## Methods

The HOVON 104 study was conducted at 15 centers in the Netherlands, Germany, and Belgium. The trial started with a randomized phase III design but due to slow accrual the dexamethasone arm closed after including 7 patients and the trial continued as a single arm phase II study.

Patients with biopsy proven, systemic, AL amyloidosis, aged between 18-70 years, with detectable M-protein and/or level of involved FLC (iFLC) > 50 mg/L, WHO performance status 0-2, NYHA stage 1-2 were included. Eligibility criteria at inclusion were corresponding to eligibility criteria for auto-SCT with the exception of measuring cardiac ejection fraction (EF). Major exclusion criteria were concurrent MM defined as Salmon Durie stage II or III, previous treatment of plasma cell dyscrasia, symptomatic orthostatic hypotension, symptomatic effusions, a NT-proBNP level > 5000 pg/ml, troponin T > 0.06 µg/l or troponin I > 2 x upper limit of normal (ULN), estimated glomerular filtration rate (eGFR) < 30 ml/min, NCI CTCAE grade sensory peripheral neuropathy (PNP) > grade 2 or > grade 1 with pain and motor PNP > grade 2, see supplementary online for complete list. Inclusion and exclusion criteria were also installed before stem cell mobilization (SCM). Inclusion criteria comprised of a WHO performance status 0-2, NYHA stage 1-2, EF > 45%. (Exclusion criteria see online supplementary). The study was approved by the ethics committee of all participating hospitals and the UMC Utrecht (IRB 10-426). All procedures were conducted in compliance with the Declaration of Helsinki. Written informed consent was provided by all patients (EudraCT number 2010-021445-42).

*Treatment design*

Four 21-day cycles of induction treatment were given consisting of bortezomib subcutaneously (sc) 1.3 mg/m$^2$ on days 1, 4, 8, 11 and dexamethasone 20 mg orally on each bortezomib and the following day. For dose adjustments see online supplementary. SCM started within 4-6 weeks after the start of last induction cycle using G-CSF 10 µg/kg divided in 2 doses, given for 5 days. Melphalan dosage was 200 mg/m$^2$ given in 2 days. Patients with an eGFR < 40 ml/min received 100 mg/m$^2$ melphalan. Hematologic response was measured after each induction cycle and all patients were planned to receive HDM. Patients not responding to induction treatment could proceed directly to SCM and auto-SCT if eligibility criteria were met.

*Hematologic and organ response criteria*

Organ involvement and hematologic and organ responses were evaluated according to the consensus criteria of the International Society of Amyloidosis published in 2005 with some modifications such as the addition of a VGPR category and addition of NT-proBNP for cardiac response.[14,15] (see supplementary online)

Both hematologic and organ responses were measured after each induction cycle, after SCM and before auto-SCT, and thereafter every 3 months for 5 years after registration. In addition, patients could participate in a side study for the measurement of minimal residual disease (MRD) with flow cytometry when a CR was reached or at the 6 months' time point after auto-SCT. Flow cytometry was performed centrally with a sensitivity level of $10^{-5}$, see details at supplementary online.

*Statistical design and endpoints*

The primary endpoint was to evaluate the efficacy of bortezomib-dexamethasone induction treatment followed by HDM and auto-SCT measured as the proportion of patients with a CR at 6 months after auto-SCT (CR6mo). For secondary endpoints and analysis effect of baseline characteristics on auto-SCT, OS and PFS see supplementary online.

## Results

### *Patient characteristics*

In the phase II part 50 patients were enrolled from March 2012 to April 2016. Patients characteristics are summarized separately in Table 1 for patients who proceeded to auto-SCT and those who did not. For all included patients the median age was 59 years (interquartile range/IQR 51-63). The median number of organs involved was 2, range 1 to 5. Multi-organ involvement with 2 or more organs involved was present in 36 patients (72%) and 3 or more organs involved in 19 (18%). Kidney involvement was most frequently present in 82% of patients, followed by cardiac involvement in 66% and liver involvement in 26% of patients. Median eGFR at inclusion was 72 ml/min (IQR 59-90) and 20, 25 and 5 patients respectively had renal stage I, II or III.[16] Mayo cardiac stage was I, II and III for respectively 16, 16 and 17 patients (1 patient missing). [17] The median NT-proBNP at start was 832 pg/ml (IQR 204-1638). In 47 patients echocardiography data were available.

Fourteen patients (28%) had 10% or more plasma cell infiltration in the bone marrow. Most patients presented with a λ light chain (80%). The median level of the iFLC was 180 mg/l (IQR 73-352) for λ light chain and 169 (IQR 61-879) mg/L for κ light chain. The median dFLC was 213 mg/L (IQR 80-397) and 50% of patients had a dFLC ≥ 180 mg/L. Of note, 7 patients were included without an M protein or dFLC value that could qualify them for the PR or VGPR category and 3 of those patients also did not have an uPEP > 0.1 g/day.

