

to the standard 3 + 7 backbone in randomized phase 2 substudies. With this design, around 100 patients per experimental arm would be needed. Results of the addition of lenalidomide and tosedostat have recently been published [5, 6]. Here, we report on the results of the investigational arm with selinexor. Selinexor is an XPO1 inhibitor. XPO1, also called exportin-1 or CRM1 is a nuclear exporter protein that is involved in the transport of several proteins and mRNA molecules from the nucleus to the cytoplasm. Among these are tumor suppressor proteins and ribosome subunits. Many tumor cell types show elevated expression of XPO1, thereby on the one hand reducing tumor suppressor protein availability in the nucleus where they e.g., normally act by keeping cell cycle progression in check, and on the other hand reducing biogenesis of mRNA molecules involved in cell cycle regulation or apoptosis induction. Inhibition of XPO1 would restore these processes, leading to reduced tumorigenesis.

In preclinical studies, the drug appeared synergistic with anthracyclines and etoposide, and in phase 1 studies, selinexor showed promising single agent activity in several tumors, with an overall response rate of 14% in AML patients with, in general, relatively little adverse effects [7, 8]. We therefore investigated the addition of selinexor to standard 3 + 7 chemotherapy in a randomized phase 2 study.

## PATIENTS AND METHODS

### Patients

Previously untreated patients, 66 years of age or older, with a cytologically confirmed diagnosis of de novo or secondary AML (not acute promyelocytic leukemia or CML blast crisis) or with refractory anemia with excess of blasts and a Revised International Prognostic Scoring System (R-IPSS) score of higher than 4.5 and a WHO performance score of 2 or less were eligible for inclusion. Except for hydroxyurea for < 2 weeks, no other previous AML treatment, including HMA therapy for previous MDS, were allowed. Exclusion criteria included clinically significant cardiovascular disease, including cerebrovascular accidents (< 6 months before randomization), myocardial infarction (< 6 months before randomization), unstable angina, New York Heart Association grade 2 or greater congestive heart failure, serious cardiac arrhythmia requiring medication and other standard general medical exclusions. The trial was approved by the institutional review boards of all participating institutions. The study was performed in accordance with the Declaration of Helsinki, and all patients provided written informed consent.

### Risk classification

Based on the karyotype and molecular genotype of the leukemic cells, patients were classified into prognostic categories according slight modifications of the ELN 2010, as described previously [6].

### Study design and chemotherapy

Selinexor was provided free of charge by Karyopharm. The study was initially planned to start with a randomized dose selection safety run-in phase with either standard chemotherapy only or oral selinexor added to standard induction chemotherapy. The starting dose of selinexor of 60 mg twice weekly, days 1–24 was meant to be increased depending on the occurrence of dose-limiting toxicities. Shortly after the start of the study however, based on external data, the Data and Safety Monitoring Board advised to keep the selinexor dose at 60 mg and to not escalate further. Therefore, all patients in the investigational arm of the study received the same selinexor dose as above. Cycle 1 consisted of daunorubicin at 60 mg/m<sup>2</sup> (3 h infusion on days 1, 2 and 3) and cytarabine at a dose of 200 mg/m<sup>2</sup> (per continuous infusion on days 1–7) with or without oral selinexor. Cycle 2 contained cytarabine 1000 mg/m<sup>2</sup> q 12 h via 6 h infusion from day 1–6 (12 doses) with or without selinexor at the same dose as in Cycle 1. Patients could be allografted off protocol according to local policy.

One interim analysis regarding efficacy was performed after enrollment of 100 patients (50 per arm) on the primary endpoint according to protocol after which the DSMB advised to close the study.

Measurable residual disease (MRD) analysis and detection was performed by immunophenotyping at the central AmsterdamUMC lab as described previously [9]. The MRD percentage was defined as the

percentage of leukemia-associated immunophenotype-positive (LAIP) cells within the white blood cell compartment. An MRD percentage  $\leq 0.1\%$  was considered negative,  $> 0.1\%$  as MRD positive. The maximal sensitivity of the MRD detection was 0.01% (i.e.,  $1 \times 10^{-4}$ ).

