 May 5;13(1):69.
134. 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. The Lancet Oncology. 2014 Aug;15(9):986–96.

135. Tardi P, Johnstone S, Harasym N, Xie S, Harasym T, Zisman N, et al. In vivo maintenance of synergistic cytarabine:daunorubicin ratios greatly enhances therapeutic efficacy. Leukemia Research. 2009 Jan;33(1):129–39.
136. Lancet JE, Cortes JE, Hogge DE, Tallman MS, Kovacsovics TJ, Damon LE, et al. Phase 2 trial of CPX-351, a fixed 5:1 molar ratio of cytarabine/daunorubicin, vs cytarabine/daunorubicin in older adults with untreated AML. Blood. 2014 May 22;123(21):3239–46.
137. 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. JCO. 2018 Sep 10;36(26):2684–92.
138. Vogler WR, Miller DS, Keller JW. 5-Azacytidine (NSC 102816): a new drug for the treatment of myeloblastic leukemia. Blood. 1976 Sep;48(3):331–7.
139. Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. Cell. 1980 May;20(1):85–93.
140. Bohl SR, Bullinger L, Rücker FG. Epigenetic therapy: azacytidine and decitabine in acute myeloid leukemia. Expert Review of Hematology. 2018 May 4;11(5):361–71.
141. Cameron EE, Bachman KE, Myöhänen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat Genet. 1999 Jan;21(1):103–7.
142. Yang H, Bueso-Ramos C, DiNardo C, Estecio MR, Davanlou M, Geng QR, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. Leukemia. 2014 Jun;28(6):1280–8.
143. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, et al. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. Cell. 2015 Aug;162(5):974–86.
144. Kaminskas E, Farrell A, Abraham S, Baird A, Hsieh LS, Lee SL, et al. Approval Summary: Azacitidine for Treatment of Myelodysplastic Syndrome Subtypes. Clinical Cancer Research. 2005 May 15;11(10):3604–8.
145. 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 Jul 16;126(3):291–9.
146. Fenaux P, Mufti GJ, Santini V, Finelli C, Giagounidis A, Schoch R, et al. Azacitidine (AZA) Treatment Prolongs Overall Survival (OS) in Higher-Risk MDS Patients Compared with Conventional Care Regimens (CCR): Results of the AZA-001 Phase III Study. Blood. 2007 Nov 16;110(11):817–817.
147. Fenaux P, Mufti GJ, Hellström-Lindberg E, Santini V, Gattermann N, Germing U, et al. Azacitidine Prolongs Overall Survival Compared With Conventional Care Regimens in Elderly Patients With Low Bone Marrow Blast Count Acute Myeloid Leukemia. JCO. 2010 Feb 1;28(4):562–9.
148. Wei AH, Döhner H, Pocock C, Montesinos P, Afanasyev B, Dombret H, et al. Oral Azacitidine Maintenance Therapy for Acute Myeloid Leukemia in First Remission. N Engl J Med. 2020 Dec 24;383(26):2526–37.
149. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013 Feb;19(2):202–8.
150. Brunelle JK, Letai A. Control of mitochondrial apoptosis by the Bcl-2 family. Journal of Cell Science. 2009 Feb 15;122(4):437–41.

