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Predicting sinusoidal obstruction syndrome after allogeneic stem cell transplantation with the EASIX biomarker panel

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Running head: EASIX predicts SOS/VOD after alloSCT

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Abstract

No biomarker panel is established for prediction of sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD), a major complication of allogeneic stem cell transplantation (alloSCT). We compared the potential of the Endothelial Activation and Stress Index (EASIX), based on lactate dehydrogenase, creatinine, and thrombocytes, with that of the SOS/VOD CIBMTR clinical risk score to predict SOS/VOD in two independent cohorts. In a third cohort, we studied the impact of endothelium-active prophylaxis with pravastatin and ursodeoxycholic acid (UDA) on SOS/VOD risk. The cumulative incidence of SOS/VOD within 28 days after alloSCT in the training cohort (Berlin, 2013-2015, n=446) and in the validation cohort (Heidelberg, 2002-2009, n=380) was 9.6% and 8.4%, respectively. In both cohorts, EASIX assessed at the day of alloSCT (EASIX-d0) was significantly associated with SOS/VOD incidence ($p<0.0001$), overall survival (OS) and non-relapse mortality (NRM). In contrast, the CIBMTR score showed no statistically significant association with SOS/VOD incidence, and did not predict OS and NRM.

In patients receiving pravastatin/UDA, the cumulative incidence of SOS/VOD was significantly lower at 1.7% ($p<0.0001$, Heidelberg, 2010-2015, n=359) than in the two cohorts not receiving pravastatin/UDA. The protective effect was most pronounced in patients with high EASIX-d0. The cumulative SOS/VOD incidence in the highest EASIX-d0 quartiles were 18.1% and 16.8% in both cohorts without endothelial prophylaxis as compared to 2.2% in patients with pravastatin/UDA prophylaxis ($p<0.0001$).

EASIX-d0 is the first validated biomarker for defining a subpopulation of alloSCT recipients at high risk for SOS/VOD. Statin/UDA endothelial prophylaxis could constitute a prophylactic measure for patients at increased SOS/VOD risk.

Introduction

Sinusoidal obstruction syndrome (SOS), also known as veno-occlusive disease (VOD), is a potentially fatal complication after allogeneic stem cell transplantation (alloSCT).[1–3] Clinical management of SOS/VOD remains challenging, since there are no standardized predictive tools[4] and diagnostic criteria are not uniform.[2,4–6] The reported incidences of SOS/VOD after alloSCT range from 5.3% to 13.7% and vary depending on conditioning regimens, type of transplant, diagnostic criteria, patient characteristics, and other factors.[7–9]

The pathophysiology of SOS/VOD is characterized by endothelial injury caused by the conditioning regimen as well as pre-transplant damage.[1,2,10,11] The resulting post-sinusoidal portal hypertension leads to the clinical syndrome of SOS/VOD.[2,5,6,11] In severe SOS/VOD, which is strongly associated with multi-organ failure, mortality remains high.[2,8,11] Early detection or prediction of SOS/VOD could allow identification of patients benefiting from prophylactic measures,[3] and preliminary data show that preemptive treatment with defibrotide might be effective,[12] making tools for prediction of SOS/VOD highly desirable.

Recently, a SOS/VOD clinical risk score (age, Karnofsky status, sirolimus use, hepatitis B/C status, conditioning regimen, disease type) has been published by the Center for International Blood and Marrow Transplant Research (CIBMTR).[13] However, this score has not been validated outside of the CIBMTR database yet. In addition, it has been suggested that serum biomarkers, including microparticles and plasminogen activator inhibitor (PAI)-1, may be useful to predict SOS/VOD,[14–18] but validation and clinical implementation of these non-routine biomarkers will be difficult due to the lack of standardization.

Previously, we have developed a standard biomarker panel to assess endothelial dysfunction and activation, termed 'Endothelial Activation and Stress Index (EASIX)'. EASIX is based on the simple formula "lactate dehydrogenase (LDH) (U/L) * creatinine (mg/dL) / thrombocytes (10^9 cells per L)" and thus calculated using three of the diagnostic parameters of thrombotic microangiopathy (TMA),[19] which is another endothelial complication after alloSCT.[20] EASIX

has also been shown to predict mortality of patients with acute graft-versus-host disease (GVHD).[19]

With this background, the aim of the current study was to test the SOS/VOD predictive capacity of EASIX compared with that of the CIBMTR score in two independent cohorts.

Methods

Study population

For this retrospective cohort analysis, a training cohort and a validation cohort comprising consecutive adult patients who had undergone alloSCT at two independent institutions were investigated. Patients from the training cohort received alloSCT at the Charité – Campus Virchow Klinikum, Berlin between 01/2013 – 12/2015. The validation cohort consisted of patients who were allografted at the University of Heidelberg between 09/2001 and 12/2009. Patients undergoing alloSCT in Heidelberg after 01/2010 received pravastatin and ursodeoxycholic acid (UDA) as prophylaxis of endothelial complications after alloSCT. To reduce confounding influences, the validation cohort was restricted to the time period before the introduction of pravastatin and UDA. The study was performed according to the declaration of Helsinki, with written informed consent obtained by all eligible patients. The study has been approved by the institutional review board of both institutions.

Definitions

SOS/VOD was defined according to the 2016 EBMT criteria for SOS/VOD diagnosis in adults.[4] The disease score we applied for the patients classifies the disease stage of the main diseases acute leukemia, myelodysplastic syndrome, chronic myeloid leukemia and non-Hodgkin lymphoma/multiple myeloma. The disease stages are assigned according to the remission status at transplant or the phases of chronic myeloid leukemia. The stages include ‘early disease stage (0), ‘intermediate disease stage’ (1) and ‘late stage disease’ (2).[21]

SOS/VOD CIBMTR risk score

The SOS/VOD CIBMTR risk score has been established to assess the risk of developing SOS/VOD after alloSCT.[13] It incorporates age, hepatitis B/C serology, Karnofsky performance status, use of sirolimus prophylaxis, disease, disease status at the time of transplant, and conditioning regimen. It was developed using the CIBMTR database.[13] We

used the 'VOD Risk Calculator'[22] and recorded the probability of SOS/VOD development for each patient in the two independent cohorts when possible.

