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Sequential therapy with allogeneic HCT in patients aged ≥70 years with active AML: a single-center retrospective analysis

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Contributions

OA, GF, and RR conceived the study, collected and interpreted the data, and drafted the manuscript. OA, GF, YM, IO, YS, DT, CK, IA, and RR contributed to the analysis, reviewed all sections critically, and approved the final version of the manuscript.

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Abstract

Patients ≥ 70 years with relapsed/refractory acute myeloid leukemia (AML) have an extremely poor prognosis. we adopted a sequential therapy approach, aiming to proceed directly to allogeneic hematopoietic cell transplantation (HCT) despite active disease. We analyzed results of all consecutive patients aged over 70 years and diagnosed with primary refractory/relapsed AML who underwent HCT with sequential therapy approach (FITCy regimen) in the Tel Aviv Sourasky Medical Center. 51 patients (median age 72 years, primary refractory, n=42/relapse, n=9). Median follow-up was 35 (range, 12-91) months. Incidences of overall and grade 3-4 acute GVHD were 39.2% (95% CI, 25.6-52.8%), and 5.9% (95% CI, 0.0-12.5%), respectively. Incidences of overall and moderate-severe chronic GVHD were 40.0% (95% CI, 25.7%-57.1%) and 29.4% (95% CI, 13.2%-46.9%), respectively. Non-relapse mortality at 3 years was 36% (95%CI 22%-49%).42/51 patients (82.4%) had CR on d+30 post HCT. Relapse incidence at 3 years was 27.8% (95% CI 14.3%-41.2%). GVHD-free relapse-free and overall survival (OS) at 3-years were 30% (95%CI 19%-47%) and 31% (95% CI 17%-55%), respectively. Multivariable analysis showed that worse ELN-2022 score, relapsed AML (vs. primary-refractory), not receiving ATG, and lower albumin prior to conditioning, were associated with higher mortality. We developed a model to predict OS that showed median OS in the low-, intermediate-, and high-risk group, not reached, 32.9 months, and 2.1 months, respectively, p<.001. We conclude that sequential therapy in elderly patients with active AML demonstrates a strong anti-leukemic effect, and age alone should not be a barrier to this strategy.

Introduction

Primary refractory acute myeloid leukemia (AML) occurs in ~30% of patients and carries a poor prognosis.(1, 2) Salvage chemotherapy, including novel agents, achieves limited responses, with median overall survival (OS) of about 12.5 months.(2, 3) Allogeneic hematopoietic cell transplantation (HCT) is the only treatment consistently associated with improved survival (4, 5), but outcomes are poor without pre-transplant remission, and many patients never reach HCT eligibility.(4, 6, 7)

In elderly patients, particularly those ≥70 years with refractory AML, prognosis is dismal: median OS with standard care may be only 2 months, and long-term survival is rare (8-10). Frailty, comorbidities, and treatment-related toxicity often preclude intensive salvage regimens, and active disease at transplant predicts inferior outcomes.

To address these challenges, several sequential approaches have been developed worldwide, the first being FLAMSA (11, 12), which combines cytoreductive chemotherapy with reduced-intensity conditioning to allow rapid progression to HCT. Our center adapted this concept into the FITCy regimen (fludarabine, cytarabine ± idarubicin, cyclophosphamide, and 4 Gy total body irradiation) for elderly patients with available donors. In a previous German–Israeli multicenter study, we reported encouraging outcomes with a FLAMSA-based sequential strategy in primary induction failure. (13)

Here, we present our single-center experience using a FITCy sequential conditioning regimen in patients ≥70 years with primary refractory or relapse AML, focusing on feasibility, disease control, and post-transplant outcomes.

Methods

Patients

Patients aged 70 years or older with either primary refractory or relapsed AML were included in this study. Patients were given FITCy conditioning regimen before HCT. Primary refractory AML was defined as unresponsiveness (at least 10% of blasts in marrow) after at least 1 course of 7+3 (defined on day 28-35 marrow) or 2 courses of venetoclax-azacitidine (VEN-AZA) regimen. Patients with relapsed AML either proceeded directly to HCT or, if a donor had not yet been identified, received salvage chemotherapy. Percentage of blasts was documented just

before starting of conditioning. No patients were a priori excluded based on comorbidities; all were evaluated for eligibility under this approach. Nevertheless, 25% of the patients were referred from other centers and were subjected to physician decision to consider sequential therapy approach. All patients gave informed consent to the planned treatment schedule as well as for reporting of transplantation outcomes and the study was approved by the local ethic committee.

Details regarding the donor search, the comprehensive treatment, supportive care, and evaluation of disease and response can be found in the Supplementary Appendix.

Statistics

Continuous variables were described as the mean, median, standard deviation and range of number observations, as applicable. Categorical data were described with contingency tables including frequency and percent. Confidence intervals were calculated at the (two-sided) 95% level of confidence. A two-sided P value of <.05 was considered to be statistically significant. Overall survival (OS) was defined as the time from HCT until the date of subject death from any cause. For subjects who have not died, survival data were censored at the subject's last known date of follow up. Disease response and disease progression were assessed according to the previously published response criteria. The probabilities of GRFS and OS were estimated using the Kaplan–Meier method, and the log-rank test was used to evaluate the differences between groups. Probabilities of GVHD were estimated with the use of cumulative incidence curves, with relapse and death treated as competing risks. Probabilities of NRM were estimated with the use of cumulative incidence curves, with relapse treated as a competing risk. Relapse incidence was evaluated with use of cumulative incidence curves, with death treated as a competing risk. The Fine and Gray's method was used to evaluate the differences between groups. Uni- and multivariable analyses were performed using a Cox proportional hazard regression model for OS, and competing risk regression by the method of Fine and Gray for GVHD, NRM, and relapse. We used SPSS version 29 and R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria) to perform the analyses.

For the comparison of patients with primary refractory AML who received sequential therapy (HCT cohort) with non-HCT series of the 3 previously published papers(14-16), restricted mean survival time (RMST) at 24 months was computed.(17) To adjust for baseline differences, a matching-adjusted indirect comparison (MAIC) using iterative proportional fitting (raking) aligned the HCT cohort distribution of sex and ELN 2022 risk with that of the Park et al. (venetoclax arm).(15) For the decitabine cohorts (14, 16), where covariate data were incomplete, exponential survival functions anchored to published medians were used for benchmarking. Hazard rates ($\lambda = \ln 2$ / median OS) were applied to derive approximate hazard ratios (HCT cohort/control). All comparator cohorts encompassed both refractory and relapsed AML; hence, the analysis likely underestimates the relative survival advantage associated with transplantation in strictly primary-refractory disease.

Results

Between May 2015 and December 2024 there were 51 patients (primary refractory, n=42; relapsed AML, n=9) that fulfilled eligibility criteria to this analysis. **Table 1** depicts the patients' characteristics. Median follow up of surviving patients was 35 (range, 12-91) months. Median age was 72 (range, 70-77) years. Median days from AML diagnosis to HCT in patients with primary refractory or relapsed disease was 86 (range, 30-188) days, and 209 (range, 125-1960) days, respectively. Majority of patients received venetoclax and ATG (63% and 88%, respectively) as part of the preparative regimen and majority (71%) received allograft from a matched unrelated donor. In all cases the graft was derived from peripheral blood stem cells (PBSC).

