lomustine is added to 3 + 7 [7]. Also CPX-351, an encapsulation in nanoscale liposomes of cytarabine and daunorubicin at a synergistic 5:1 molar ratio, has been shown to be superior to 3 + 7 in secondary and therapy related AML of older age [8].

Moreover, reduced intensity allogeneic hematopoietic stem cell transplantation (HSCT) is reasonably well tolerated in terms of early toxicities and is currently broadly applied in patients at ages even over 70, mainly after attainment of CR [9].

We investigated the value of the addition of lenalidomide to standard chemotherapy in a randomized Phase 2 study. Lenalidomide belongs to the class of immunomodulatory drugs, which are orally available compounds, that modulate the immune system and other biologically important targets through antineoplastic, immunomodulatory, and anti-angiogenic properties. Effects both on the malignant clone and the microenvironment have been suggested [10]. In a published small single arm phase 2 study older adults with AML received lenalidomide as monotherapy at 50 mg daily for up to two 28-day cycles followed by maintenance therapy. Overall CR/CRi rate was 30, and 53% in patients completing high-dose lenalidomide with a median duration of 10 months (range, 1 to >17 months) [11]. Main toxicity was myelosuppression. Dosages as low as 10–15 mg given as monotherapy in R/R myeloid malignancies proved not to be effective [12]. Also in combination with ARA-C lenalidomide at a dosage of 10 mg did not appear to result in improved CR over single agent cytarabine [13]. Recently the safety and tolerability of lenalidomide with mitoxantrone, etoposide, and cytarabine (MEC) in relapsed/refractory AML were tested in a Phase I dose-escalation study. The MTD of lenalidomide combined with MEC was 50 mg/day 1–10. Results were promising in 17 patients treated at the MTD of which seven attained CR (41%) [14]. The aforementioned studies were all conducted in patients with relapsed/refractory (R/R) AML. No randomized controlled trials have been performed with lenalidomide in newly diagnosed older adults with AML.

Here we report the results of a Phase 2 randomized study concerning the addition of lenalidomide to standard chemotherapy in 222 patients with newly diagnosed AML or high-risk MDS.

Methods

Patients

Previously untreated patients, 66 years of age or older, with a cytologically confirmed diagnosis of de novo AML (excluding acute promyelocytic leukemia) or with refractory anemia with excess of blasts and an International Prognostic Scoring System score of 1.5 or higher and a WHO performance score of 2 or less were eligible for inclusion. Patients with secondary AML progressing from antecedent myelodysplasia were also eligible. No previous treatment was allowed except for a short period of hydroxyurea. 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, and serious cardiac arrhythmia requiring medication. Other standard general medical exclusions were also applied (detailed inclusion and exclusion criteria are presented in the Supplementary File 1). 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 [15].

Study design and chemotherapy

Lenalidomide was provided free of charge by Celgene. The study was divided into two parts. The study started with a randomized dose selection run-in phase with 10 mg/day 1–21, 15 mg/day 1–21, and 20 mg/day 1–21 dose levels of lenalidomide and after evaluation of the toxicity profiles after each dose level the study was continued with lenalidomide at 20 mg/day as an open label randomized Phase 2 trial. A dose limiting toxicity (DLT) was defined as death within 31 days of start of cycle I and before initiation of start of cycle II, irrespective of the cause of death. In the HOVON/SAKK AML 43 the incidence of DLT defined in this way had been 13% [4, 5]. A patient was considered evaluable for DLT if the patients have had started cycle I after registration. A patient was considered to have no DLT if the patient was still alive at day 31 after start of cycle I or at start of cycle II (whichever comes first) The decision rules for dose selection are depicted in the Supplementary File 2.

In the second part of the study the safety, tolerability, and efficacy were assessed at the highest feasible dose lenalidomide. One interim analysis was performed on the primary endpoint according to protocol. Patients were randomly assigned to remission induction regimens with or without

lenalidomide. Cycle I included daunorubicin at 45 mg/m^2 (3-h infusion on days 1, 2, and 3) and cytarabine at a dose of 200 mg/m^2 (per continuous infusion on days 1–7) with or without lenalidomide at the assigned dose level. Cycle II contained cytarabine 1000 mg/m^2 q 12 h via 6 h infusion from day 1 to 6 (12 doses) with or without lenalidomide at 20 mg/day 1–21. Patients could be allotransplanted off protocol according to local policy. MRD analysis and detection was performed as previously described [16].