In the group of 7 patients enrolled in the dexamethasone arm of the closed phase III part of the trial one patient was not eligible. In the remaining 6 patients the median age was 57 and also

other baseline characteristics were comparable to the 50 patients in the phase II part (data not shown). Three patients received an auto-SCT and the estimated 3 years PFS was 83%. These 6 patients are not included in this final analysis.

*Treatment characteristics*

All patients started with induction treatment. Most patients (88%) received 4 cycles of bortezomib-dexamethasone, 2 patients 3 cycles, 2 only 2 cycles and 2 patients had to discontinue after the first cycle. Half of the patients had dose modifications in the administered cycles as prescribed per protocol: in 50% of patients for bortezomib and 44 % for dexamethasone. The reasons for dose modifications for bortezomib were mostly neurotoxicity, both sensory and autonomic PNP (9 pts) and infection (3 pts) and for dexamethasone heart failure (4 pts), edema (3 pts), infection (3 pts) and myopathy (2 pts).

Eleven patients did not have their stem cells collected after induction therapy. Five patients did not fulfill the eligibility criteria, including 2 patients that stopped during induction due to progressive heart failure and kidney failure, respectively. Two patients died, one during induction cycle 1, 3 patients experienced bortezomib related toxicity and were taken of the trial during induction treatment (1 patient with bronchial hyper reactivity, 1 patient with gastro intestinal necrosis and 1 patient with CTCAE grade 4 sensory PNP) and 1 patient refused stem cell collection.

The median number of stem cells collected was $6.3x10^6$ CD34/kg (IQR 4.6-9.3). Ten patients needed 2 days of apheresis and cyclophosphamide was given in 9 patients according to local policy. After stem cell collection, 4 patients did not proceed to HDM and stem cell reinfusion. All

4 had clinical deterioration consisting of symptomatic pleural effusions in 2 patients, one patient started dialysis and one patient had several toxicities, worsening of the clinical condition and was taken off the trial by decision of the treating physician.

In total 35 patients (70%) received HDM and stem cell reinfusion as an inpatient procedure. Thirty-one patients were given full dose of HDM, per protocol this dose was reduced to 100 $mg/m^2$ in 4 patients due to an eGFR below 40 ml/min. All patients engrafted without G-CSF support with a median time of 13 days for white blood cells and 16 days for platelets. A consort diagram summarizes the treatment course of all 50 patients (Figure 1).

Based on the univariate logistic regression model we could not identify a prognostic baseline characteristic such as NYHA stage, NT pro BNP, MAYO stage, ≥ 10% plasmacells, dFLC ≥ 180 mg/L number of involved organs, that was related to proceeding to auto-SCT after induction therapy. As shown in Table 1 the baseline MAYO stage was not statistically different between the groups with or without auto-SCT (p-value 0.80). In the 35 patients who received an auto-SCT, 22 received full dose bortezomib and 23 full dose dexamethasone. In contrast in the 15 patients that did not proceed to auto-SCT only 3 received full dose bortezomib and 5 full dose dexamethasone. Because troponin levels were only measured at baseline a MAYO stage assessment could not be calculated after induction treatment and before auto-SCT.

*Hematologic responses*

Hematologic responses are summarized in Table 2. The overall response rate (ORR) after induction treatment was 80%, including 38% of patients with a VGPR and 20% with a CR. The median time to first response was 28 days (IQR 21-43) and the median time to best response

was 67 days (IQR 28-240). Within the first 3 months from start 80% of responses were detected (Figure 2). Ten patients (20%) had a CR after induction treatment and 8 of them proceeded to auto-SCT. Responses assessed 6 months after auto-SCT, which was the primary endpoint of the study, improved but were assessed in 35 patients only, since 15 patients did not proceed to auto-SCT. In these 35 patients, the CR rate doubled from 23% after induction treatment to 46% at 6 months after auto-SCT. The ORR increased to 86% at 6 months after auto-SCT. On intention to treat analysis the CR rate at 6 months after auto-SCT was 32% and therefore the primary endpoint of the study was not met. In the group of 15 patients who did not proceed to auto-SCT, 10 (67%) had a hematologic response. In an univariate logistic regression analysis a dFLC ≥ 180 mg/L or bone marrow plasmacells ≥ 10% at diagnosis were not related to depth of hematologic response.