### Statistical analysis

The primary endpoint of the second part of the study was the rate of complete remission after induction treatment. A patient was considered to have a response if the best response to remission induction therapy (cycle 1 and/or 2) was a CR/CRi. Secondary endpoints were considered as exploratory and included: overall survival (OS), event free survival (EFS), disease free survival (DFS), the prognostic value of leukemic molecular markers and gene expression profiles and the prognostic value of minimal residual disease measurements following therapy. The definition of endpoints was according to the ELN 2017 recommendations [10]. A planned futility interim analysis was incorporated after 100 patients were randomized.

At final analysis, selinexor was considered not effective as addition to standard chemotherapy if no difference in CR/CRi rate in favor of selinexor was seen i.e., when the upper limit of the 80% confidence interval (CI) of the difference in CR rate would be less than 15%, which is the case if the observed difference in complete response rates was less than 2% in favor of the investigational arm. Otherwise, we would consider continuing as Phase III. Kaplan-Meier survival curves and Cox tests were used to compare the survival distributions between the treatment arms.

## RESULTS

The study was activated in 2017 and closed after an interim efficacy analysis in 2019. Median FU of patients still alive is 19 months (range: < 0.1–30). In total, 105 patients were registered and randomized. Three patients were subsequently excluded from analysis as they were later found to be non-eligible and one patient in the investigational arm went off-protocol before the drug was given, but was included in the final analysis of the study. The intention-to-treat analysis presented here therefore includes 51 patients eligible for the investigational arm and 51 patients in the control arm who received standard treatment. See CONSORT diagram shown in Fig. 1.

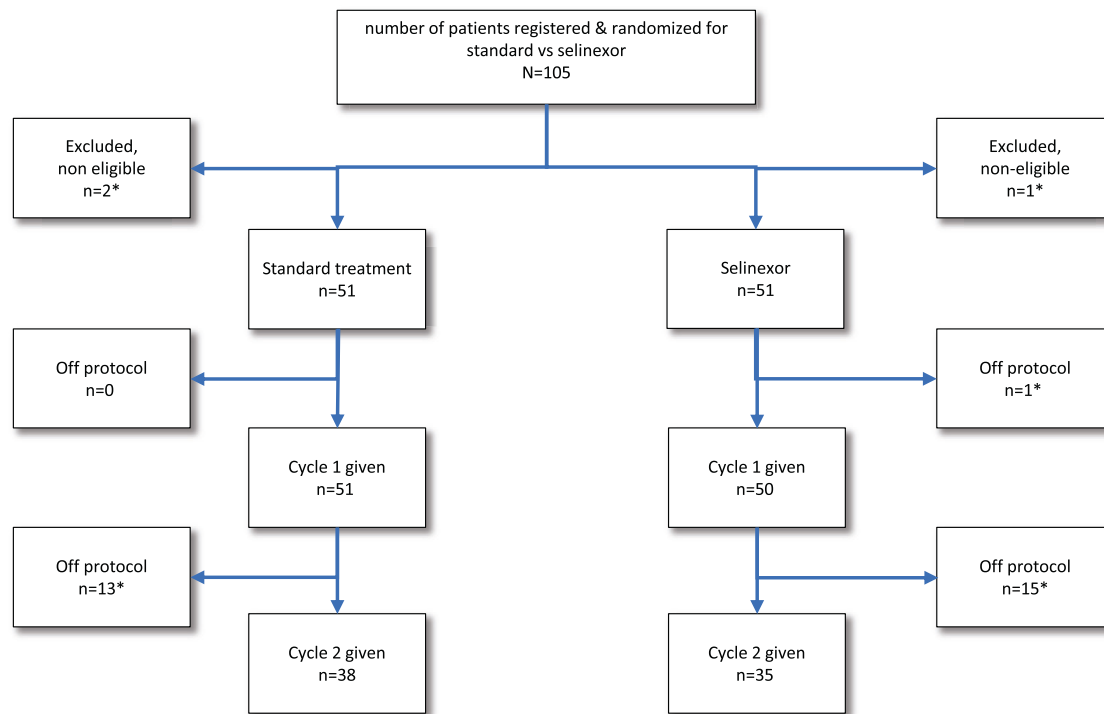
### Patients

Patient characteristics at diagnosis by treatment arm are shown in Table 1. Median age of the patients was 69 years in both arms with slightly more patients being > 70 years of age in the control arm. In the investigational arm, the AML of 79% of patients classified as poor or very poor risk, whereas this was 59% in the control arm. Other major known risk factors were well-balanced over both arms.