Early Transplant Course and Clinical Events

Seventeen patients (33%) developed microbiologically documented infections (MDI), including 8 with gram-negative rods (GNR) and 10 with gram-positive cocci (GPC); one patient had a mixed infection. Among the GNR cases, 2 involved extended-spectrum β-lactamase (ESBL)-producing organisms and 1 involved a carbapenemase-producing Enterobacterales (CPE). None of the GPC isolates exhibited antibiotic resistance. Two patients (4%) developed invasive fungal infections — one probable *Aspergillus* infection and one *Candida fermentati* infection. Two cases of GNR bacteremia were fatal. Among the 17 patients with MDI, only 1 patient (6%) had a

preconditioning-absolute neutrophil count above 0.5x10^3/microL. Seven patients (14%) developed mucositis (grade 1, n=2; grade 2, n=5). None developed grade 3-4. Five patients (10%) developed SOS (mild, n=1; moderate, n=3; severe n=1).

Median time to neutrophil engraftment was 11 (range, 8-21) days and median time to platelet engraftment was 18 (range, 11-46) days. Two patients with early progression of AML (within the first 2 weeks) were not evaluated for engraftment. Two additional patients had neutrophil engraftment but never attained sustained platelet engraftment; both subsequently relapsed at 2.7 and 3.2 months post-HCT. None of the 49 patients had primary or secondary graft failure. Whole marrow chimerism at day 30 post HCT showed median of 100% (range, 56%-100%) of donor-derived cells. Among the 6 patients who had donor chimerism levels below 90% on day +30 post-HCT without evidence of disease, three (50%) subsequently relapsed at 2.7, 3.2, and 32.2 months after HCT.

Graft vs. Host disease

Median onset of grade 2-4 acute GVHD was 41 (range, 7-140) days. Three cases were identified as late acute GVHD, emerging after cyclosporine tapering had begun. Among the 20 patients who developed acute GVHD, involvement of skin, gut, and liver occurred in 14 (70%), 9 (45%), and 0 (0%) of the patients, respectively. By day 200, the cumulative incidences of overall and grade 3-4 acute GVHD were 39.2% (95% CI, 25.6–52.8%), and 5.9% (95% CI, 0.0–12.5%), respectively **(Fig 1A)**. In univariable analyses, none of the candidate predictors were significantly associated with overall aGVHD (**Table 2)**. The low number of events precluded multivariable analysis.

Median time for the development of chronic GVHD was 6.7 (range, 4.1-11.7) months. At 12 and 36 months after HCT, the cumulative incidences of overall and moderate-severe chronic GVHD were 40.0% (95% CI, 25.7%-57.1%) and 25.8% (95% CI, 11.0%-42.1%), and 40.0% (95% CI, 25.7%-57.1%) and 29.4% (95% CI, 13.2%-46.9%), respectively **(Fig 1B)**. In univariable analysis, administration of ATG was associated with a lower incidence of overall chronic GVHD (HR=0.43, 95%CI 0.3-0.8, p=0.004), whereas age, donor type, female-to-male transplantation, and patient sex were not predictive. The low number of events precluded multivariable analysis.

Non relapse mortality

Eighteen patients died because of NRM (prior GVHD, n=8; sepsis with no prior GVHD, n=8; head injury, n=1; undetermined cause, n=1). Six deaths occurred within 30 days post HCT – all sepsis-related with no evidence of GVHD; 2 with prior moderate SOS.
The cumulative incidences of NRM at 1, 3 months, 1 year, and 3 years were 11.8% (95%CI 2.8%-20.7%), 26% (95%CI 13.4%-37.6%), 33% (95%CI 20%-47%), and 36% (95%CI 22%-49%) (**Fig 1C**). Higher albumin prior to HCT was associated with lower NRM (HR=0.86, 95%CI 0.80-0.97, p=0.017) and increased days to HCT was associated with higher NRM (HR=1.1, 95%CI 1.03-1.4. p=0.04, while age, sex, HCTCI, WBC prior to HCT, and primary vs. relapsed AML were not predictive. The low number of events precluded multivariable analysis.

Disease Response and Relapse Incidence

At day +30 after HCT, in an intent-to-treat analysis, 42/51 patients (82.4%, 95% CI 69.7%-90.4%) demonstrated CR. Seven patients (NRM, n=6 and relapse, n=1) died before day +30, and 2 additional patients experienced relapse by that time. Among the 44 patients who were alive at day +30, 42 (95.5%, 95% CI 84.9%-98.7%) demonstrated CR.
There were 14 cases of relapse. Relapse incidence at 3 months, 6 months 1 year, and 3 years post HCT were 11.8% (95% CI 2.8%-20.7%), 15.7% (95% CI 5.6%-25.8%), 19.7% (95% CI 8.6%-30.8%), and 27.8% (95% CI 14.3%-41.2%), respectively (**Figure 1D)**. There were 2 cases of late relapses, that occurred 32- and 64-months post HCT. Among the relapsing patients, 7 patients (50%) had AML with MDS related gene mutations. Lower chimerism test on day +30 and relapsed AML (as opposed to primary refractory) were both associated with increased relapse risk (HR=1.1, 95%CI 1.05-1.28, p=0.04, and HR=2.5, 95%CI 1. 3-7.9, p=0.03, respectively), while ATG, age, percentage of blasts prior to HCT, first line treatment prior to HCT, and days to HCT, did not impact risk of relapse. The low number of events precluded multivariable analysis.

Intervention at the time of relapse was chemotherapy (n=5), chemotherapy with DLI (n=4). One patient received chemotherapy with stem cell boost and one patient proceeded to a second allogeneic HCT. Median time from relapse to death was 2.98 (range, 0.1-85.2) months. Median time from relapse to death in patients given cellular therapy was 5.9 (range, 0.6-33.3) months.

Survival analysis

At the time of data analyses, 21 (41%) patients were alive. GRFS at 6- 12- and 36-months post HCT were 58% (95%CI 46%-73%), 38% (95%CI 27%-54%), and 30% (95%CI 19%-47%) **(Figure 2A)**. Patients with primary refractory disease had better GRFS at 6 and 12-months post HCT compared to patients with relapsed AML (66%, 95%CI 53%-82% vs. 22%, 95%CI 7%-75% and 44% 95%CI 31%-62% vs. 11% 95%CI 2%-71%, p=.003) (**Figure 2B**). Multi-variable analysis identified adverse ELN 2022 score, relapsed disease (vs. primary refractory) associated with worse GRFS (HR=2.02 95%CI 1.1-3.7, p=0.02, and HR=3.7 95%CI 1.6-9.1, p=0.003, respectively) and ATG as associated with better GRFS (HR=0.32, 95%CI 0.1-0.9, p=0.03) (**Table 2)**.

Incidences of overall survival at 3 months, 1 year, 3, and 5 years post HCT were 69% (95% CI 57%-83%), 53% (95% CI 41%-69%), 42% (95% CI 30%-60%), and 31% (95% CI 17%-55%), respectively (**Figure 2C)**. Subgroup analysis showed that patients with primary refractory disease had better 3 and 12-months OS compared to those with relapsed AML (74%, 95%CI 62%-88% vs. 44%, 95%CI 21%-92% and 60%, 95%CI 46%-76% vs. 22%, 95%CI 7%-75%, respectively, p=.008) **(Figure 2D**).

Multivariable analysis showed that worse ELN-2022 score and relapsed AML following were associated with increased mortality (HR=2.58 (95%CI 1.2-5.6; p=0.02) and HR=2.65 (95%CI 1.1-6.6; p=0.04), respectively). Conversely, ATG and higher albumin (per 1g/dL) were associated with lower mortality (HR=0.32 (95%CI 0.1-0.9; p=0.03) and HR=0.9 (95%CI 0.8-0.99; p=0.044), respectively). The model discriminated well (C-index = 0.745) and met proportional hazards assumptions.

