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Distinguishing AML from MDS: A fixed blast percentage may no longer be optimal

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Abstract:
Patients with acute myeloid leukemia (AML) have conventionally received more "intense" therapy than patients with myelodysplastic syndromes (MDS). Although less intense therapies are being used more often in AML, the AML-MDS dichotomy remains, with the presence of ≥ 20% myeloblasts in marrow or peripheral blood generally regarded as defining AML. Consequently, patients with 19% blasts are typically ineligible for AML studies, with patients with 21% blasts ineligible for MDS studies. Here we cite biologic and clinical data to question this practice. Biologically, abnormalities in chromosome 3q26,and mutations in *NPM1*, and *FLT3*, regarded as AML-associated, also occur in MDS. The genetic signatures of MDS, particularly cases with 10-19% blasts (MDS-EB2), resemble those of AML following a preceding MDS ("secondary AML"). Mutationally, secondary AML appears at least as similar to MDS-EB2 as to de novo AML. Patients presenting with de novo AML but with secondary-type AML mutations, appear to have the same poor prognoses associated with clinically defined secondary AML. Seattle data indicate that after accounting for European LeukemiaNet (ELN) 2017 risk, age, performance status, clinically secondary AML, and treatment including allogeneic transplant, patients with WHO-defined AML (n=769) have similar rates of OS, EFS and CR/CRi as patients with MDS-EB2 (n=202). We suggest defining patients with 10-30% blasts ("AML/MDS") as eligible for either AML or MDS studies. This would permit empirical testing of the independent effect of blast percentage on outcome, allow patients access to more therapies, and potentially simplify the regulatory approval process.

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Distinguishing AML from MDS: A fixed blast percentage may no longer be optimal

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Running Title: 20% blast cut-point to distinguish AML vs. MDS

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Key Points

Eligibility for clinical trials in AML or MDS is typically determined by whether a patient has ≥ 20% blasts (“AML”) or < 20% blasts (“MDS”). Hence, for example, a patient with 19% blasts is often ineligible for an AML trial, and a patient with 21% blasts ineligible for an MDS trial.

However, biologic and clinical data, discussed here, suggest the 20% cut-point is arbitrary. Although also arbitrary, defining patients with 10-30% blasts (“AML/MDS”) as eligible for either AML or MDS studies would permit empirical testing of the independent effect of blast percentage on outcome, allow patients access to more therapies, and potentially simplify the regulatory approval process. The 30% could be raised, or the 10% lowered, depending on results in the 10-30% group.

Abstract

Patients with acute myeloid leukemia (AML) have conventionally received more “intense” therapy than patients with myelodysplastic syndromes (MDS). Although less intense therapies are being used more often in AML, the AML-MDS dichotomy remains, with the presence of ≥ 20% myeloblasts in marrow or peripheral blood generally regarded as defining AML. Consequently, patients with 19% blasts are typically ineligible for AML studies, with patients with 21% blasts ineligible for MDS studies. Here we cite biologic and clinical data to question this practice. Biologically, abnormalities in chromosome 3q26,and mutations in *NPM1*, and *FLT3*, regarded as AML-associated, also occur in MDS. The genetic signatures of MDS, particularly cases with 10-19% blasts (MDS-EB2), resemble those of AML following a preceding MDS (“secondary AML”). Mutationally, secondary AML appears at least as similar to MDS-EB2 as to de novo AML. Patients presenting with de novo AML but with secondary-type AML mutations, appear to have the same poor prognoses associated with clinically defined secondary AML. Seattle data indicate that after accounting for European LeukemiaNet (ELN) 2017 risk, age, performance status, clinically secondary AML, and treatment including allogeneic transplant, patients with WHO-defined AML (n=769) have similar rates of OS, EFS and CR/CRi as patients with MDS-EB2 (n=202). We suggest defining patients with 10-30% blasts (“AML/MDS”) as eligible for either AML or MDS studies. This would permit empirical testing of the independent effect of blast percentage on outcome, allow patients access to more therapies, and potentially simplify the regulatory approval process.

Treatment for patients with acute myeloid leukemia (AML) and for myelodysplastic syndromes (MDS), including those MDS patients with excess blasts, has historically differed, with more "intense" regimens reserved for AML. Although less intense induction is now increasingly used in AML, the AML-MDS therapeutic dichotomy remains, largely based on the requirement, first put forth in the 2001 World Health Organization (WHO) classification of myeloid neoplasms[1], for ≥20% morphologic myeloblasts in either bone marrow or peripheral blood to diagnose AML. Retained in the 2008 and 2016 revisions [2, 3], the 20% blast criterion has affected patients' ability to receive new drugs in clinical trials. Despite the approval of several new drugs for AML [4-12],current therapy for AML and for MDS with 10-19% blasts (MDS-EB2) remains unsatisfactory[12,13] .Hence many physicians and patients would prefer participation in a trial. However, the 20% threshold means a patient is ineligible for an "AML-trial" with 19% blasts, but eligible with 21% blasts, and, conversely, eligible for an "MDS-trial" with 19%, but not 21%, blasts. However even if 500 cells are enumerated perfectly accurately, the 95% confidence intervals about 19% blasts (16-23%) and 21% blasts (18-25%) overlap significantly[14]. Reproducibility is also problematic. Assessing concordance among four experienced academic hematology cytologists regarding whether the blast count was 10-19% or <10% in 50 patients with MDS, Senent et al. found a kappa statistic value of 0.60, conventionally denoting only "moderate" concordance[15]. Although any blast percentage is arbitrary, a lower blast threshold would permit more patients to be treated on AML trials. Eligibility criteria for trials originating in academic centers often permit patients with 10-19% blasts to enroll on AML trials. However, the Food and Drug Administration's (FDA) and other regulatory agencies' strict separation of AML from MDS, based on the 20% blast threshold, continues to influence pharmaceutical companies, the sponsors of many innovative trials. Similarly, if the blast threshold for a diagnosis of AML were, for example, ≥ 30% (the theshold for AML used until 2001), patients with 21-29% blasts, who today are considered as AML, would be considered as MDS and become eligible for MDS trials.