EASIX Score

The EASIX score was calculated using the formula: "LDH (U/L) * creatinine (mg/dL) / thrombocytes (10^9 cells per L)" as described previously, assessed at the day of alloSCT.[19]

Statistical analyses

The primary objective was prediction of SOS/VOD occurrence. Primary analysis was performed for the binary endpoint "cumulative incidence of SOS/VOD within 28 days after alloSCT" and the time-to-event endpoint "time to VOD (TTV)" which is defined as time from alloSCT to diagnosis of SOS/VOD. Secondary objectives were the prediction of OS and time to NRM measured from the day of alloSCT. TTV respectively NRM were analyzed using competing event models. The competing events are "non-SOS/VOD-mortality" defined as time from alloSCT to death without prior SOS/VOD respectively time to relapse defined as time from alloSCT to relapse of disease. Further details on statistical analyses are given in the online supplement.

Results

Patient characteristics

The training cohort and the validation cohort consisted of 446 and 380 patients, respectively. The baseline characteristics are presented in Table 1. The patient cohorts were similar in regards to the characteristics age, sex, conditioning regimen and SOS/VOD incidence. However, there were significant differences in the categories donor type (more matched unrelated donors in the training cohort), underlying disease (most frequent disease: acute myeloid leukemia, 51% and 27% in the training and validation cohort, respectively), stem cell source (use of bone marrow more frequently in the validation cohort), and use of anti-T-cell globulin (ATG) (more commonly used in the training cohort).

EASIX-d0 and SOS/VOD risk

SOS/VOD was diagnosed in 43 patients (9.7%, median onset d +9) in the training cohort and in 32 patients (8.4%, median onset d +7) in the validation cohort. In the training cohort, median EASIX-d0 in patients who later on developed SOS/VOD was significantly higher as compared to patients who did not develop SOS/VOD (Figure 1A, $p < 0.0001$, 40.26; IQR 14.72 – 80.38 vs. 16.06; IQR 6.00 – 36.54). These findings were confirmed in the validation cohort, where median EASIX-d0 in patients who subsequently developed SOS/VOD was also significantly higher as compared to patients who did not develop SOS/VOD (Figure 1B, $p < 0.0001$, 8.64; IQR 3.38 – 15.40 vs. 2.28; IQR 0.92 – 7.48).

Increasing EASIX-d0 was significantly associated with SOS/VOD incidence in the training cohort in both univariable (OR per log2 increase 1.45, 95% CI 1.23-1.73, $p < 0.0001$) and multivariable analysis with the CIBMTR score as confounder (incorporating information on 6 clinical variables) (OR per log2 increase 1.39, 95% CI 1.15-1.69, $p = 0.0008$) (Figure 2A). Similarly, EASIX-d0 was strongly associated with the incidence of SOS/VOD in the validation cohort (univariable analysis: OR per log2 increase 1.50, 95% CI 1.22-1.88, $p = 0.0002$; multivariable analysis: OR per log2 increase 1.57, 95% CI 1.26-2.01, $p = 0.0001$) (Figure 2B).

Based on this data, we have created the EASIX-d0 SOS/VOD calculator for free public use (http://biostatistics.dkfz.de/EASIX/).

Association of EASIX-d0 with OS and NRM

In the training cohort, EASIX-d0 was significantly associated with OS and NRM in univariable analysis (OS: HR per log2 increase 1.20, 95% CI 1.12-.1.29, $p < 0.0001$; NRM: cause-specific hazard ratio (CSHR) per log2 increase 1.25, 95% CI 1.14-1.38, $p < 0.0001$) (Figure 3A and 3C). Likewise, EASIX-d0 was significantly associated with OS and NRM in the validation cohort in univariable analysis (OS: HR per log2 increase 1.10, 95% CI 1.02-1.18, $p = 0.0124$.; NRM: CSHR per log2 increase 1.18, 95% CI 1.06-1.32, $p = 0.0024$) (Figure 3B and 3D).

Association of the CIBMTR clinical risk score with SOS/VOD incidence, OS and NRM

In the training cohort, a non-significant trend towards a higher median CIBMTR score was observed in patients who subsequently developed SOS/VOD as compared to patients who did not develop SOS/VOD (1.51; IQR 0.82 – 2.37 vs 1.01; IQR 0.76 – 1.80, $p = 0.069$). In the validation cohort, the median CIBMTR score in patients with SOS/VOD was significantly higher as compared to patients without SOS/VOD (1.92; IQR 1.44 – 3.09 vs 1.20; IQR 0.89 – 2.15, $p = 0.00053$). On time-to-event analysis, however, the association of the CIBMTR score with SOS/VOD incidence was not statistically significant in the training cohort (Figure 4A, univariable analysis: OR per log2 increase 1.21, 95% CI 0.91-1.57, $p = 0.152$) nor in the validation cohort (Figure 4B, univariable analysis: OR per log2 increase 1.07, 95% CI 0.92-1.20, $p = 0.308$).

Brier score based on observed SOS/VOD incidence in the training cohort (null model) is 0.0774 in the validation cohort. Inclusion of EASIX-d0 leads to a reduction of the quadratic prediction error (0.0735) of approximately 5%. In contrast to this, Brier score of the CIBMTR score model (0.0771) approximately coincides with Brier score of null model.

In both cohorts, the CIBMTR score was not predictive of OS or NRM (univariable analyses, training cohort, OS: HR per log2 increase 1.08, 95% CI 0.95-1.22, p = 0.2264; NRM: CSHR per log2 increase 1.15, 95% CI 1.00-1.33, p = 0.0565 / validation cohort, OS: HR per log2 increase 1.03, 95% CI 0.97-1.03, p = 0.4126; NRM: CSHR per log2 increase 0.95, 95% CI 0.84-1.08, p = 0.4648).

