

## ORIGINAL ARTICLE

# A prospective randomized controlled trial comparing PCR-based and empirical treatment with liposomal amphotericin B in patients after allo-SCT

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**We compared the efficacy and safety of empirical plus PCR-based vs empirical liposomal amphotericin B treatment after Allo-SCT. Allo-SCT recipients were randomized to receive either PCR-based preemptive therapy (group A; n = 198) or empirical antifungal therapy (group B; n = 211) with liposomal amphotericin B. In group A, therapy was started after one positive PCR result or after 120 h of febrile neutropenia refractory to broad-spectrum antibacterial therapy. In group B, liposomal amphotericin B was started after 120 h of refractory febrile neutropenia. Demographic and clinical characteristics were well balanced. A total of 112 (57.1%) patients in group A and 76 (36.7%) patients in group B received antifungal therapy ( $P < 0.0001$ ). Twelve patients in group A and 16 patients in group B developed proven invasive fungal infection (IFI). Survival curves showed better survival until day 30 when close PCR monitoring was performed (mortality 1.5 vs 6.3%;  $P = 0.015$ ), but there was no difference at day 100. At day 100, no difference was observed in the incidence of IFI (primary end point) and survival between the two arms. Further studies are required to assess the benefit of using PCR in patients after SCT.**

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## Introduction

Up to 15% of patients undergoing Allo-SCT develop invasive fungal infections (IFIs) after transplantation.<sup>1</sup> IFIs are associated with high mortality. A systematic review of the literature calculated a case fatality rate of 86.7% for SCT recipients with invasive aspergillosis.<sup>2</sup> A prospective multicenter surveillance study showed a mortality rate of about 40% within 30 days after the onset of candidemia in patients with leukemia.<sup>3</sup> Major risk factors for IFI in SCT patients include long-lasting neutropenia, compromised granulocyte and macrophage functions, depression of cellular and humoral immunity as a consequence of severe GVHD and/or immunosuppression, breakdown of natural antimicrobial barriers owing to chemotherapy-related mucositis, indwelling venous catheters and/or alterations of the endogenous microflora.<sup>1</sup>

The most common pathogens that cause deep fungal infections are *Candida* and *Aspergillus* spp., but with increasing frequencies, uncommon fungal pathogens such as *Trichosporon* spp., Zygomycetes and *Fusarium* spp. are reported as underlying pathogens.<sup>4</sup> To reduce the high mortality rate, antifungal therapy should be initiated early in SCT patients, but rapid diagnosis of systemic fungal infections is difficult owing to frequent delays in culture detection of fungal species. Furthermore, up to 75% of disseminated candidiasis cases are missed and invasive pulmonary aspergillosis is often diagnosed at autopsy only.<sup>5</sup> With the exception of the *Aspergillus*-directed galactomannan enzyme immunoassay and 1,3- $\beta$ -glucan detection assays, the serological kits available for the detection of fungal antigens have inconsistent sensitivity, specificity or both.<sup>6–11</sup> Thus, techniques with higher sensitivity and specificity for detection of fungal pathogens, such as fungus-specific DNA probes and the PCR assays, are urgently needed.

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PCR-based assays of blood samples make the early identification of patients at risk for IFIs possible.<sup>12</sup> High sensitivity (100%) and specificity (73%) of a new panfungal PCR assay were shown in febrile neutropenic patients without a history of IFI.<sup>12</sup> A French investigation confirmed the improvement of early diagnosis of proven or probable invasive aspergillosis by PCR assays of serum samples showing a sensitivity of 63.6% and a specificity of 89.7%.<sup>13</sup> Frequent PCR testing in patients with a high risk of IFI (for example, patients after SCT) may enable a diagnosis of IFI before the onset of symptoms and an earlier start of treatment.<sup>12</sup> However, PCR is not considered as a microbiological tool for the diagnosis of IFI in the new European Organization for Research and Treatment of Cancer/Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions.<sup>14</sup>