The WHO has noted that "the 20% blast threshold is not a mandate to treat the patient as having AML or blast transformation: therapeutic decisions must always be based on the clinical situation after all information is considered"[2]. For example, as noted since the 2001 edition[1], "patients with the clonal, recurring cytogenetic abnormalities t(8;21)(q22;q22), inv(16)(p13q22), or t(16;16)(p13;q22) should be considered to have AML regardless of the blast percentage". Inclusion of these patients within AML likely reflects their responsiveness to intensive therapy usually reserved for patients with ≥ 20% blasts. The French-American-British (FAB) system, the predecessor of the WHO, considered the threshold for AML to be 30% blasts[16], with patients with 20-29% blasts classified in the MDS category "refractory anemia with excess blasts in transformation" (RAEB-t) [17]. However, the observation that administration of intensive AML-type therapy to patients with FAB-defined AML (≥30% blasts) or FAB RAEB-t (20-29% blasts) resulted in similar outcomes after accounting for cytogenetics, age, de novo vs. secondary AML, and treatment, again suggested the value of clinical data in informing classification[18]. This observation influenced 2001 WHO's reduction of the blast threshold for AML from 30% to 20%[1]. Here we suggest, that the 20% threshold is as arbitrary and as problematic as the prior 30%.

Biologic data

1) *AML-associated abnormalities can present as MDS*

Core-binding factor (CBF) rearrangements (as well as *PML-RARA* rearrangements) are considered AML-defining, irrespective of blast count[1]. Likewise, although also considered an AML-associated abnormality, *NPM1* mutations can rarely present as MDS or CMML[19-20]. Such cases appear biologically different from the more common *NPM1* wildtype MDS or CMML[20]. Inv(3)/t(3;3) can also present as either AML or MDS[21]. Based on analyses of 2,043 patients, Bersanelli et al. classified MDS into 8 distinct groups defined by specific genomic features[22]. Group 7 comprised 174 patients with "AML like mutations" occurring in *DNMT3A, NPM1, FLT3, IDH1*, and *RUNX1* genes; 83% of these cases presented with 15-19% blasts[22]. Rather than classifying solely on blast percentage, some cases might better be classified based on common genetic features, such as '*NPM1*-mutated myeloid neoplasm" or "myeloid neoplasm with inv(3)/t(3:3)".

2) *Genetic overlap between high-grade MDS and secondary AML*

Defining the "chromatin/spliceosome" class of AML by mutations in genes regulating RNA splicing (*SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*), chromatin modification (*ASXL1*, *STAG2*, *BCOR*, KMT2a ^{PTD}, *EZH2*, and *PHF6*), or transcription (*RUNX1*), Papaemmanuil et al. [23] noted AML patients in this class were older, often presented with an antecedent hematologic disorder and/or dysplastic marrow morphology, and had inferior outcomes. The same gene mutations of the Papaemmanuil et al. "chromatin/spliceosome" AML class or the Lindsley et al secondary AML pattern[24] (see below) have also been described in high-grade MDS[25,26], suggesting that secondary AML and high-grade MDS represent biologically very similar myeloid neoplasms transcending the morphologic 20% blast threshold.

Furthermore, Menssen and Walter[27] have that noted mutations in genes involved in at least 6 major pathways are shared between MDS and secondary AML. These entities also share cytogenetic abnormalities resulting in copy number alterations, in contrast to the balanced translocations more common in de novo AML. Indeed, a group of MDS-associated cytogenetic abnormalities are diagnostic of the WHO category "AML with myelodysplasia-related changes" (AML-MRC) even in clinically de novo cases without a prior MDS diagnosis or significant morphologic dysplasia. These abnormalities are also common in MDS, and are often considered 'poor' or 'very poor' risk in the MDS Cytogenetic Scoring System[28] and the revised International MDS Prognostic Scoring System (IPSS-R)[29], each of which combined MDS patients with AML patients with 20-29% blasts developing after MDS[28,29].

3*) MDS progression to AML evaluated using paired samples*

Menssen and Walter[27] identified 60 patients with paired MDS/secondary AML samples. Mutations in *TP53*, splicing factor, and epigenetic modifying genes occurred in both MDS and secondary AML stages, but the proportion of patients with these mutations was higher in the MDS stage; at AML progression, these mutations often persisted, but became less prominent than mutations in transcription factors (e.g. *RUNX1*, *CEBPA*) and activating signaling genes (e.g. *NRAS*/*KRAS*, *FLT3*), suggesting that AML progression is largely driven by de novo/pan AML mutations arising in

pre-existing MDS clones. Other studies[30-32] have similarly supported mutations in epigenetic regulating genes as early founder events followed by progression events (e.g. mutations in signaling genes or *NPM1*). However, months to years before progression, progression-associated mutations can often be identified at low levels at the MDS stage[26], with preleukemic mutations persisting in AML remission[33]. These data suggest a complex relationship between blast percentage and underlying mutation signature , defying simple categorization as “MDS” or “AML” based on a single blast percentage cutoff.

4) *Clinically secondary vs clinically de novo AML*

Lindsley et al. [24] compared mutation patterns in 93 patients with secondary AML (defined by histologic documentation of MDS or chronic myelomonocytic leukemia [CMML] ≥ 3 months before AML diagnosis) with mutation patterns in 180 patients with clinically de novo AML[34]. Mutations in eight genes (*SRSF2*, *ZRSR2*, *SF3B1*, *ASXL1*, *BCOR*, *EZH2*, *U2AF1*, and *STAG2*) were >95% specific for secondary AML while 3 alterations (*NPM1* mutations, *KMT2a* rearrangements, and CBF gene fusions) were >95% specific for de novo AML. Sixteen genes had less than 95% specificity and were considered “pan-AML” mutations.

Extending these comparisons to include MDS Chen et al. compared mutation incidence in 36 genes among 102 patients with MDS-EB (5-19% blasts), 69 (non-paired) patients with WHO-defined AML-MRC (n=61) or therapy-related AML (t-AML, n=8), and 64 patients with de novo AML [35]. Mutations in spliceosome genes occurred in 35% of MDS-EB, 32% of AML-MRC/t-AML, and 25% of de novo AML ($p = 0.38$). *TP53* mutations were seen in 39% of MDS-EB, 29% of AML-MRC/t-AML, and 2% of de novo AML ($p < 0.00001$). *NPM1* mutation frequency was 6% in MDS-EB, closer to the frequency in AML-MRC/t-AML (13%, $p = 0.17$) than to that in *de novo* AML (41%, $p < 0.001$). Likewise, the frequency of *FLT3*-ITDs was closer comparing MDS-EB and AML-MRC/t-AML (0% versus 6% , $p = 0.025$), than comparing AML-MRC/t-AML and de novo AML (6% vs 22%, ($p = 0.007$) .

