# Introduction

About 75% of patients with acute myeloid leukemia (AML) are aged ≥60 years.[1] The optimal treatment of older AML patients in daily clinical practice remains challenging and is dependent on patient characteristics (ie, age, performance, comorbidities), disease characteristics (ie, cytogenetic and molecular abnormalities, white blood cell [WBC] count), and the preference of the patient.[2] The hypomethylating agents (HMAs) azacitidine and decitabine have relatively mild side effects and are particularly feasible for the treatment of older patients and patients with comorbidities. Although not in their primary analyses, recent phase 3 trials have shown the superiority of azacitidine and decitabine compared with conventional care for older patients with AML in a prespecified analysis (censoring patients who received AML treatment after discontinuing study drug [azacitidine]) or after an unplanned analysis (after more events [decitabine]).[3-5] Results of treatment with modifications of the standard azacitidine (7 days, 75 mg/m^2 subcutaneously; every 4 weeks) and decitabine (5 days, 20 mg/m^2 IV; every 4 weeks) schedules have been reported. Initial studies evaluating the 10-day decitabine schedule have shown promising results. In a hallmark study, 52 older patients newly diagnosed with AML were treated with the 10-day decitabine schedule; 21 patients (40%) achieved a complete remission (CR), and the median overall survival (OS) was 318 days.[6] Various retrospective analyses have confirmed these favorable data.[7,8] A phase 3 trial (#NCT02172872) in older patients with AML (aged ≥60 years) that compares conventional intensive chemotherapy based on cytarabine combined with an anthracycline ("3 + 7") with the 10-day decitabine schedule, followed by allogeneic hematopoietic cell transplantation, is currently in progress.

It remains an important clinical challenge to further improve the cumulative CR/complete remission with incomplete hematologic recovery (CR/CRi) rates and survival of the 10-day decitabine schedule. Based on preclinical scientific evidence, we hypothesized that ibrutinib, an oral inhibitor of Bruton tyrosine kinase (BTK) that is approved by the US Food and Drug Administration for the treatment of mantle cell lymphoma, chronic lymphocytic leukemia, and Waldenström macroglobulinemia, could be an interesting new drug to add to decitabine.[9] Although BTK is critical to the B-cell receptor signaling cascade, AML cell lines also have high expression of BTK,
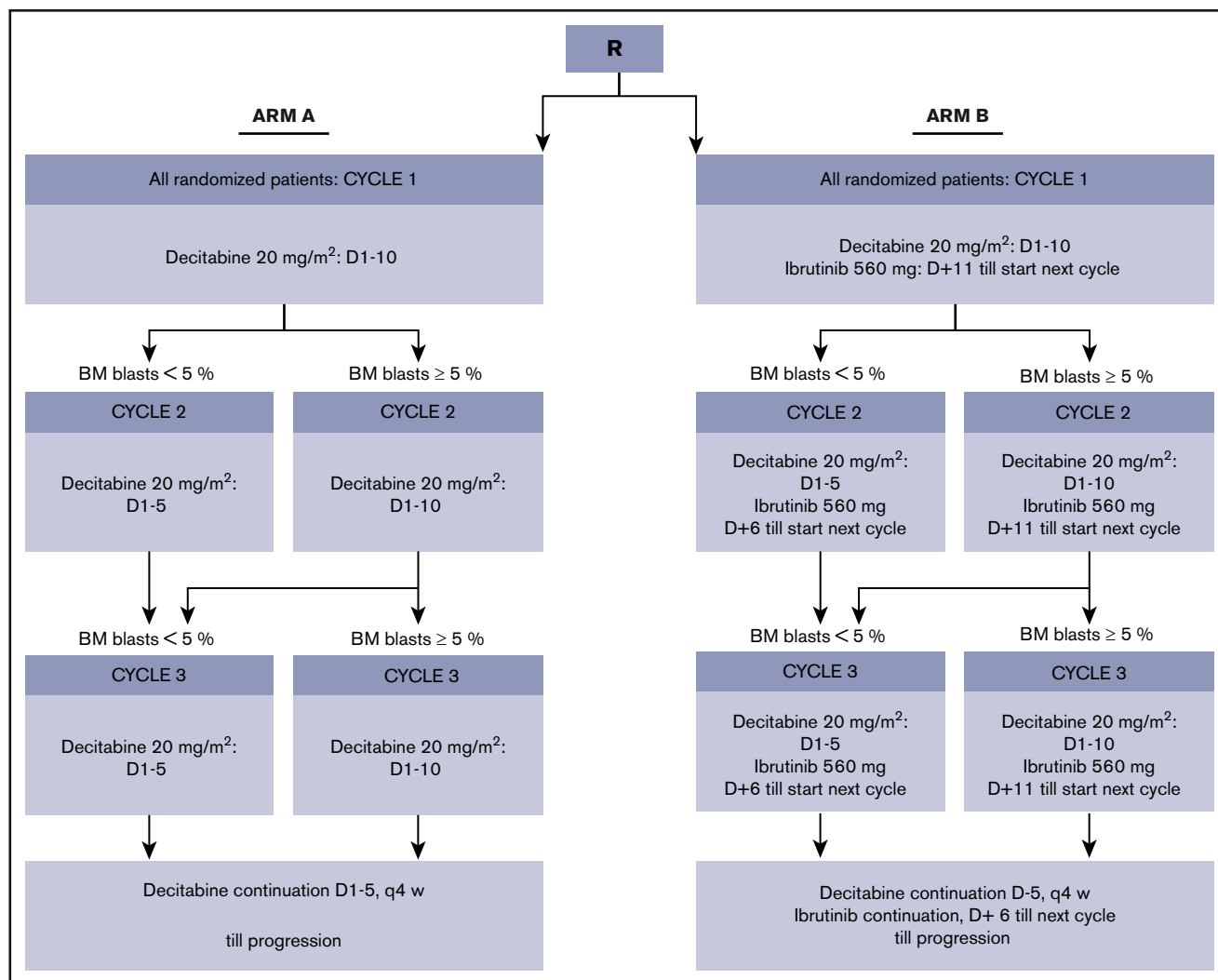
Figure 1. **Study scheme.** BM, bone marrow; D, day.