*Flow cytometry*

Samples of 26 patients at diagnosis were available. Of this group 20 patients proceeded to auto-SCT. The median % of clonal plasmacells detected at baseline was 1.4 % (range 0.17-4.9). At 6 months post auto-SCT in total 7 samples were collected. Six of these patients had a CR and were also MRD negative. Additional analyses was performed such as previous reported by Perez-Persona et al in which the ratio of clonal plasmacells within the total of bone marrow plasmacells (aPCs/BMPC ) of ≥ 95% had prognostic value for progression to MM in patients with monoclonal gammopathy of unknown significance and smoldering MM.[18] In the current study patients with aPCs/BMPC ≥ 95% at baseline had a shorter PFS (HR 8.44, 95% CI 1.05-67.96) and

a lower chance to proceed to auto-SCT, however due to small sample size this was not statistically significant (OR 0.16, 95% CI 0.02- 1.67).

*Organ responses*

Organ responses were already seen after induction treatment and improved after HDM + auto SCT and are summarized in Figure 3 for both patients who proceeded to auto-SCT and those who did not. Kidney response improved from 24 to 69% at 2 years after auto-SCT and cardiac responses from 24 to 78%. On intention to treat analysis 61% of patients achieved a renal response, 72% a cardiac response and 62% a liver response. During the HDM and auto-SCT procedure 2 patients had a detoriation of their renal function, defined as a decrease of 25% in eGFR, which persisted during follow up. Median time to first cardiac organ response was 222 days (IQR 125-395) and 318 (IQR 91-615) days for kidney. Organ progression was seen in 6 out of 33 patients with heart involvement, 13 out of 41 patients with kidney involvement, and 3 out of 13 patients with liver involvement.

During the study new renal response criteria were developed ( ≥ 30% decrease in proteinuria or drop of proteinuria < 0.5 g/day in the absence of renal progression defined as ≥ 25% decrease in eGFR). According to these criteria 14 patients (48%) that proceeded to auto SCT had a renal response after induction treatment and this increased to 22 (76%) after auto-SCT (Figure 3).

Patients with a deep hematologic response (CR/VGPR) at 6 months after auto-SCT and in further time points had a higher renal response rate (72% vs 28%, P-value= 0.03). Although more patients in this group also had a higher cardiac response rate (65% vs 35%), this was not statistically significant for cardiac response at any time point after auto-SCT.

*OS and PFS*

The median and IQR follow up of the 43 patients still alive is 38.3 (34-46) months and 36.9 (29-46) months for all 50 patients. Five patients died during follow up, mostly due to amyloid related organ failure. None of the transplanted patients have died. Kaplan-Meier PFS curves for 50 patients and for 35 transplanted patients are shown in Figure 4. The median OS and PFS were not reached. The 3 year estimated OS and PFS are 86% and 63%, respectively. In total 7 patients had progression of their plasma cell dyscrasia after auto-SCT, including 1 patient with a previous CR, 3 with VGPR, 2 with a PR and one patient with no response. None of the 8 patients with a CR before auto-SCT have progressed compared to 8 patients in the non CR group before auto-SCT (p=0.13). Using univariate Cox regression prognostic baseline characteristics such as type of hospital (high vs low number of included patients), eGFR >30 and < 50 vs eGFR ≥50 ml/min, NYHA I vs II, NT- proBNP as continuous variable, plasma cells < 10% vs ≥ 10% , dFLC < vs ≥ 180 mg/L, number of organs involved ≤2 vs >2, MAYO stage, nervous system involvement and cardiac involvement were tested but none of these variables were statistically related to OS.

*Adverse events and mortality*

Adverse events (AEs) were commonly seen during induction treatment in all patients. The most frequent AEs seen during induction treatment are summarized in Table 3. The most common experienced AEs were neurotoxicity, gastro intestinal, infections and cardiac. Although dizziness, orthostatic hypotension and syncope may also have cardiac origins, all these events were grouped together as autonomic neuropathy events if no cardiac cause, such as arrhythmias or

deterioration in EF, was detected. In total 10% of patients experienced autonomic neuropathy and 34% had sensory neuropathy related to bortezomib. Interestingly, patients with nervous system involvement at start did not require more dose adjustments for bortezomib compared to patient without nervous system involvement. There was no engraftment syndrome seen after auto-SCT.

In total 47 serious adverse events (SAEs) were reported in 29 patients, 34 during induction treatment, 3 during SCM and 10 following 30 days after HDM and auto-SCT. The SAEs were mostly due to hospitalization (81%) and 28 were considered related to the treatment. Twenty-nine SAEs resolved completely, the others were ongoing. In total, 2 patients died during the study treatment phase. One patient had a sudden cardiac death during induction cycle 1, probably related to the cardiac amyloidosis, 1 patient died due to hepato-renal syndrome after receiving 4 cycles of induction treatment. There was no transplant related mortality of the HDM + auto-SCT procedure.