5*) Relation between blast percentage and tumor burden assessed by variant allelic frequencies*

Chen et al. also reported that the distribution of variant allelic frequencies (VAFs) of individually mutated genes did not differ between MDS-EB and AML-MRC/t-AML, despite the difference in blast percentages between these entities[35]. Toth et al. reported similar results [36].

Walter et al.[31] pursued this topic using paired bone marrow samples from 7 patients at the MDS stage (mean blast count <10%) and subsequently at AML progression (mean blast count approximately 45%). They assessed tumor burden as the percentage of clonal cells, based on VAFs of various mutations. Despite the increase in morphologic blast count, approximately 85% of the cells were clonal at both the MDS and secondary AML stages.

These biologic data suggest that secondary AML arising from prior MDS, and even clinically-defined de novo AML exhibiting a secondary-type AML gene signature, bear more resemblance to MDS-EB than to de novo AML lacking MDS-type genetics. “MDS-EB” and “AML” essentially form a continuum. Blast percentage is an imperfect guide to tumor burden since in both MDS and AML, a

similarly high proportion of hematopoietic cells are part of the mutated clone. Rather than blast percentage, disease categorization may be more accurate if based on biologic features. One possibility would classify disease as (1) "true" MDS with <5% blasts without known high risk mutations (e.g. *TP53*) or cytogenetic abnormalities(e.g. inv(3)/t(3;3) and thus with low risk of progression to AML; examples include MDS with isolated del(5q)[37] or SF3B1-mutated MDS[38], (2) true" AML with *PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11* gene fusions*, NPM1 mutations, KMT2A* gene re-arrangments or bi-allelic *CEBPA* mutations, regardless of blast % and (3) cases with high-risk mutations (e.g. *TP53*, *ASXL1, RUNX1*) or cytogenetic abnormalities (e.g. inv(3)/t(3;3) that are common to both AML and MDS, and other cases with > 5% blasts. Patients with t-AML could belong to either group 2 or group 3 but only rarely to group 1.

Clinical data

1) *Dominance of genetic ontogeny over clinical ontogeny*

Among 42 patients aged ≥ 60 years with clinically de novo AML, those patients with secondary-type mutations had poorer outcomes, resembling those seen in patients with documented secondary AML[24]. Outcome in t-AML was also poorer in the presence of a secondary mutation pattern[24].

2) *Comparative importance of specific genetic abnormalities vs. AML/ MDS distinction*

a) *RUNX1-RUNX1T1; t(8;21)(q22.q22.1)* and *CBFB-MYH11* inv(16)(p13.1q22) or t(16;16)(p13.1;q22) – WHO considers these patients to have "AML" regardless of blast count given the lack of dysplasia in those with < 20%[1] blasts and the similarly favorable outcomes following AML-type therapy regardless of blast percentage[1]. We believe this example provides a compelling precedent for defining AML based on genetic features, rather purely on a rigid blast percentage..

b) *NPM1* - despite a blast count < 20%, *NPM1*-mutated MDS/CMML appears sensitive to AML-type induction chemotherapy. Montalban-Bravo et al.[39] compared AML-type induction therapy (typically anthracyclines + cytarabine +/- fludarabine or cladribine) and MDS-type therapy (typically hypomethylating agents [HMA] azacitidine or decitabine) in 31 patients with *NPM1*-mutated MDS or CMML. Median marrow blast count was 10% (range 0-19%); 19 patients had MDS EB-2 or CMML-2 (10-19% blasts). The 11 patients given AML induction were younger than the 20 given HMAs, but distributions of IPSS-R scores were similar. CR rates were 90% with AML induction and 28% with HMAs (p=0.004). Seven patients given AML-type therapy and 6 given HMA received allogeneic hematopoietic cell transplant (HCT). With a 30-month median follow-up, AML-type induction was associated with longer progression-free survival (p= 0.023) and overall survival (p =0.047). The number of events/deaths was too small to support a multivariate analysis, nor was there a comparison with AML patients with *NPM1* mutations given intensive induction . However, the results suggest a focus on the 20% cut-point may lead to potentially efficacious therapy being withheld from *NPM1*-mutated MDS/CMML patients and their exclusion from clinical trials specifically targeting *NPM1* mutations but intended only for patients with AML.
c) *GATA2*,*MECOM*(*EVI1*); inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2);– although more commonly found in AML, these entities share the same biology and dismal outcome whether treated as MDS or AML[40,41].

d) *TP53* – this mutation is typically associated with extraordinarily poor outcomes regardless of whether classified as AML[42] or MDS[43].

e) *FLT3*-ITD: *FLT3*-ITD and TKD mutations occur, although rarely, in MDS. Xu et al. have reported that *FLT3*-ITD mutations are an adverse prognostic factor in de novo MDS patients[44], mirroring findings in *FLT3*-ITD mutated AML[42].

3) Seattle data – Fred Hutchinson Cancer Research Center/University of Washington (FH/UW)considers patients with MDS-EB2 (10-19% morphologic blasts) eligible for AML-type therapy, in locally-initiated studies[45]. Other such patients have received conventional MDS-type therapy, particularly HMAs. Patients with ≥ 20% morphologic blasts have also received both types of therapy, although few received HMAs plus venetoclax, which became available only relatively recently.

We analyzed outcomes in 769 patients with WHO-defined AML (APL excepted) and 202 patients with MDS-EB2 . AML patients were considered secondary if they had bone marrow documentation of antecedent MDS or myeloproliferative neoplasm (MPN) (n=123), therapy-related disease (n=72) or both (n=21). In 137 of the 144 of cases AML developing after marrow documentation of MDS or MPN the marrow showing MDS or MPN was obtained > 3 months prior to AML diagnosis . MDS patients were considered secondary only if they had therapy-related disease (n=23). Patients were treated between 2008 and 2016 and median follow-up in patients remaining alive or alive in remission was 4.2 years. 729/769 (95%) of WHO-defined AML cases had ≥ 20% morphologic blasts in marrow (n=535), or, if marrow was inadequate, in peripheral blood (n=194). The remaining 5% were considered AML because, of CBF abnormalities or biopsy-proven granulocytic sarcoma. The AML and MDS-EB2 patients did not differ in age or performance status (table 1). ELN 2017 favorable risk disease[42] was more common in AML while ELN intermediate and adverse risk[42] were more common with MDS-EB2(table 1). Reflecting the different criteria for secondary AML vs. secondary MDS, secondary disease was more common with AML (28% vs. 11%,table 1). Receipt of low-intensity induction (typically HMAs) was more common with MDS EB-2. We combined patients receiving either 7+3 or induction containing cytarabine in doses ≥ 1g/m^2 into a high-intensity group since induction with either 7+3 or high-dose cytarabine appears equally efficacious[42]. Receipt of allogeneic hematopoietic cell transplant (HCT)was more common with MDS EB-2(table 1). Statistical analyses were not adjusted for multiple testing.