Amphotericin B has antifungal properties against a wide range of pathogens including *Candida* and *Aspergillus* species. However, its use is limited by acute toxicity. The encapsulation of amphotericin B into the membrane of liposomes offers an interesting approach to reduce toxicity while maintaining or even enhancing efficacy.<sup>15,16</sup> A randomized double-blind trial comparing liposomal amphotericin B (Gilead Sciences, Martinsried, Germany) with conventional amphotericin B in 787 patients with fever of unknown origin showed similar efficacy in both groups, but fewer breakthrough fungal infections and less nephrotoxicity in patients receiving liposomal amphotericin B.<sup>17</sup> In a further randomized study, liposomal amphotericin B in the empirical setting of fever of unknown origin was shown to be effective even at a dose level of 1 mg/kg body weight.<sup>18</sup> Furthermore, the dosage of liposomal amphotericin B can be increased safely to dosages higher than 3 mg/kg if necessary.<sup>19</sup>

To investigate the impact of PCR as a diagnostic tool for guiding preemptive antifungal therapy, we compared the incidence of IFIs as well as the overall and IFI-related mortality in patients after Allo-SCT randomized to PCR-based preemptive as opposed to empirical treatment with liposomal amphotericin B.

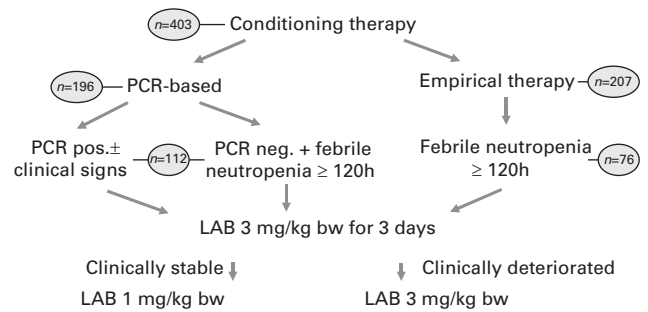
## Materials and methods

### Patients

Study patients were required to receive Allo-SCT (BMT or peripheral blood progenitor cell transplantation from related and unrelated donors). Patients with documented allergy against liposomal amphotericin B, history of IFI at the time of randomization or before the study entry were excluded.

### Settings and design

This prospective randomized controlled multicenter study was conducted between July 1998 and June 2001 in five different centers. Patients were randomized centrally and stratified according to the study center and type of transplant (matched-unrelated and HLA-mismatched donor vs allogeneic sibling donor). Written informed



**Figure 1** Course of the study with 403 patients (ITT population) undergoing Allo-SCT randomized to PCR-based or empirical antifungal therapy. bw = body weight; ITT = intention-to-treat; LAB = liposomal amphotericin B.

consent was given by all patients. The protocol was approved by independent ethics committees in all participating centers.

### Treatment

After informed consent, which had to be obtained before SCT, patients were randomly assigned to PCR-based antifungal therapy (group A) or to empirical antifungal therapy (group B) (Figure 1). SCT was performed according to local guidelines.

Patients in group A were treated with liposomal amphotericin B after one positive PCR result. If patients in group A had febrile neutropenia for more than 120 h not responding to broad-spectrum antibacterial therapy, they also received liposomal amphotericin B, even if PCR remained negative. Patients in group B received empirical antifungal therapy after 120 h of febrile neutropenia not responding to broad-spectrum antibacterial therapy. Furthermore, treatment with liposomal amphotericin B was started in both groups after the detection of a pulmonary infiltrate. The recommendation was to give liposomal amphotericin B i.v. at a dose of 3 mg/kg body weight for 3 days followed by 1 mg/kg body weight in clinically stable patients. If the patient's condition deteriorated, the dosage was kept at 3 mg/kg body weight. It was at the discretion of the physician to deviate from the recommended dose if the condition of the patient deteriorated. Antifungal treatment lasted for a minimum of 7 days. PCR-based liposomal amphotericin B therapy was stopped as soon as the granulocyte count had recovered ( $> 500/\mu\text{l}$  for 3 consecutive days), or if the patient had been afebrile for a minimum of 3 days, presented no signs and symptoms of IFI, and two consecutive PCR results were negative. Empirical liposomal amphotericin B was stopped if the granulocytes had recovered ( $> 500/\mu\text{l}$  for 3 consecutive days), or if the patient had been afebrile for a minimum of 3 days and presented no radiological signs of invasive aspergillosis.