Table 1: Baseline characteristics of 50 patients, separated for group that did or did not proceed to auto-SCT

| CHARACTERISTIC | NUMBER (%) OR MEDIAN (IQR) | | |
|---|---|---|---|
| | Patients who proceeded to auto SCT N = 35 | Patients who not proceeded to auto SCT N = 15 | Total group of patients N = 50 |
| Age (years) | 59 (50-63) | 60 (53-63) | 59 (51-63) |
| Sex (female) | 14 (40%) | 6 (40%) | 20 (40%) |
| WHO performance status | | | |
| 0 | 17 (49%) | 6 (40%) | 23 (46%) |
| 1 | 16 (46%) | 5 (33%) | 21 (42%) |
| 2 | 1 (3%) | 4 (27%) | 5 (10%) |
| Clonal disease | | | |
| Lambda | 27 (77%) | 13 (87%) | 40 (80%) |
| Kappa | 8 (23%) | 2 (13%) | 10 (20%) |
| Involved FLC level | 167 (63-341) | 205 (64-493) | 181 (73-35) |
| Difference FLC (dFLC) | 214 (103-342) | 212 (68-461) | 213 (80-397) |
| Number of patients dFLC ≥ 180 mg/L | 17 (49%) | 8 (53%) | 25 (50%) |
| % plasmacells bone marrow | 6 (4-11) | 5 (2-8) | 6 (0-33) |
| Number of patients with ≥ 10% plasmacells | 12 (34%) | 2 (13%) | 14 (28%) |
| Median number of organs involved | 2 (1-3) | 2 (1-3) | 2 (1-3) |
| Number of organs involved | | | |
| ≥ 2 | 26 (74%) | 10 (67%) | 36 (72%) |
| ≥ 3 | 12 (34%) | 7 (47%) | 19 (38%) |
| Organ involvement, n % | | | |
| Kidney | 29 (83%) | 12 (80%) | 41 (82%) |
| Heart | 23 (66%) | 10 (67%) | 33 (66%) |
| Nervous system | 4 (11%) | 3 (20%) | 7 (14%) |
| Gastro intestinal | 4 (11%) | 0 (0%) | 4 (8%) |
| Liver | 8 (23%) | 5 (33%) | 13 (26%) |
| Soft tissues | 5 (14%) | 3 (20%) | 8 (16%) |
| MAYO classification | | | |
| I | 12 (34%) | 4 (27%) | 16 (32%) |
| II | 10 (29%) | 6 (40%) | 16 (32%) |
| III | 12 (34%) | 5 (33%) | 17 (34%) |
| NYHA stage | | | |
| I | 22 (63%) | 6 (40%) | 28 (56%) |
| II | 12 (34%) | 9 (60%) | 21 (42%) |
| NT pro BNP level (ng/l) | 675 (166-1638) | 1110 (264-2292) | 832 (204-1638) |
| Echo cardiography | | | |
| Mean left ventricular wall thickness | 12 mm (11-15) | 13 mm (12-14) | 13 mm (11-15) |
| Ejection fraction | 63% (55-71). | 58% (53-66). | 60% (55-68). |
| Diastolic dysfunction | 60% present | 60% present | 60% present |

| | | | |
|---|---|---|---|
| eGFR | 68 (58-87) | 90 (60-95) | 72 (59-90) |
| Renal stage | | | |
| I | 12 (34%) | 8 (53%) | 20 (40%) |
| II | 19 (54%) | 6 (40%) | 25 (50%) |
| III | 4 (11%) | 1 (7%) | 5 (10%) |

Table 2, Percentage (%) of hematologic responses during treatment phases.

First percentage concerns patients who received auto SCT (35 in total), second percentage between brackets is the response rate assessed as intention to treat analysis

| | After induction therapy | +6 months post auto SCT (35 pts) | +12 months post auto SCT (34 pts) | +24 months post auto SCT (33 pts) |
|---|---|---|---|---|
| Hematologic response (intention to treat) | | | | |
| ≥PR (ORR) | 83 (80) | 86 (60) | 89 (60) | 91 (60) |
| ≥VGPR | 66 (58) | 72 (50) | 77 (52) | 76 (50) |
| CR | 23 (20) | 46 (32) | 56 (38) | 58 (38) |
| | | | | |
| Median time to first response | 28 days | | | |
| Median time to max response | 67 days | | | |

Table 3, percentage (%) of most common treatment related adverse events of bortezomib-dexamethasone induction treatment

| ADVERSE EVENT | GRADE 1 | GRADE 2 | GRADE 3 | GRADE 4 | GRADE 5 | TOTAL |
|---|---|---|---|---|---|---|
| Nervous system, total | 12 | 24 | 12 | 2 | - | 50 |
| - sensory PNP | 12 | 14 | 6 | 2 | - | 34 |
| - autonomic (syncope, dizziness, orthostatic hypotension) | - | 6 | 4 | - | - | 10 |
| - motoric | - | - | 2 | - | - | 4 |
| - neuropathic pain | - | 4 | - | - | - | |
| Gastro intestinal (constipation, diarrhea, nausea, vomiting) | na | 30 | 10 | 4 | - | 44 |
| Infections | na | 20 | 10 | - | - | 30 |
| Cardiac | na | 12 | 12 | 2 | 2 | 28 |
| Metabolic/nutrition | na | 18 | 6 | - | - | 24 |
| Fatigue | na | 10 | 2 | - | - | 12 |