Effect of pravastatin/UDA on SOS/VOD incidence

Patients undergoing alloSCT in Heidelberg after 01/2010 received pravastatin and UDA as routine prophylaxis of endothelial complications after alloSCT. In this cohort of 359 consecutive patients transplanted in Heidelberg between 01/2010 and 12/2015, the SOS/VOD incidence was significantly lower than in the training and validation cohorts treated without endothelial prophylaxis (Figure 5A, p < 0.0001; 1.7%, vs 9.6% and 8.4%). Next, we focused on the effect of pravastatin/UDA prophylaxis on SOS/VOD incidence, NRM and OS in a population at increased risk for SOS/VOD, defined by the highest EASIX-d0 quartile in each of the three cohorts. The patient cohort at increased risk that received pravastatin/UDA showed a significantly lower SOS/VOD incidence (Figure 5B), lower NRM (Figure 5C), and higher OS (Figure 5D) as compared to the high-risk patient populations in the training and validation cohorts.

Discussion

In this retrospective cohort analysis, EASIX-d0 was found to be an independent predictor of SOS/VOD risk, OS and NRM in adult patients receiving alloSCT. EASIX-d0 constitutes the first validated biomarker for defining a subpopulation of alloSCT recipients at high risk for SOS/VOD. It consists of routine laboratory parameters enabling easy implementation in any transplant center. EASIX-d0 seems to be a readily available tool for stratifying patients into high- and low-risk populations, which could be useful to improve clinical management of SOS/VOD, and for identifying patient subsets for clinical trials on SOS/VOD prophylaxis. Outside of clinical studies, patients with high EASIX-d0 scores might benefit from closer monitoring of emerging clinical SOS/VOD signs and early interventions.

EASIX-d0 has to be put in perspective with the CIBMTR SOS/VOD clinical risk score, which has been recently described as a predictive tool to identify patients at high risk of developing SOS/VOD.[13] The CIBMTR score has been established using a large sample from the CIBMTR database and consists of select baseline parameters which are partly fixed (age, hepatitis B/C serology, Karnofsky performance score, diagnosis, disease status at transplant) and partly subject to intervention (use of sirolimus, conditioning regimen). It has been shown to be predictive for SOS/VOD but not for NRM and OS.[13] In the present study, the CIBMTR score exhibited only a weak association with SOS/VOD incidence and no association with NRM or OS. One explanation for this discrepancy might be that the two European cohorts investigated in the current study were in some respects different from the CIBMTR cohort. First, in vivo T-cell depletion with ATG was administered in most patients in the two European cohorts, whereas the majority of the CIBMTR patients did not receive ATG for GVHD prophylaxis.[13] Second, most patients in our two cohorts were conditioned with reduced intensity conditioning (RIC), whereas only a minority of patients from the CIBMTR database received a RIC regimen.[13] Third, sirolimus was administered in 8% of the patients from the CIBMTR database, [13] whereas none of our patients received sirolimus prophylactically. This is relevant because sirolimus was a risk factor for SOS/VOD development in the CIBMTR analysis. In addition, registry data might be prone to

consistency deficits, while we had immediate access to the primary data ensuring high quality of data. Of note, the CIBMTR score has been primarily validated in a large population. We have validated the current EASIX-VOD score in smaller cohorts. Therefore, further validation is necessary. The EBMT is currently conducting a prospective non-interventional study on the value of the EASIX score for prediction of alloSCT-related endothelial complications. Furthermore, we expect that data from several retrospective cohorts in different centers will be available soon.

Based on previous publications on the efficacy of UDA and statins in the protection of the endothelium,[23,24] the Heidelberg alloSCT group decided to prophylactically administer pravastatin and UDA to alloSCT recipients transplanted after 01/2010. UDA is a synthetic bile acid that reduces the incidence of SOS/VOD, and is associated with less liver toxicity and better survival rates.[3,25] Statins have not been extensively studied for the prevention of endothelial complications. However, its pleiotropic effects include, besides the inhibition of cholesterol synthesis, improvement of endothelial function, reduction of oxidative stress and inflammation, and decrease of thrombogenic properties.[26,27] Statins may therefore be of beneficial effect in the prevention of endothelial complications. Accordingly, we observed a reduction of endothelial post-transplant complications, as previously shown in TMA[20] and refractory GVHD[28] upon implementation of statin/UDA endothelial prophylaxis.

In the present study, the SOS/VOD incidence was markedly reduced after the introduction of pravastatin/UDA prophylaxis. These protective effects, both in terms of SOS/VOD risk reduction and lower NRM and overall mortality, were specifically pronounced in patients with high EASIX-d0 scores, as compared to high-risk patients that did not receive pravastatin/UDA. This suggests that patients at high risk for SOS/VOD may benefit most from prophylactic SOS/VOD strategies.

Limitations of our study are its retrospective design and the validation in only one independent cohort of patients with similar patient characteristics, which are typical for adult

European alloSCT transplant centers. Therefore, the results cannot be extrapolated to pediatric alloSCT populations. Since EASIX-d0 is very easy to assess, any transplant center now has the opportunity to evaluate its potential in their respective patient population and we hope that more data on different patient populations, including pediatric patients or haploidentical transplantation, will be available soon. To facilitate this process, we have created the EASIX-d0 SOS/VOD calculator for free public use (http://biostatistics.dkfz.de/EASIX/).

In conclusion, EASIX-d0 seems to be an easy-to-use biomarker for identifying patient populations at high risk for SOS/VOD, and thus could be a promising tool both for clinical trials and tailored monitoring strategies. Statin/UDA endothelial prophylaxis could be an option to overcome the increased SOS/VOD risk indicated by high EASIX-d0 scores.

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Tables

Table 1: Baseline characteristics of the three patient cohorts Berlin (training), Heidelberg no statins/UDA (validation), and Heidelberg with statins/UDA.