Rates of CR or CRi, and of CR without measurable residual disease (CR_{MRD-}, determined by multi-parameter flow cytometry), were higher with AML (table 1) and overall survival (OS), event-free survival (EFS), and relapse-free survival (RFS) in patients achieving CR/CRi (but not CR_{MRD-}) also were superior in AML (fig 1). However, after accounting for the covariates listed in table 1, whether a patient had AML or MDS EB-2 did not affect OS or EFS (table 2), likely reflecting that AML patients were less likely to be in the ELN 2017 adverse group ($p= 0.03$) or the intermediate group($p=0.009$) and more likely to be in the favorable group ($p< 0.001$) (table 1), with ELN 2017 having a major impact on both survival and EFS(table 2). Although the discrepancies were not as great as with ELN 2017, AML patients were more likely than MDS EB-2 patients to receive intensive induction (table 1), which was also associated with improved OS and EFS (table 2).

OS is the endpoint most commonly used for new drug approvals by FDA, with EFS also commonly used because it estimates the effect of new drugs independent of therapy given after relapse, or for refractory AML[46] . Achievement of CR or CRi was not affected by the AML-MDS EB2 distinction, although RFS in patients achieving CR/CRi was longer in AML(table 2). In contrast, AML patients were more likely to achieve $CR_{MRD\ neg.}$, but RFS was similar in such patients regardless of the AML-MDS EB2 distinction. Notably however, the effect of AML rather than MDS-EB2 on RFS in CR/CRi (HR 0.66) was less than that of intermediate or adverse risk ELN (HRs 2.15 and 3.07) and of receipt of HCT (HR 0.29). Similarly, the effect of AML rather than MDS-EB2 on the rate of $CR_{MRD\ neg}$ (OR 1.13) was less than that of ELN intermediate or adverse risk (ORs 0.82 and 0.71) and similar to that of secondary AML (OR 0.88). Results were essentially the same (not shown) when blast percentage was examined in deciles: 10-19, 20-20, 30-39… 90-99%, thus providing a clinical counterpart to Walter et al.'s observation of a lack of relation between blast percentage and tumor burden assessed using genetic methods to estimate per cent clonal cells by genetic methods.[31].

The expected prognostic impacts of ELN 2017 risk group, age, receipt of HCT, performance status, and secondary AML suggest the Seattle dataset is representative of usual experience. Median follow-up of our patients remaining alive in remission was about 4 years. Although the probability of relapse or death from AML appears to decline sharply after remission lasting for 3 years[47], there were patients lost to follow up, and these may have had worse OS, EFS, or RFS than other patients. The proportion of our MDS-EB2 patients who received high-intensity induction(68%) is likely higher than the proportion at other centers. Furthermore ,we found that, after accounting for covariates, high intensity therapy was associated with better OS and EFS, which is not necessarily the general experience. However, the lack of more widespread use of intensive therapy in EB2 is precisely the practice that we are challenging, and we hope our data stimulate comparison of intensive vs. non-intensive therapy for MDS-EB2 patients in randomized prospective trials. HCT, particularly in CR1, may also have been more commonly used in Seattle than elsewhere. However, within the limits of patient numbers and events, interaction terms indicated the effects of the AML vs MDS-EB2 distinction on outcomes were similar regardless of intensity of induction or receipt of HCT(p-values for all outcomes > 0.05). A related important question is whether differences in outcome between AML and MDS-EB2 might be more obvious in other subgroups defined for example by age or ELN.

We cannot retrospectively assess the possible role of "latent variables", e.g. selection bias, in determining which patients received AML-type and which MDS-type therapy, although we attempted to adjust for this by including variables such as age and performance status. A trial randomizing patients with WHO-defined AML or MDS-EB2 to the same therapies would be needed to evaluate the effect of such "latent variables", recognizing that several such trials might be needed to account for various subgroups defined by ELN, age, and other variables. In the shorter term, European studies which have included MDS-EB2 patients in their AML trials might serve as validation cohorts for the Seattle cohort to possibly reduce the effect of selection bias.

Discussion

We observed that the AML/ MDS-EB2 distinction had no effect OS or EFS, arguably the two most important clinical endpoints[46]. There were only variable/inconsistent effects (a) on CR/CRi and RFS among those achieving CR/CRi and (b) on $CR_{MRD\ neg}$ and RFS among those achieving CR_{MRD}

$_{neg}$(table 2). The effects of ELN, HCT, and in most cases, age, performance status and secondary AML, were greater than those of AML vs MDS-EB2(table 2). Hence,particularly given the biologic data described above, we see no compelling reason to determine eligibility for either an AML or an MDS trial therapy based solely on a 20% blast cut-point,with the burden of proof resting on those who advocate for this cut-point.

Nonetheless the 20% blast cut-point continues to play a key role, with important consequences for patients. For example, despite sharing the same genetic lesions, patients considered as MDS-EB2 may be ineligible to receive agents approved only for AML. Examples include gemtuzumab ozogamicin (GO) in *NPM1*-mutated disease[48] or gilteritinib in *FLT3*-ITD mutated disease[7]. Formally, the use of GO or gilteritinib in MDS represents off-label use and is thus not reimbursed in most health care systems. Patients diagnosed with MDS-EB2 are similarly ineligible for important trials of therapies being conducted exclusively in AML. Examples are phase 1 studies of the SYK inhibitor entospletinib[49] (NCT03013998), the menin-KMT2a inhibitors KO-539[50](NCT04067336),and SNDX-5613[51] (NCT04065399). Likewise, patients with AML are typically ineligible to receive novel lower intensity therapies under investigation in MDS. The AML-MDS dichotomy has become so pronounced that separate AML and MDS protocols are used to investigate drugs , such as eprenetapopt (APR 246), which reactivates TP53 [52,53] or magrolimab, which restores macrophage checkpoint inhibition[54] ,despite the similar biology and clinical implications of TP53 mutations and macrophage checkpoint inhibition in AML and MDS[42,43].