Statistical analysis

The primary endpoint of the second part of the study is response rate. A patient is considered to have a response if the best response to remission induction therapy (cycle I and/or II) is a CR/CRi. Secondary endpoints are considered as exploratory and included: overall survival (OS), event-free survival (EFS), relapse free survival (RFS), early death (ED), and hematopoietic recovery. The definitions which are standard are according the ELN recommendations [17]. A planned futility interim analysis was incorporated after 100 patients were randomized

At this analysis, the study was to be stopped because of inefficacy if no difference in CR/CRi rate in favor of lenalidomide is to be expected i.e., upper limit of the 80% confidence interval (CI) of the difference in CR rate <15%, which is the case if the observed difference in response rate is <6% in favor of the lenalidomide treatment arm. Otherwise we would consider not to continue 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 2010 and closed after completion of accrual in 2014. Median FU of patients still alive is 51 months. The number of patients randomized to 10 and 15 mg lenalidomide were 58 and 51, respectively. According to the decision rules of the protocol no excess of DLTs was observed in comparison with the control arm. The same accounted for the 20 mg dosage. A summary of the run-in-phase study with Lenalidomide 10 and 15 mg is provided in the Supplementary Files 3 and 4.

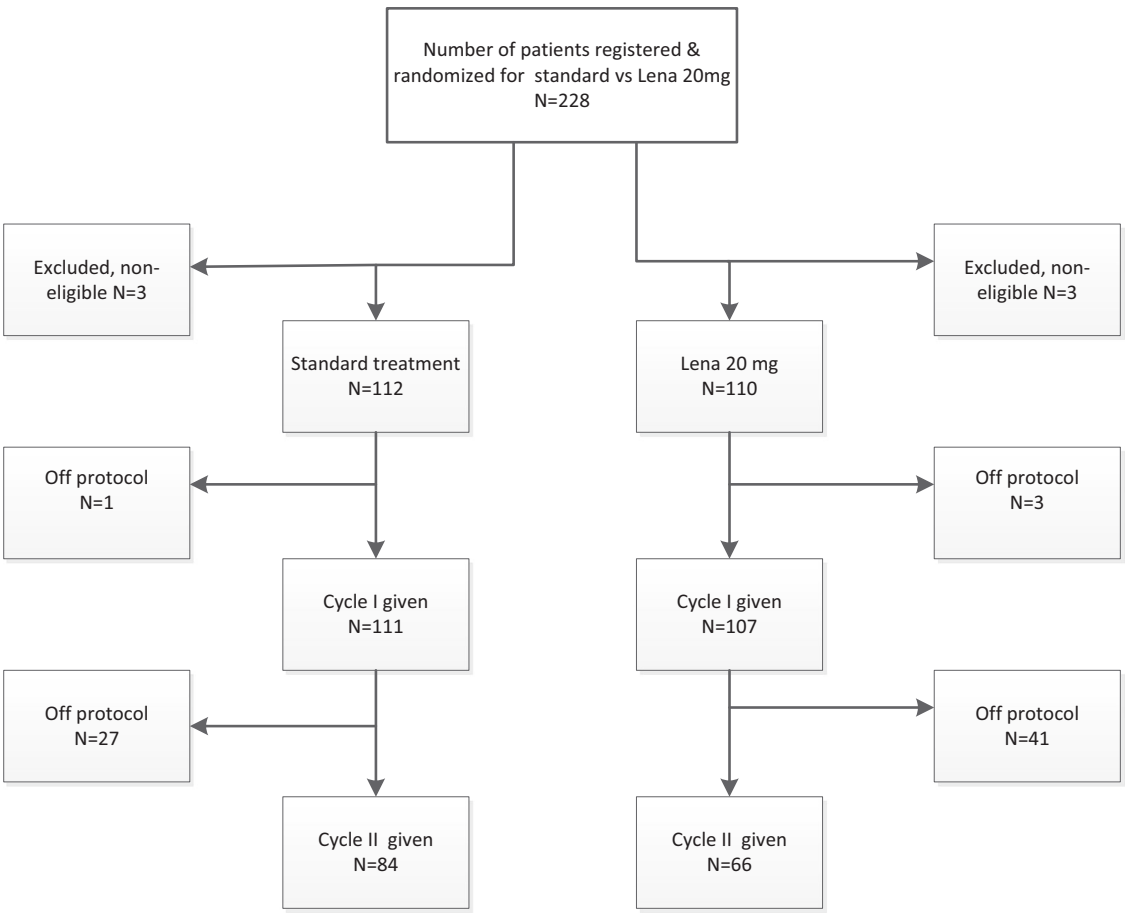

Fig. 1 Consort diagram.