Based on the multivariable Cox model, we derived a patient-level OS risk score as the model's linear predictor and then grouped patients by tertiles of this score into Low, Intermediate, and High risk. The following equation was used –

Risk score (linear predictor) = 0.948×(ELN step) + 0.975×(Relapse=1, Primary-refractory=0) − 1.139×(ATG Yes=1, No=0) - 0.117×(Albumin in g/dL). Kaplan–Meier curves demonstrated clear separation across the three groups. Median OS was not reached in the Low-risk group (NR), 32.9 months (95% CI 1.2-63.8) in the Intermediate-risk group, and 2.1 months (95% CI 0.3-6.5) in the High-risk group, p<0.001 **(Figure S1)**.

<u>Comparative Survival Analysis with External Relapsed/Refractory Cohorts</u>

In the composite Kaplan–Meier panel, the HCT cohort of primary-refractory AML patients aged ≥70 showed early and durable separation from the non-HCT comparators (**Figure S2)**. The venetoclax-combination cohort from Park et al. comprised relapsed/refractory AML (median age=69 years; 37% ≥70; 57% primary-refractory in the VEN arm) treated mainly with venetoclax+azacitidine ± FLT3/IDH inhibitors; after ranking our cohort to match sex and ELN distributions, the approximate HR favored sequential therapy approach (HR=0.52 vs VEN-combination and HR=0.33 vs the HCT-censored VEN curve). We then compared our cohort to the 10-day decitabine comparators (Ritchie et al: median age=70 years; Bouligny et al.: median age 67.5 years; ELN intermediate 53%, adverse 44%). Relative to the 10-day decitabine salvage approach, the sequential therapy cohort showed a lower hazard of death (HR=0.38 vs decitabine).

Discussion

In this single-center study we evaluated a sequential therapy platform (FITCy protocol) prior to allogeneic HCT in patients aged ≥70 years with active AML, mostly primary-refractory. We showed that early disease control was frequent (day +30 CR 82% by ITT; 95.5% among day +30 survivors), with a relative short time to engraftment (neutrophils/platelets at 11/18 days), and complete donor chimerism by day +30 was common. Acute and chronic GVHD were in the expected range for PBSC-RIC HCT. However, NRM clustered early (26% by day 90 and 36% at 3 years) and was largely associated with infectious. 3-year GRFS and OS were at the range of 30% and were stratified by a parsimonious multivariable model (ELN-2022 risk, relapse vs primary-refractory, ATG use, albumin), with a clear separation of 3 Kaplan-mayer curves groups. Together, these data support the biological premise of sequential therapy in elderly patients with chemo-resistant disease. The concept is coherent with the original FLAMSA experience showing feasibility and long-term survival in younger patients with refractory AML, and with later sequential backbones that reproduce these features in poor-risk settings.(12) Randomized evidence now suggests that delaying HCT to achieve CR may be unnecessary in refractory or relapsed AML. In the phase 3 ASAP trial, an “immediate HCT” strategy after brief disease-control measures was non-inferior to salvage induction followed by HCT, reinforcing

approaches that compress cytoreduction and move directly to the curative modality.(18) Nevertheless, median age in ASAP was 61 years and while the study supports proceeding rapidly to transplant in fit adults, many septuagenarians are not eligible to myeloablative conditioning and face higher transplant-related toxicity, so sequential reduced-intensity platforms tailored to older adults offer a safer and pragmatic path to curative therapy. In our exploratory overlays, the ≥70 years primary-refractory HCT cohort separated early and durably from non-HCT comparators both when compared to a VEN-combination salvage series (15) (HR=0.52 vs VEN-combination) or when compared to a 10-day decitabine comparators (HR=0.38), consistent with reported R/R outcomes on HMA schedules where CR/CRi rates are modest and median OS typically 4-6 months. (14, 16) Although baseline alignment was achieved for ELN risk and sex (weighted matching), our sequential therapy cohort was older and composed exclusively of primary-refractory patients, whereas external series included both relapsed and refractory cases. Consequently, the observed survival advantage of HCT should be interpreted with caution. Other retrospective studies reinforces the same message in older adults: VEN-based salvage produces responses but median OS is generally short (4-7 months) outside of successful bridging to HCT, and post-VEN failure outcomes are particularly poor, underscoring that, for fit septuagenarians with active AML, curative intent remains closely tied to timely transplantation rather than prolonged non-transplant salvage. (15, 19)

Against this backdrop, toxicity profiles in the elderly are nontrivial: In an EBMT ALWP analysis of AML not in remission (n=360; median age 72 years), 2-year NRM varied by donor and ranged from 17.5%- 43.9%, while GRFS ranged from 19-35%. (20) Earlier work in the elderly reported 2-year chronic GVHD near 36-40% under RIC. (21) Against those benchmarks, our acute GVHD (day-200 overall 39.2%; grade 3-4 5.9%) and chronic GVHD (overall 40%; moderate–severe 29%) sit within elderly RIC ranges; our 1- and 3-year NRM are comparable to outcomes reported for non-remission disease with unrelated donors at this age, suggesting that case-mix (active disease, MUD predominance, PBSC source) is a principal driver rather than regimen idiosyncrasy. (22)

Majority of NRM cases occurred in the first 90 days and many cases were associated with infections. Although this observation is based on a relatively small subgroup within the cohort, a non-neutropenic state prior to conditioning was associated with a lower rate of infections. There are several options to improve this domain. First, modifying GVHD prophylaxis to post-

transplant cyclophosphamide strategy, based on BMT CTN 1703, may result in superior GRFS, lower steroid exposure and mucosal injury, thereby cutting serious infections, the dominant pattern in our early NRM. (23) Second, CMV prevention with letermovir, should be standardized. (24) We have started to use standard letermovir prophylaxis only in 2023. Presumably, with generalization of the use of letermovir and perhaps also with the extending of the treatment beyond day 100, may be a reasonable default for septuagenarians. Third, antifungal prophylaxis should be tuned in cases of prolong cytopenia and when anticipating steroid exposure. (25)

Based on the mutivaraible analysis for overall survival we developed a risk model, which is clinically intuitive and may help tailor the platform. ELN-2022 risk group integrates genomic risk; disease status (relapse vs primary-refractory) showed consistently in our cohort impact on outcome; ATG plausibly mitigates chronic GVHD and improves GRFS/OS; and hypoalbuminemia captures frailty and infection susceptibility. Given the observed early infectious NRM, prioritizing the current FITCy-sequential approach for low- and intermediate-risk strata is reasonable while we iterate safety features in the high-risk tertile. To raise results in that high-risk group, two parallel tracks are sensible. One is to make the platform safer with possible adoption of PTCy-based GVHD prophylaxis and rigorous infection control. In this context, and based on emerging clinical data, further reduction of post-transplant cyclophosphamide dosing in older adults may be feasible and safe. Given the limited prospective evidence in this age group, careful dose optimization in future studies will be important to define the minimal effective PTCy intensity that maintains GVHD control while reducing toxicity. The other is to enhance efficacy without adding substantial toxicity: embed measurable residual disease and early chimerism monitoring with pre-emptive immunomodulation triggers (IST taper and donor lymphocyte infusion) when day +30 to +60 donor chimerism is low or MRD is positive, and deploy molecularly targeted maintenance in appropriate subsets (e.g., FLT3 or IDH inhibitor maintenance). These measures are compatible with the sequential paradigm and directly address the two dominant failure modes - early infection-related NRM and later relapse.

