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Christian Koenecke, Philipp Kümpers, Alexander Lukasz, Elke Dammann, Willem Verhagen, et al.. Shedding of the endothelial receptor tyrosine kinase Tie2 correlates with leukemic blast burden and outcome after allogeneic hematopoietic stem cell transplantation for AML. *Annals of Hematology*, 2010, 89 (5), pp.459-467. <10.1007/s00277-009-0869-5>. <hal-00535110>

HAL Id: hal-00535110

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Submitted on 11 Nov 2010

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Shedding of the endothelial receptor tyrosine kinase Tie2 correlates with leukemic blast burden and outcome after allogeneic hematopoietic stem cell transplantation for AML

Christian Koenecke · Philipp Kümpers · Alexander Lukasz · Elke Dammann · Willem Verhagen · Gudrun Göhring · Stefanie Buchholz · Jürgen Krauter · Matthias Eder · Brigitte Schlegelberger · Arnold Ganser

Received: 10 November 2009 / Accepted: 16 November 2009 / Published online: 7 January 2010
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Abstract Angiogenesis plays an important role in the growth and viability of hematologic malignancies. Emerging data suggest a crucial involvement of the endothelial-specific Tie2 receptor and its antagonistic ligand Angiopoietin-2 (Ang-2) in this process. The purpose of this study was to elucidate whether the soluble domain of the Tie2 receptor (sTie2) predicts outcome in patients with acute myeloid leukemia (AML) undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Serum levels of sTie2 and Ang-2 were measured by ELISA in 181 AML patients before conditioning for HSCT. The median follow-up time was 22 months after

HSCT. Pre-HSCT sTie2 levels were significantly higher in patients (median 2.2 (range 1.8–3.0) ng/mL) compared to healthy controls (1.3 (0.9–1.6); $p < 0.0001$). Elevated sTie2 levels were independently associated with active AML but did not relate to cytogenetics/mutational status before transplantation. Logistic regression analysis identified elevated sTie2 (odds ratio (OR) 3.07 (95% confidence interval (CI); 1.56–6.04), $p = 0.001$) as a strong predictor for disease relapse and poor overall survival after HSCT. In a multi-marker approach the highest risk for relapse was observed in patients with both elevated sTie2 and elevated Ang-2 (OR 4.07, (95% CI 1.79–9.25) $p < 0.0001$), as well as patients with both elevated Ang-2 and elevated bone marrow blast count (OR 4.16, (95% CI 1.88–7.36) $p < 0.0001$). Elevated serum sTie2 levels were related to active leukemia, correlated with the percentage of leukemic blasts in the bone marrow, and independently predicted relapse in AML patients after allogeneic HSCT. Furthermore, our data indicate that Tie2 shedding and Ang-2 release seem to reflect overlapping, but nevertheless distinctive features in leukemia-associated neoangiogenesis.

Part of this data was presented at the 50th Annual Meeting of the American Society of Hematology 2008, San Francisco, USA.

Christian Koenecke and Philipp Kümpers contributed equally to the manuscript and are both considered first authors.

C. Koenecke · E. Dammann · S. Buchholz · J. Krauter · M. Eder · A. Ganser (✉)
Department of Hematology, Hemostasis, Oncology and Stem-Cell Transplantation, Hannover Medical School,
Carl-Neuberg-Str. 1,
30625 Hannover, Germany
e-mail: ganser.arnold@mh-hannover.de

P. Kümpers · A. Lukasz
Department of Nephrology, Hannover Medical School,
Hannover, Germany

W. Verhagen
Institute of Virology, Hannover Medical School,
Hannover, Germany

G. Göhring · B. Schlegelberger
Institute of Cellular and Molecular Pathology,
Hannover Medical School,
Hannover, Germany

Keywords Angiopoietin · Soluble Tie2 · Acute myeloid leukemia · Allogeneic stem cell transplantation · Relapse · Biomarker

Introduction

Relapse after hematopoietic stem cell transplantation (HSCT) occurs frequently in patients with acute myeloid leukemia (AML) depending on the disease stage and the type of transplant [1]. The objective of defining the relapse

probability has gained increasing attention since the reduction or withdrawal of immunosuppression early after transplantation [2] and the use of donor leukocyte infusions (DLI) [3] are options to diminish the risk of relapse and improve outcome after HSCT. However, there are no established markers available that predict the risk of relapse independent from cytogenetic/mutational status [4, 5] or disease stage [6, 7].