comparable to B-cell lines (www.broadinstitute.org/ccle). It has been reported that BTK is phosphorylated in the leukemic blasts in >90% of AML patient samples but not in healthy CD34$^+$ cells.[10-12] Furthermore, it seems that ibrutinib (alone or combined with cytarabine or daunorubicin) inhibits blast proliferation from human AML in vitro.[10] The in vitro cytotoxicity of ibrutinib in AML is comparable to the in vitro cytotoxicity of primary chronic lympho-cytic leukemia cells (ie, 50% inhibitory concentration of ~5 μM). BTK-targeted RNA interference knockdown reduces colony-forming capacity of primary AML blasts and proliferation of AML cell lines. Silencing of BTK inhibits protein kinase B (AKT) and extracellular signal-regulated kinase (ERK) pathways in AML cells. It has been shown that ibrutinib's antiproliferative effects in AML are mediated via an inhibitory effect on downstream NF-κB survival pathways.[11] Moreover, ibrutinib seems to inhibit AML cell adhesion to bone marrow stroma.[13] These experimental results provide a biological rationale for the clinical evaluation of BTK inhibition in patients with AML.

Here we present the final analysis of the HOVON135/SAKK-30/15 study. In this phase 2 study, older patients (aged ≥66 years) with AML or higher risk myelodysplasia (MDS) (Revised International Prognostic Scoring System score >4.5) considered not suitable for intensive cytotoxic treatment were randomized to receive 10-day decitabine with or without ibrutinib. The aim was to assess the value of adding ibrutinib to 10-day decitabine with respect to CR/CRi after 3 cycles of treatment (primary end point) and OS (secondary end point).

Methods

Study design, patients, and treatment

This was a randomized phase 2 multicenter study (HOVON135; SAKK30/15; EudraCT #2015-002855-85) (protocol is available at: www.hovon.nl). Previously untreated adults who were ≥66 years old and not considered eligible for intensive chemotherapy, with a cytopathologically confirmed diagnosis of AML or with higher risk MDS (Revised International Prognostic Scoring System score >4.5), a World Health Organization (WHO) performance status ≤2, and written informed consent, were eligible. Patients were considered not suitable for intensive chemotherapy if they had comorbidities that would put them at high risk for early death when treated with intensive chemotherapy (defined as hematopoietic cell transplantation comorbidity index [HCT-CI] ≥3) or if the patient declined to receive intensive chemotherapy. The limit for HCT-CI ≥3 was chosen because this value has been shown to be associated with an early mortality rate (<30 days) of ~30% in older patients with AML who are treated with intensive chemotherapy.[14] Based on the karyotype and molec-ular genotype of the leukemic cells, patients were classified into prognostic categories according to the European LeukemiaNet (ELN) 2017 criteria.[15]