Our study is limited by several factors. First, this is a retrospective, single-center design and modest sample size; second, some endpoints (e.g., acute/chronic GVHD and relapse) had limited events for multivariable modeling, increasing type II error risk. Supportive-care practices and

ATG use may have evolved during the study period, and our exploratory overlays versus non-HCT comparators, despite raking on ELN and sex, remain ecological rather than fully adjusted. The risk model we presented is internally derived and requires external calibration and validation of cut points across centers, donor sources, and supportive-care eras. Nonetheless, the consistency of core signals (rapid early CR, manageable GVHD, and clean model-based survival separation) and their biological coherence argue for pragmatic adoption with concurrent optimization.

In conclusion, in carefully selected septuagenarians with active AML, a sequential FITCy platform can compress cytoreduction, expedite access to the curative modality, and harness graft-versus-leukemia while maintaining GVHD and quality of life at acceptable levels. NRM has remained a significant domain and may require further future modifications in protocol. Prospective, multi-center work comparing sequential backbones (including treosulfan-anchored variants), hard-wiring contemporary infection prevention, and validating the OS risk score should clarify optimal patient selection and consolidate gains in this growing population.

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Table 1 – Characteristics of Patients

Domain	All patients (n=51)	Primary refractory (n=42)	Relapsed (n=9)
Sex, N Male (%)	32 (63%)	27 (64%)	5 (56%)
Age, Median (range)	72 (70-77)	72 (70 -77)	73 (70-74)
Median days from AML diagnosis to HCT (range)	125 (30-1960)	86 (30-188)	209 (125-1960)
ELN 2022 risk			
Favorable, n (%)	5 (10%)	3 (7%)	2 (22%)
Intermediate, n (%)	19 (37%)	19 (45%)	0 (0%)
High, n (%)	27 (53%)	20 (48%)	7 (78%)
Prior therapy			
Prior refractory to – 7+3-based induction, n (%)	n/a	23 (55%)	n/a
Prior refractory to – Azacitidine+Venetoclax, n (%)	n/a	19 (45%)	n/a
n lines prior (median, range)	n/a	n/a	2 (1-3)
% blasts in marrow prior to HCT (median, range)	30 (13-92)	34 (13-92)	24 (15-81)
Preparative regimen – Venetoclax included, n (%)	32 (63%)	25 (60%)	7 (78%)
Preparative regimen – ATG included, n (%)	45 (88%)	37 (88%)	8 (89%)
Hb gr/dL (median, range)	8.2 (6.7-11.9)	8.2 (6.7-11.9)	8.3 (7.3-10.1)
ANC 10^3/microL (median, range)	0.2 (0-1.4)	0.2 (0-1.2)	0.3 (0-1.4)
WBC 10^3/microL (median, range)	1.7 (0-20.3)	1.8 (0-14.6)	1.6 (0.1-20.3)
Albumin g/L (median, range)	36.4 (24-48)	35.7 (24-48)	36.6 (32.7-41)
LDH U/L (median, range)	354 (107-2968)	351 (107-2968)	394 (238-893)
CRP mg/L (median, range)	16 (0.3-295)	13 (0.3-245)	26 (1.4-295)
Karnofsky score (median, range)	80 (50-100)	80 (50-100)	90 (80-100)
HCT-CI (median, range)	3 (0 -7)	3 (0-7)	4 (0-6)
High modified AL-EBMT score, n (%)	13 (25%)	13 (31%)	0 (0%)
Donor's characteristics			
Matched-related, n (%)	8 (16%)	7 (17%)	1 (11%)
Matched-unrelated, n (%)	36 (71%)	30 (71%)	6 (67%)
Haploidentical, n (%)	7 (14%)	5 (12%)	2 (22%)
Female-to-Male, n (%)	2 (4%)	2 (5%)	0 (0%)
CMV Status			
D+/R+, n (%)	41 (80%)	34 (81%)	7 (78%)
D+/R-, n (%)	1 (2%)	1 (2%)	0 (0%)
D-/R+, n (%)	7 (14%)	6 (14%)	1 (11%)
D-/R-, n (%)	2 (4%)	1 (2%)	1 (11%)

AL-EBMT = acute leukemia–European Bone Marrow Transplantation risk score; AML = acute myeloid leukemia; ANC = absolute neutrophil count; ATG = anti-thymocyte globulin; CMV = cytomegalovirus; CRP = C-reactive protein; D = donor; ELN = European LeukemiaNet; HCT = hematopoietic cell transplantation; HCT-CI = hematopoietic cell transplantation–comorbidity index; Hb = hemoglobin; LDH = lactate dehydrogenase; N = number; R = recipient; U/L = units per liter; WBC = white blood cells

Table 2 – Univariable and multivariable analyses

		Univariable analysis			Multivariable analysis		
Domain	Parameter	HR	95%CI	p value	HR	95%CI	p value
Acute GVHD	Age	1.02	0.8-1.4	0.89			
	Sex (Female vs. male)	0.46	0.2-1.2	0.11			
	ATG	0.55	0.1-2.2	0.4			
	Donor (non- vs. matched sibling)	1.22	0.4-3.9	0.83			
	First line treatment	0.67	0.3-1.6	0.36			
	Female-to-male	1.6	0.2-2.7	0.68			
	Relapsed (vs. primary refractory)	0.83	0.2-2.9	0.77			
Chronic GVHD	Age	0.85	0.7-1.1	0.23			
	Sex (Female vs. male)	0.96	0.3-2.7	0.94			
	ATG	**0.43**	**0.3-0.8**	**0.004**			
	Donor (non- vs. matched sibling)	0.62	0.2-2.3	0.47			
	Female-to-male	1.86	0.2-2.8	0.63			
Non-relapse mortality	Age	1.04	0.8-1.4	0.79			
	Sex (Female vs. male)	0.75	0.3-1.9	0.55			
	Relapsed (vs. primary refractory)	1.6	0.5-4.9	0.43			
	Days-to-HCT	**1.1**	**1-1.4**	**.04**			
	Albumin prior to conditioning	**0.86**	**0.8-0.97**	**.017**			
	White blood cells prior to conditioning	0.98	0.8-1.1	0.80			
	ANC prior to conditioning	1.03	0.94-1.15	0.41			
	HCTCI	0.95	0.8-1.2	0.68			
Relapse	Age	0.97	0.7-1.3	0.84			
	First line treatment (Ven-based vs. 7+3)	1.04	0.4-2.9	0.94			
	Relapsed (vs. primary refractory)	**2.5**	**1.3-7.9**	**0.03**			
	Days-to-HCT	1	0.98-1.1	0.66			
	% blasts prior to conditioning	1.2	0.1-5.7	0.35			
	D+30 donor chimerism (<90% vs. higher)	**1.1**	**1.05-1.3**	**0.04**			
GRFS	Age	0.95	0.7-1.2	0.62			
	Sex (Female vs. male)	2.7	1.4-5.3	0.005	1.3	0.8-1.7	0.13