	Berlin cohort (training cohort) (n = 446)	**Heidelberg no statins/UDA cohort (validation cohort) (n = 380)**	**Heidelberg with statins/UDA cohort (n = 359)**	***P* Berlin vs Heidelberg no statins/UDA**	***P* Berlin vs Heidelberg with statins/UDA**	***P* Heidelberg no statins/UDA vs Heidelberg with statins/UDA**
Date of alloSCT	01/2013 – 12/2015	09/2001 – 01/2010	01/2010 – 12/2015			
Age at transplant in years - median (range)	54 (18-75)	50 (17-70)	56 (19-75)			
Recipient sex (n, %)				0,668	0,942	0,764
- Female	172 (39%)	153 (40%)	140 (39%)			
- Male	274 (61%)	227 (60%)	219 (61%)			
Donor sex				0,877	0,437	0,584
- Female	124 (28%)	121 (32%)	122 (34%)			
- Male	274 (61%)	259 (68%)	237 (66%)			
- NA	48 (11%)					
Donor				**<0,001**	**0,034**	**<0,001**
- Matched related donor	88 (20%)	141 (37%)	94 (26%)			
- Matched unrelated donor	263 (59%)	140 (37%)	196 (55%)			
- Mismatched related donor	6 (1%)	14 (4%)	11 (3%)			
- Mismatched unrelated donor	89 (20%)	85(22%)	58 (16%)			
Disease				**<0,001**	**<0,001**	**<0,001**
- AML	229 (51%)	101 (27%)	126 (35%)			
- MDS/MPN	82 (18%)	53 (14%)	69 (19%)			
- ALL	35 (8%)	39 (10%)	20 (6%)			
- lymphoma	41 (9%)	96 (25%)	100 (28%)			
- MM	39 (9%)	75 (20%)	36 (10%)			
- Other	20 (4%)	16 (4%)	8 (2%)			
Disease score				**0,012**	**<0,001**	**<0,001**

- 0	142 (32%)	131 (34%)	122 (34%)			
- 1	47 (11%)	65 (17%)	122 (34%)			
- 2	248 (56%)	184 (48%)	115 (32%)			
- NA	9 (2%)					
Stem-cell source				**<0,001**	**<0,001**	0,566
- Peripheral blood	443 (99%)	351 (92%)	336 (94%)			
- Bone marrow	2 (1%)	29 (8%)	23 (6%)			
- NA	1 (0%)					
Conditioning				0,999	0,055	0,069
- RIC	341 (76%)	291 (77%)	294 (82%)			
- MAC	105 (24%)	89 (23%)	64 (18%)			
Use of ATG				**<0,001**	**<0,001**	**<0,001**
- Yes	399 (89%)	193 (51%)	259 (72%)			
- No	47 (11%)	187 (49%)	100 (28%)			
SOS/VOD development (EBMT criteria)				0,472	**<0,001**	**<0,001**
- SOS/VOD	43 (10%)	32 (8%)	6 (2%)			
- No SOS/VOD	401 (90%)	348 (92%)	353 (98%)			
Onset of SOS/VOD (median, range)	d+9 (d+3 to d+30)	d+8 (d0 to d+24)	d+10 (d+1 to d+17)			
Median CIBMTR score (range, IQR)						
SOS/VOD	*n = 32, rest of data NA:* 1.51 (0.56 – 4.48; 0.82 – 2.37)	*n = 29, rest of data NA:* 1.9 (0.3 – 8.7; 1.4 – 3.1)	*n = 5, rest of data NA:* 1.7 (0.7 – 3.7; 1.0 – 2.7)			
No SOS/VOD	*n = 338, rest of data NA:* 1.01 (0.32 – 9.72; 0.76 – 1.80)	*n = 333, rest of data NA:* 1.2 (0.4 – 20.6; 0.9 - 2.1)	*n = 347, rest of data NA:* 1.0 (0.3 – 9.1; 0.8– 1.7)			
Median EASIX-d0 (range, IQR)						
SOS/VOD	*n = 41, rest of data NA: 40.26:* (5.23 – 865.06; 14.72 – 80.38)	*n = 32, rest of data NA:* 8.6 (0.2 – 41.0; 3.4– 15.4)	*n = 6 rest of data NA:* 7.4 (4.0 – 17.1; 5.8-14.8)			
No SOS/VOD	*n = 376, rest of data NA:* 16.06 (0.38 – 575; 6.00 – 36.54)	*n = 348, rest of data NA:* 2.3 (0.2 – 99.2; 0.9 – 7.5)	*n = 353, rest of data NA:* 7.5 (0.2 – 195.8; 2.1 – 14.9)			

Abbreviations:

UDA = ursodeoxycholic acid, alloSCT = allogeneic stem cell transplantation, NA = not available, AML = acute myeloid leukemia, MDS = myelodysplastic syndrome, ALL = acute lymphocytic leukemia, CLL = chronic lymphocytic leukemia, MPN = myeloproliferative neoplasms, CML = chronic myeloid leukemia, MM = multiple myeloma, RIC = reduced intensity conditioning, MAC = myeloablative conditioning, ATG = anti-thymocyte globulin, KPS = Karnofsky Performance Score, IQR = interquartile range, CIBMTR = Center for International Blood and Marrow Transplant Research

Figure Legends

Figure 1: **EASIX-d0 in patients without SOS/VOD vs EASIX-d0 in patients with SOS/VOD.** Box plot of EASIX-d0 in patients without SOS/VOD (No VOD) vs EASIX-d0 in patients with SOS/VOD in (A) the training and (B) the validation cohort.