PNP = polyneuropathy, na = not assessed

**Figure Legends**

Figure 1:

Consort diagram of 50 included patients

Figure 2:

Upper line denotes time to first response, defned as partial respons (PR) or better, and lower line time to first complete response (CR)

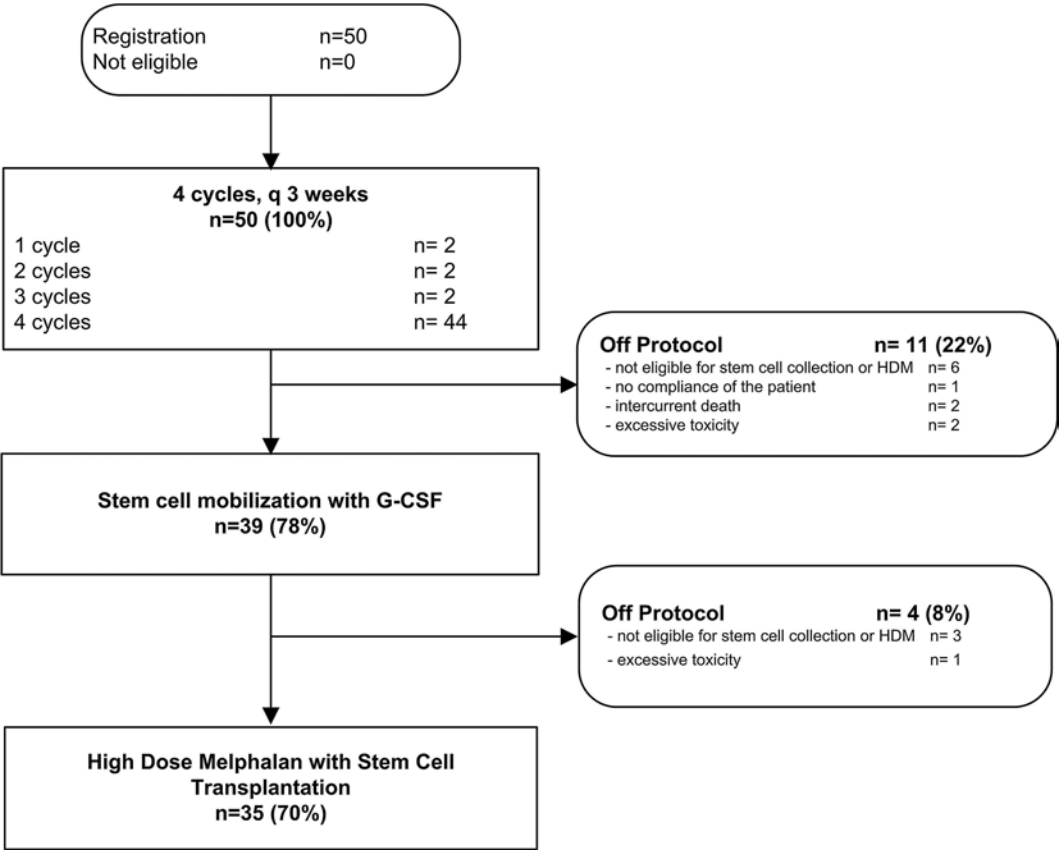
Figure 3:

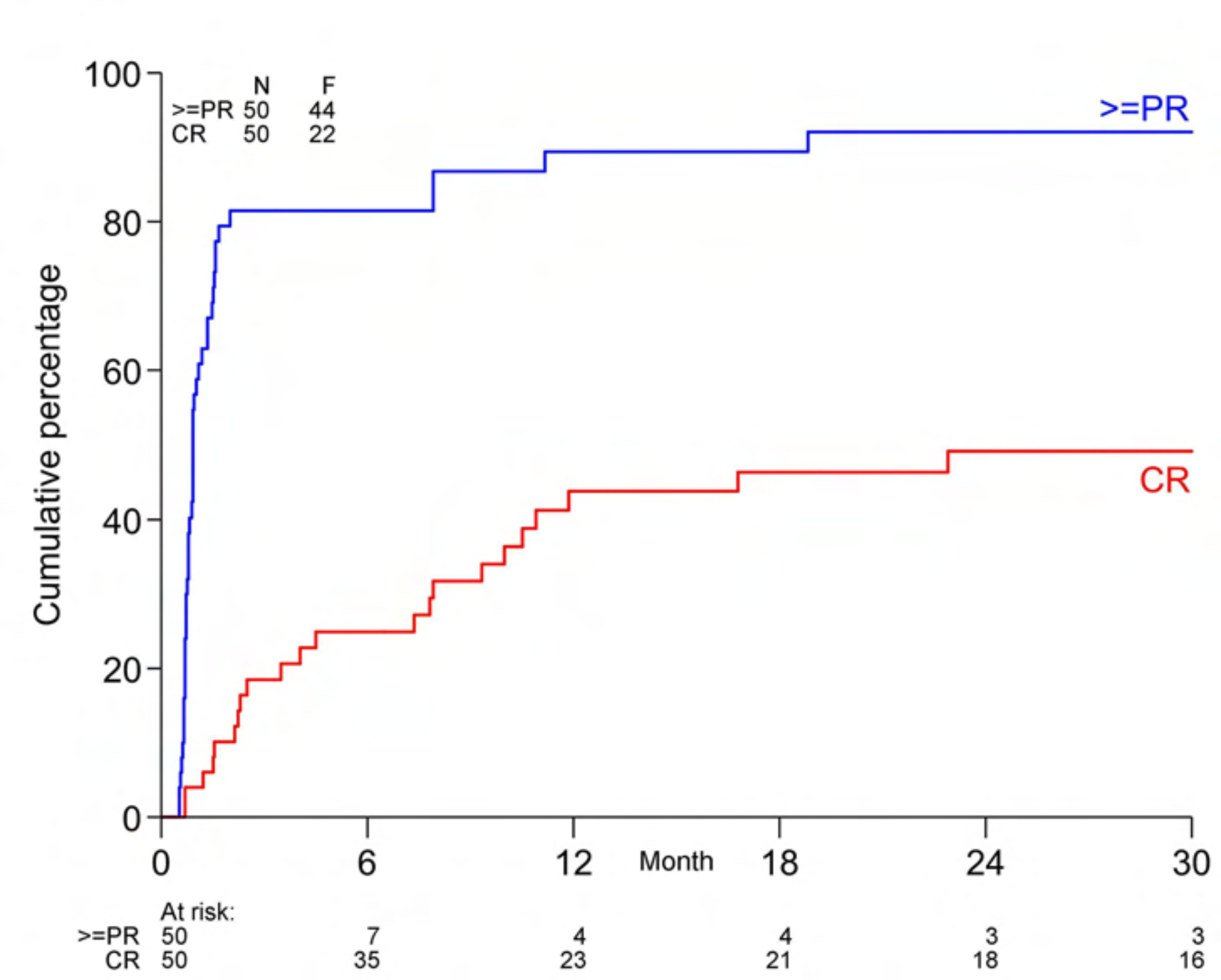
Organ responses depicted separately for patients proceeding to auto-SCT or not. Organ responses were assessed according to consensus criteria after induction and as best responses achieved during study treatment and follow up. BD = bortezomib-dexamethason, SCT = stemcell transplantation

Figure 4:

Overall survival (OS) and progression free survival (PFS) of total cohort and 35 patients with auto-SCT.
A: PFS and OS from date of registration for 50 patients included, B: PFS from auto-SCT of 35 transplanted patients according to response achieved after induction therapy. PR = partial remission, VGPR = very good partial remission, SCT = stem cell transplantation