	Higer ELN 2022 score	**2.1**	**1.2-3.8**	**0.009**	**2.02**	**1.1-3.7**	**0.02**
	Relapsed (vs. primary refractory)	**3.3**	**1.4-7.4**	**0.005**	**3.7**	**1.6-9.1**	**0.003**
	First line treatment (Ven-based vs. 7+3)	0.46	0.2-0.9	0.03			
	Days-to-HCT	1.0	1.0-1.0	0.13			
	Donor (non- vs. matched sibling)	1.1	0.4-2.6	0.87			
	Albumin prior to conditioning	0.9	0.8-0.98	0.01	0.93	0.9-1.02	0.14
	ATG	**0.24**	**0.1-0.6**	**0.002**	**0.32**	**0.1-0.9**	**0.03**
	Venetoclax added to condtioning	0.71	0.4-1.4	0.32			
	HCTCI	1.02	0.9-1.2	0.79			
	AL EBMT score	1.3	0.6-2.9	0.46			
Overall Survival	Age	0.95	0.8–1.2	0.62			
	Sex (Female vs. male)	2.7	1.4–5.3	0.005	1.4	0.8-1.9	0.15
	Higer ELN 2022 score	**2.1**	**1.2–3.8**	**0.009**	**2.58**	**1.2–5.6**	**0.02**
	Relapsed (vs. primary refractory)	**3.3**	**1.4–7.4**	**0.005**	**2.65**	**1.1–6.6**	**0.04**
	First line treatment (Ven-based vs. 7+3)	0.46	0.2–0.9	0.03			
	Days-to-HCT	1.03	0.99–1.1	0.12			
	Donor (non- vs. matched sibling)	1.1	0.5–2.6	0.87			
	Albumin prior to conditioning	**0.9**	**0.8–0.98**	**0.01**	**0.9**	**0.8–0.99**	**0.04**
	ATG	**0.24**	**0.1–0.6**	**0.002**	**0.32**	**0.1–0.9**	**0.03**
	ANC prior to conditioning	0.84	0.4-1.2	0.25			
	Venetoclax added to conditioning	0.71	0.4–1.4	0.32			
	HCTCI	1.02	0.9–1.2	0.79			
	AL EBMT score	1.3	0.6–2.9	0.46			

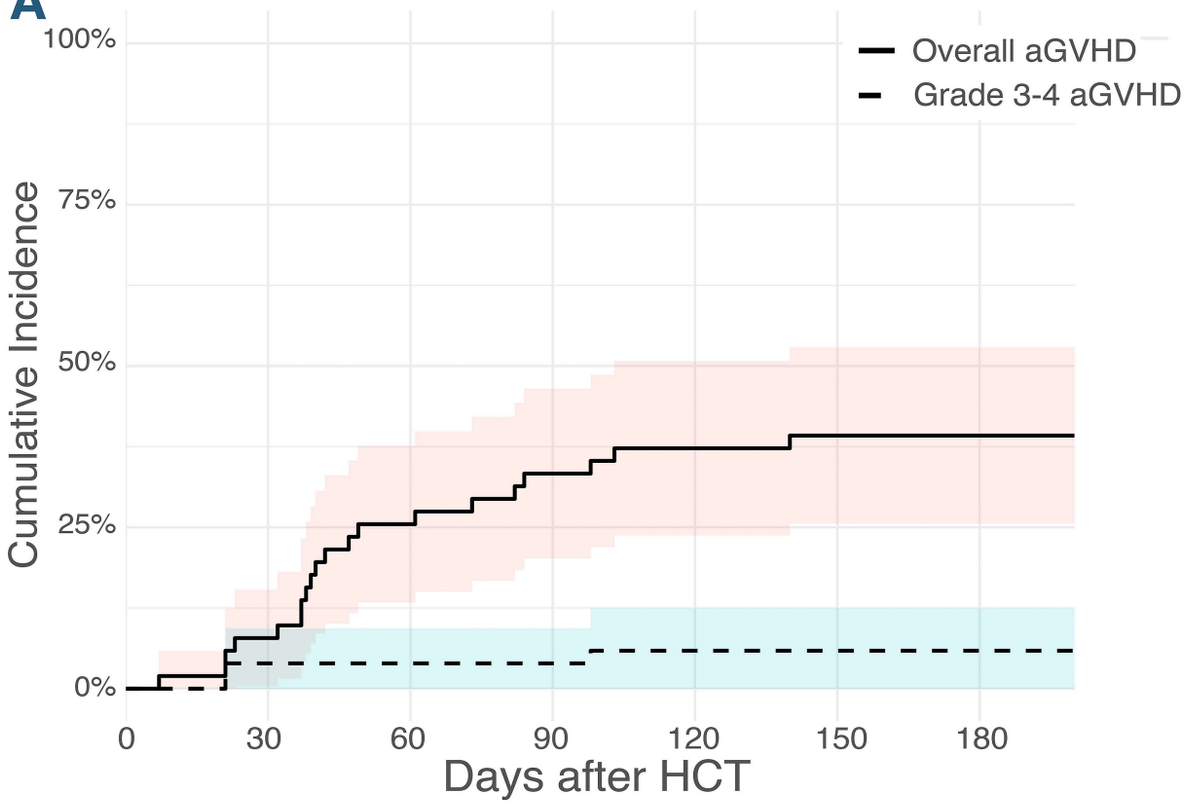
AL-EBMT = acute leukemia–European Bone Marrow Transplantation risk score; ANC = Absolute neutrophil count; ATG = anti-thymocyte globulin; D = donor; D+30 = day +30 post-transplant; ELN = European LeukemiaNet; GRFS = GVHD-free/relapse-free survival; GVHD = graft-versus-host disease; HCT = hematopoietic cell transplantation; HCT-CI = hematopoietic cell transplantation–comorbidity index; OS = overall survival; R = recipient; Ven = venetoclax; WBC = white blood cells.

Figure Legends

Figure 1 – Toxicity and relapse after treatment – A) overall and grade 3-4 acute graft vs. host disease (GVHD); B) overall and moderate-severe chronic GVHD; C) Non-relapse mortality; D) Relapse

Figure 2 – Survival analyses after treatment – A) Graft vs. host and relapse-free survival (GRFS); B) GRFS in patients with primary refractory vs relapse AML; C) Overall survival; D) Overall Survival in patients with primary refractory vs relapse AML