Angiogenesis, the development of new blood vessels from pre-existing vessels, is essential for tumor growth and maintenance [8]. While this has long been accepted for solid tumors, there is now emerging evidence that links the extent of leukemia-associated (neo-)angiogenesis observed in bone marrow (BM) biopsies with disease burden, prognosis, or response to treatment in leukemia [9–11]. Consequently, several proangiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) have been identified as prognostic factors in AML and other hematologic malignancies [12, 13].

Recently, the vascular-specific Angiopoietin-Tie2 ligand-receptor axis emerged as a nonredundant gatekeeper of leukemia-associated angiogenesis in AML. Angiopoietins are angiogenic factors essential for vascular development and maturation [14]. As circulating or matrix-bound molecules, angiopoietin-1 (Ang-1) and its antagonist angiopoietin-2 (Ang-2) bind to the extracellular domain of the receptor tyrosine kinase Tie2, which is almost exclusively expressed on endothelial cells [15–17]. Produced by vascular smooth muscle cells (SMCs) and precursor pericytes, Ang-1-induced activation of the Tie2 receptor promotes quiescence and structural integrity of mature vessels, thus protecting the endothelium from activation by cytokines and growth factors [18–23]. In contrast, Ang-2 disrupts the constitutive Ang-1/Tie2 signaling by preventing Ang-1 from binding to the common receptor [15, 24–26]. Increased Ang-2 release from endothelial Weibel–Palade bodies upon stimulation of the endothelium with for instance hypoxia [24] seems to be essential for tumor angiogenesis [27]. Consistently, increased Ang-2-expression (and subsequent Tie2 deactivation) is only observed at sites of active angiogenesis [27], and high levels of Ang-2 in both tissue and in the circulation have been associated with advanced stages of solid tumors [28, 29] and hematologic malignancies [30, 31]. We could recently show that circulating Ang-2 is a strong positive predictor of disease-free survival (DFS) in patients undergoing HSCT for high-risk AML and myelodysplastic syndrome (MDS) [32].

It is believed that the quantitative balance between Ang-1 and Ang-2 determines the grade of endothelial Tie2 phosphorylation [14, 15, 18, 33–35]. However, experimental data suggest that this balance is influenced by a soluble form of the Tie2 receptor (sTie2) which results from cleavage of the extracellular domain of the membrane

bound full length Tie2 receptor by matrix metalloproteases [36] upon VEGF or bFGF stimulation [36, 37] (Fig. 1). Hence, enhanced sTie2 production (i.e., cleavage of the full length Tie2 receptor) by matrix metalloproteases may further shift the Ang-2/Ang-1 ratio in favor of Ang-2 by binding Ang-1 with ~20-fold higher affinity than Ang-2 [35]. Indeed, Tie2 is strongly upregulated during tumor angiogenesis [38, 39], and circulating sTie2 levels are elevated in malignant diseases, such as renal cell carcinoma [40] and AML [30].

We hypothesized that sTie2 is a marker of disease activity and relapse in the setting of allogeneic HSCT for AML. Therefore, we compared pre-HSCT levels of sTie2 with clinicopathologic features and outcome in 181 adult AML patients.