151. Stilgenbauer S, Eichhorst B, Schetelig J, Coutre S, Seymour JF, Munir T, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. The Lancet Oncology. 2016 Jun;17(6):768–78.
152. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med. 2016 Jan 28;374(4):311–22.
153. Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T, et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. Cancer Discovery. 2016 Oct 1;6(10):1106–17.
154. Delia D, Aiello A, Soligo D, Fontanella E, Melani C, Pezzella F, et al. bcl-2 proto-oncogene expression in normal and neoplastic human myeloid cells. Blood. 1992 Mar 1;79(5):1291–8.
155. Vo TT, Ryan J, Carrasco R, Neuberg D, Rossi DJ, Stone RM, et al. Relative Mitochondrial Priming of Myeloblasts and Normal HSCs Determines Chemotherapeutic Success in AML. Cell. 2012 Oct;151(2):344–55.
156. Niu X, Zhao J, Ma J, Xie C, Edwards H, Wang G, et al. Binding of Released Bim to Mcl-1 is a Mechanism of Intrinsic Resistance to ABT-199 which can be Overcome by Combination with Daunorubicin or Cytarabine in AML Cells. Clinical Cancer Research. 2016 Sep 1;22(17):4440–51.
157. Tsao T, Shi Y, Kornblau S, Lu H, Konoplev S, Antony A, et al. Concomitant inhibition of DNA methyltransferase and BCL-2 protein function synergistically induce mitochondrial apoptosis in acute myelogenous leukemia cells. Ann Hematol. 2012 Dec;91(12):1861–70.
158. Bogenberger JM, Kornblau SM, Pierceall WE, Lena R, Chow D, Shi CX, et al. BCL-2 family proteins as 5-Azacytidine-sensitizing targets and determinants of response in myeloid malignancies. Leukemia. 2014 Aug;28(8):1657–65.
159. 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 Aug 13;383(7):617–29.
160. Pratz KW, Jonas BA, Pullarkat VA, Thirman MJ, Garcia JS, Fiedler W, et al. Long-Term Follow-up of the Phase 3 Viale-a Clinical Trial of Venetoclax Plus Azacitidine for Patients with Untreated Acute Myeloid Leukemia Ineligible for Intensive Chemotherapy. Blood. 2022 Nov 15;140(Supplement 1):529–31.
161. Othman J, Amer M, Amofa R, Anderson L, Austin MJ, Bashford A, et al. Venetoclax with Azacitidine or Low Dose Cytarabine As an Alternative to Intensive Chemotherapy in Fit Adults during the COVID19 Pandemic: Real World Data from the UK National Health Service. Blood. 2021 Nov 5;138(Supplement 1):2321–2321.
162. Morsia E, McCullough K, Joshi M, Cook J, Alkhateeb HB, Al-Kali A, et al. Venetoclax and hypomethylating agents in acute myeloid leukemia: Mayo Clinic series on 86 patients. Am J Hematol. 2020 Dec;95(12):1511–21.
163. 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: IDH mutations in AML. Am J Hematol. 2015 Aug;90(8):732–6.
164. Losman JA, Looper RE, Koivunen P, Lee S, Schneider RK, McMahon C, et al. (R)-2-Hydroxyglutarate Is Sufficient to Promote Leukemogenesis and Its Effects Are Reversible. Science. 2013 Mar 29;339(6127):1621–5.

165. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 Mutations Result in a Hypermethylation Phenotype, Disrupt TET2 Function, and Impair Hematopoietic Differentiation. Cancer Cell. 2010 Dec;18(6):553–67.
166. DiNardo CD, Stein EM, De Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable Remissions with Ivosidenib in *IDH1* -Mutated Relapsed or Refractory AML. N Engl J Med. 2018 Jun 21;378(25):2386–98.
167. Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, et al. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. Blood. 2017 Aug 10;130(6):722–31.
168. Watts JM, Baer MR, Yang J, Prebet T, Lee S, Schiller GJ, et al. Olutasidenib alone or with azacitidine in IDH1-mutated acute myeloid leukaemia and myelodysplastic syndrome: phase 1 results of a phase 1/2 trial. The Lancet Haematology. 2023 Jan;10(1):e46–58.
169. 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 Apr 21;386(16):1519–31.
170. Pollyea DA, DiNardo CD, Arellano ML, Pigneux A, Fiedler W, Konopleva M, et al. Impact of Venetoclax and Azacitidine in Treatment-Naïve Patients with Acute Myeloid Leukemia and *IDH1/2* Mutations. Clinical Cancer Research. 2022 Jul 1;28(13):2753–61.
171. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory *FLT3* -Mutated AML. N Engl J Med. 2019 Oct 31;381(18):1728–40.
172. Zhao JC, Agarwal S, Ahmad H, Amin K, Bewersdorf JP, Zeidan AM. A review of FLT3 inhibitors in acute myeloid leukemia. Blood Rev. 2022 Mar;52:100905.
173. Xuan L, Wang Y, Huang F, Fan Z, Xu Y, Sun J, et al. Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. The Lancet Oncology. 2020 Sep;21(9):1201–12.
174. Burchert A, Bug G, Fritz LV, Finke J, Stelljes M, Röllig C, et al. Sorafenib Maintenance After Allogeneic Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia With *FLT3* –Internal Tandem Duplication Mutation (SORMAIN). JCO. 2020 Sep 10;38(26):2993–3002.
175. Lainez-González D, Serrano-López J, Alonso-Domínguez JM. Understanding the Hedgehog Signaling Pathway in Acute Myeloid Leukemia Stem Cells: A Necessary Step toward a Cure. Biology. 2021 Mar 24;10(4):255.
176. Irvine DA, Copland M. Targeting hedgehog in hematologic malignancy. Blood. 2012 Mar 8;119(10):2196–204.
177. Cortes JE, Heidel FH, Hellmann A, Fiedler W, Smith BD, Robak T, et al. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. Leukemia. 2019 Feb;33(2):379–89.
178. Hillestad LK. Acute promyelocytic leukemia. Acta Med Scand. 1957 Nov 29;159(3):189–94.
179. Thomas X. Acute Promyelocytic Leukemia: A History over 60 Years—From the Most Malignant to the most Curable Form of Acute Leukemia. Oncol Ther. 2019 Jun;7(1):33–65.
180. Dinmohamed AG, Visser O. Incidence of acute promyelocytic leukemia across Europe: results of RARECAREnet—a population-based study. Stem Cell Investig. 2019 Nov;6:37–37.