All patients received oral antifungal prophylaxis with fluconazole 200 mg/day and/or  $4 \times 5$  ml/day of amphotericin B suspension. During treatment with liposomal amphotericin B, other antifungal drugs were not allowed except oral nystatin.

### PCR screening

Beginning prior to transplantation until day 100, post-transplant EDTA-anticoagulated blood samples (10 ml) were drawn in group A twice weekly until day 30 and once weekly after day 30 until day 90. Blood samples were analyzed by PCR for fungal DNA on the following day. The same PCR screening was established and performed in three different centers.<sup>20</sup>

The designs of the oligonucleotide primer pair (5'-ATTGGAGGGCAAGTCTGGTG and 59-CCGATCCC-TAGTCGGCATAG) and the DNA probes for *C. albicans* (TCTGGGTAGCCATTTATGGCGAACCAGGAC), *C. glabrata* (TTCTGGCTAACCCCAAGTCCTTGTGGCTTG), *C. krusei* (GTCTTTCCTTCTGGCTAGCCTCGGGCGAAC), *C. tropicalis* (GTTGGCCGGTCCATCTTTCTGATGCGTACT) and *C. parapsilosis* (TTTCTTCTGGCTAGCCTTTTTGGCGAACC), as well as the *Aspergillus* DNA probe (CATGGCCTTCACTGCTGTGGGGGAACCA) and the DNA probe specifically hybridizing with *A. fumigatus*, *A. flavus* and *A. versicolor* (TGGGGAACCTCATGGCCTTCACTGGCTGTG) used in this study were derived from a comparison of the sequences of 18S rRNA genes (ribosomal DNA) in the GenBank database (EMBL and DDBJ databases). The primers target a consensus sequence for a variety of fungal pathogens. Amplification with the primers described above yields a 482- to 503-bp fragment, depending on the fungal pathogen tested.<sup>21</sup> It detects and identifies the clinically relevant *Candida* and *Aspergillus* species at a reproducible sensitivity of 10 colony forming units (CFUs) per ml blood.

### Evaluations

Investigations included the history of the disease, physical examinations and daily assessments of the performance status as well as laboratory variables, such as blood count, differential blood count, creatinine, bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase. If IFI was suspected, CT scans of the lungs, contrast-enhanced CT scans of the liver and spleen, sequential fungal cultures from the central venous line and peripheral blood were performed to confirm the diagnosis. All radiological examinations were reviewed by radiologists 'blinded' to the treatment group of the patient.

For tolerability assessment, adverse events possibly related to liposomal amphotericin B were documented, and vital signs, performance status and changes of laboratory parameters were reported.

### Criteria of evaluation

The primary study objective was the incidence of IFIs during the observation period of 100 days after transplantation. Secondary objectives were survival at day +100 (overall mortality), survival at day +30, IFI-related mortality, incidence and spectrum of adverse drug reactions.

The study was designed before the publication of the definition of the criteria for IFI by the Invasive Fungal Infections Cooperative Group of the EORTC/MSG.<sup>22</sup> Therefore, IFI were classified *a posteriori* as proven,

probable or possible according to the criteria of the Invasive Fungal Infections Cooperative Group of the EORTC. The classification of fungal infections was not based on the results of the PCR testing.

### Study drug

For antifungal treatment, liposomal amphotericin B (AmBisome) was applied as a 1- to 3-h infusion.

### Statistical analysis

Statistical analysis was performed in two steps. Demography, IFI incidence and survival were evaluated by the Institute for Medical Information Processing of the Tuebingen University (step 1) according to the statistical plan in the study protocol. The analysis of antifungal treatment with liposomal amphotericin B, of IFI severity score according to the newly introduced EORTC/MSG classification, and of IFI-related mortality was performed by two MD students as part of their medical thesis (step 2).

The study was designed to test the incidence of IFIs during the observation period of 100 days after transplantation. Analysis was performed for the intention-to-treat (ITT) population.