### Treatment, response, and outcome

All 51 eligible patients in the standard arm and 50 of 51 patients in the investigational arm received the first treatment cycle. Ninety-three (92%) received full doses of daunorubicin according to the protocol and 100 (98%) received full doses of cytarabine in cycle 1. Twenty-six of 51 patients (51%) completed the full series of doses of selinexor according to protocol in cycle 1. The majority of the patients who did not receive the protocol-specified dosages of selinexor discontinued prematurely or received reduced dosages due to toxicity (specified below). Length of stay in the hospital was on average 2 days longer in the investigational arm than in the standard arm (mean 30 days compared to 28 days).

In cycle 2, cytarabine was administered at full dose in 35 of 38 patients (92%) in the control arm and in 30/35 (86%) of the investigational arm. Selinexor was given at full dose in 10 of 35 patients (29%), with 20/35 patients (57%) stopping early or interrupting treatment because of toxicity. Length of hospital stay for the second cycle was prolonged by 5 days in the investigational arm compared to the control arm (mean 35 days compared to 30 days). Sixteen patients (42%) in the control arm and 11 (31%) in the investigational arm proceeded to alloSCT.



\*: reasons for going off protocol are given in Table S1

**Fig. 1 Consort diagram.** Distribution of patients over both treatment arms and number of patients going off-protocol shown in the boxes below.

CR/CRI rate on induction in the control arm was 80% (95%-CI: 69–91%) and 59% (95%-CI: 45–72%) in the investigational arm ( $p = 0.018$ ). With a median follow-up time of patients still alive of 19 months, the overall survival in the control arm was significantly higher than in the investigational arm (Cox- $p = 0.009$ , OS at 18 months 58% vs. 33%, see Fig. 2a), as was event-free survival ( $p = 0.01$ ; EFS at 18 months 45% versus 26%, see Fig. 2b) and disease-free survival ( $p = 0.15$ ; DFS at 18 months 53 vs 39%, not shown). Also in the subgroup of patients with poor or very poor risk AML, results of the investigational arm were worse than those in the control arm (see Fig. S1). Due to the limited number of patients, no separate survival analyses were done for the individual molecular subgroups.

Although early death rates within 30 days were comparable between both arms, the death rate within 60 days in the investigational arm exceeded that in the control arm (18% versus 8%). See Table 2 for an overview of these results.

#### Adverse events and hematological recovery

In Supplemental Tables 3, 4, the number of AEs in cycles 1 and 2 by diagnosis category, common toxicity criteria (CTC) grade, and treatment arm of randomization are given. The frequencies of toxicities were higher in the investigational arm than in the control arm, with grade 3 nervous system AEs in 12 vs 2% in the first cycle, and, in the second cycle, grade 3–4 cardiac AEs in 11% vs 5%, grade 3–4 gastrointestinal AEs in 43% vs 26%, infectious AEs grade 3–4 in 57% vs 37% and metabolic and nutritional disorders AEs in 46% vs 29%. In the control arm, 19 patients (37%) of patients experienced at least 1 SAE, whereas this was 23 (45%) in the investigational arm, and of these SAEs, 7 in the control arm and 14 in the investigational arm were life-threatening or resulted in death, the majority due to various infections.

After the first cycle, time to neutrophil recovery  $> 0.5$  and  $1.0 \times 10^9/L$  was delayed in the investigational arm (median 29

versus 25 days,  $p = 0.007$ ; 37 versus 29 days,  $p < 0.001$ , respectively), whereas platelet recoveries were not significantly different (see Fig. 3a, b). After the second cycle no significant differences in hematological recovery times were noted between the arms. (See Suppl Fig. S2).

#### Measurable residual disease (MRD)

In 45 patients (30 in the control arm and 15 in the investigational arm) MRD was assessed after the second cycle. MRD negativity rates were not different between the two arms. Overall, OS at 2 years was 75% for patients who became MRD-negative and 34% for MRD-positive patients. Disease-free survival at 2 years was 58% and 12%, respectively. Because of the limited numbers of patients, no  $p$ -values are given. (See Suppl Figure S3).