181. Lehmann S, Deneberg S, Antunovic P, Rangert-Derolf Å, Garelius H, Lazarevic V, et al. Early death rates remain high in high-risk APL: update from the Swedish Acute Leukemia Registry 1997–2013. Leukemia. 2017 Jun;31(6):1457–9.
182. Mantha S, Tallman MS, Soff GA. What's new in the pathogenesis of the coagulopathy in acute promyelocytic leukemia?: Current Opinion in Hematology. 2016 Mar;23(2):121–6.
183. Park JH, Qiao B, Panageas KS, Schymura MJ, Jurcic JG, Rosenblat TL, et al. Early death rate in acute promyelocytic leukemia remains high despite all-trans retinoic acid. Blood. 2011 Aug 4;118(5):1248–54.
184. Stahl M, Tallman MS. Acute promyelocytic leukemia (APL): remaining challenges towards a cure for all. Leuk Lymphoma. 2019 Dec;60(13):3107–15.
185. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood. 2008 Mar 1;111(5):2505–15.
186. Lin RJ, Sternsdorf T, Tini M, Evans RM. Transcriptional regulation in acute promyelocytic leukemia. Oncogene. 2001 Oct 29;20(49):7204–15.
187. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, et al. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature. 1998 Feb 19;391(6669):815–8.
188. Zhang JW, Gu J, Wang ZY, Chen SJ, Chen Z. Mechanisms of all-trans retinoic acid-induced differentiation of acute promyelocytic leukemia cells. J Biosci. 2000 Sep;25(3):275–84.
189. Yan J, Wang K, Dong L, Liu H, Chen W, Xi W, et al. PML/RARα fusion protein transactivates the tissue factor promoter through a GAGC-containing element without direct DNA association. Proc Natl Acad Sci USA. 2010 Feb 23;107(8):3716–21.
190. Menell JS, Cesarman GM, Jacovina AT, McLaughlin MA, Lev EA, Hajjar KA. Annexin II and Bleeding in Acute Promyelocytic Leukemia. N Engl J Med. 1999 Apr;340(13):994–1004.
191. Li T, Ma R, Zhang Y, Mo H, Yang X, Hu S, et al. Arsenic trioxide promoting ETosis in acute promyelocytic leukemia through mTOR-regulated autophagy. Cell Death Dis. 2018 Jan 23;9(2):75.
192. Choudhry A, DeLoughery TG. Bleeding and thrombosis in acute promyelocytic leukemia. Am J Hematol. 2012 Jun;87(6):596–603.
193. Manji GA, Khan Manji S, Karne S, Chao J. Time to ATRA in suspected newly diagnosed acute promyelocytic leukemia and association with early death rate at a non-cancer center institution: Are we meeting the target? JCO. 2012 May 20;30(15_suppl):6615–6615.
194. Gill H, Yung Y, Chu HT, Au WY, Yip PK, Lee E, et al. Characteristics and predictors of early hospital deaths in newly diagnosed APL: a 13-year population-wide study. Blood Adv. 2021 Jul 27;5(14):2829–38.
195. Sanz MA, Lo Coco F, Martín G, Avvisati G, Rayón C, Barbui T, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood. 2000 Aug 15;96(4):1247–53.
196. Sanz MA, Fenaux P, Tallman MS, Estey EH, Löwenberg B, Naoe T, et al. Management of acute promyelocytic leukemia: updated recommendations from an expert panel of the European LeukemiaNet. Blood. 2019 Apr 11;133(15):1630–43.
197. Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Iacobelli S, et al. Retinoic Acid and Arsenic Trioxide for Acute Promyelocytic Leukemia. N Engl J Med. 2013 Jul 11;369(2):111–21.