A


Overall aGVHD
Grade 3-4 aGVHD
Cumulative Incidence
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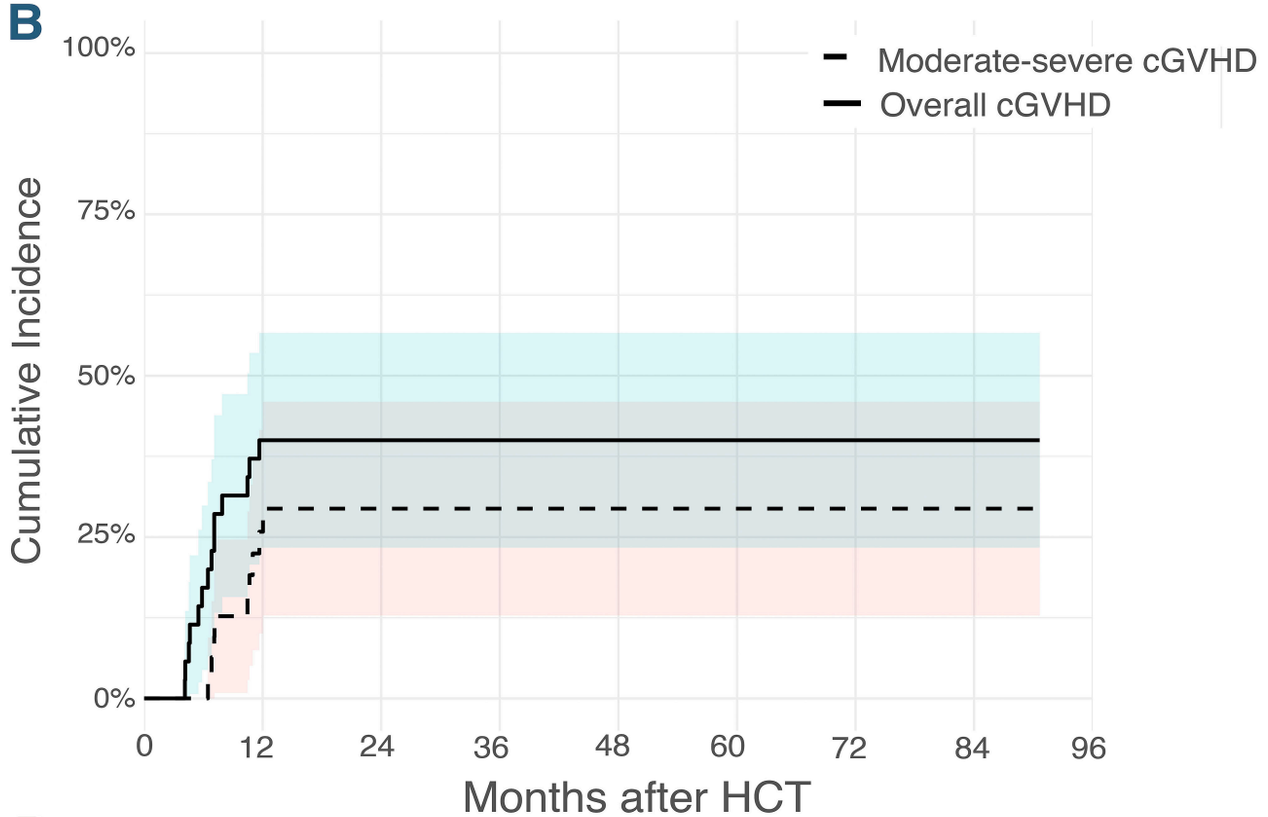
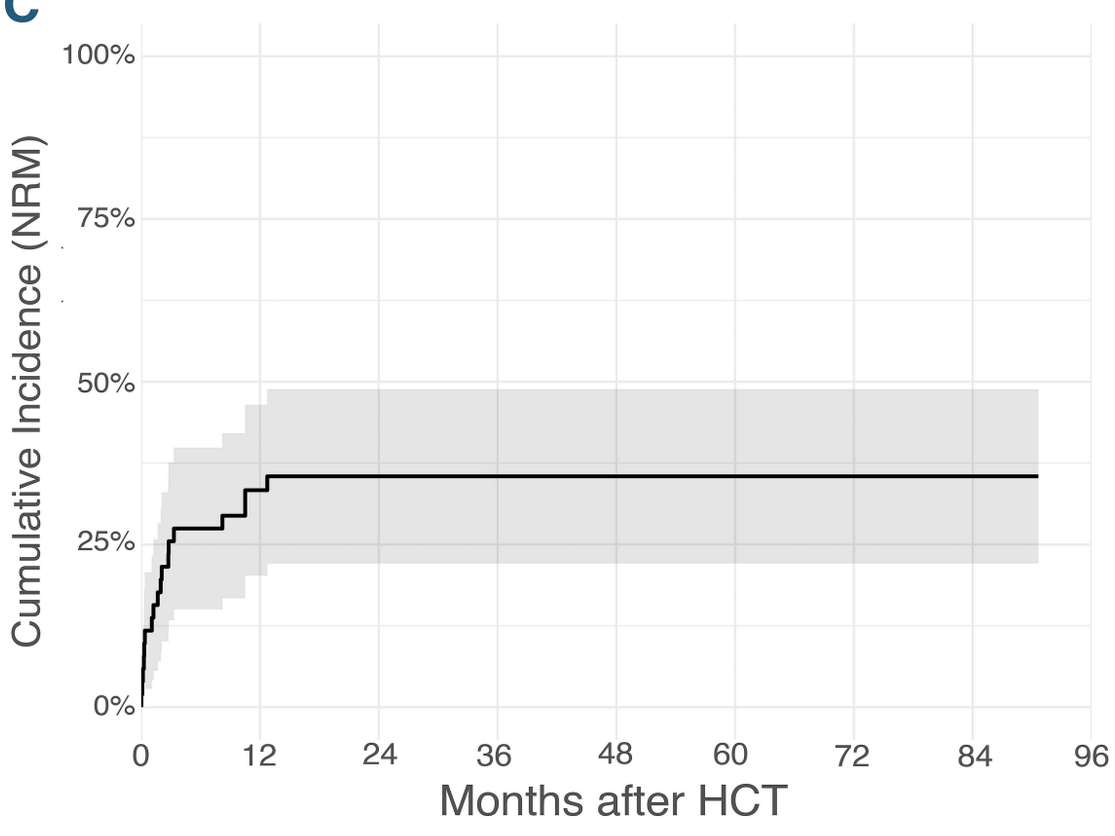
75%
50%
25%
0%
0
30
60
90
120
150
180
Days after HCT