Patients were randomly assigned (1:1) to 10-day decitabine without (arm A) or with (arm B) ibrutinib 560 mg (Figure 1). In arm B, ibrutinib was given sequentially with decitabine, starting the day after the last dose of decitabine until the day before the start of the next cycle of decitabine. (Decitabine and ibrutinib were provided free of charge by Janssen Pharmaceutics.) The duration of decitabine in the second and third cycle (ie, 5 or 10 days) was dependent on the percentage of bone marrow blasts (ie, <5% or ≥5%), as determined by cytomorphology, at the day 28 bone

Table 1. Patient characteristics

| Characteristic	10-d decitabine (n = 72)	10-d decitabine + ibrutinib (n = 72)
Sex, male/female	47/25 (65/35)	42/30 (58/42)
Age, median (range), y	76 (68-84)	75 (66-89)
WHO performance		
WHO 0	26 (36)	25 (35)
WHO 1	38 (53)	35 (49)
WHO 2	8 (11)	12 (17)
Comorbidity		
HCT-CI 0-2	42 (58)	42 (58)
HCT-CI ≥3	30 (42)	30 (42)
ELN risk group (AML)		
Favorable	15 (21)	10 (14)
Intermediate	12 (17)	11 (15)
Adverse	34 (47)	37 (51)
Unknown	0 (0)	2 (3)
Primary AML	48 (67)	45 (63)
Secondary AML	13 (18)	15 (21)
MDS	11 (15)	12 (17)
WBC, median (range), ×10^9/L	2.95 (0.70-29.9)	3.20 (0.60-30.0)

Data are n (%) except as noted.

marrow evaluation. Cycle 2 started within 1 week after the day 28 bone marrow evaluation, independent of recovery of peripheral counts. Cycle 3 started if neutrophils were ≥0.5 × 10^9/L or platelets were ≥25 × 10^9/L, with a maximum delay of 2 weeks. From cycle 4, treatment could only be continued with 5-day decitabine (with or without ibrutinib).

Molecular analyses and flow cytometry

Bone marrow aspirations or peripheral blood samples at di-agnosis were taken. Blasts and mononuclear cells at diagnosis were purified by Ficoll-Hypaque (Nygaard, Oslo, Norway) density gradient centrifugation and cryopreserved. After thawing, cells were lysed in RLT solution with the addition of dithiothreitol (Qiagen, Venlo, The Netherlands). High-quality DNA and RNA were extracted by using QIAsymphony (Qiagen). DNA concentration was measured by Qubit Fluorometric Quantitation (Thermo Fisher Scientific, Wilmington, DE) and RNA concentration using the Implen NanoPhotometer (Westburg, Leusden, The Netherlands). CBFB-MYH11, RUNX1-RUNX1T1, and FLT3 internal tandem duplication mutations were determined as previously described.[16,17] Mutations in 38 of 54 genes frequently mutated in hematologic malignancies were determined by targeted next-generation sequencing (NGS) with the TruSight Myeloid Sequencing panel, following the manufacturer's protocol (Illumina, San Diego, CA). The NGS libraries were paired-end sequenced (2 × 221 bp) on a MiSeq System (Illumina). Variants were called as described.[18]