We continued according protocol with lenalidomide 20 mg in the second part of the study. Although the DLT in this population was not reached we decided not to escalate because of toxicity results noted in a parallel HOVON study in young patients (HOVON132, Eudract number: 2013-002843-26) at a dosage of Lenalidomide 20 mg in combination with intensive treatment. The analysis presented here is restricted to the 228 patients randomized for treatment with the 20 mg dose of lenalidomide (CONSORT diagram shown in Fig. 1), from which 222 eligible patients are included in the analyses.

Patients

Patient characteristics at diagnosis by treatment arm are shown in Table 1. Median age of the patients in both arms is 69 years (range 65–84). The arms are well balanced for major prognostic factors, slightly more patients were above 70 years in the investigational arm (39 vs. 29%), but with no difference in mean age. A prior hematological disease was present in 8% of the population.

Treatment, response, and outcome

Of 222 eligible patients, 218 patients received the first treatment cycle and 216 (99%) received full doses of daunorubicin according to the protocol and 214 (98%) received full doses of cytarabine in cycle 1. However only 69 patients out of 107 (64%) completed the full series of doses of lenalidomide according to schedule. The majority of the patients without dosage according to protocol stopped early due to toxicity. This may have been due to the fact that this was not a blinded study and toxicities could be attributed to the experimental drug which is a known phenomenon in non-blinded studies associated already with a relatively substantial level of toxicity. In case a patient prematurely discontinued lenalidomide administration the median numbers of days between first and last dose was 12.

In cycle 2 cytarabine could be administered full dose in 77 of 84 patients (92%) in the standard arm and in 62 of 66 patients (94%) of the experimental arm while lenalidomide was administered according to schedule in 37 of 66 patients (56%). Median number of days given in those patients that stopped early was 12.

Patients assigned to the standard treatment arm and lenalidomide treatment arm respectively showed similar CR/CRi rates on induction 69%; (95% CI: 60–77%) versus 66%; (95% CI: 58–75%). Twenty-one patients in the standard arm versus 12 in the experimental arm received an alloHSCT. There were no significant differences with respect to OS, EFS, RFS, ED between both arms (see Table 2 and Fig. 2). A subanalysis of Lenalidomide compliant patients showed neither significant differences in comparison to the standard arm in any of the clinical outcome parameters (not shown). Statistically significant

Table 1 Baseline characteristics of patients assigned to intensive induction chemotherapy with or without lenalidomide 20 mg.

	Standard arm	Lenalidomide 20 mg	Total
Sex			
M	71 (63%)	63 (57%)	134 (60%)
F	41 (37%)	47 (43%)	88 (40%)
Age groups			
≤70 years	79 (71%)	67 (61%)	146 (66%)
>70 years	33 (29%)	43 (39%)	76 (34%)
Age			
Mean; SD	69.6; 3.16	70; 3.49	69.8; 3.33
Median; range	69; 66–84	69; 65–81	69; 65–84
WHO performance			
0	52 (46%)	43 (39%)	95 (43%)
1	43 (38%)	55 (50%)	98 (44%)
2	13 (12%)	8 (7%)	21 (9%)
3	0	1 (1%)	1 (0%)
?	4 (4%)	3 (3%)	7 (3%)
Diagnosis			
MDS	12 (11%)	10 (9%)	22 (10%)
AML	100 (89%)	100 (91%)	200 (90%)
Prior HM			
No	104 (93%)	96 (87%)	200 (90%)
Yes	7 (6%)	11 (10%)	18 (8%)
Risk AML (ELN 2010)			
Good	14 (13%)	18 (16%)	32 (14%)
Intermediate	16 (14%)	14 (13%)	30 (14%)
Poor	56 (50%)	56 (51%)	112 (50%)
Very poor	26 (23%)	22 (20%)	48 (22%)
NPM1 mutation			
Neg	67 (60%)	69 (63%)	136 (61%)
Pos	20 (18%)	23 (21%)	43 (19%)
FLT3ITD			
Neg	78 (70%)	82 (75%)	160 (72%)
Pos	10 (9%)	13 (12%)	23 (10%)
EVI1 overexpression			
Neg	72 (64%)	72 (65%)	144 (65%)
Pos	9 (8%)	12 (11%)	21 (9%)
CEBPA DM			
Neg	70 (63%)	79 (72%)	149 (67%)
Pos	4 (4%)	3 (3%)	7 (3%)
FLT3ITD × NPM1 mutation			
Pos × Pos	7 (6%)	9 (8%)	16 (7%)
Pos × neg	2 (2%)	4 (4%)	6 (3%)
Neg × Pos	11 (10%)	14 (13%)	25 (11%)
Neg × Neg	65 (58%)	65 (59%)	130 (59%)

Gene mutations.