Academic trials may be becoming less proscriptive. Eligibility for a HOVON and SAKK trial , investigating the addition of clofarabine to 7+3, included patients with RAEB (MDS-EB in today's nomenclature)[55]. Subgroup analyses showed no differences in outcome between AML and MDS-EB[55]. Patients with MDS-EB were also eligible for randomization between 7+3 +/- lenalidomide in a study conducted by the same groups, with potential differences in outcome between AML and MDS not reported[56]. Ongoing HOVON/ AMLSG trials examining 7+3 +/- ivosidenib/enasidenib (NCT03839771), and 7+3 + midostaurin or gilteritinib (NCT04027309) allow patients with either AML or MDS EB2. FH/UW often enrolls patients with MDS EB-2 into trials for newly-diagnosed AML[45,], as does MD Anderson Cancer Center[57] , which found little evidence that marrow blast % considered as 10-19%, 20-29%, or ≥ 30% had independent effects on survival in either patients age < 60,60-69, or ≥70 years[58]. However, U.S. cooperative groups generally restrict trials to either AML or MDS based on the 20% blast count criterion. Pharmaceutical-company sponsored trials, particularly important as the source of many novel treatments, have similarly adhered to the 20% cut-point, likely reflecting FDA's continued emphasis on this cut-point.

We propose that patients with *NPM1*-, *FLT3*-, or *TP53*-mutations, with *KMT2a* rearrangements or with inv(3)/t(3:3), be eligible for AML trials regardless of blast count, much as is currently done for CBF disease. Recognizing that any blast cut point is arbitrary, we further propose that patients with 10-30% blasts ("AML/MDS") be routinely eligible for "AML" or "MDS" trials. This would allow formal testing of the effect of blast percentage on outcome, especially if such AML/MDS patients were randomized between AML and MDS therapies. Some therapies may be more effective against higher or lower blast count disease, while others' effectiveness may depend on genetic profile irrespective of blast percentage, as suggested for AML therapy in *NPM1*-mutated disease[39] .Although arbitrary, the 10% lower limit and 30% upper limit are based on similar risks of death in

patients with >10%-20% and 21-30% blasts in the revised IPSS classification of MDS[29] and similar reductions in risk of death in patients receiving azacitidine compared to conventional care regimens regardless of whether patients had MDS-EB2 or 20-30% blasts[59]. The 30% upper limit could be increased, or the 10% lower limit decreased, based on the results of initial trials. In principle, all patients with >5% blasts might be considered eligible for either AML or MDS studies. However, our clinical data are limited to patients with ≥ 10% blasts; further study is needed on MDS patients with 5-9% blasts. Defining patients as MDS versus AML based on mutation profile is another possibility. However, many centers around the world do not have access to the extensive molecular testing that would be required or do not receive results in a timely manner. Nonetheless, a uniform genetic evaluation of the current "MDS", "MPN", or "AML" might eventually allow harmonization of trials and comparison among them.

We believe that creating a 10-30% "AML/MDS" category would give more patients access to a wider variety of treatments, and would potentially simplify the regulatory approval process, with potential extension of drugs approved for AML to MDS-EB2, while allowing patients with low blast count AML access to drugs used to treat MDS-EB2. We hope our data will stimulate discussion regarding the criteria used to define AML and MDS in future disease classification schemes , such as the WHO Classification[60].

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Authorship

Contributions

EE, RPH, and HD jointly formulated the topic, collected and interpreted data, and wrote, critically reviewed, and approved the manuscript before submission.

Conflict of Interest Disclosures

EE

Consultancy AbbVie, Bristol Meyers Squibb, Pfizer, Up-to-date

Clinical Research Funding to Institution: AbbVie, Amgen, Aptevo, Bayer, Biosight, ImmunoGen, Janssen, Kura, Macrogenics, Menarini Ricehrche, Pfizer

RPH

No relevant conflicts of interest

HD

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Figure 1: The Y-axes show the probabilities of the indicated outcomes. The univariate log rank p-values are as indicated.