The last observation was carried forward if patients had died or were lost to follow-up. By means of a two-sided Mantel-Haenszel test, the null hypotheses of equal incidence rates in both treatment groups were tested for  $\alpha = 0.01$  (global level of the trial, one-sided). Furthermore, explorative analyses of other parameters of efficacy, such as survival at day 30, IFI incidence until day 30, duration of antifungal therapy, the number of courses given, tolerability, adverse events and toxicity were carried out. Survival rates were compared by using the Kaplan-Meier estimator and the log-rank test. Laboratory parameters were described by statistical characteristics stratified for treatment (changes vs baseline included). The last values before randomization were used as baseline values for the above mentioned tests.

$\alpha = 0.01$  (one-sided) was determined as the global significance level of the trial for the primary end point. As one interim analysis was carried out, the level for each test was  $\alpha = 0.005$  (one-sided, Bonferroni-adjusted). Experience with earlier trials suggested an incidence of IFI of approximately 15% in the empirically treated group.<sup>23-25</sup> In the PCR-treated group, an IFI incidence of 5% was expected. To detect a reduction in the incidence of IFI by 66% under PCR-based therapy, 442 patients (221 per treatment group) were required ( $\beta = 0.15$  for a one-sided Fisher test). As the comparison was tested statistically by means of a Mantel-Haenszel test, which allows for adjustments for stratification variables, the power might be slightly lower. As all efforts had to be undertaken to follow the patients for the above mentioned primary end point, a low dropout rate of about 5% seemed achievable. Thus, about 464 patients were intended to be included in the trial.

The results of statistical tests for the comparison of the secondary end points and for subgroup analysis are presented with two-sided *P*-values not adjusted for multiple testing. These results were not interpreted in a confirmatory sense.

In a prespecified interim analysis, a low incidence of IFI was documented. The analysis showed an incidence of proven and probable IFI of 8.2% in both groups. Thus, the statistical calculation indicated a much higher number of patients to be recruited for achieving statistically significant differences. Therefore, the study was terminated prematurely.

## Results

A total of 403 patients were included in the ITT population and evaluated for safety. Six randomized patients (two randomized to group A and four randomized to group B) were excluded from the ITT analysis because of different reasons (no SCT, patient wished to withdraw and exclusion/inclusion criteria not fulfilled).

At baseline, demographic and clinical characteristics such as age, sex, Karnofsky score, HLA type of donor, type of graft and underlying disease characteristics were well balanced between the two groups (Table 1). As expected, the percentage of patients treated with liposomal amphotericin B was higher in group A, which received PCR-based antifungal therapy or classical empirical treatment in PCR-negative patients who remained in febrile neutropenia for  $\geq 5$  days despite broad spectrum antibacterial therapy (112 of 196 patients; 57.1%), compared with group B, which received empirical antifungal therapy only (76 of 207 patients; 36.7%;  $P < 0.0001$  with a risk ratio of 1.5, 95% CI: 1.2–1.9).

Compliance until 5 weeks post transplant was as follows: 47 of 196 (24%) patients had blood samples analyzed by PCR twice weekly until the end of the fifth week in respect of the end of their initial hospital stay, if discharge was before the fifth week. In the other patients, one or more PCR tests were missed because of various reasons.

## Treatment

A total of 112 patients in group A received a total of 162 antifungal treatment courses with liposomal amphotericin B (1.45 courses per patient), whereas in group B, 76 patients received 93 treatment courses (1.22 courses per patient). The most frequent indications for antifungal therapy with liposomal amphotericin B were positive PCR tests (49.7%), fever (27.2%) and pulmonary infiltrates (12.4%) in group A and fever (58.0%) and pulmonary infiltrates (22.6%) in group B. Liposomal amphotericin B was dosed as recommended (3 mg/kg/day for 3 days and 1 mg/kg/day thereafter) in 80 of 162 (49.4%) treatment courses of group A and in 47 of 93 (50.5%) treatment courses of group B. In both groups, liposomal amphotericin B was given for a median duration of 10 days. Further details of treatment with liposomal amphotericin B are presented in Table 2.