Flow diagram of the 50 eligible patients in Bor-Dex arm of HO104 AL amyloidosis




N F
>=PR 50 44
CR 50 22
>=PR
CR
Cumulative percentage
100
80
60
40
20
0
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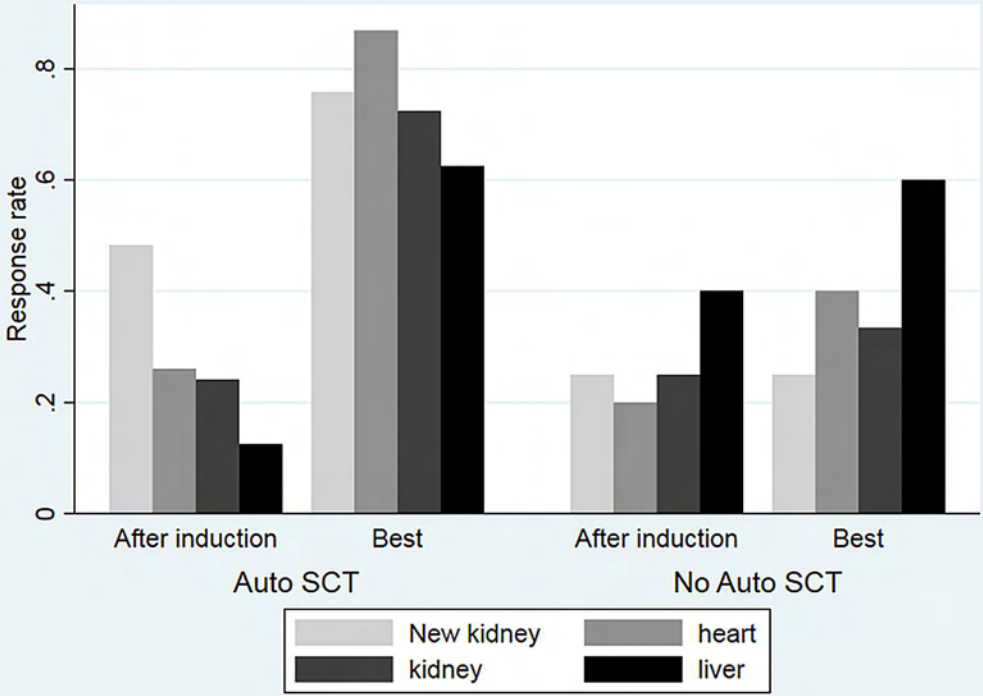
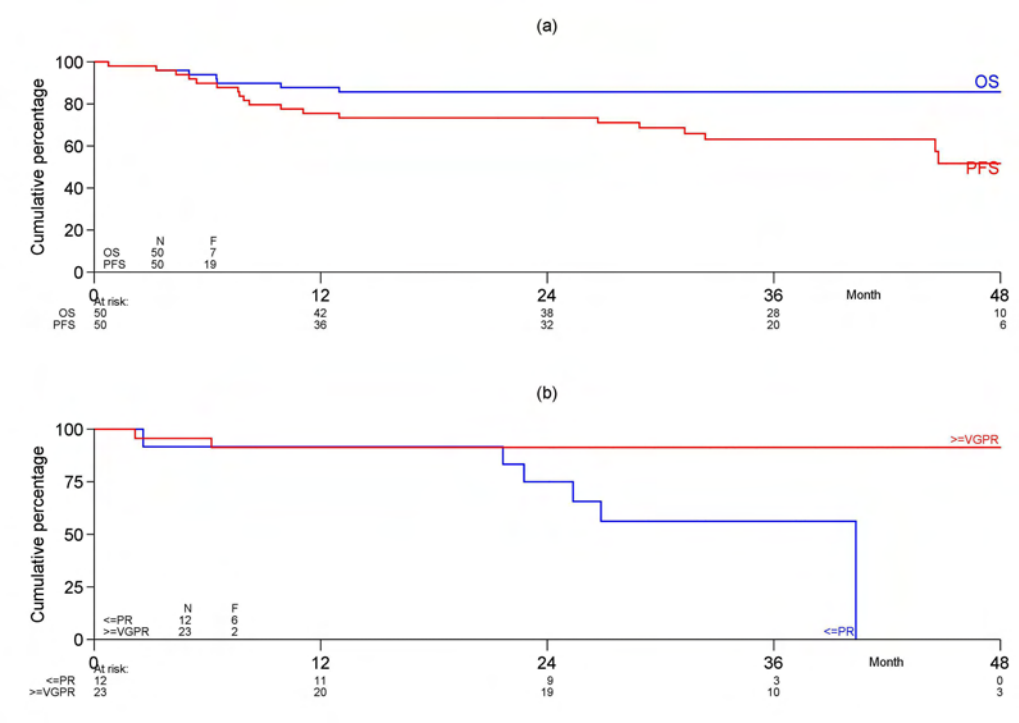
30
Month
At risk:
>=PR 50 7 4 4 3 3
CR 50 35 23 21 18 16


Response rate
0
.2
.4
.6
.8
After induction
Best
After induction
Best
Auto SCT
No Auto SCT
New kidney
heart
kidney
liver


(a)
Cumulative percentage
100
80
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48
At risk:
OS 50 42 38 28 10
PFS 50 36 32 20 6
(b)
Cumulative percentage
100
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25
0
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<=PR 12 11 9 3 0
>=VGPR 23 20 19 10 3

HOVON 104, supplementary data

Methods

The study was approved by the ethics committees of the UMC Utrecht, the University of Heidelberg, and all participating sites. All patients gave written informed consent. The study was conducted in accordance with the European Clinical Trial Directive and the Declaration of Helsinki (registered at *www.trialregister.nl*; NTR3220).