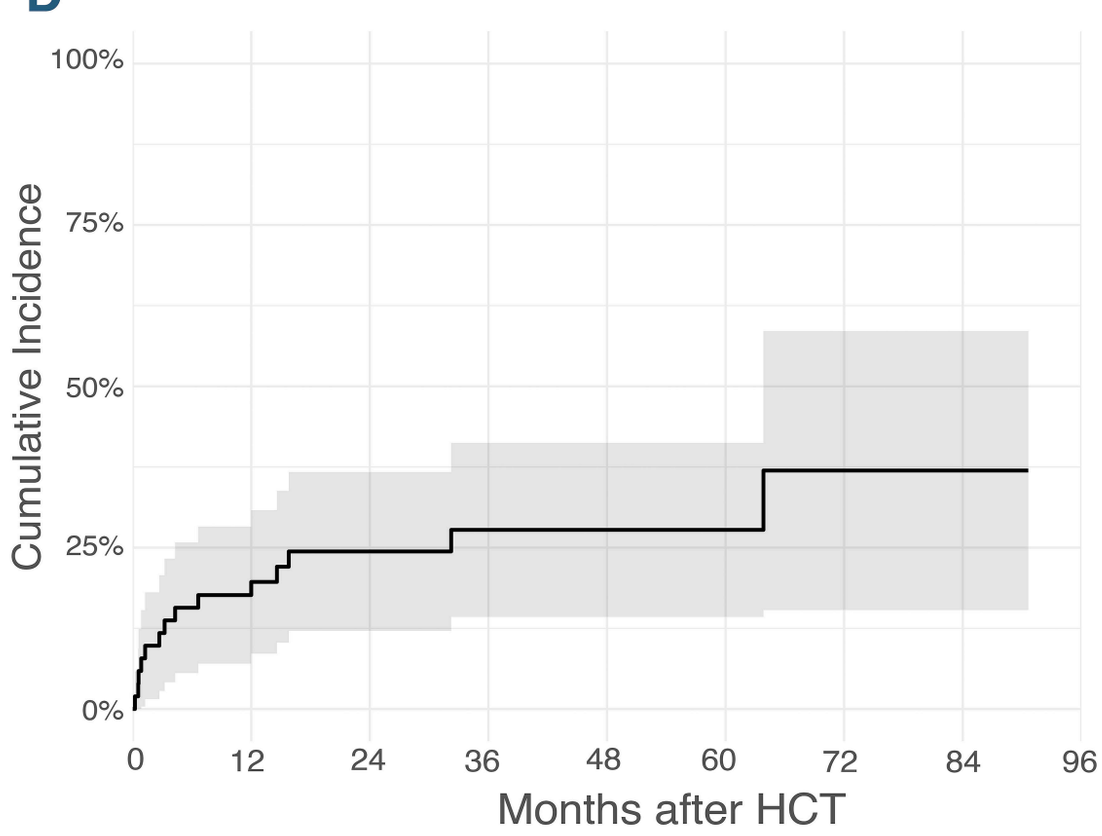
B

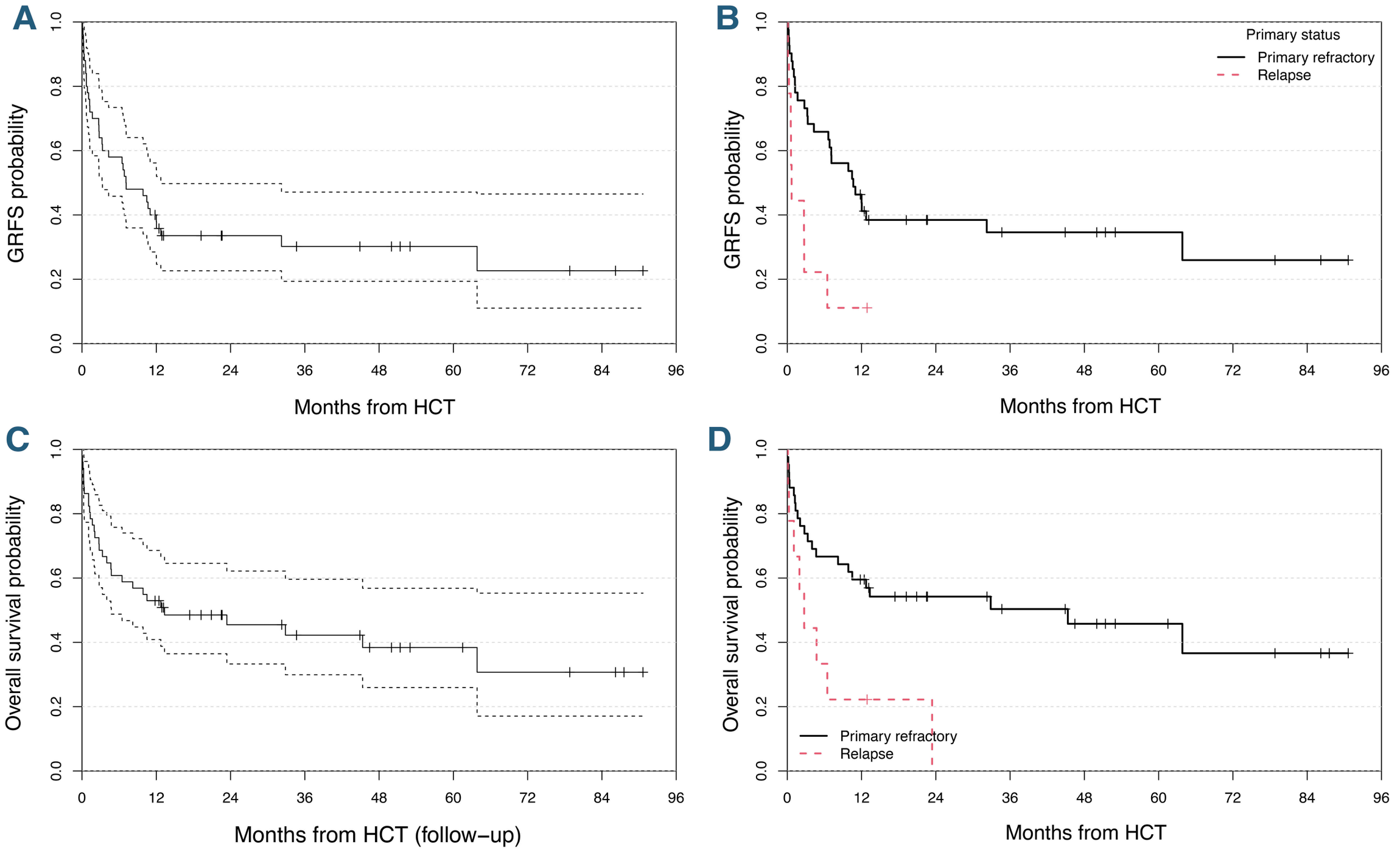

Moderate-severe cGVHD
Overall cGVHD
Cumulative Incidence
100%
75%
50%
25%
0%
0
12
24
36
48
60
72
84
96
Months after HCT

C


Cumulative Incidence (NRM)
100%
75%
50%
25%
0%
0
12
24
36
48
60
72
84
96
Months after HCT

D


Cumulative Incidence
100%
75%
50%
25%
0%
0
12
24
36
48
60
72
84
96
Months after HCT


A
GRFS probability
0.0
0.2
0.4
0.6
0.8
1.0
0
12
24
36
48
60
72
84
96
Months from HCT
B
GRFS probability
Primary status
Primary refractory
Relapse
Months from HCT
C
Overall survival probability
Months from HCT (follow−up)
D
Overall survival probability
Primary refractory
Relapse
Months from HCT

Methods

Donor Search

Blood for HLA typing was obtained from each patient upon starting induction treatment. High-resolution molecular typing using polymerase chain reaction (PCR) in the sampled DNA with sequence-specific primers was performed for HLA A, B, and C as well as for class II alleles (HLA DRB1 and DQB1). In the case of potential matched siblings, the goal was to achieve HLA results by day 14 after starting induction chemotherapy and at least low/ intermediate resolution for unrelated donor search results by day 21. In addition, the "Donor Search Centre" was notified once the patient was identified as having a "probably refractory disease" according to suggestive findings in the day 14 marrow (if patients received 7+3) or day 21 marrow (in case received VEN-AZA), unrecovered blood count by day 28, or evidence of progression early during treatment. This notification was done to shorten donor identification time in cases no results were finalized by 14-21 days. In specific cases when donor clearance has not finalized, patients could have started the first block prior to full clearance, but were given the second block only after full clearance of the donor was approved.

Treatment

The FITCy protocol is a modified version of the FLAMSA protocol, essentially omitting amsacrine. In detail, patients were initially treated with fludarabine (30 mg/m2/d) and cytarabine (2 g/m2/d <65 years or 1 g/m2/d if ≥ 65 years) for 5 consecutive days from day -13 to day -9. This block was followed by a 3-day break. The RIC part was based on 2 Gy twice daily TBI (day -5) and cyclophosphamide (40 mg/kg/d in case of a matched related donor and 60 mg/kg/d in case of a matched unrelated donor) from day -4 to day -3. In cases of haplo-identical donor, the pre-transplant cyclophosphamide dose was reduced to 25.5 mg/kg/d and post-transplant cyclophosphamide 14.5 mg/kg/d was administered on days +3 and +4. Anti-thymocyte globulin, ATG (Grafalon, Neovii) was given at 5 mg/kg BW/d for both matched siblings and unrelated donors (the protocol was amended on January 2018 to include ATG for all patients after interim analysis showed a high rate of GVHD). Venetoclax was added for the first 12 days of preparative regimen since January 2020 to increase efficacy. As a graft source,

G-CSF mobilized peripheral blood stem cells (PBMC) were preferred and bone marrow (BM) was accepted at the donor's preference. No graft manipulation was performed.