Table 1: Patient Characteristics and Response

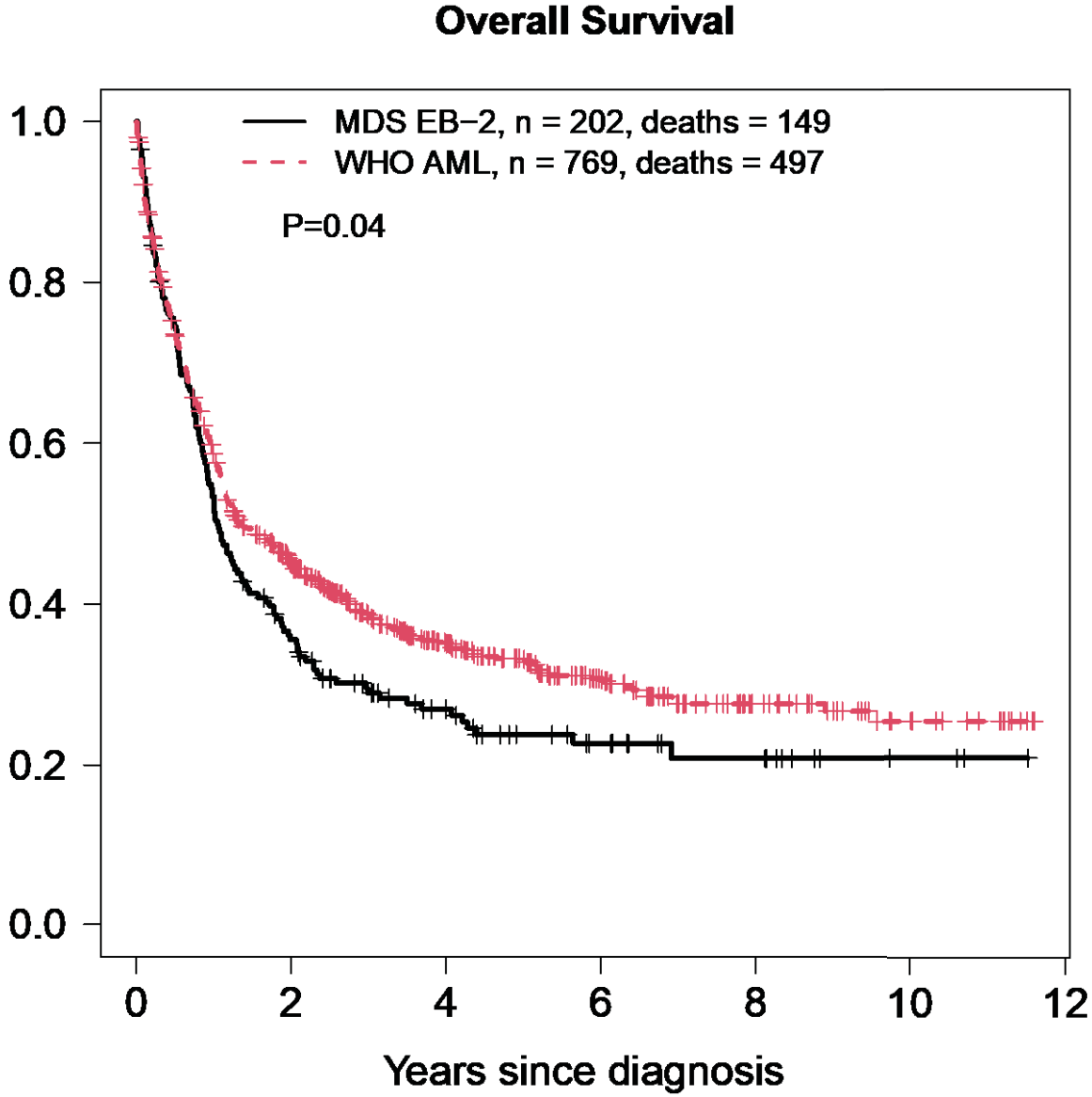
Factor	MDS-EB2 (n=202)	WHO AML (n=769)	All (n=971)	P-value MDS-EB2 vs. WHO AML
Mean age(range)	62 (22-85)	63 (18-91)	62 (18-91)	0.36
Performance status				0.85(PS 0-1 vs 2-4)
0-1	159 (79%)	598 (78%)	757 (78%)	
2-4	43 (21%)	171 (22%)	214 (22%)	
Disease status				
De novo	179 (89%)	553 (72%)	732 (75%)	
Secondary	23 (11%)	216 (28%)	239 (25%)	
Subcategories of secondary				
Prior marrow documenting MDS or MPN (antecedent hematologic disorder; AHD)	Not applicable (N/A)	123 (16%)	123 (13%)	N/A
Prior cytotoxic therapy, no AHD	23 (11%)	72 (9%)	95 (10%)	0.53
Both AHD and prior cytotoxic therapy	N/A	21 (3%)	21	N/A
Mean % morphologic blasts (range)	14 (10-19.8)	45 (0-100)	34 (0-100)	<0.001
ELN 2017 risk				<0.001
"favorable"	11(5%)	189 (25%)	200 (21%)	<0.0001
"intermediate"	98 (49%)	293 (38%)	391 (40%)	0.009
"adverse"	89 (44%)	274 (36%)	363 (37%)	0.03
unknown	4 (2%)	13 (2%)	17 (2%)	0.76
Induction intensity				0.038
"high"	137(68%)	577 (75%)	714(75%)	
"low"	65(32%)	192 (25%)	257 (26%)	
CR_{MRD-}	59 (29%)	359 (47%)	418 (43%)	<0.001
CR or CRi	117 (58%)	530 (69%)	647 (67%)	0.0043
Received allogeneic HCT	90 (45%)	270 (35%)	960 (37%)	0.02