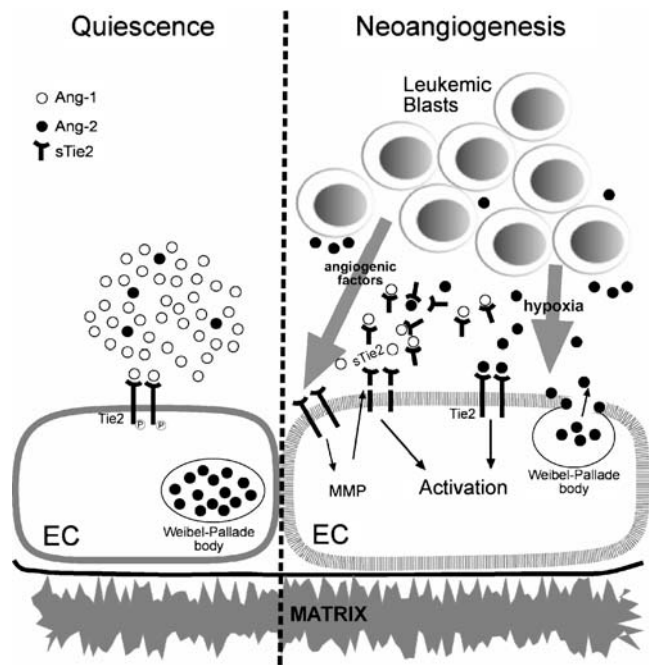


Fig. 1 Proposed mechanism that forms the basis of our study. Ang-1, when secreted by pericytes and smooth muscle cells, induces phosphorylation of the Tie2 receptor (*p*) in endothelial cells, resulting in quiescence. During acute leukemia, angiopoietin-2 (*Ang-2*) can be released rapidly from the endothelial Weibel–Palade bodies upon stimuli such as hypoxia. Ang-2 then prevents Ang-1 from binding to the endothelial Tie2 receptor, resulting in endothelial activation. Once activated, the vasculature is highly responsive to angiogenic factors, such as VEGF, which are released from leukemic blasts. High levels of VEGF and several other angiogenic growth factors increase MMP-induced proteolytic cleavage of the extracellular domain of Tie2, resulting in the release of sTie2. Enhanced sTie2 production may be a marker of endothelial stimulation by leukemic blasts and may inhibit angiopoietin-Tie2 signaling. sTie2 may shift the Ang-2/Ang-1 ratio in favor of Ang-2 by binding Ang-1 with ~20-fold higher affinity than Ang-2. This may further enhance a presumed effect of Ang-2 on leukemia-associated angiogenesis in the bone marrow

Material and methods

Patients—eligibility criteria

This observational single-center study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board. The cohort consisted of 181 consecutive patients undergoing allogeneic HSCT for AML at Hannover Medical School from 1991 to 2008. Ang-2 was analyzed before in a subgroup of patients ($n=84$), although with a shorter follow-up period [32]. Risk stratification of cytogenetics (good, intermediate, and high-risk karyotype) was carried out in accordance with SWOG/ECOG [41] criteria. *FLT3* mutations were considered as unfavorable cytogenetics in patients with normal karyotype [4, 42, 43]. Patient characteristics are shown in Table 1. Serum samples were taken within 1 week before the beginning of cytoreductive/conditioning therapy, stored at -80°C , and analyzed in a blinded fashion. Thirty volunteers from the Hannover Medical School staff served as healthy controls.

Soluble Tie2 and angiopoietin-2 enzyme-linked immunosorbent assay

Serum levels of sTie2 were measured in duplicates by enzyme-linked immunosorbent assay (ELISA) methodology (R&D Systems) according to the manufacturer's instructions. Serum levels of Ang-2 were measured by in-house ELISA methodology as described previously [32]. Antibodies were obtained from R&D Systems (R&D, Oxon, UK).

Statistical analysis

Continuous variables are expressed as medians with corresponding 25th and 75th percentiles (interquartile percentile) and were compared by using the Mann–Whitney rank sum test. The Kruskal–Wallis one-way analysis of variance followed by Dunn's multiple comparison test (posttest) analyzed the difference between patient groups according to diagnosis and disease status at HSCT. Correlations between variables were assessed by Pearson's correlation after logarithmic transformation.