Residual disease detection by using multiparameter flow cytom-etry was performed as previously described.[19] The residual disease percentage was defined as the number of leukemia-associated immunophenotype (LAIP) cells within the bone marrow compartment. The threshold between residual and no
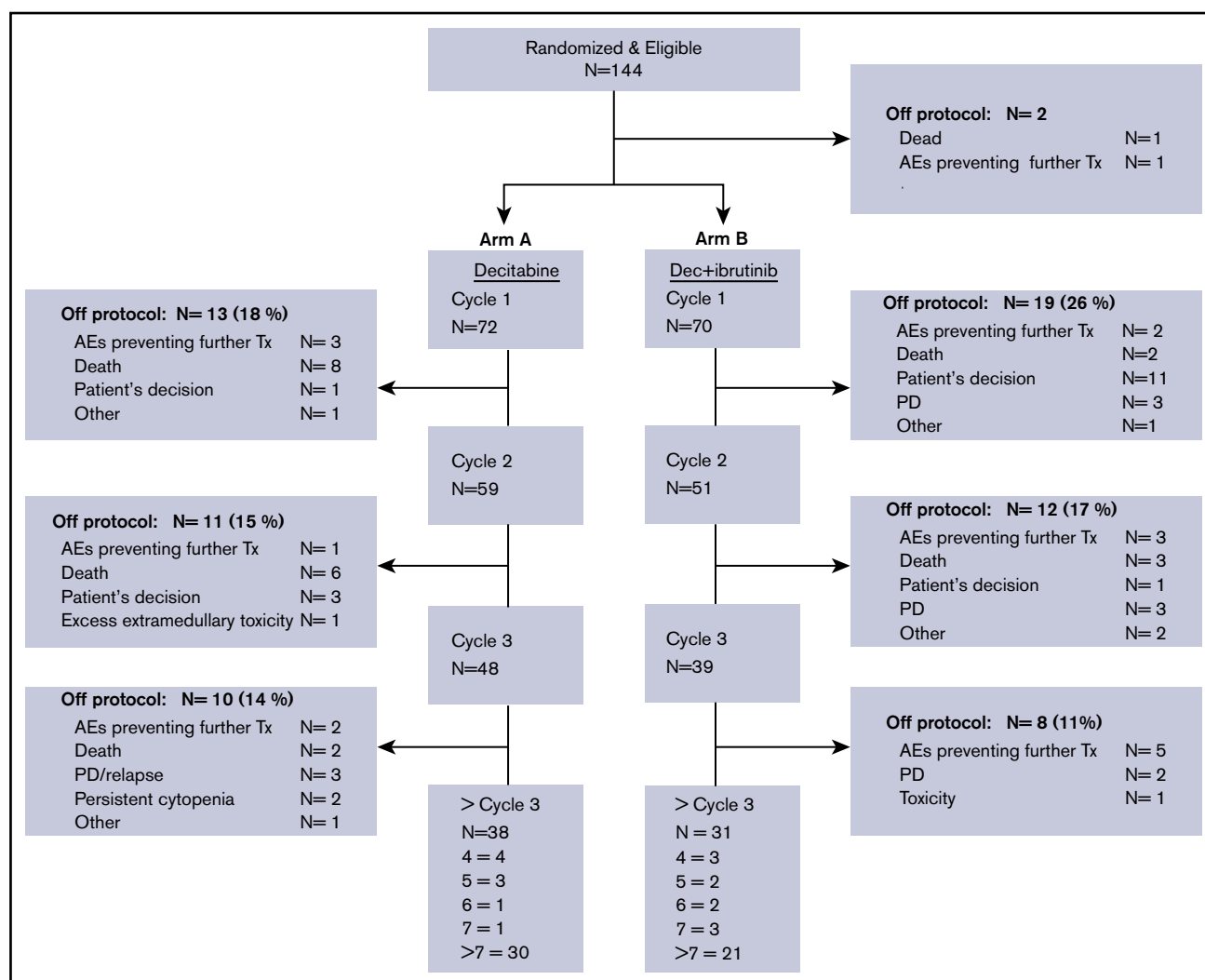
Figure 2. CONSORT study diagram. Arm A = 10-day decitabine; arm B = 10-day decitabine plus ibrutinib. PD, progressive disease; Tx, treatment.

residual disease based on flow cytometry was established and validated on 0.1%.[19,20] Information on clones and commercial sources of all monoclonal antibodies used is provided in supplemental Table 1.

Study end points and statistical analysis

The aim of this study was to decide whether the addition of ibrutinib to standard induction treatment could be sufficiently effective to warrant continuation to a phase 3 study. The observed difference in CR/CRi rates between investigational arm B and standard arm A was used as the criterion in decision rules. At final analysis, the decision to stop because of inefficacy (ie, not to continue with phase 3) was based on observed difference in CR/CRi rates ≤0 or if the upper limit of the 80% confidence interval (CI) was <25%.

The primary end point of the study was cumulative CR/CRi after 3 cycles (for all patients [MDS and AML]). A patient was considered to have a CR/CRi if this end point was attained within the first 3 induction cycles and the patient did not relapse on a consecutive cycle (within these 3 induction cycles). All other patients without "CR/CRi after 3