#### DISCUSSION

With a median age at diagnosis of around 70 years, AML is primarily a disease of the elderly. Although tolerance to intensive induction regimens diminishes with age, large registry data show that a majority of patients up to the age of 79 years old can tolerate intensive chemotherapy with over half of them attaining CR [11]. However, as survival in elderly AML patients is clearly below 30% at two years, better treatments are urgently needed. We therefore evaluated the addition of the XPO1 inhibitor selinexor in newly diagnosed elderly AML patients who were deemed fit enough for intensive chemotherapy, as part of the HOVON 103 study where several promising investigational agents are successively examined in combination with an intensive chemotherapy backbone. This chemotherapy regimen includes a relatively high cytarabine dose of  $1000 \text{ mg/m}^2$  for 6 days as a second induction cycle, which has been the standard of care since many years in subsequent HOVON/SAKK AML trials for patients 60 years and above and it has remained so for the ongoing major

**Table 1.** Baseline patient characteristics.

	Control arm (n = 51)	Selinexor 60 mg (n = 51)	Total
<b>Sex</b>			
M	28 (55%)	38 (75%)	66 (65%)
F	23 (45%)	13 (25%)	36 (35%)
<b>Age groups</b>			
≤ 70 years	31 (61%)	34 (67%)	65 (64%)
> 70 years	20 (39%)	17 (33%)	37 (36%)
<b>Age (years)</b>			
Mean; SD	69.9; 2.9	69.7; 3.5	69.8; 3.2
Median; range	69; 65–78	69; 65–80	69; 65–80
<b>WHO performance</b>			
0	21 (41%)	27 (53%)	48 (47%)
1	28 (55%)	23 (45%)	51 (50%)
2	2 (4%)	1 (2%)	3 (3%)
<b>Diagnosis</b>			
MDS (RAEB)	3 (6%)	10 (20%)	13 (13%)
AML	48 (94%)	41 (80%)	86 (87%)
<b>Prior HM*</b>			
No	49 (96%)	47 (92%)	96 (94%)
Yes	2 (4%)	3 (6%)	5 (6%)*
Unknown	0	1 (2%)	1 (1%)
<b>AML risk group (acc. to HOVON 103 protocol)</b>			
Good	3 (6%)	3 (6%)	6 (6%)
Intermediate	18 (35%)	8 (16%)	26 (25%)
Poor	24 (47%)	29 (57%)	53 (52%)
Very poor	6 (12%)	11 (22%)	17 (17%)
<b>IPSS-R risk score (if RAEB)</b>			
5.0	2	1	3
5.5	0	2	2
6.0	0	1	1
7.5	0	2	2
8.0	0	1	1
unknown	1	3	4
<b>WBC at diagnosis [<math>\times 10^9/l</math>]</b>			
Mean (SD)	20.0 (35.5)	15.8 (40.8)	17.9 (38.1)
Median	2.20	3.45	3.20
Range	0.60–121	0.60–245	0.60–245
<b>Blasts in BM [%]</b>			
Mean (SD)   43.5;25.8 41.5;27.4   42.5;26.5	43.5 (25.8)	41.5 (27.4)	42.5 (26.5)
Median	36	31	34
Range	4–96	2–99	2–99
<b>NPM1 mutation</b>			
Neg	20 (39%)	22 (43%)	42 (41%)
Pos	6 (12%)	5 (10%)	11 (11%)
NA	25 (49%)	24 (47%)	49 (48%)
<b>FLT3 ITD</b>			
Neg	26 (51%)	27 (53%)	53 (52%)
Pos	4 (8%)	2 (4%)	6 (6%)
NA	21 (41%)	22 (47%)	43 (42%)