Relapse after HSCT was the primary outcome studied and was calculated from the day of transplantation to day of relapse. Patients who did not suffer from relapse within the follow-up were censored at the date of last contact. To identify predictors of relapse, Cox's proportional hazards regression analysis was performed using backward elimination (Wald's test); the results are presented as hazard ratios (HR) with corresponding 95% confidence intervals. Variables found to be statistically significant at a 10% level in the univariate analysis were included into the multivar-

Table 1 Patient characteristics

Characteristics	Values
No. of patients	181
Male	94
Female	87
Median age, years	48 (36–55)
Disease	
De novo AML	140
Secondary AML	41
Risk according to cytogenetics/mutational status	
High	68
Good	4
Standard	74
Unknown	35
Stage at transplantation	
Untreated	10
Primary induction failure	28
Progressive disease	2
Partial remission	3
First CR	110
Second CR	4
First relapse after CR	19
Second relapse after CR	3
Third relapse after CR	2
Donor type no.	
MRD	89
MMRD	7
MUD	59
MMUD	26
Reduced intensity conditioning no.	96
Stem cell source	
PBSCs	153
Bone marrow	23
PBSC + BM	5
Median follow-up, months	22 (6–42)

Abbreviations: AML acute myeloid leukemia, CR complete remission, MRD matched related donor, MMRD mismatched related donor, MUD matched unrelated donor, MMUD mismatched unrelated donor, PBSC peripheral blood stem cell

iate Cox's model. Relapse-free survival was visualized by Kaplan–Meier method with logrank test. Prognostic relevant cutoff values of Ang-2 and sTie2 were derived from receiver operating characteristic (ROC) curve analysis. Univariable logistic regression analysis was used to explore the prognostic relevance of different dichotomous variables, such as Ang-2 cut-off values and several multimarker combinations, with regard to relapse. Odds ratios and 95% confidence intervals were calculated.

Overall survival was calculated from the day of HSC infusion to death from any cause. Treatment-related

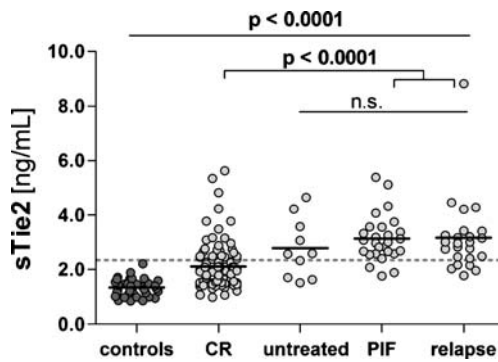


Fig. 2 Pre-HSCT serum levels of sTie2 in healthy controls and patients stratified to disease stage at HSCT. Serum levels of sTie2 were analyzed by ELISA in 30 apparently healthy controls and 181 patients undergoing allogeneic HSCT for AML within 1 week before the beginning of conditioning therapy. Horizontal bars indicate median values. A gray dotted line indicates the cutoff levels for sTie2 (2.24 ng/mL) that best segregated patients with relapse from patients without relapse by ROC curve analysis

mortality was defined as death other than by relapse during the first 100 days after HSCT. All tests were two-sided and used a significance level of $p < 0.05$. All of the above described statistical analyses were performed with the SPSS package (Chicago, IL, USA). Figures were prepared with GraphPad Prism (San Diego, California, USA).

Results

Soluble Tie2 levels before HSCT in patients and healthy controls

Serum levels of sTie2 were analyzed by ELISA methodology in 181 patients undergoing allogeneic HSCT for AML within 1 week before the beginning of conditioning therapy. Median sTie2 serum levels were significantly elevated in

patients compared to 30 healthy controls (2.2 (1.8–3.0) vs 1.3 (0.9–1.6); $p < 0.0001$; Fig. 2).

Soluble Tie2 levels before HSCT in relation to cytogenetics/mutational status and disease status

The cytogenetics/mutational status of the 181 patients were good risk in four, standard risk in 74, and high risk in 68 patients (SWOG/ECOG criteria and *FLT3* mutations [4, 41]. Pre-HSCT sTie2 levels were not different between patients with good/standard and high-risk cytogenetics ($p = 0.069$).

Patients in complete remission (CR) had lower sTie2 levels (2.0 (1.6–2.4) ng/ml) compared to untreated patients (2.6 (1.7–3.7) ng/ml), patients with primary induction failure (PIF; 3.0 (2.6–3.6) ng/ml), and patients with relapse (2.9 (2.4–3.4) ng/ml; all < 0.0001 , respectively; Fig. 2). Patients' BM was infiltrated by leukemic blasts ($> 5\%$, non CR) in 62 out of 181 analyzed patients prior to HSCT (34.3%). Soluble Tie2 correlated positively with the percentage of BM infiltration by leukemic blast ($r = 0.463$, $p < 0.0001$) using Pearson's correlation after logarithmic transformation.