cycles" (including partial remission and morphologic leukemia-free state [MLFS]) were classified as "induction failures."
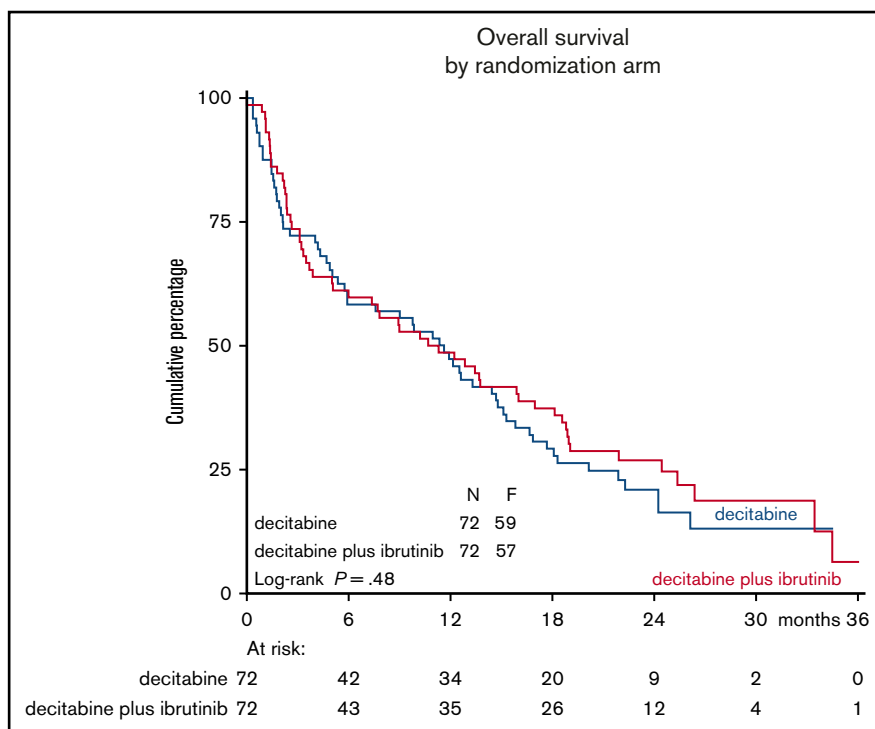
Secondary end points included safety, tolerability, and efficacy profile (response rate [CR, CRi, MLFS, partial remission]), event-free survival (EFS), and OS. OS is defined as the time from the date of randomization to the date of death, whatever the cause. The follow-up of patients still alive was censored at the moment of last visit/contact. EFS was defined as the time from registration to induction failure, death, or relapse, whichever occurred first. Kaplan-Meier survival curves and Cox regression tests were used to compare the survival distributions between the treatment arms. For statistical comparison of mutation frequencies and clonal characteristics between individuals with and without a response, Fisher's exact test and Mann-Whitney U tests were used.

Results

Study patients and treatment

From 1 September 2016 until 13 June 2018, a total of 144 patients were registered for the study. The database was closed on 17

Figure 3. Kaplan-Meier estimates for OS. Arm A = 10-day decitabine arm; arm B = 10-day decitabine plus ibrutinib. Median OS was almost 12 months in both arms.

Overall survival by randomization arm

	N	F
decitabine	72	59
decitabine plus ibrutinib	72	57

Log-rank $P = .48$

At risk:

	0	6	12	18	24	30	36
decitabine	72	42	34	20	9	2	0
decitabine plus ibrutinib	72	43	35	26	12	4	1

February 2020. The median follow-up for all patients is 11.3 months, and the median follow-up for patients still alive is 23.5 months. Patient characteristics are described in Table 1. The main patient-related factors (sex, age, performance, and comorbidities) and disease-related factors (2017 ELN risk, secondary AML, MDS, and WBC count) were equally distributed over both arms. In the decitabine arm (arm A), 65% were male, median age was 76 years (range, 68-84 years), 89% had performance score WHO 0 to 1, and 42% had an HCT-CI ≥3. In the decitabine plus ibrutinib arm (arm B), 58% were male, median age was 75 years (range, 66-89 years), 84% had performance score WHO 0 to 1, and 42% had an HCT-CI ≥3. In arm A, 47% had 2017 ELN adverse risk and 18% secondary AML; in arm B, 53% had 2017 ELN adverse risk and 21% secondary AML.

All 72 patients allocated to arm A started 10-day decitabine; 59 patients (82%) received a second cycle, and 48 patients (67%) received a third cycle. In arm B, 2 patients (of 72) went off protocol before the start of the first 10-day decitabine cycle; 51 patients (82%) received a second cycle, and 39 patients (54%) received a third cycle. Main reasons to discontinue treatment were death, adverse events (AEs) preventing further treatment, and progressive disease/relapse (Figure 2). "Full-dose" decitabine (including "delayed") was given in arm A in 99%, 97%, and 98% and in arm B in 100%, 92%, and 92% in the first 3 cycles. The full-dose ibrutinib was given in 53%, 45%, and 38% during the first 3 cycles; "dose reduced >10%" and/or "delayed" in 38%, 33%, and 44% during the first 3 cycles; "interrupted and resumed" in 6%, 12%, and 5% during the first 3 cycles; and "not given" in 3%, 10%, and 13% during the first 3 cycles. The relatively high number of patients whose dose was "reduced by >10%" and/or "delayed" might be explained by the study protocol, which required reducing the dose of ibrutinib from 560 mg to 140 mg in case moderate or