## IFI incidence

Twelve proven and five probable IFIs were detected in 16 of the 196 patients of the ITT population randomized to PCR

**Table 1** Demographic and clinical characteristics of 403 patients undergoing Allo-SCT randomized to PCR-based or empirical antifungal therapy

Characteristics	All patients (n = 403)	PCR-based therapy (n = 196)	Empirical therapy (n = 207)
Male	227 (56.3)	106 (54.1)	121 (58.5)
Female	176 (43.7)	90 (45.9)	86 (41.5)
Age (mean; range)	32.8 (0.1–66.7)	32.2 (0.1–66.7)	33.4 (0.6–65.7)
Age < 20 years	125 (31.0)	66 (33.7)	59 (28.5)
<i>Karnofsky score</i>			
< 80	16 (4.0)	10 (5.1)	6 (2.9)
80	48 (11.9)	22 (11.2)	26 (12.6)
90	283 (70.2)	138 (70.4)	145 (70.0)
100	56 (13.9)	26 (13.3)	30 (14.5)
<i>HLA-type of donor</i>			
HLA-identical sibling	170 (42.2)	82 (41.8)	88 (42.5)
Identical twin	1 (0.2)	0	1 (0.5)
HLA-matched unrelated donor	161 (40.0)	83 (42.3)	78 (37.7)
HLA-mismatched unrelated donor	40 (9.9)	17 (8.7)	23 (11.1)
Other relative	31 (7.7)	14 (7.1)	17 (8.2)
<i>Type of transplant</i>			
BM	160 (39.7)	80 (40.8)	80 (38.6)
Peripheral blood	241 (59.8)	115 (58.7)	126 (60.9)
BM plus peripheral blood	2 (0.5)	1 (0.5)	1 (0.5)
<i>Underlying disease</i>			
AML	98 (24.3)	44 (22.4)	54 (26.1)
ALL	86 (21.3)	46 (23.5)	40 (19.3)
CML	80 (19.9)	45 (23.0)	35 (16.9)
MDS	30 (7.4)	17 (8.7)	13 (6.3)
Other	109 (27.0)	44 (22.4)	65 (31.4)

Abbreviation: MDS = myelodysplastic syndrome.  
Data are presented as number (%).

**Table 2** Treatment with liposomal amphotericin B in 403 patients (ITT population) undergoing Allo-SCT randomized to PCR-based or empirical antifungal therapy

Treatment with liposomal amphotericin B	Group A (PCR-based treatment) (n = 196)			Group B (empirical treatment) (n = 207)		
	All treatments	Until day 30	After day 30	All treatments	Until day 30	After day 30
Patients treated <sup>a</sup>	112 (57.1)	89	23	76 (36.7)	64	12
Start of treatment (days after SCT; mean)	21.0	11.4	57.6	19.5	11.2	63.3
Treatment courses	162	103	59	93	69	24
Dose reduction at day 4	80	49	31	47	34	13
No dose reduction at day 4	79	54	25	46	35	11
Dose unknown	3	0	3	0	0	0
Total amount of liposomal amphotericin B given (median (mean; mg/kg))		18.1 (29.2)			19.5 (22.9)	

Abbreviation: ITT = intention-to-treat.

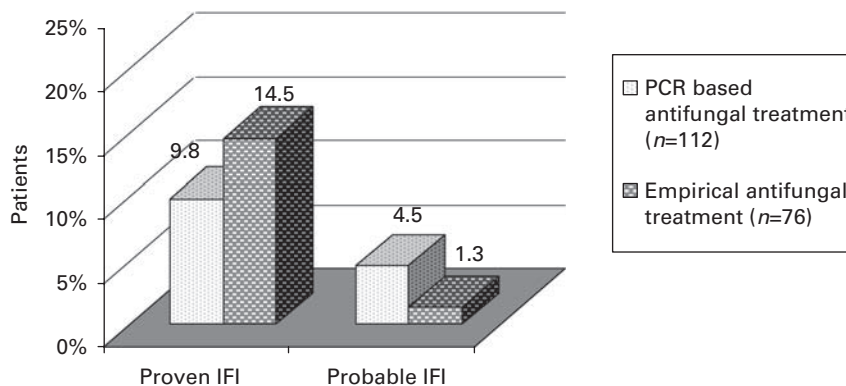
<sup>a</sup>Number (%).