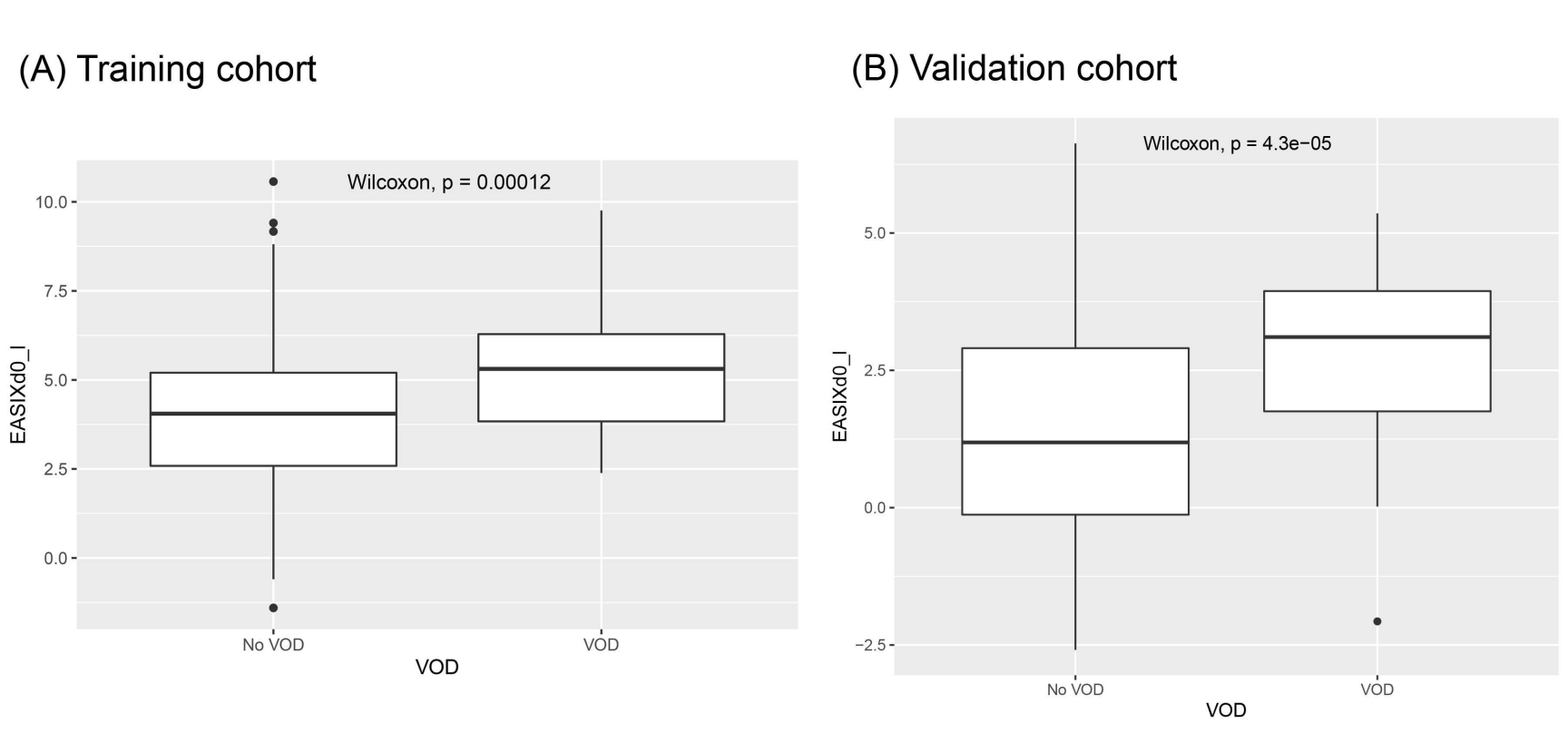
Figure 2: **Time to SOS/VOD depending on EASIX-d0 quartiles.** (A) Training and (B) validation cohort.

Figure 3: **Univariable effect of EASIX-d0 on overall survival and non-relapse mortality.** Univariable effect of EASIX-d0 on overall survival in (A) the training cohort and (B) the validation cohort. Univariable association of EASIX-d0 with non-relapse mortality in (C) the training cohort and (D) the validation cohort.

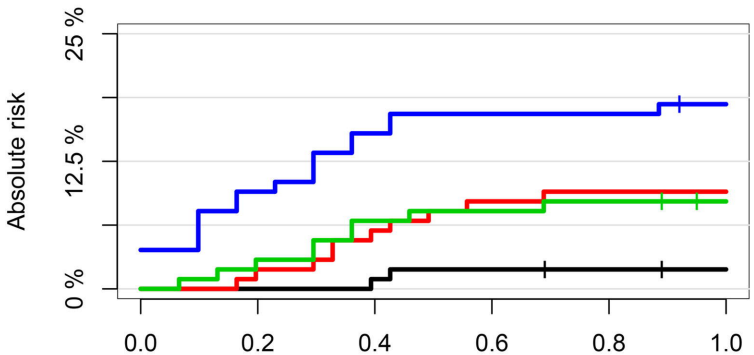
Figure 4: **Time to SOS/VOD depending on CIBMTR score quartiles.** (A) Training and (B) validation cohort.

Figure 5: **Effects of pravastatin/UDA prophylaxis (blue) compared to the training cohort (green) and the validation cohort (red).**

(A) Time to SOS/VOD in the three cohorts.
(B) Time to SOS/VOD in patients with the highest EASIX-d0 quartiles in the three cohorts.
(C) Time to NRM in patients with the highest EASIX d0 quartile in the three cohorts.
(D) OS in patients with the highest EASIX-d0 quartile in the three cohorts.

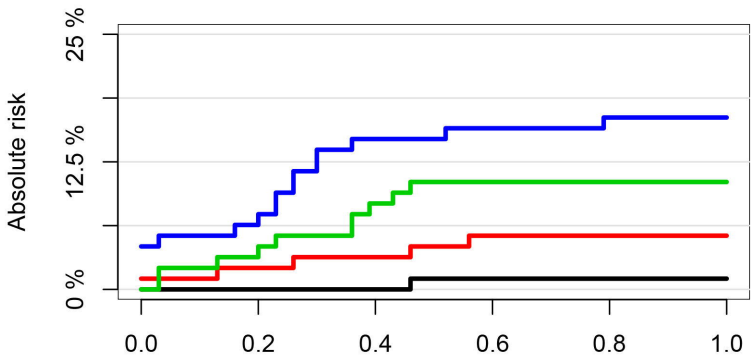

(A) Training cohort
Wilcoxon, p = 0.00012
EASIXd0_l
10.0
7.5
5.0
2.5
0.0
No VOD
VOD
VOD
(B) Validation cohort
Wilcoxon, p = 4.3e−05
EASIXd0_l
5.0
2.5
0.0
−2.5
No VOD
VOD
VOD