strong cytochrome P450 3A inhibitors were used (eg, ciprofloxacin, posaconazole, voriconazole). The median number of decitabine cycles applied was 4 in arm A (range, 1-26) and 3 (range, 1-29) in arm B. Three patients received an allogeneic hematopoietic cell transplantation (1 in arm A, 2 in arm B).

Treatment outcome according to randomization

The primary end point of this study was the cumulative CR/CRi rate during the first 3 cycles, which was attained in 29 of 72 patients (40%; 80% CI, 33-48) in arm A and 22 of 72 patients (31%; 80% CI, 24-38) in arm B. Thus, there is no difference in CR/CRi between arms B and A. Some patients obtained a CR/CRi after the first 3 cycles (the primary end point), resulting in a total CR/CRi rate (on protocol) of 50% in arm A and 41% in arm B.

At time of the final analysis, a total of 116 patients had died. Median OS was 11.5 months in arm A and 11.0 months in arm B. OS at 12 and 24 months was similar between arms (A vs B): 47% (95% CI, 35-58) vs 49% (95% CI, 37-60) and 21% (95% CI, 12-31) vs 27% (95% CI, 17-38), respectively (log-rank test, $P = .48$) (Figure 3).

The CR/CRi rates within the first 3 cycles were 40% for favorable, 35% for intermediate, and 35% for adverse risk AML according to ELN 2017 criteria, and 35% for the higher risk MDS patients. The OS was not different for the favorable, intermediate, and adverse risk AML and higher risk MDS subgroups (log-rank test, $P = .08$) (supplemental Figure 1).

Tolerability of treatment

During cycle 1, the median number of nights in the hospital was 13 (range, 0-42) in arm A and 17 (range, 0-56) in arm B; during cycle 2, the median number of nights in the hospital was 9 (range, 0-32) in arm A and 10 (range, 0-33) in arm B (Table 2). During the third cycle, the median number of nights in the hospital for both arms was

Table 2. Tolerability of treatment per treatment arm

Variable	10-d decitabine (n = 72)	10-d decitabine + ibrutinib (n = 72)
Cycle 1, median		
Nights in hospital	13	17
No. of RBC transfusions	5	5
No. of platelet transfusions	3	3
Cycle 2, median		
Nights in hospital	9	10
No. of RBC transfusions	4	4
No. of platelet transfusions	1	1
Cycle 3, median		
Nights in hospital	0	0
No. of RBC transfusions	2	2
No. of platelet transfusions	0	0
AEs, any, n (%)		
Grade 2	50 (9)	58 (11)
Grade 3	81 (14)	92 (18)
Grade 4	30 (5)	21 (4)
Patients with SAEs, n (%)		
0 SAE	14 (19)	9 (13)
1 SAE	25 (35)	32 (44)
2 SAEs	12 (17)	21 (29)
3 SAEs	13 (18)	6 (8)
>3 SAEs	8 (11)	4 (6)

RBC, red blood cell; SAE, serious AE.

Table 3. Molecular diagnostics and response

Gene	No. of individuals with mutations	No. (%) CR/CRi after 3 cycles
DNMT3A	46	18 (39)
TET2	36	10 (28)
ASXL1	35	7 (20)
SRSF2	34	8 (24)
RUNX1	32	9 (28)
TP53	27	15 (56)
IDH2	23	4 (17)
NRAS	21	8 (38)
NPM1	19	5 (26)
STAG2	17	2 (12)
U2AF1	15	8 (53)
SF3B1	15	6 (40)
JAK2	12	6 (50)
BCOR	12	5 (42)
EZH2	11	2 (18)
CEBPA	10	2 (20)
FLT3	10	4 (40)

Data from 140 patients with available extensive molecular analyses are considered.

0. The median number of red blood cell transfusions in arms A and B were comparable: 5, 4, and 2, respectively, during cycles 1, 2, and 3. The median number of platelet transfusions in arms A and B was also comparable: 3, 1, and 0 during cycles 1, 2, and 3.