In line, multivariate logistic regression analysis demonstrated that among all available baseline parameters (Table 1), only disease stage at time of transplantation (CR, PIF, untreated, relapse) independently predicted elevated sTie2 levels ($p < 0.0001$).

Soluble sTie2 levels and outcome

Using univariate Cox proportional hazards analysis, cytogenetics ($p = 0.028$), disease stage at HSCT ($p = 0.009$), the conditioning regimen ($p = 0.008$), the percentage of BM blasts ($p = 0.002$), circulating Ang-2 ($p = 0.058$), and sTie2 ($p = 0.004$) predicted relapse after HSCT (Table 2), whereas age, sex, donor type, CMV status, or graft type did not (data

Table 2 Predictors of relapse in univariate and multivariate Cox regression analysis

Variables	Univariate model			Multivariate model		
	HR	95% CI	P value	HR	95% CI	P value
Cytogenetics (others vs high risk)	1.22	1.02–1.47	0.028			
Disease stage at HSCT (others vs 1.CR)	2.07	1.20–3.58	0.009			
Conditioning (standard vs RIC)	2.19	1.23–3.89	0.008			
BM blasts (per 10%↑)	1.33	1.12–1.50	< 0.001	1.26	1.11–1.44	< 0.001
Ang-2 (per 1 ng/mL ↑)	1.04	1.00–1.08	0.058	1.07	1.02–1.13	0.046
sTie2 (per 1 ng/mL ↑)	1.41	1.11–1.77	0.004	1.30	1.01–1.68	0.011

Estimated hazard ratios, 95% confidence intervals, and P values were calculated by Cox regression analysis. Variables found to be statistical significant at a P value of < 0.1 in the univariate model were included into the multivariate model using stepwise backward elimination. A two-sided P value of < 0.05 was considered statistically significant in the multivariate model

Abbreviations: HR hazard ratios, CI confidence intervals, CR complete remission, RIC reduced intensity conditioning, BM bone marrow, Ang-2 angiopoietin-2, sTie2 soluble Tie2 receptor

not shown). Ang-2 was included in the analysis since it was identified previously as a strong predictor of DFS in patients undergoing HSCT for high-risk AML and MDS by our group [32]. Of note, in the present cohort, sTie2 performed slightly better on univariate analysis than Ang-2 did.

By multivariate Cox regression analysis, the percentage of BM blasts ($p<0.001$), circulating Ang-2 ($p=0.046$), and sTie2 ($p=0.011$) emerged as independent predictors for relapse. Neither disease stage nor conditioning regimen remained significant in the multivariate setting (Table 2). Figure 3 illustrates relapse-free survival after HSCT stratified to the percentage of BM blasts, circulating Ang-2 and sTie2 levels. Consistently, patients with BM blast $\geq 5\%$ (logrank

test $p=0.004$), Ang-2 levels above the median ($p<0.006$), and sTie2 levels above the median ($p<0.0006$) had a significantly poorer prognosis, respectively (Fig. 3).

Similarly, sTie2 levels above the median were associated with unfavorable overall survival (logrank test $p=0.011$). However, sTie2 was not independently associated with overall survival in a multivariate Cox regression model. Neither Ang-2 nor sTie2 did predict treatment-related mortality within 100 days post HSCT using univariate Cox regression ($p=0.283$ and $p=0.806$).

Multimarker approach for prognostic assessment of relapse after HSCT

ROC curve analysis was used to identify best cutoff levels for Ang-2 and sTie2 in our study population. An Ang-2 value of 2.18 ng/mL was associated with a prognostic sensitivity of 55% and a specificity of 76% (AUC 0.64, $p=0.002$). A sTie2 concentration of 2.24 ng/mL (indicated as dotted line in Fig. 2) was associated with a prognostic sensitivity of 69% and a specificity of 49% (AUC 0.62, $p=0.01$).