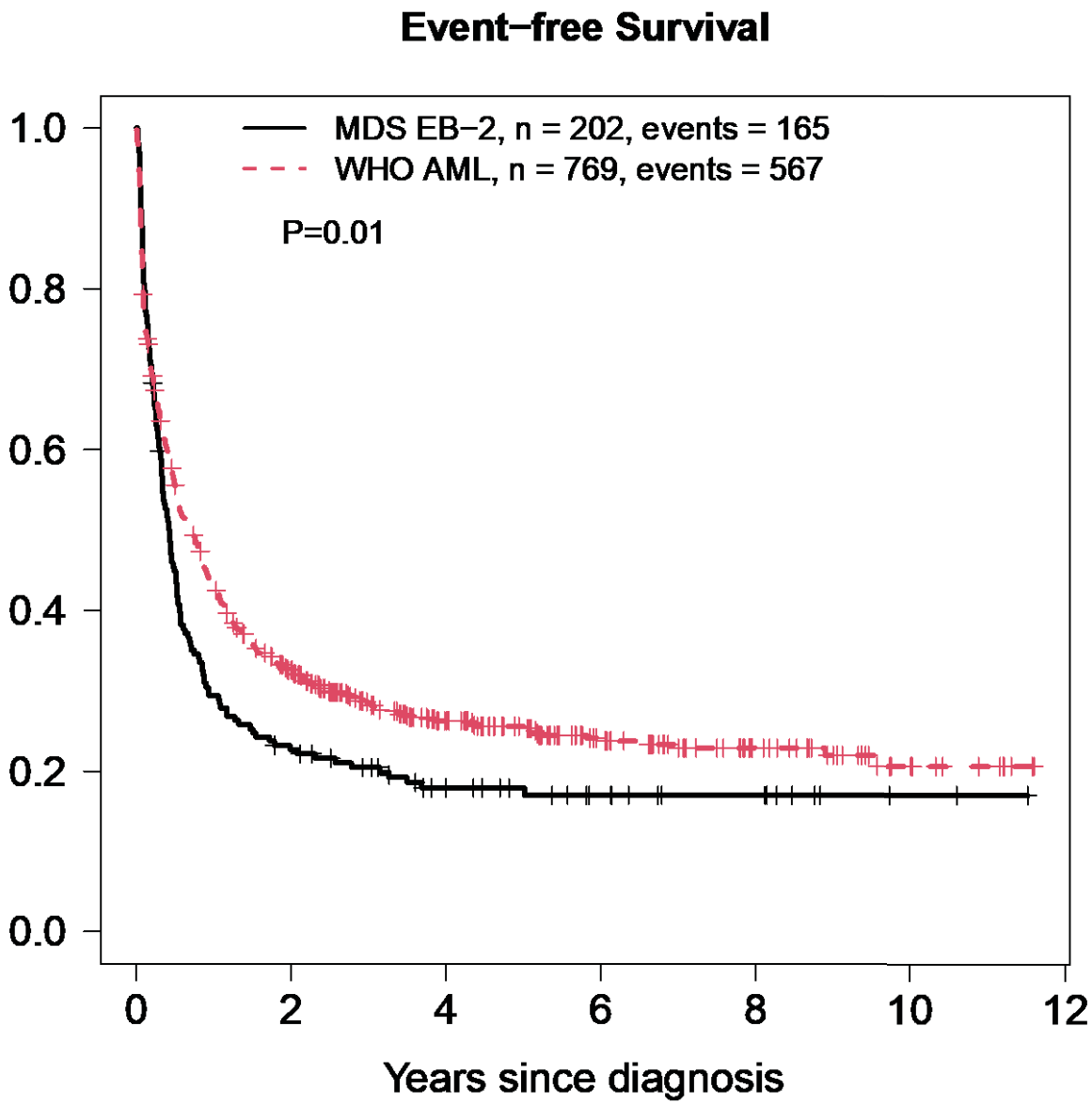
Table 2: Multivariable Models

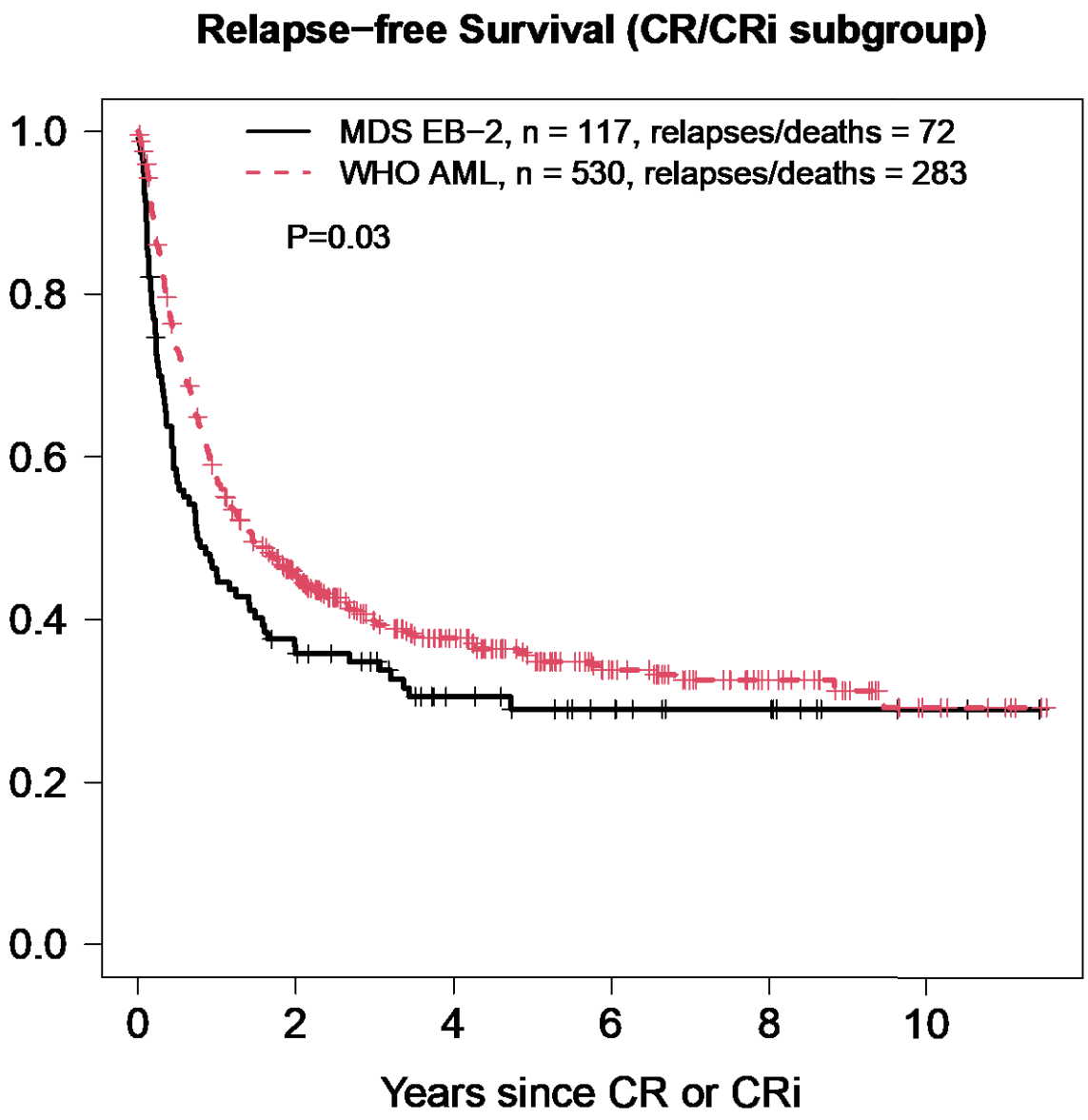
Variable	Overall Survival HR(95% CI); p-value	Event Free Survival HR(95% CI);p-value	CR or CRi OR(95% CI;p-value)	RFS if CR/CRi HR(95%CI);p-value
WHO AML (ref =MDS EB2)	0.89(0.74-1.07) p = 0.21	0.89(0.75-1.06) P = 0.2	1.06(0.99,1.13) P = 0.11	0.66(0.53,0.83) P<0.001
Age (per 10 years)	1.3(1.22-1.38) P < 0.001	1.19(1.13,1.26) P < 0.001	0.98(0.96,1) P = 0.02	1.13(1.05,1.2) P<0.001
PS 2-4 (ref PS 0-1)	2(1.68,2.37) P < 0.001	1.68(1.42,1.99) P < 0.001	0.87(0.82,0.93) P< 0.001	1.21(0.96,1.51) P=0.11
ELN 2017 int. risk (ref favorable risk)	1.7(1.34,2.15) P < 0.001	1.72(1.38,2.14) P<0.001	0.86(0.8,0.93) P<0.001	2.15(1.67,2.76) P<0.001
ELN 2017 adverse risk (ref = favorable risk)	2.28(1.8,2.88) P < 0.001	2.29(1.84,2.85) P< 0.001	0.78 (0.73,0.84) P<0.001	3.07(2.35,4) P< 0.001
Secondary (ref = de novo)	1.3 (1.1,1.55) P = 0.002	1.28(1.08, 1.5) P=0.004	0.93((0.87,0.99) P=0.02	1.16(0.93,1.43) P=0.18
Low intensity induction (ref= high intensity)	1.3 (1.08,1.55) P = 0.004	1.62(1.36,1.93) P < 0.001	0.7(0.66,0.75) P<0.001	1.07(0.82,1.38) P=0.63
Allogeneic HCT (ref = no allo. HCT)	0.48(0.39,0.6) P < 0.001	0.39(0.31,0.47) P< 0.001	Not applicable	0.29(0.23,0.36) P< 0.001