The total number of grade 2 to 4 AEs was 577 (72 of which were serious AEs) in arm A and 524 (72 of which were serious AEs) in arm B (supplemental Table 2). The number of any Common Terminology Criteria for Adverse Events grade 2, 3, or 4 AEs was 9%, 14%, and 5% in arm A and 11%, 18%, and 4% in arm B, respectively. Grade 3/4 infections and infestations were 9% in both arms. The early death rate (within 30 days from registration) was 13% in arm A and 3% in arm B.

Treatment outcome according to molecular profile

Comprehensive molecular analysis at diagnosis was performed in 140 cases; 7 (5%) of these had no detectable gene mutations. The most frequently mutated genes were DNMT3A (33%), TET2 (26%), ASXL1 (25%), SRSF2 (24%), RUNX1 (23%), and TP53 (19%) (Table 3). The typical molecular abnormalities in (younger) AML, mutated NPM1 and FLT3 internal tandem duplication, were both observed in only 14% of these older patients. The various mutations were generally equally distributed over both treatment groups (supplemental Figure 3A).

The response rates (CR/CRi) after 3 cycles of treatment were not different for most of the molecular abnormalities (supplemental Figure 3B). The response rates (CR/CRi within 3 cycles) for the most frequently mutated genes in the evaluable 140 patients are depicted in Table 3. Of those mutations that were observed at least 10 times, only mutations in TP53 were associated with a significantly higher CR/CRi rate (56%) during the first 3 cycles (Figure 4A). This favorable CR/CRi rate did not translate into superior OS of patients with mutated TP53 (supplemental Figure 4). Indeed, the inferior OS of patients with mutated TP53 was borderline significant compared with those without mutated TP53 ($P = .05$). Among the mutations that were observed at least 10 times, patients with mutations in STAG2, IDH2, and ASXL1 showed significantly reduced CR/CRi rates within 3 cycles of decitabine (12% for STAG2, 17% for IDH2, and 20% for ASXL1). Although numbers were low, the reduced CR/CRi rates for these genes did not result in inferior OS of patients with these genes mutated (supplemental Figure 5).

In addition, to address the question of whether the size of the (dominant) clone or the number of (sub) clones affects response to decitabine, we analyzed the relation between variant allele frequency, the number of mutations, and outcome. This analysis revealed that the highest variant allele frequency per individual, the number of mutations per individual (median, 3; range, 0-12), and the number of mutated genes per individual (median, 3; range, 0-9) did not correlate with CR/CRi rates within 3 cycles (Figure 4B-D). With respect to the most common cytogenetic abnormalities, only the presence of complex cytogenetic abnormalities (ie, ≥3) was associated with a greater probability of response (Figure 4A).