The full protocol can be seen at the HOVON site: http://www.hovon.nl/studies/studies-per-ziektebeeld/mm.html?action=showstudie&studie_id=82&categorie_id=3

Additional exclusion criteria;

- Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form,
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule,
- Pregnant or breast feeding females,
- Presence of other active malignancy or a history of active malignancy during the past 5 years, with the exception of nonmelanoma skin cancer, stage 0 cervical carcinoma, or treated early-stage prostate cancer provided that prostate-specific antigen is within normal limits,
- Hypersensitivity to boron or mannitol,
- Uncontrolled infection,
- Positive for HIV or infectious hepatitis, B or C (screening obligatory),
- bilirubin > 2x ULN
- Absolute neutrophil count < $1.0 \times 10^9$/L,

- Concurrent diagnosis of B-cell NHL or B-CLL,
- Previous organ transplantation,
- Unwilling or unable to use adequate contraception

Exclusion criteria before stem cell mobilization were symptomatic pleural effusions, uncontrolled infection, symptomatic orthostatic hypotension, ANC < $1.0x10^9$/L, bilirubin > 2x ULN, and the start of SCM > 12 weeks after start of the last course of bortezomib treatment.

Treatment design

Patients with pre-existing PNP grade 2 without neuropathic pain or PNP grade 1 with neuropathic pain at study entry started with a 25% dose reduction of bortezomib, i.e. 1.0 mg/$m^2$ and once per week schedule in a 5 week cycle. Also patients with a moderate or severe hepatic dysfunction at study entry or during treatment received a lower dose of bortezomib of 0.7 mg/$m^2$. Guidelines for dose reductions of both bortezomib and dexamethasone were installed for hematologic and non-hematologic toxicities.

*Hematologic and organ response criteria*

CHR definition consisted of a negative serum and urine M protein immunofixation (IF) test and a normal FLC ratio with a normal absolute value of the iFLC. Very good partial response (VGPR) was defined as the difference between involved and uninvolved FLC (dFLC) of < 40 mg/l. All values were calculated from baseline, or from lowest value reached (nadir) and all progression categories require two consecutive assessments.

*Flow cytometry protocol*

For the analysis of PC in BM, 1 x $10^6$ cells were incubated with titrated monoclonal antibodies (CD19, CD27, CD38, CD45, CD56, cytKappa, cytLambda and CD138) . In short, sample incubation was performed in a total volume of 180 µl. Cells were incubated for 15 minutes at room temperature in the dark. After washing, the cells were fixed, (reagents A, IntraStain, Dako Cytomation) washed and incubated with intra-cytoplasmic VS38c, lambda and kappa and permeabilisation reagents (reagents B, IntraStain, Dako Cytomation) for 15 minutes at room temperature. After the final washing step cells were directly analyzed. IntraStain was used according to manufacturer's procedure for simultaneous detection of surface and intracellular antigens. PC were identified as described by Rawstron et al. Using CD38, CD138 and light scatter characteristics PC's were identified and sequentially analyzed for CD56, CD19 and cytoplasmic kappa or lambda light chain expression. Total amount of plasmacells from the whole nucleated bone marrow cellularity as well as total amount of abnormal plasmacells were calculated. .Cell analysis was performed on a 3-laser Canto II flowcytometer (Becton Dickinson).

*Statistical design and endpoints*

For the sample size calculation the following assumptions were made. The null hypothesis was a CR rate at 6 months after SCT (CHR6mo) of 30% and we expected an increase of the CHR6mo to 50%. With an 80% power and 2-sided significance level of $\alpha = 0.05$, 44 eligible patients were required based on sample size calculation of one-sample proportion. In order to overcome possible dropouts due to ineligibility, 50 patients were registered.

Secondary endpoints were overall survival (OS), which was measured from the time of registration until death, patients still alive or lost to follow up were censored at the day they were last known to be alive, progression free survival (PFS), which was measured from time of registration until hematologic progression, relapse or death, whichever occurs first, hematologic response rate after induction therapy, maximal response rate, both hematologic and organ, time to response, both hematologic (defined as ≥PR) and organ, duration of response, both hematologic and organ, safety (type, frequency, and severity of adverse events (CTCAE grade ≥ 2 except for neurotoxicity for which also grade 1 was recorded), relationship of AE to study drug, and evaluation of prognostic factors for survival. OS and PFS analysis were performed using a Kaplan-Meier method from date of registration till cutoff date on 28 August 2018.

Potential baseline characteristics that may affect patients not proceeding to SCT after induction therapy were tested via a univariate logistic regression model. These baseline characteristics were type of hospital (high vs low number of included patients), eGFR >30 and < 50 vs eGFR ≥50 ml/min, NYHA I vs II, NT- proBNP as continuous variable, plasma cells < 10% vs ≥ 10% , dFLC < vs ≥ 180 mg/L, number of organs involved ≤2 vs >2, MAYO stage, nervous system involvement and cardiac involvement. The impact of aforementioned baseline characteristics were also tested on OS and PFS via univariate Cox regression. For flow cytometry data, we computed aPCs/BMPC ≥ 95% as a binary variable and tested the association of this binary variable with not proceeding to auto-SCT, PFS, and OS. For these analyses we also used a logistic regression model and a univariate Cox regression.

A 2-sided p-value of <0.05 was considered statistically significant and all analysis were performed using Stata 15.1.