**Table 1.** continued

	Control arm (n = 51)	Selinexor 60 mg (n = 51)	Total
<b>FLT3 TKD835</b>			
Neg	11 (22%)	14 (27%)	25 (25%)
Pos	2 (4%)	1 (2%)	3 (3%)
NA	38 (75%)	36 (71%)	58 (73%)
<b>CEBPA DM</b>			
Neg	27 (53%)	26 (51%)	53 (52%)
Pos	0	1 (2%)	1 (1%)
NA	24 (47%)	24 (47%)	48 (47%)
<b>FLT3ITD × NPM1 mutation</b>			
Pos × Pos	2 (4%)	1 (2%)	3 (3%)
Pos × neg	2 (4%)	1 (2%)	3 (3%)
Neg × Pos	4 (8%)	3 (6%)	7 (7%)
Neg × Neg	18 (35%)	20 (39%)	38 (37%)
NA	25 (49%)	26 (51%)	51 (50%)

\*Exclusive of previous MDS

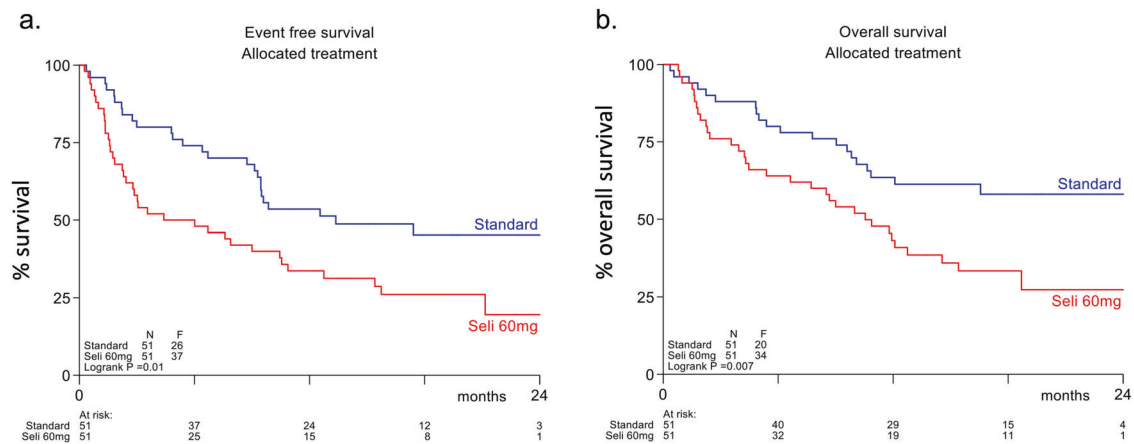
SD Standard Deviation, WHO World Health Organization, MDS Myelodysplastic syndrome, RAEB Refractory anemia with excess of blasts, AML Acute myeloid leukemia, HM Haematological malignancy, HOVON Hemato-Oncologie voor Volwassenen Nederland, IPSS-R International Prognostic Scoring System-Revised, WBC White Blood cell Count, NPM1 Nucleophosmin-1, FLT3 FMS-like tyrosine kinase 3, ITD Internal tandem duplication, TKD Tyrosine kinase domain, CEBPA CCAAT-enhancer binding protein-alpha, NA Not available.

HOVON 150 and 156 studies. The 30- and 60-day mortality rates, 4 and 8% in the standard arm of the current study, compare favorably with those from other collaborative groups using lower cytarabine dosages, or with fewer administrations. Of note, for the older age category that was enrolled in this study, 1000 mg/m<sup>2</sup> cytarabine b.i.d. days 1–6, is single agent treatment in the second cycle.

Nevertheless, the results of the current study are disappointing, with comparatively reduced overall and disease-free survival for the investigational selinexor treatment arm. This seems to have mainly been caused by a lower CR/CRi rate, increased toxicities and infection rates in relation to the addition of selinexor, and may in part also relate to the higher proportion of patients with a high or very high disease risk that were randomized to the experimental arm, although results seem equally poor in this category of patients.

Our choice for selinexor was based on positive results of preclinical studies and of a clinical phase I dose-escalation study with single agent selinexor in 95 relapsed/refractory AML patients, which showed an objective response rate of 14% with 31% of patients obtaining at least a 50% reduction in blast counts [8, 12]. Recently, in a randomized phase II study in 118 selinexor (single agent) treated relapsed/refractory AML patients, an overall response rate of 14% was obtained, compared to 9% in the control arm treated with either best supportive care alone (BSC), BSC plus low-dose cytarabine or BSC plus a hypomethylating agent [13].