Treatment outcome according to minimal residual disease

LAIP was determined by multiparameter flow cytometry in all 144 patients using bone marrow samples before the start of study
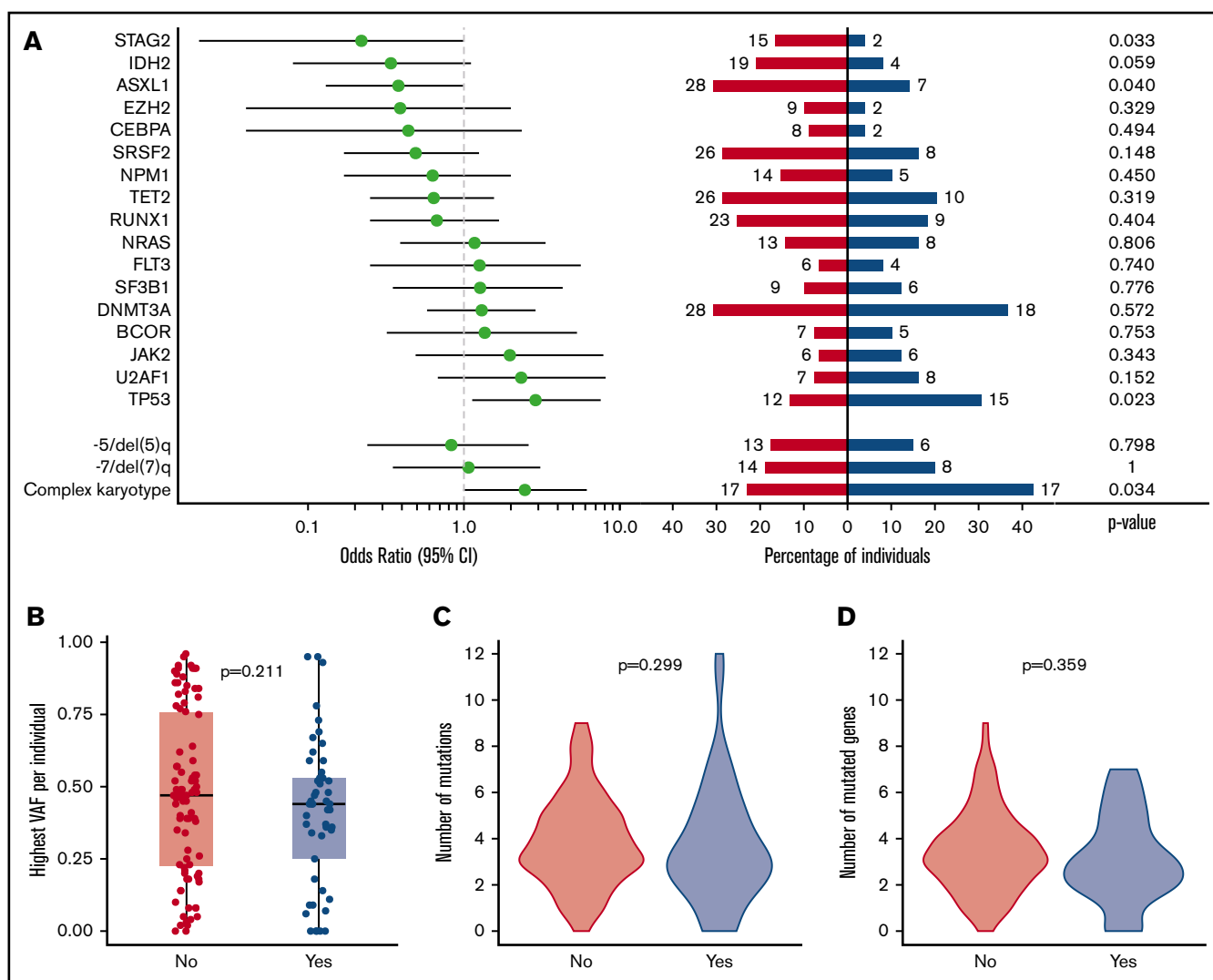
Figure 4. Associations between molecular and cytogenetic abnormalities (with prevalence ≥10) and response. (A) The association between individual frequently mutated genes (≥10 times) and frequently observed cytogenetic abnormalities (≥10 times) and response (CR/CRi) after 3 cycles. Odds ratios are displayed on a $\log_{10}$ scale, with lines extending to the 95% CI. Horizontal bars indicate the proportion with a mutation in the respective gene for patients with (blue) and without (red) response, with absolute numbers given for each gene. Data on molecular abnormalities were available for 140 individuals, whereas cytogenetic abnormalities were assessed in a subset of 114 individuals with identified cytogenetic abnormalities. *P* values are derived from Fisher's exact test. The association between response (CR/CRi) within 3 cycles and highest variant allele frequency (VAF) per individual (B), number of mutations per individual (C), and number of mutated genes per individual (D). *P* values are from Wilcoxon signed-rank test.

treatment. Minimal residual disease (MRD) was assessed in bone marrow samples after cycle 3 in 57 patients, of whom 42 were in CR/CRi/MLFS (34 in CR/CRi; 8 in MLFS). Median MRD was 0.12% (range, 0%-47.9%). Of these 57 patients, 28 (49%) became MRD negative (<0.1% LAIP/WBC count). Interestingly, the EFS and OS curves of these 57 patients were independent of their MRD status (positive, n = 29; negative, n = 28) (Figure 5). Also, when only considering the 34 patients in CR/CRi or the 42 patients in CR/CRi/MLFS, the OS was independent of MRD status (supplemental Figure 6). Although the 0.1% threshold was not able to identify patients with superior survival, lowering the cutoff point to 0.05% (resulting in 3 more MRD-positive patients) was not able to discriminate OS between patients with and without MRD.

Discussion

HMAs are an important treatment modality in older, unfit patients with AML and in higher risk MDS patients. Here we report on the therapeutic value of ibrutinib added to 10-day decitabine regimen. Recent preclinical insights regarding the inhibitory effects of ibrutinib on AML blasts led us to perform a randomized phase 2 clinical trial in older patients with newly diagnosed AML. However, this study, which compared a 10-day decitabine schedule with a combination of decitabine plus ibrutinib, revealed no therapeutic advantage for the ibrutinib combination. No improvement in response was noted for the ibrutinib arm. The results of the study also did not suggest indications for a therapeutic benefit in a particular molecular subset of AML (eg,