For each parameter: if not 100% the remaining % is unknown.

NPM1 nuclephosmin-1, *FLT3* fms-like tyrosine kinase-3, *CEPBA* CCAAT/enhancer-binding protein alpha, *EVI1* ecotropic virus integration 1 gene.

Table 2 Outcome of treatment of patients assigned to intensive induction chemotherapy with or without lenalidomide 20 mg.

Outcomes	Standard treatment	Lena 20 mg	Logistic/cox regression		
			OR/HR	95% CI	*P* value
Complete remission (CR/CRi) CI	77 (69%)	73 (66%)			
CR/CRi (after cycle I)	54 48%	58 (54%)			
Early death					
Early death (<30 days)	11 (10%)	10 (9%)			
Death within 60 days	20 (20%)	22 (20%)			
OS at 3 years	35% ± 5	30% ± 4	1.01	0.74–1.36	0.96
By age groups	≤70 >70	≤70 >70			
	41% ± 6 21% ± 7	38% ± 6 17% ± 6			
EFS at 3 years	19% ± 4	21% ± 4	1.0	0.75–1.33	1.0
RFS at 3 years	24% ± 5	28% ± 5	0.97	0.68–1.39	0.89

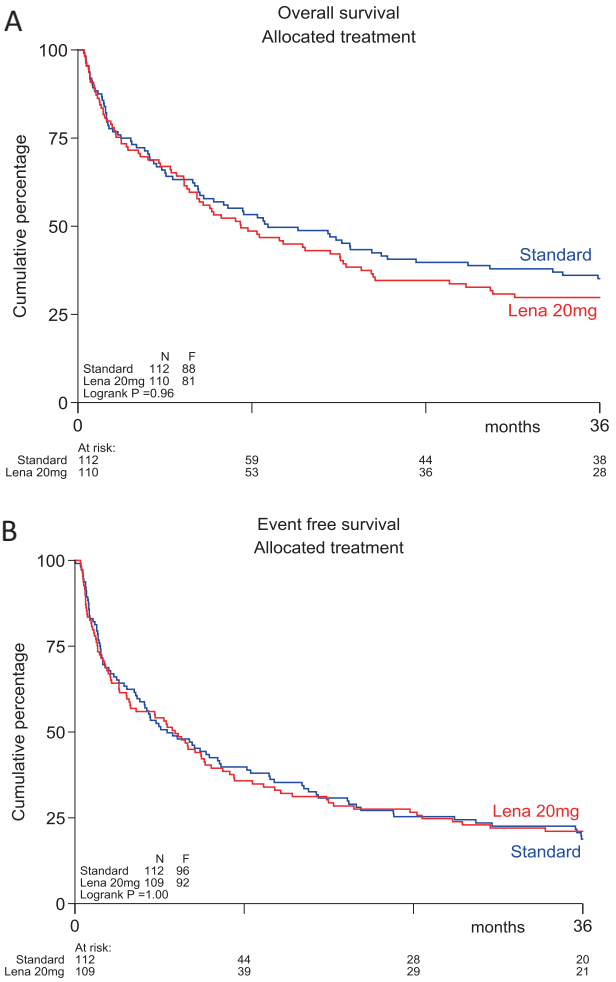

Fig. 2 Effect of addition of lenalidomide to a standard regimen of daunorubicin and cytosine-arabinoside. Shown are OS(2A) and EFS (2B).

differences for OS between patient above and below 70 years of age ($p < 0.001$) as well as between good/intermediate versus poor/very poor risk AML ($p < 0.001$) were apparent (Supplementary Figs. 1 and 2).

Adverse events and hematological recovery

In Supplementary Table 1, the number of adverse events (AEs) in cycles 1 and 2 by diagnosis category, common toxicity criteria (CTC) grade, and arm of randomization are given. The frequencies of grade 3 and 4 CTCs appear generally similar in both. The two treatment arms were comparable with respect to the maximum grade of events and serious AEs (Table 3). A more detailed analysis of events known to be associated with lenalidomide showed no difference in thromboembolic complications (Table 3).