The drug was also evaluated in combinations with daunorubicine/cytarabine, cladribine/cytarabine, FLAG-IDA and high dose cytarabine/mitoxantrone, and with decitabine in a 10 days regimen, in small (n = 14–40) phase I studies, mostly with relapsed or refractory, elderly AML patients [14, 15]. Although some signals of additive activity of selinexor were suggested, these studies showed increased toxicity of the combination with, amongst

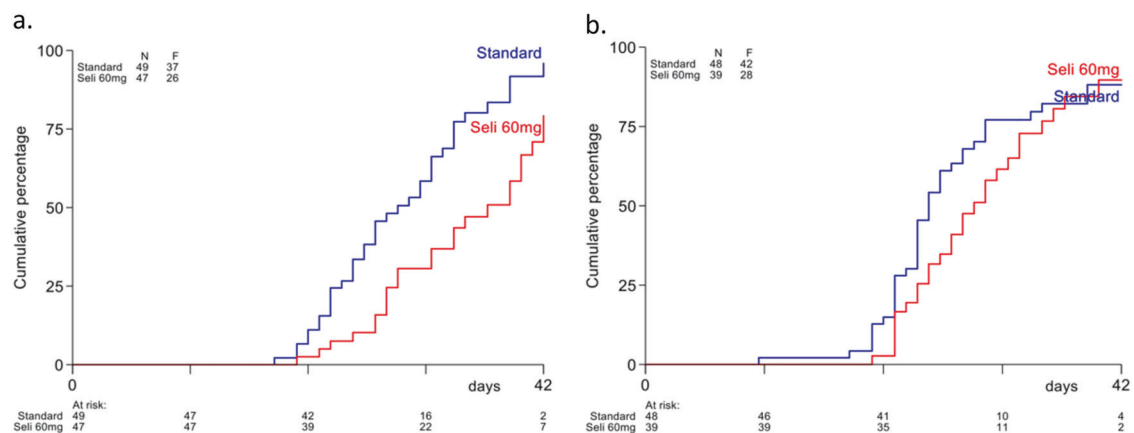


**Fig. 2** Overall survival and event-free survival. **a** overall survival. **b** event-free survival.

**Table 2.** Treatment outcome of patients randomized to standard chemotherapy with or without selinexor.

	Control treatment	Selinexor 60 mg	HR (95% C.I.)	<i>p</i>
Complete remission/CRi	80%	59%		0.018
95% C.I. (%)	69–91	(45–72)		
CR/CRi (after cycle I)	71%	46%		
Death within 30 days	4%	6%		
Death within 60 days	8%	18%		
OS at 18 months	58	33	2.10 (1.21–3.65)	0.009
EFS at 18 months	45	26	1.91 (1.15–3.16)	0.01
DFS at 18 months	53	39	1.59 (0.85–2.98)	0.15

HR Hazard ratio, C.I. Confidence interval, CR Complete remission, CRi Complete remission with incomplete hematologic recovery, OS Overall survival, EFS Event-free survival, DFS Disease-free survival.



**Fig. 3** Hematological recovery after cycle 1. **a** Recovery time to ANC  $> 1.0 \times 10^9/L$ . **b** Recovery time to platelets  $> 50 \times 10^9/L$ .

others, many electrolyte disturbances consisting of hyponatremia, hypophosphatemia, hyperglycemia and anorexia, nausea and vomiting. This limited dosing of selinexor to 60 mg twice weekly.

The results of our study are especially unsatisfactory, as the drug has been shown to be effective in other hematological malignancies, like relapsed/refractory diffuse large-cell B-cell lymphoma, and in combination with dexamethasone and bortezomib in relapsed multiple myeloma, where it was recently approved by the FDA. In the first study however, selinexor was

given as a single drug, whereas co-treatment with dexamethasone and bortezomib proved to be tolerable for the majority of patients in the latter study [16, 17]. Nevertheless, cytopenias and gastrointestinal adverse effects were common in both studies and, like in our study, infectious adverse events were more frequent in the selinexor containing arm of the myeloma study. Moreover, gastro-intestinal adverse effects were common in these studies, corresponding with our experience. Apparently, any added toxicity on top of that caused by the 3 + 7 regimen is