198. Platzbecker U, Avvisati 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. JCO. 2017 Feb 20;35(6):605–12.
199. Wang M, Lindberg J, Klevebring D, Nilsson C, Mer AS, Rantalainen M, et al. Validation of risk stratification models in acute myeloid leukemia using sequencing-based molecular profiling. Leukemia. 2017 Oct;31(10):2029–36.
200. Wang M, Lindberg J, Klevebring D, Nilsson C, Lehmann S, Grönberg H, et al. Development and Validation of a Novel RNA Sequencing-Based Prognostic Score for Acute Myeloid Leukemia. J Natl Cancer Inst. 2018 Oct 1;110(10):1094–101.
201. Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, Savage SL, et al. Functional genomic landscape of acute myeloid leukaemia. Nature. 2018 Oct;562(7728):526–31.
202. Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, et al. A survey of best practices for RNA-seq data analysis. Genome Biol. 2016 Dec;17(1):13.
203. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. CP Molecular Biology. 2015 Jan;109(1).
204. Yan F, Powell DR, Curtis DJ, Wong NC. From reads to insight: a hitchhiker's guide to ATAC-seq data analysis. Genome Biol. 2020 Feb 3;21(1):22.
205. Moran S, Arribas C, Esteller M. Validation of a DNA methylation microarray for 850,000 CpG sites of the human genome enriched in enhancer sequences. Epigenomics. 2016 Mar;8(3):389–99.
206. Pidsley R, Zotenko E, Peters TJ, Lawrence MG, Risbridger GP, Molloy P, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome Biol. 2016 Dec;17(1):208.
207. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.
208. Pavlou M, Ambler G, Seaman SR, Guttmann O, Elliott P, King M, et al. How to develop a more accurate risk prediction model when there are few events. BMJ. 2015 Aug 11;h3868.
209. Ambler G, Seaman S, Omar RZ. An evaluation of penalised survival methods for developing prognostic models with rare events. Statist Med. 2012 May 20;31(11–12):1150–61.
210. Hothorn T, Hornik K, Zeileis A. Unbiased Recursive Partitioning: A Conditional Inference Framework. Journal of Computational and Graphical Statistics. 2006 Sep;15(3):651–74.
211. Sardá-Espinosa A, Subbiah S, Bartz-Beielstein T. Conditional inference trees for knowledge extraction from motor health condition data. Engineering Applications of Artificial Intelligence. 2017 Jun;62:26–37.
212. Rubin DB. Estimating Causal Effects from Large Data Sets Using Propensity Scores. Ann Intern Med. 1997 Oct 15;127(8_Part_2):757.
213. Church DM, Schneider VA, Graves T, Auger K, Cunningham F, Bouk N, et al. Modernizing Reference Genome Assemblies. PLoS Biol. 2011 Jul 5;9(7):e1001091.

214. Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D, et al. The ExAC browser: displaying reference data information from over 60 000 exomes. Nucleic Acids Res. 2017 Jan 4;45(D1):D840–5.
215. Schneider VA, Graves-Lindsay T, Howe K, Bouk N, Chen HC, Kitts PA, et al. Evaluation of GRCh38 and de novo haploid genome assemblies demonstrates the enduring quality of the reference assembly. Genomics; 2016 Aug.
216. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014 Dec;15(12):550.
217. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, et al. Model-based Analysis of ChIP-Seq (MACS). Genome Biol. 2008 Nov;9(9):R137.
218. Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature. 2012 Jan;481(7381):389–93.
219. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Molecular Cell. 2010 May;38(4):576–89.
220. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016 Jan 4;44(D1):D733–45.
221. Mujahed H, Miliara S, Neddermeyer A, Bengtzén S, Nilsson C, Deneberg S, et al. AML displays increased CTCF occupancy associated with aberrant gene expression and transcription factor binding. Blood. 2020 Jul 16;136(3):339–52.
222. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics. 2014 May 15;30(10):1363–9.
223. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015 Apr 20;43(7):e47–e47.
224. Gillespie M, Jassal B, Stephan R, Milacic M, Rothfels K, Senff-Ribeiro A, et al. The reactome pathway knowledgebase 2022. Nucleic Acids Research. 2022 Jan 7;50(D1):D687–92.
225. Oestreich M, Holsten L, Agrawal S, Dahm K, Koch P, Jin H, et al. hCoCena: horizontal integration and analysis of transcriptomics datasets. Bioinformatics. 2022 Oct 14;38(20):4727–34.
226. Pilarczyk M, Fazel-Najafabadi M, Kouril M, Shamsaei B, Vasiliauskas J, Niu W, et al. Connecting omics signatures and revealing biological mechanisms with iLINCS. Nat Commun. 2022 Aug 9;13(1):4678.
227. Lou Y, Ma Y, Sun J, Suo S, Tong H, Qian W, et al. Effectivity of a modified Sanz risk model for early death prediction in patients with newly diagnosed acute promyelocytic leukemia. Ann Hematol. 2017 Nov;96(11):1793–800.
228. Cai P, Wu Q, Wang Y, Yang X, Zhang X, Chen S. An effective early death scoring system for predicting early death risk in *de novo* acute promyelocytic leukemia. Leukemia & Lymphoma. 2020 Jul 2;61(8):1989–95.
229. Lou Y, Ma Y, Suo S, Ni W, Wang Y, Pan H, et al. Prognostic factors of patients with newly diagnosed acute promyelocytic leukemia treated with arsenic trioxide-based frontline therapy. Leukemia Research. 2015 Sep;39(9):938–44.

230. Zhu HH, Ma YF, Yu K, Ouyang GF, Luo WD, Pei RZ, et al. Early Death and Survival of Patients With Acute Promyelocytic Leukemia in ATRA Plus Arsenic Era: A Population-Based Study. Front Oncol. 2021;11:762653.
231. Eisfeld AK, Kohlschmidt J, Mrózek K, Blachly JS, Walker CJ, Nicolet D, et al. Mutation patterns identify adult patients with de novo acute myeloid leukemia aged 60 years or older who respond favorably to standard chemotherapy: an analysis of Alliance studies. Leukemia. 2018 Jun;32(6):1338–48.
232. Prassek VV, Rothenberg-Thurley M, Sauerland MC, Herold T, Janke H, Ksienzyk B, et al. Genetics of acute myeloid leukemia in the elderly: mutation spectrum and clinical impact in intensively treated patients aged 75 years or older. Haematologica. 2018 Nov;103(11):1853–61.
233. Weller S, Toennießen A, Schaefer B, Beigl T, Muenchow A, Böpple K, et al. The BCL-2 inhibitor ABT-199/venetoclax synergizes with proteasome inhibition via transactivation of the MCL-1 antagonist NOXA. Cell Death Discov. 2022 Apr 20;8(1):215.
234. Jaramillo S, Schlenk RF. Update on current treatments for adult acute myeloid leukemia: to treat acute myeloid leukemia intensively or non-intensively? That is the question. haematol. 2023 Feb 1;108(2):342–52.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1954

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-501512

ACTA UNIVERSITATIS
UPSALIENSIS
2023