Time to neutrophil or platelet recovery between the two groups did not significantly differ after cycle I nor after cycle II but a trend to delayed recovery was observed in the lenalidomide arm for both neutrophil and platelet regeneration (Fig. 3 and Supplementary Fig 3) and also more nights were spent in the hospital by patients treated with lenalidomide.

Measurable residual disease (MRD)

In 60 patients (29 in the standard arm and 31 in the experimental arm) MRD was assessed. These limited numbers do not allow for a meaningful comparative survival analysis. In the control arm 72% became MRD negative as compared with 81% in the lenalidomide arm. OS at 2 years was 61% for the MRD negative patients and 50% for the MRD positive patients ($p = 0.31$) (Supplementary Fig. 4).

Discussion

Intensive treatment remains the standard of care in older "fit" patients with AML but outcome is much worse as compared with the younger patient. Various previous therapeutic developmental attempts to improve outcome for these patients have failed. The HOVON-SAKK consortium

Table 3 Toxicities in patients assigned to intensive induction chemotherapy with or without lenalidomide during and after Cycle 1 and 2.

	Cycle 1 ($n = 111$)		significance	Cycle 2 ($n = 107$)		Significance
	Standard	Lenalidomide 20 mg	$P = 0.374$	Standard	Lenalidomide 20 mg	$P = 0.667$
Maximum grade events[a], no. of patients						
0	6	2		3	0	
2	9	6		7	4	
3	74	69		53	46	
4	16	24		13	10	
5	6	6		8	6	
Toxicites of special interest, no. of patients						
Myocardial infarction	0	0		0	2	
CVA	1	0		0	1	
Pulmonary embolism	0	0		0	0	
Thrombosis	3	5		2	1	
Serious adverse events, no. per patient Cycle 1 and 2 combined						
0	71 (63%)	58 (53%)				
1	31 (28%)	40 (36%)				
2	6 (5%)	7 (6%)				
3	3 (3%)	4 (1%)				
4	1 (1%)	1 (1%)				
Outcome last SAE if no. of SAEs >1, no. of patients Cycle 1 and 2 combined						
Resolved	5 (50%)	6 (50%)				
Death	3 (30%)	6 (50%)				
Ongoing at death	2 (20%)	6 (50%)				
No of nights in hospital						
Mean; SD	29;10	32;9		31; 11	30; 11	
Median(range)	28 (13–92)	31 (12–64)		28 (0–70)	31 (2–59)	

[a]Details of toxicities in Supplementary Table 1.

has decided already quite some years ago instead of performing large randomized trials to embark on a new trial design adding promising drugs to a standard chemotherapy backbone in a Phase 2 randomized setting with the possibility to proceed to a phase III study depending on the results defined by strict criteria.

Here we report on the study approach dealing with the question of the added therapeutic value of lenalidomide to standard chemotherapy. Unfortunately, as many other studies conducted in the setting of AML in patients of older age, also the current study failed to demonstrate a clear benefit for the investigational treatment approach. The CR/CRi rates and survival values were similar in both arms.

It is interesting to see how well patients with intermediate and good risk performed. Around 60–70% are alive at 3 years and notably around 20–25% of patients with a poor and very poor risk profile were still alive after 3 years. Most of these patients are probably cured, a phenomenon that has not been observed in the majority of patients that are treated with low intensity cytotoxic and hypomethylating therapy. These observations underline and confirm the value of intensive treatment in properly selected older adult patients.

Whether lenalidomide should be regarded as an inactive drug in the setting of AML cannot be entirely definitively concluded from the results of the current study. The schedule of only two short cycles of application of lenalidomide in the current study may have been inadequate. It is to be noted that lenalidomide in other treatment indications where it has shown therapeutic activity (e.g., in MDS, myeloma) is usually applied during prolonged intervals. Also the dosage could have been too low although 20 mg day 1–21 in combination with intensive chemotherapy seems quite high. Therefore we cannot exclude the possibility that lenalidomide may exert a beneficial anti-AML effect in case of a