(A) Training cohort



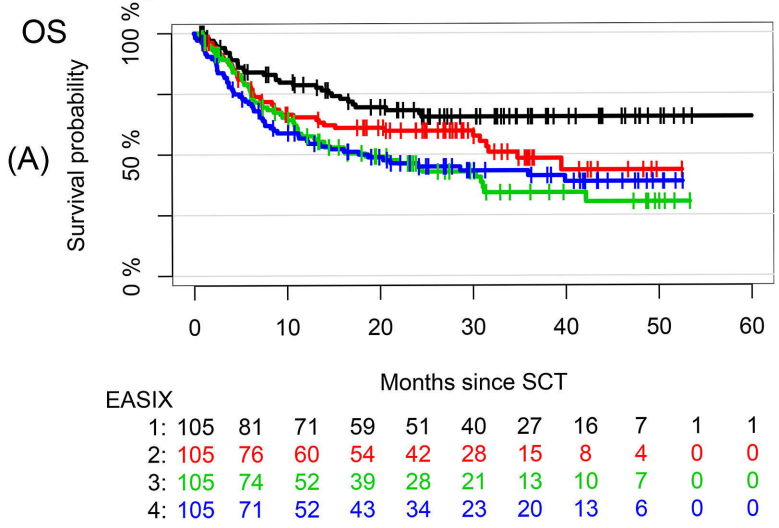
EASIX											
1:	105	105	105	105	104	103	103	102	102	100	100
2:	105	105	103	102	99	96	95	94	94	94	93
3:	105	104	102	100	98	97	96	95	95	94	93
4:	104	97	94	90	88	86	86	86	86	84	82

(B) Validation cohort

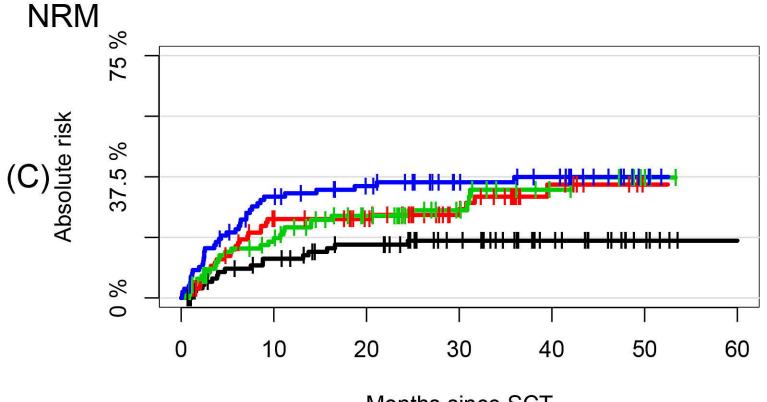


EASIX											
1:	95	95	95	95	95	94	94	94	94	94	93
2:	95	94	93	91	91	90	89	89	89	89	88
3:	95	93	92	90	86	83	83	83	83	83	83
4:	95	90	88	81	80	79	78	78	77	77	76

Training cohort

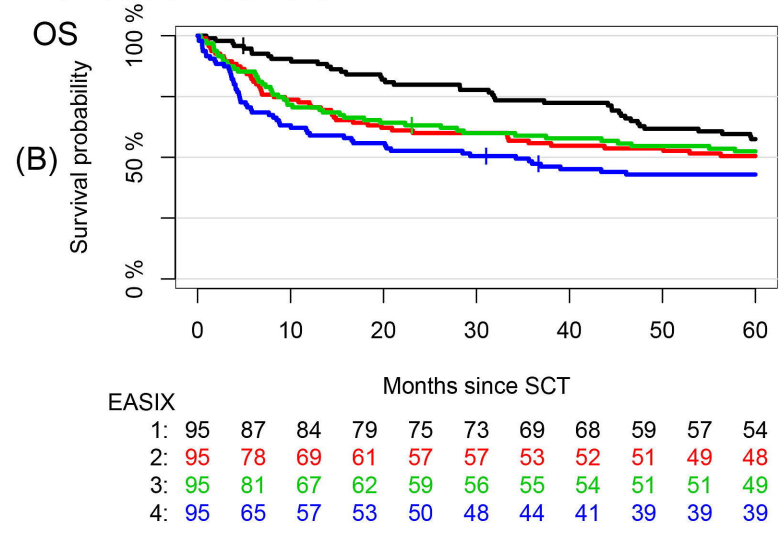


EASIX											
1:	105	81	71	59	51	40	27	16	7	1	1
2:	105	76	60	54	42	28	15	8	4	0	0
3:	105	74	52	39	28	21	13	10	7	0	0
4:	105	71	52	43	34	23	20	13	6	0	0

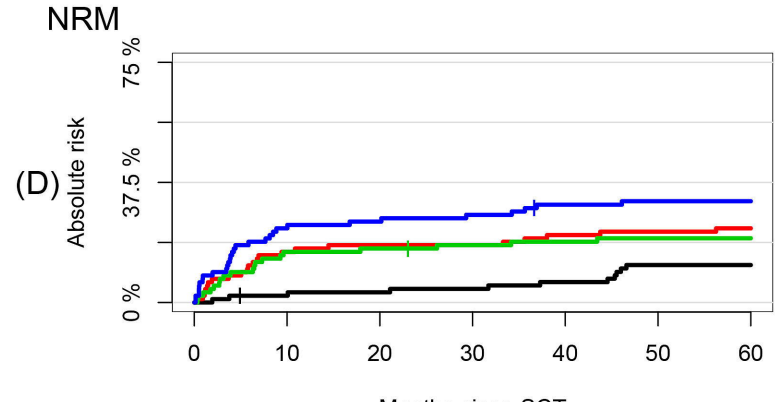


EASIX											
1:	105	81	71	59	51	40	27	16	7	1	1
2:	105	76	60	54	42	28	15	8	4	0	0
3:	104	73	51	39	28	21	13	10	7	0	0
4:	105	71	52	43	34	23	20	13	6	0	0