Supportive care

All patients were hospitalized in a designated ward in single-bed rooms equipped with HEPA filters. All patients receiving FITCy were given prophylaxis with ciprofloxacin. Antifungal prophylaxis consisted of posaconazole (300 mg/d) or voriconazole (200 mg, twice daily) and HSV/VZV prophylaxis of valacyclovir (1000 mg/d) or acyclovir (1600 mg/d). Pneumocystis jirovecii prophylaxis consisted with co-trimoxazole 3 days per week. Since 2023, all CMV-pos patients were given letermovir. Weekly monitoring of peripheral blood CMV-DNA by PCR was performed. In case of CMV reactivation, valganciclovir or intravenous ganciclovir treatment was initiated. During the period of neutropenia, a weekly monitoring of galactomannan antigen in the peripheral blood was performed. Prophylaxis of graft versus host disease (GvHD) consisted of cyclosporine A (CsA, given from day −1 to +100 adjusted to serum level (200–350 ng/ml), tapered from day +100 and discontinued up to day +180, if no signs of GvHD were present and mycophenolate mofetil (MMF, 2 g/day in matched related donors and 3 gr/day in unrelated donors given from day 0 to +30, tapered from day +30 and discontinued from day +50). In case of CsA side effects or non-tolerability, CsA was replaced by tacrolimus adjusted to serum level (5-15 ng/ml). During hospitalization, clinical status, adverse events, haematological as well as biochemistry parameters were monitored on a daily basis. After discharge, patients were followed in the outpatient clinic at least twice per week until day +60 with gradually longer intervals thereafter. Regimen-related toxicities were graded according to the CTCAE 5.0. Acute and chronic GvHD were graded and staged by the standard MAGIC and NIH criteria, respectively.(1, 2)

Evaluation of disease and response

Since majority of patients had refractory disease, the disease stage and time-from-diagnosis components of the AL-EBMT score were not applicable.(3)We therefore calculated a modified score including four domains: age (70–74 vs. ≥75 years), Karnofsky performance status, donor

type, and female-to-male donor–recipient mismatch. Each adverse factor was assigned 1 point (range 0–4), and patients were categorized as low risk (0–1) or high risk (2–4).

Engraftment was defined as the first of 3 days with a neutrophil count of more than 0.5×10^9/L and a non-transfused platelet count of more than 20×10^9/L. Disease response and donor whole marrow chimerism were assessed at day +30 and day +100 in BM. Complete remission (CR) was defined as less than 5% blast cells in BM by cytomorphology and flow-cytometry, and neutrophils of more than 1500/μL. Hematologic relapse was defined by the reappearance of blast cells in the PB, or by more than 5% blast cells in BM. Death from leukemia was defined as death with refractory disease after transplantation or death from any cause after post-transplantation relapse. Non-relapse mortality (NRM) was defined as death from any cause other than refractory disease or relapse. GVHD–relapse–free survival (GRFS) was defined as the time from transplantation to the first occurrence of grade III–IV acute GVHD, systemic therapy–requiring chronic GVHD, relapse, or death from any cause, whichever occurred first.

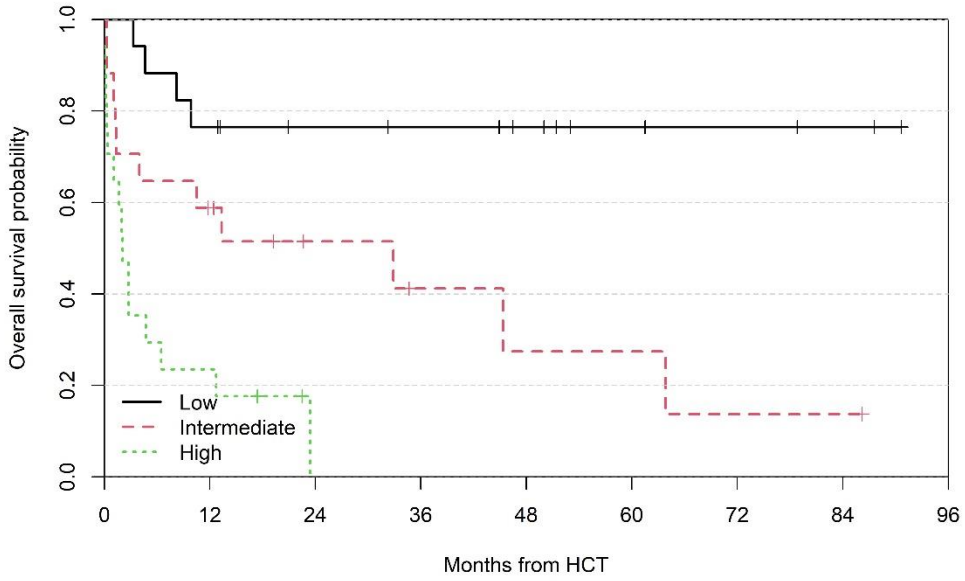
Because no randomized studies have evaluated HCT in elderly patients with primary refractory AML, survival was benchmarked against contemporary non-transplant salvage cohorts reported in comparable age groups and disease settings. Three published cohorts were selected for comparison: Park et al.(4) - 88 patients with relapsed/refractory AML (median age 69 years; 37% ≥ 70) treated with venetoclax + azacitidine ± FLT3/IDH inhibitors or intensive chemotherapy; Bouligny et al. (5) - AML treated with a 10-day decitabine regimen (mixed upfront and relapsed settings; median age 67.5 years); and Ritchie et al. (6) - 102 relapsed/refractory AML patients treated with repeated 10-day decitabine cycles (median age for the salvage subset not reported).

Legends

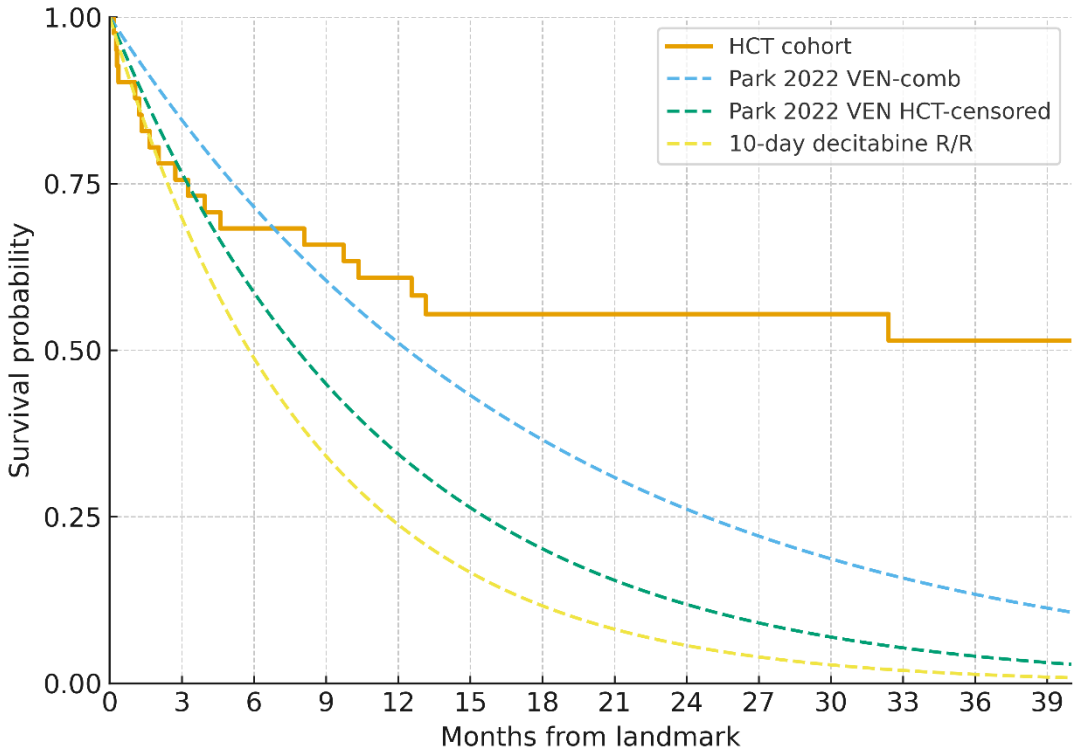
Supplemental Figure 1 - OS According to Multivariable Risk Score

Supplemental Figure 2 - Kaplan–Meier comparison: Patients with primary refractory AML who received sequential therapy vs heuristic comparators (exponential medians)

Supplemental Figure 1



Supplemental Figure S2



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