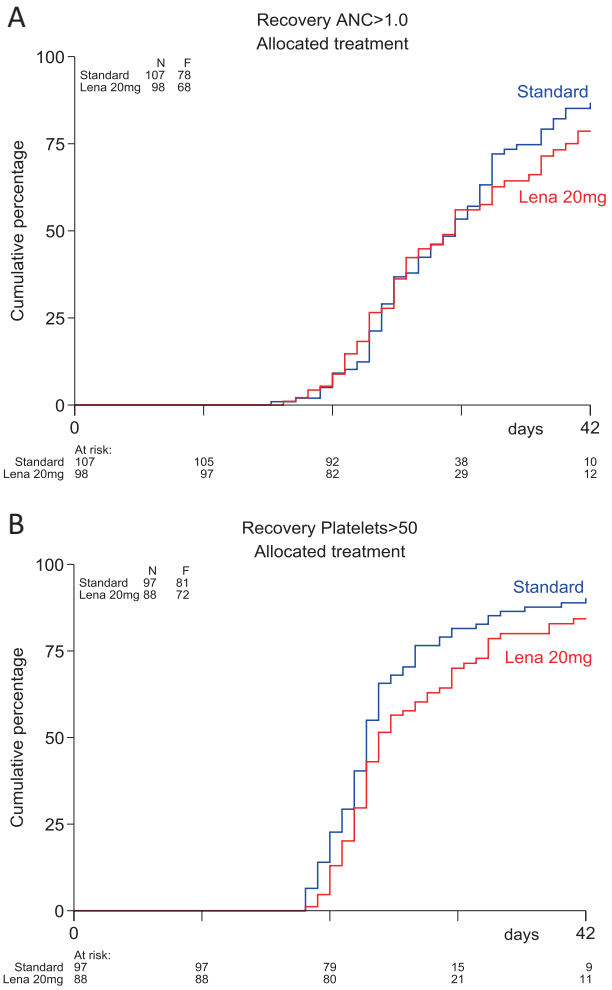

Fig. 3 Recovery time in days to achieve a neutrophil count of $>1 \times 10^9$/l (3A) and platelet count of $>50 \times 10^9$/l (3B) in Cycle 1.

regimen of administration that enables both induction and postinduction exposure to the drug. Furthermore we did not evaluate the possibility of a beneficial effect of lenalidomide in the maintenance setting.

Numerous new drugs are currently emerging and suggest new avenues of treatment. Especially the data in unfit patients or relapse/refractory AMLs for the IDH1,2 inhibitors (enasidenib and ivosidenib), FLT3 inhibitors and the bcl2-inhibitor Venetoclax in combination with LDAC or HMAs are very promising and most probably will be game changing for AML treatment [18–22]. Also new immunotherapeutic drugs will most likely enrich our therapeutic arsenal in the near future [23]. Hopefully intelligent designed combinations of these drugs either added to intensive treatment or administered as combo's or triplets will change the prospects for these AML patients who in terms of frequencies represent the highest medical need.

Study design, data analysis, preparation of publication

The study was designed by the Leukemia Working Group of the HOVON/SAKK Cooperative Groups, the HOVON Data Center was responsible for the central datamanagement and YvN performed the analysis of the data. The decision to publish was made by the cooperative group. GO and subsequently BL and YvN produced the first version of the manuscript, which was circulated for comments to the other authors.

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The Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) and Swiss Group for Clinical Cancer Research (SAKK) D. A. Breems[30], Havelange[31], M-C Vekemans[31], I. Moors[32], F. van Obberg[33], J. A. Maertens[34], B. Hodossy[35], S. Vansteenweghen[35], L. Lammertijn[35], D. Deeren[36], C. Graux[37], A. Sonet[37], A. Triffet[38], B. T. Gjertsen[11], M. Bargetzi[5], J. Passweg[39], D. Heim[39], San Giovanni[40], Georg Stuessi[40], T. Pabst[28], D. Betticher[41], Y. Chalandon[42], O. Spertini[43], M. Gregor[44], U. Hess[45], M. Fehr[45], M. G. Manz[46], S. K. Klein[17], B. J. Biemond[6], G J Ossenkoppele[47], A. van de Loosdrecht[47], J J W M Janssen[47], J. W. J. van Esser[48], M. Van der Klift[48], R. E. Brouwer[49], D. Van Lammeren-Venema[50], M. D. Levin[51], L. W. Tick[52], M. C. J. C. Legdeur[53], G. Huls[54], E. Vellenga[54], M. Hoogendoorn[55], J. H. Veelken[56], P. A. von dem Borne[56], H. C. Schouten[57], O. de Weerdt[58], W. J. F. M. van der Velden[59], J. Cornelissen[15], M. Jongen-Lavrencic[15], B. Wouters[15], H. G. M. Raaijmakers[15], B. Löwenberg[15], J. Kuball[60], A. Van Rhenen[60], M. Van Marwijk Kooy[61]

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Compliance with ethical standards

Conflict of interest The authors declare no relevant competing financial interest.