**Table 3** Incidence of IFIs in 403 patients (ITT population) after SCT according to treatment groups and treatment with liposomal amphotericin B

	Randomized to PCR testing (n = 196)			Randomized to empirical therapy only (n = 207)		
	Patients	Proven IFI	Probable IFI	Patients	Proven IFI	Probable IFI
Total	196	12	4 <sup>a</sup>	207	16	1
With Amph therapy	112	11	4 <sup>a</sup>	76	12	1
No Amph therapy	84	1	0	131	4	0

Abbreviations: Amph = amphotericin; IFI = invasive fungal infections.

<sup>a</sup>one patient with a probable and a proven IFI was counted as proven.



**Figure 2** Comparison of IFI incidence in patients randomized to PCR-based antifungal treatment (group A) and empirical antifungal treatment (group B). IFI = invasive fungal infection.

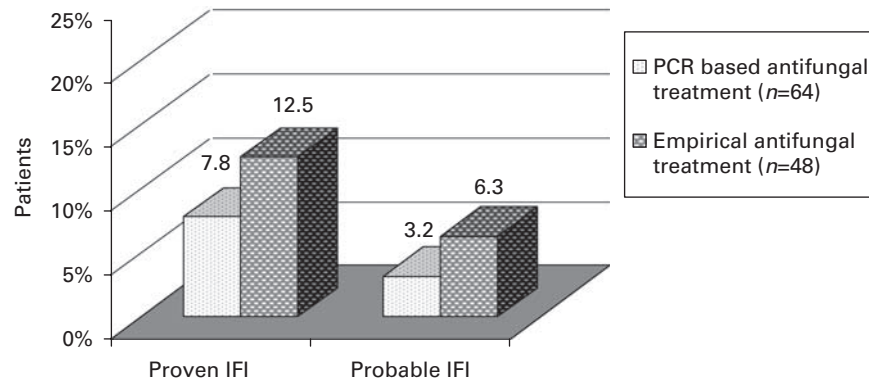
testing (8.2%) (one patient presented two IFI episodes: one episode was classified as proven; the other was classified as probable.). In the 207 patients of the ITT population randomized to empirical treatment, 16 proven and one probable IFI were detected (8.2%; Table 3). In the PCR group, only one patient with a proven IFI (1 of 12; 8.3%) was missed in contrast to 4 of 16 (25.0%) patients in the empirical group.

In the 112 treated patients in group A, 16 cases of IFI (14.3%) were diagnosed. Eleven IFIs were classified as proven and five as probable (one proven IFI episode occurred in a patient who was not treated with liposomal amphotericin B). Of the 76 treated patients in group B, 12 IFIs (15.8%) were diagnosed. Eleven IFIs were classified as proven and one as probable (Figure 2). Seven proven IFIs

in the PCR group and eight proven IFIs in the empirical group occurred before day 30. Patients in group A who had undergone at least one PCR-based treatment cycle tended to a lower incidence of IFI (Figure 3). The most frequent species among proven fungal infections were *Candida* (four cases in group A and 11 cases in group B), *Aspergillus* (four cases in group A and four cases in group B) and combined infections with *Candida* and *Aspergillus* (four cases in group A, and one case in group B).

### Survival

After a mean observation period of 91 days after transplantation, 66 deaths were reported, 32 (16.3%) in group A and 34 (16.4%) in group B. Survival did not differ



**Figure 3** IFI incidence in group A. Comparison of patients who received at least one PCR-based antifungal treatment cycle and patients who received empirical antifungal treatment only. The difference between the two groups (seven IFI in 64 patients with PCR-based antifungal treatment vs nine IFIs in 48 patients with empirical antifungal treatment) was not statistically significant ( $P=0.244$ ). IFI = invasive fungal infection.

**Table 4** Elevations of clinical laboratory values ( $\geq$ double baseline value) in patients undergoing Allo-SCT who received liposomal amphotericin B treatment

	All courses	Group A PCR-based treatment	Group B empirical treatment	Patients with dose reduction at day 4	Patients without dose reduction at day 4
$n^a$	244	152	92	123	121
Creatinine; $n$ (%)	67 (27.5)	39 (25.7)	28 (30.4)	29 (23.6)	38 (31.4)
$n^a$	235	149	86	118	117
Bilirubin; $n$ (%)	74 (31.5)	44 (29.5)	30 (34.9)	23 (19.5)	51 (43.6)
AST; $n$ (%)	121 (51.5)	70 (47.0)	51 (59.3)	49 (41.5)	72 (61.5)
ALT; $n$ (%)	109 (46.4)	65 (43.6)	44 (51.2)	44 (37.3)	65 (55.6)

Abbreviations: ALT = alanine amino transferase; AST = aspartate amino transferase.