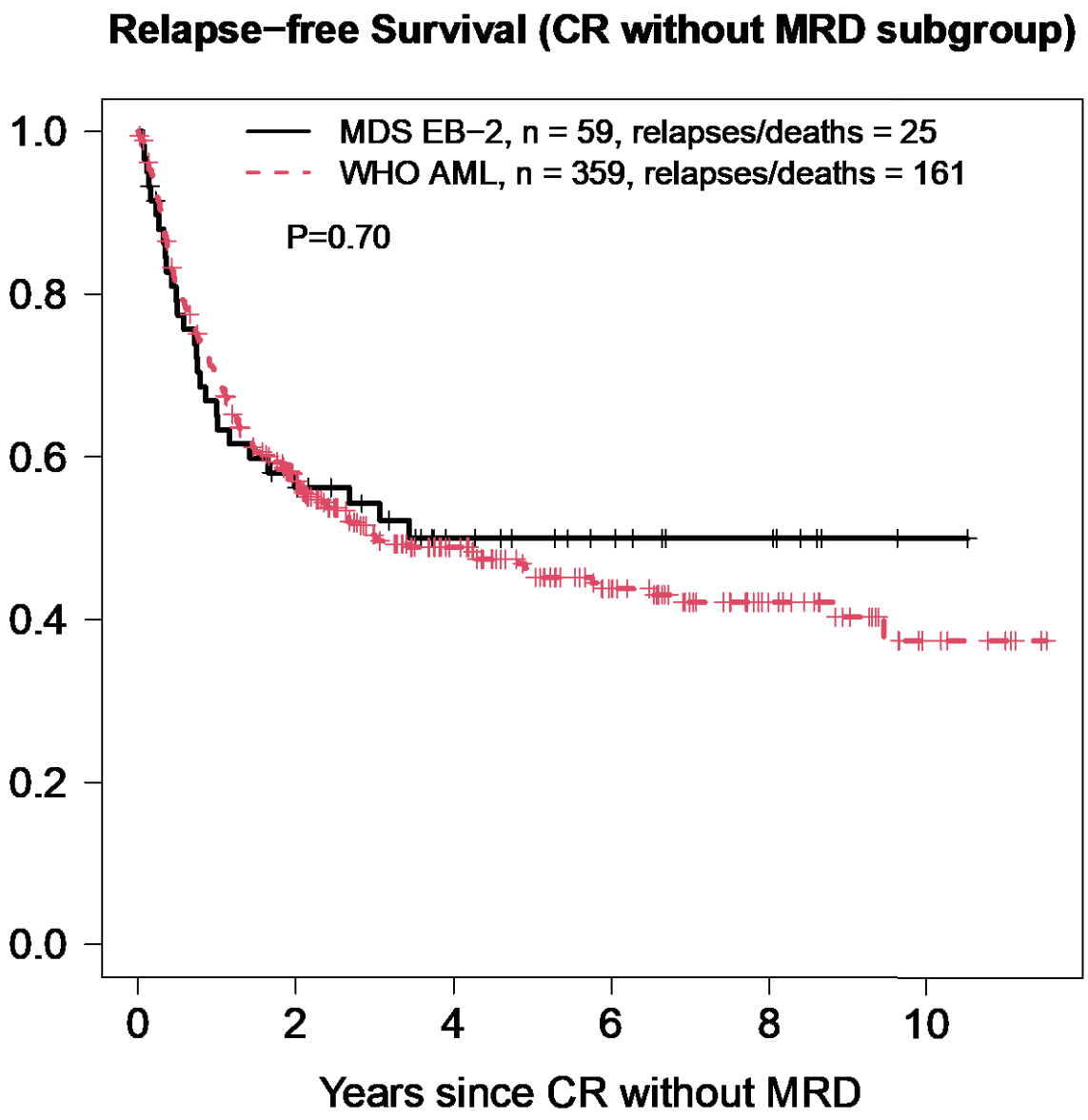
Variable	CR without MRD OR(95% CI; p-value)	RFS if CR without MRD HR(95% CI;p-value)
WHO AML (ref =MDS EB2)	1.13(1.05,1.21) P<0.001	0.8(0.56,1.15) P = 0.23
Age (per 10 years)	0.97(0.95,0.99) P=0.004	1.18(1.08,1.3) P<0.001
PS 2-4 (ref PS 0-1)	0.92(0.86,0.98) P= 0.01	1.12(0.82,1.53) P=0.48
ELN 2017 int. risk (ref favorable risk)	0.82(0.75,0.88) P< 0.001	2.14((1.57,2.93) P<0.001
ELN 2017 adverse risk (ref = favorable risk)	0.71(0.65,0.77) P<0.001	2.58(1.8,3.7) P<0.001
Secondary (ref = de novo)	0.88(0.82,0.94) <0.001	0.85(0.61,1.18) P=0.33
Low intensity induction (ref= high intensity)	0.76(0.71,0.82) P<0.001	1.18(0.81,1.72) P= 0.39
Allogeneic HCT (ref = no allo. HCT)	Not applicable	0.27(0.2,0.38) P<0.001

Time-dependent Cox regression for OS,EFS,RFS, HR = hazard ratio; Logistic regression for CR/CRi, CR w/o MRD,OR= odds ratio


Overall Survival
MDS EB−2, n = 202, deaths = 149
WHO AML, n = 769, deaths = 497
P=0.04
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Years since diagnosis


Event−free Survival
MDS EB−2, n = 202, events = 165
WHO AML, n = 769, events = 567
P=0.01
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Years since diagnosis


Relapse−free Survival (CR/CRi subgroup)
MDS EB−2, n = 117, relapses/deaths = 72
WHO AML, n = 530, relapses/deaths = 283
P=0.03
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
Years since CR or CRi


Relapse−free Survival (CR without MRD subgroup)
MDS EB−2, n = 59, relapses/deaths = 25
WHO AML, n = 359, relapses/deaths = 161
P=0.70
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
Years since CR without MRD