Finally, we investigated whether Ang-2 or sTie2 can provide prognostic information additive to that of disease status or cytogenetics/mutational status for risk assessment at time of HSCT. As shown in Table 3, the presence of BM blasts $\geq 5\%$ was no significant risk factor for relapse in our cohort (odds ratio (OR) 1.78, $p=0.06$). High-risk cytogenetics were associated with a significant but only modest increase in the risk of relapse after HSCT compared to intermediate and standard risk cytogenetics (OR 1.25, $p=0.04$).

However, the risk of relapse predicted by either variable was higher when Ang-2 elevation (>2.18 ng/ml) was also taken into account (OR 3.08, $p=0.004$ and OR 4.16, $p<0.0001$; Table 3). Interestingly, the same was true for the combination of Ang-2 with sTie2 (OR 4.07, $p<0.0001$). Figure 4a visualizes the relapse incidence according to the combinations of Ang-2 with sTie2 (high vs low as defined by ROC cutoff values). In line, the Kaplan–Meier curves of different Ang-2/sTie2 combinations showed clearly distinguishable risk groups that separated early after HSCT and continued to diverge during follow-up (logrank test [Mantel Cox] = $p<0.0001$; Fig. 4b).

Discussion

This is the first study investigating the predictive value of sTie2 in patients undergoing HSCT for AML. Elevated serum sTie2 levels correlated with the percentage of leukemic blasts in the BM and were independently related to active leukemia at baseline. Furthermore, Cox proportional hazards regression analysis identified sTie2 as an independent predictor for relapse in our cohort.

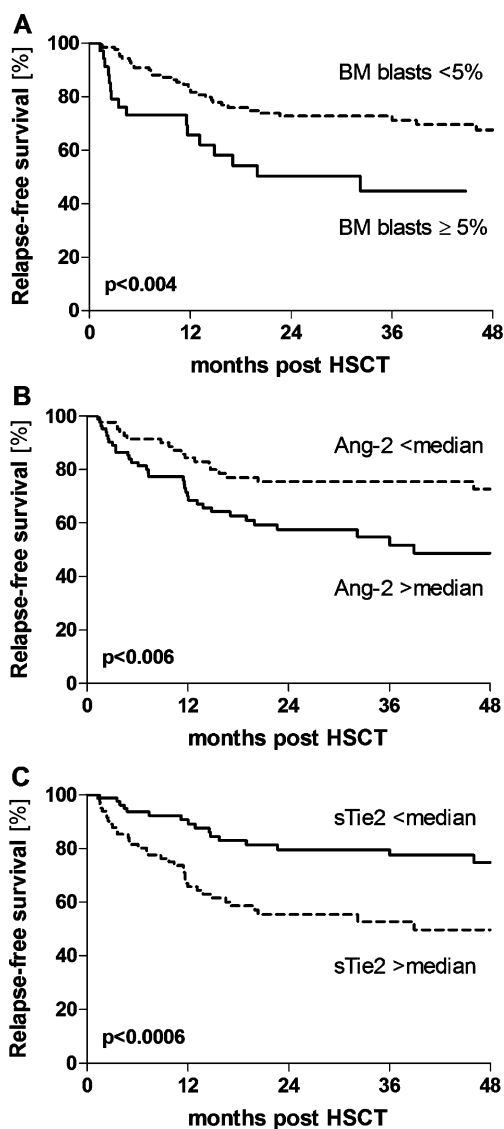


Fig. 3 Independent predictors of relapse after HSCT. Kaplan–Meier curves showing relapse-free survival after HSCT stratified to **a** bone marrow blasts (less vs greater than 5%), **b** Ang-2 (less vs greater than median [1.7 ng/ml]), and **c** sTie2 (less vs greater than median [2.2 ng/ml]) prior to allogeneic HSCT for AML. Statistical significance was confirmed by logrank test