<sup>a</sup>Number of treatment courses.

at day 100 post transplantation. However, during the regular PCR monitoring until day 30, a survival advantage in the group with PCR-based therapy was reported when compared with the empirically treated group (mortality: 1.5 vs 6.3%;  $P=0.015$ ). In group A, three patients died before day 30 of infection/multiorgan failure and one patient of hemorrhage; in group B, nine patients died of infection/multiorgan failure, two patients of veno-occlusive disease and one patient each of hemorrhage and GVHD. One patient of group A and five patients of group B who died before day 30 had a proven IFI ( $P=0.103$ ; Fisher's exact test).

Mortality caused by fungal infections was 6.3% (seven of 112 patients; four cases of *Aspergillus*, two cases of *Candida* and one case not specified) in patients with PCR-based liposomal amphotericin B therapy (group A) compared with 13.2% (10 of 76 patients; six cases of *Aspergillus*, four cases of *Candida*,  $P=0.106$ ) in patients who received empirical antifungal treatment (group B). None of the 47 patients in group A with dose reduction of liposomal amphotericin B to 1 mg/kg on day 4 died of fungal infections, whereas 7 of 64 (10.9%) patients who needed higher doses of liposomal amphotericin B died of fungal infections (no data available for two patients.). In group B, four of 36 (11.1%) patients treated according to the protocol and 6 of 40 (15%) patients who did not respond and were maintained on higher dosages of liposomal amphotericin B died of fungal infections. A total of 22 (19.6%) patients in group A and 18 (23.7%) patients in

group B died during ongoing therapy with liposomal amphotericin B.

### Safety

Generally, liposomal amphotericin B was well tolerated. Treatment was discontinued in 28 of 255 (11.0%) treatment cycles. In 24 cases, treatment was discontinued because of adverse events, in two cases because of insufficient efficacy and in two patients because of progression of the underlying disease. The laboratory variables are presented in Table 4.

### Discussion

To our knowledge, this is the first randomized controlled study that compares PCR-based antifungal treatment vs empirical treatment in patients after Allo-SCT. Even if the incidence of IFI and survival at day 100 post transplant did not differ between the groups and the main study goal was not reached, there are several important findings: (1) During the period with frequent PCR testing until day 30, overall survival was better in the PCR group, (2) It was shown that PCR testing could be performed successfully in three laboratories including patients from five clinical institutions, (3) Treatment with liposomal amphotericin B is safe and well tolerated. However, the study has several limitations. The primary objective (reduction of IFI until

day 100) could not be achieved and the incidence of IFI as well as mortality at day 100 post transplant was not reduced in group A, probably because of a lower than expected incidence of IFI in the empirical treatment group and the resulting underpowering of the study.

The lower incidence of IFI compared with earlier studies<sup>1</sup> might be caused by the high efficacy of liposomal amphotericin B treatment and/or the recent progress in general care of SCT patients. After the introduction of liposomal amphotericin B in one of our centers, the death rate by IFI decreased from 11 to 5%.<sup>26</sup> Furthermore, the low incidence could be related to the considerable number of CML patients (about 20%) who are known to have a lower IFI incidence than patients with acute leukemia. The better survival of patients with proven and probable IFI suggests that the earlier start of antifungal treatment may have influenced the outcome of the fungal infection. This finding seems to be important, but further clinical studies are needed for confirmation.

Various new potential antifungal compounds, such as azols or echinocandins, have been introduced into clinical practice since the study period. We can only speculate about the potential results of a similar study using newer compounds. These compounds as well as combinations of newer compounds with liposomal amphotericin B are options for future studies investigating PCR in high-risk SCT patients.