Validation cohort

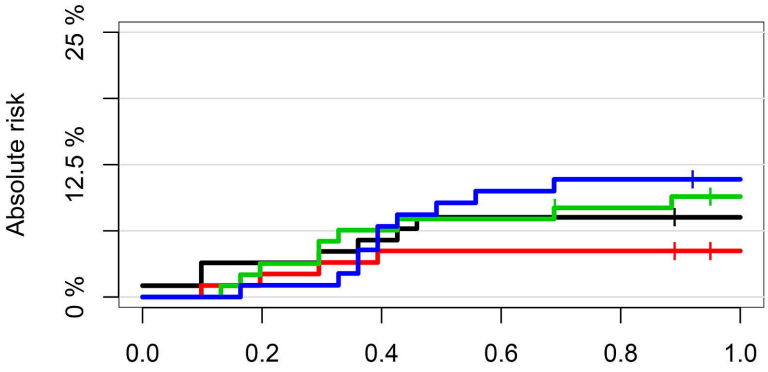


EASIX											
1:	95	87	84	79	75	73	69	68	59	57	54
2:	95	78	69	61	57	57	53	52	51	49	48
3:	95	81	67	62	59	56	55	54	51	51	49
4:	95	65	57	53	50	48	44	41	39	39	39



EASIX											
1:	95	78	65	61	57	56	50	48	42	41	41
2:	95	68	55	52	49	48	42	41	40	40	39
3:	95	69	58	55	52	50	48	48	46	45	45
4:	95	63	50	48	45	44	41	39	38	38	38

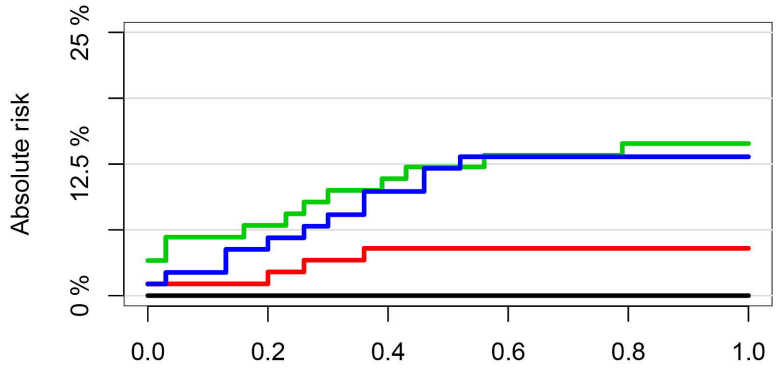
(A) Training cohort



CIBMTR

1:	93	90	90	89	88	86	86	86	86	84	83
2:	92	91	90	89	88	88	88	88	88	86	85
3:	95	95	92	90	89	88	88	86	86	85	84
4:	90	90	89	89	84	82	81	80	80	80	78

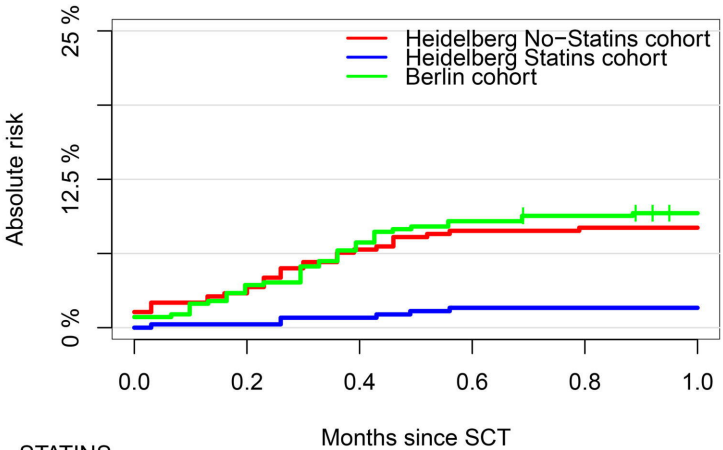
(B) Validation cohort



CIBMTR

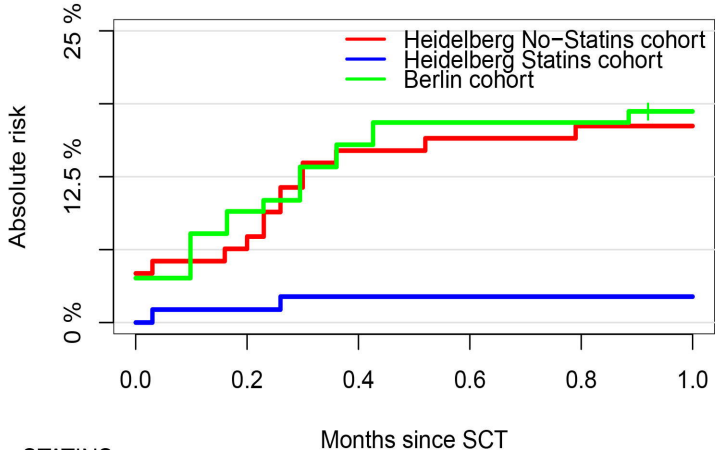
1:	92	92	92	92	92	92	92	92	92	92	92
2:	89	88	88	86	85	85	85	85	85	85	84
3:	90	85	84	81	80	78	77	77	76	76	75
4:	91	89	87	84	82	80	79	79	79	79	78

(A) Time to SOS/VOD



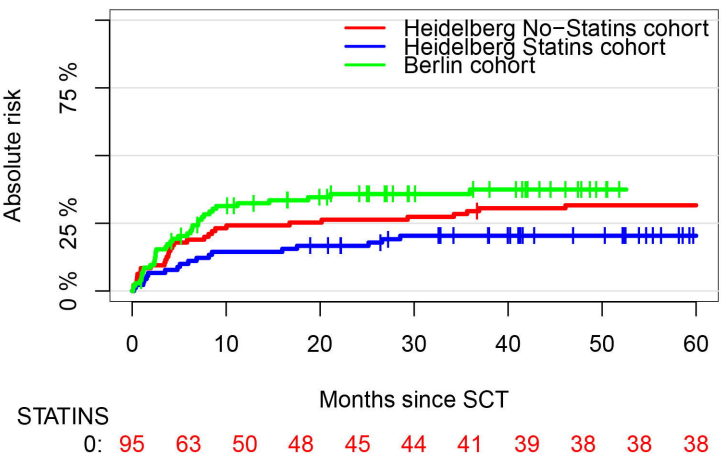
STATINS											
0:	380	372	368	357	352	346	344	344	343	343	340
1:	359	358	358	355	353	351	350	350	350	349	348
2:	445	437	429	422	413	406	403	400	400	395	390