Table 3 Multimarker approach for relapse prediction after HSCT

Variables	OR	95% CI	P value
BM blasts (>5%)	1.78	0.97–3.67	0.061
Cytogenetics (high risk)	1.25	1.01–1.56	0.042
Cytogenetics (high risk) and BM blasts (>5%)	1.42	1.01–1.88	0.013
Ang-2 elevation	2.38	1.23–4.60	0.01
Ang-2 elevation and cytogenetics (high risk)	3.08	1.43–6.66	0.004
Ang-2 elevation and BM blasts (>5%)	4.16	1.88–7.36	<0.0001
sTie2 elevation	3.07	1.56–6.04	0.001
Tie2 elevation and cytogenetics (high risk)	3.14	1.41–7.01	0.005
Tie2 elevation and BM blasts (>5%)	1.42	1.08–1.88	0.013
Ang-2 elevation and sTie2 elevation	4.07	1.79–9.26	<0.0001

OR, 95% confidence intervals (CI), and *P* values for different dichotomous variables, different cutoff values, and several multimarker combinations were calculated by binary logistic regression analysis. A two-sided *P* value of <0.05 was considered statistically significant

Abbreviations: OR odd ratios, CI confidence intervals, BM bone marrow, Ang-2 angiotensin-2, sTie2 soluble Tie2 receptor

Tie2 shedding is enhanced in diseases characterized by angiogenesis or vascular remodeling [39]. Although the pathophysiologic correlation of elevated sTie2 in hematologic malignancies has not been elucidated, most authors

consider the endothelial cell-derived sTie2 receptor and its circulating ligand Ang-2 as indicators for the extent of tumor-driven neoangiogenesis [30, 44, 45]. Accordingly, we found high levels of sTie2 in HSCT patients, suggesting ongoing Tie2 shedding. More specific, multivariate logistic regression analysis demonstrated that elevated sTie2 were independently related to active leukemia (untreated, PIF, relapse) in our cohort of patients. In turn, high pretransplant levels of sTie2 (and Ang-2) predicted a higher rate of relapse and poorer overall survival in a multivariate Cox regression model. These observations contrast two recent observations by Schliemann and Lee [30, 31] who found that, although elevated sTie2 levels correlated with overall survival in newly diagnosed AML patients, this association was not significant in a multivariate model. These data might suggest that the prognostic information provided by sTie2 might be somewhat similar to that of Ang-2 or BM blasts, respectively. However, the combination of sTie2 with Ang-2 outperformed “classical” risk factors (and the combinations thereof), such as cytogenetics and remission status in a multimarker approach in the current study. Thus, the highest rate of relapse after HSCT (>50%) occurred in patients with both elevated sTie2 and elevated Ang-2 levels, indicating that sTie2 and Ang-2 seem to reflect overlapping but nevertheless distinctive features in leukemia-associated neoangiogenesis. In line, sTie2 and Ang-2 levels in our healthy control group (in the absence of neoangiogenesis) were below the identified best cutoff levels for sTie2 and Ang-2.

Unfortunately, previous studies in de novo AML lack data on the prognostic impact of Ang-2 in patient subgroups with high or low sTie2 expression, respectively [31, 46]. An explanation for the additional prognostic impact of sTie2 may be that sTie2 more likely binds circulating Ang-1 compared to Ang-2, since sTie2 has a

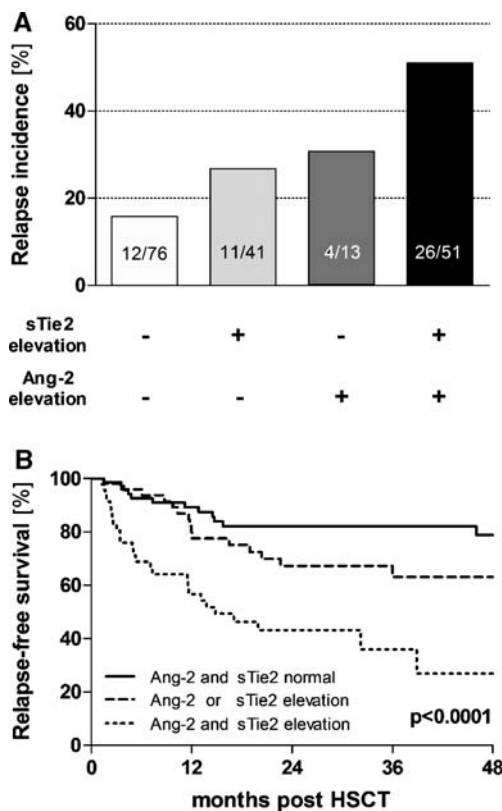


Fig. 4 Integration of sTie2 and Ang-2 for risk prediction after HSCT. ROC curve analysis was used to identify best cutoff levels for sTie2 (2.24 ng/mL) and Ang-2 (2.18 ng/mL). **a** Bar charts visualize the relapse incidence in the presence of elevated Ang-2 and/or elevated sTie2 levels, respectively. The number of patients with relapse and the overall number of patients are given for each column