One of our assumptions was that frequent PCR testing until day 100 and beyond can detect IFI early enough to achieve reduction of mortality by the immediate start of treatment. After day 30, regular PCR testing was difficult because most patients had already been discharged from hospital. Even until 5 weeks of post transplant, only 24% of patients had blood samples analyzed by PCR twice weekly (10 PCR analyses). However, this low compliance resulted partly from the fact that patients with eight or nine analyses were classified as non-compliant.

The frequency of PCR testing may partly explain the survival advantage until day 30 that was lost on day 100. Other authors tested more frequently. In a study that investigated a galactomannan EIA, diagnostic tests were performed daily.<sup>27</sup> *Aspergillus* infections were detected effectively, but the investigators failed to detect infections with other filamentous fungi and with *Candida* spp. Recently, the greater sensitivity of galactomannan EIA or PCR as compared with culture in the detection of *Aspergillus* spp. in bronchoalveolar fluid was confirmed by another group.<sup>28</sup> The high sensitivity of a PCR assay in the detection of *Aspergillus* spp. was shown in patients with cerebral aspergillosis.<sup>29</sup> The feasibility of PCR testing to detect *Candida* and *Aspergillus* spp. was further shown in blood samples and other body fluids.<sup>30</sup>

Treatment with liposomal amphotericin B was discontinued because of adverse events in 24 of 255 (9.4%) treatment cycles in this study. Treatment was discontinued only four times because of nephrotoxicity. This low rate of nephrotoxicity of liposomal amphotericin B, despite treatment with concomitantly administered nephrotoxic drugs, confirms the findings of a randomized double-blind comparison with conventional amphotericin B.<sup>17</sup>

In our study, 11 of 12 patients assigned to the PCR screening arm received antifungal therapy before IFI was proven in contrast to only 75% of the patients assigned to the empirical treatment arm (12 of 16). It seems that some patients who urgently needed antifungal treatment were missed by the criteria of empirical therapy but could possibly be detected by PCR. On the other hand, 50% of all treatment courses in the PCR group were started for the criteria of empirical therapy. At that time, we did not dare to withhold antifungal therapy in a PCR-negative patient with  $\geq 5$  days of antibiotic- refractory febrile neutropenia. It is not clear if all persistently febrile patients lacking a positive PCR result really needed antifungal treatment. If febrile patients with negative PCR had been excluded, the number of patients and the number of treatment cycles would have been similar in both groups.

Mean treatment duration with liposomal amphotericin B in this study was 10 days. If the incidence of proven or probable IFI is around 8%, both PCR-based and febrile neutropenia therapies result in significant overtreatment. However, early treatment might be life-saving, possibly by a reduced mortality owing to IFI.<sup>26</sup>

Our study shows that 1–3 mg/kg (body weight) liposomal amphotericin B can be administered without significant adverse effects even in patients after Allo-SCT. Liposomal amphotericin B can be reduced safely in clinically stable patients to 1 mg/kg on day 4, even in high-risk patients (allograft recipients). No IFI-related death occurred in patients with reduced dosage at day 4, whereas 7 of the 64 patients who were not clinically stable and remained on the higher dose died of IFI.

## Conclusions

This study was not able to show a benefit on the reduction of IFI in Allo-SCT by the use of PCR, and further studies are required to assess the benefit of using PCR in such settings, especially with PCR screenings  $\geq$  twice per week beyond day +30. At day 100, the incidence of IFI (primary end point) and the survival did not differ between the two arms. However, the group receiving PCR-based therapy had better survival at day 30. The reason for this difference is unknown and merits further study. Liposomal amphotericin B is a safe and effective treatment option in SCT patients with a high risk of IFIs. Dose reduction of liposomal amphotericin B from 3 to 1 mg/kg on day 4 seems to be feasible in clinically stable patients without the risk of breakthrough infections.

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### Conflict of interest

Hermann Einsele declares that he has received research support for performing this study from Gilead. All decisions regarding the study, including the premature termination, were made independently by the investigators and were not influenced by the sponsor. Per Ljungman declares that he has no conflict of interest during the design of the study. Later, he had been the chair of the DSMB for the AmBiload study sponsored by Gilead. All other authors declare no conflicts of interest.

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