(B) Time to SOS/VOD in patients with the highest EASIX-d0 quartile



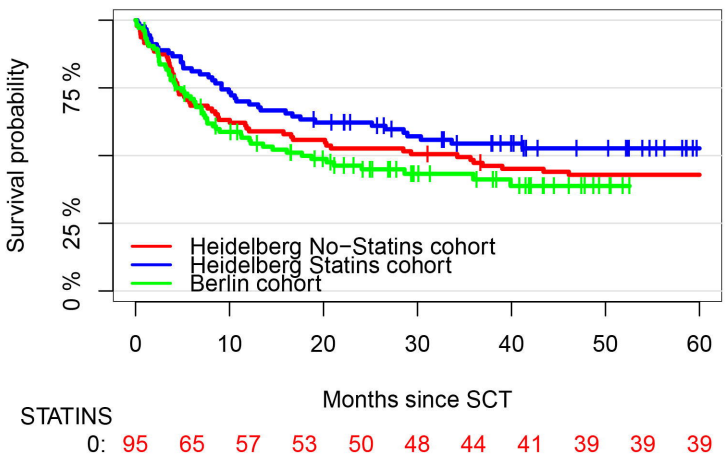
STATINS											
0:	95	90	88	81	80	79	78	78	77	77	76
1:	90	89	89	87	86	86	86	86	86	86	85
2:	104	97	94	90	88	86	86	86	86	84	82

(C) Time to NRM in patients with the highest EASIX-d0 quartile



STATINS											
0:	95	63	50	48	45	44	41	39	38	38	38
1:	90	62	54	50	44	38	35	27	25	20	12
2:	105	71	52	43	34	23	20	13	6	0	0

(D) OS in patients with the highest EASIX-d0 quartile



STATINS											
0:	95	65	57	53	50	48	44	41	39	39	39
1:	90	73	63	57	51	44	38	28	26	21	13
2:	105	71	52	43	34	23	20	13	6	0	0

Supplementary methods

Methods

Study population

Patient, laboratory and clinical data were accessed retrospectively using the clinical data management software SAP and COPRA.

Definitions

EBMT criteria for SOS/VOD diagnosis in adults: These criteria differentiate between classical SOS/VOD, which occurs in the first 21 days after stem cell transplantation, and late-onset SOS/VOD, which occurs beyond 21 days after stem cell transplantation. Classical SOS/VOD diagnosis requires bilirubin levels to be ≥ 2 mg/dL and two of the following criteria to be present: painful hepatomegaly, weight gain > 5%, and ascites. Late onset SOS/VOD can be diagnosed if the classical criteria are met, SOS/VOD is histologically proven, or if hemodynamical or/and ultrasound evidence of SOS/VOD are present and at least two of the four EBMT criteria are met.

Statistical analyses

The primary objective was prediction of SOS/VOD occurrence. Primary analysis was performed for the binary endpoint “cumulative incidence of SOS/VOD within 28 days after alloSCT” and the time-to-event endpoint “time to VOD (TTV)” which is defined as time from alloSCT to diagnosis of SOS/VOD. Secondary objectives were the prediction of OS and time to NRM measured from the day of alloSCT. TTV respectively NRM were analyzed using competing event models. The competing events are “non-SOS/VOD-mortality” defined as time from alloSCT to death without prior SOS/VOD respectively time to relapse defined as time from alloSCT to relapse of disease.

Categorical variables are presented as numbers and percentages. Continuous variables are presented as medians and ranges or interquartile ranges (IQR). For the primary statistical

analysis of EASIX-d0, the log2 transformed index, log2(EASIX) = log2(LDH) + log2(creatinine) – log2 (thrombocytes) was used.

Median follow-up time was estimated using the reverse Kaplan-Meier method. The analyses of the binary endpoint SOS/VOD within 100 days after alloSCT were performed using logistic regression models. We report estimated odds ratios (OR) and corresponding confidence intervals. The OR is the ratio of the estimated odds for a SOS/VOD event given a defined risk factor and the odds for a SOS/VOD event in the absence of this risk factor. Survival and incidence curves are based on the Kaplan-Meier estimator respectively Aalen-Johanson estimator for competing risk scenarios. For univariable and multivariable analyses of OS and NRM, (cause-specific) Cox proportional hazards models were used. Hazard ratios (HR) were calculated to demonstrate the prognostic effect of biomarkers. To check whether EASIX-d0 improves individual risk prediction in the presence of the well-established CIBMTR score, we trained a multivariable model with log2(EASIX) and the individual risk prediction from the CIBMTR calculator as covariates.

Validation of univariate EASIX-d0 and CIBMTR SOS/VOD model (Berlin) was performed in an external validation cohort (Heidelberg, no pravastatin/UDA). Discriminative ability was assessed using ROC-curves and AUC. Additionally, the Brier score was included which is a function measuring the accuracy of predictions. In contrast to the c-index, the Brier score checks for both, discrimination as well as calibration of the model. If the Brier score of a statistical model (including EASIX) is lower than the Brier score of the null model (without EASIX), a better prediction (of SOS/VOD) is indicated.

Pravastatin and UDA were routinely applied in the Heidelberg cohort starting in 01/2010, whereas the training cohort did not regularly receive pravastatin and UDA as prophylaxis. To assess differences in the prognostic effect of EASIX-d0 between subgroups of patients with pravastatin/UDA prophylaxis or no pravastatin/UDA prophylaxis, separate subset Cox regression models for the Heidelberg cohort were performed (Heidelberg no pravastatin/UDA vs Heidelberg with pravastatin/UDA). Estimates based on the univariate logistic training

cohort model can be obtained via an online calculator which is available on http://biostatistics.dkfz.de/EASIX/. The tool provides an estimate of the probability for SOS/VOD within 28 days after alloSCT and a corresponding confidence interval given a certain EASIX-d0 value.