higher affinity for Ang-1 than Ang-2 (Fig. 1). Therefore, the Ang-2/Ang-1 ratio is shifted further in favor of Ang-2 in the presence of elevated sTie2 levels [35, 47].

Our study has several limitations. First, leukemic blasts have also been shown to express Tie2 and Ang-2 mRNA and, therefore, could serve as an alternative source of circulating Ang-2 and sTie2 protein [12, 30]. However, in sharp contrast to the unfavorable prognostic value of elevated sTie2 and Ang-2 protein in the circulation, a high blast expression of Tie2 and Ang-2 was consistently identified as an independent predictor of a favorable prognosis in AML patients [12, 46]. In addition, the prognostic significance of high cellular Ang-2 expression was more obvious in a subgroup of patients with low (but not high) Tie2 expression [45], whereas in the present study, sTie2 and Ang-2 were increased in parallel. Thus, it is conceivable that the predominant proportion of circulating Ang-2 in our cohort was derived from BM endothelial cells in the face of extensive neoangiogenesis [31, 46]. Second, in the current study, there were only serum (but not plasma) samples available. However, since platelets are a rich source of Ang-1 and VEGF (REF 15367176), we cannot provide data on Ang-1 and VEGF levels, respectively. Finally, high levels of sTie2 [48] and Ang-2 are also detected in disease stages that present themselves with an inflammatory endothelial phenotype, such as severe infections [49–51].

In this study, we could exclude this confounder to some degree, since patients with severe infections rarely proceed to HSCT and, therefore, should be free of additional Ang-2. Of note, neither sTie2 nor Ang-2 did predict treatment-related mortality in the present study.

Increased BM angiogenesis might not only serve as a prognostic marker but has been recognized as a central player in disease initiation, maintenance, and progression and will become a crucial target in the treatment of hematologic malignancies [10]. Moreover, given its key role in angiogenesis, the Ang-Tie2 axis would appear as an attractive therapeutic target as well [10]. Very recently, AMG 386, the first (unselective) angiopoietin inhibitor drug, appeared to impact tumor vascularity and demonstrated a significant antitumor activity in patients with advanced solid cancer [52]. Collectively, these data constitute the intriguing concept that pre-HSCT levels of sTie2 and Ang-2 cannot only identify patients at risk for relapse but might provide a rationale for angiopoietin-targeted antiangiogenic therapy at the same time.

In summary, the results from this observational study indicate that circulating sTie2 and Ang-2, measured before the start of conditioning, are promising new biomarkers to identify patients with a high risk of relapse and poor outcome. Given that very few predictors for relapse exist in clinical practice, sTie2 and Ang-2 might become readily

available biomarkers that improve risk stratification and might help to tailor individualized immunosuppression schedules and DLI management and eventually antiangiogenic treatment in AML patients undergoing HSCT.

Acknowledgments We thank all the doctors and nurses on our transplant unit and in the outpatient clinic for their dedicated work. We thank Julian Hellpap for excellent technical assistance.

Authorship C.K. and P.K. had the initial idea, designed and performed the research, analyzed the results, prepared the figures, and wrote the manuscript. A.L. established the immunoassay and performed the measurements. W.V. stored and provided samples and reviewed the manuscript. E.D., S.B., J.K., M.E. and identified patients provided clinical data, participated in the design of the study, and reviewed the manuscript. G.G. and B.S. contributed cytogenetic data and reviewed the manuscript. A.G. supervised the project, participated in the design and interpretation of the study, and reviewed the manuscript.

Conflict of interest The authors declare no competing financial interests.

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