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BRIEF REPORT

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High β-1,4-Galactosyltransferase-I expression in peripheral T-lymphocytes is associated with a low risk of relapse in germ-cell cancer patients receiving high-dose chemotherapy with autologous stem cell reinfusion

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ABSTRACT

Survival of patients with germ-cell cancer (GCC) and primary progression or relapse after cisplatin-based first-line chemotherapy is highly heterogeneous, ranging from close to zero to more than 70%. We investigated β-1,4-Galactosyltransferase-I (*B4GALT1*) expression levels in peripheral lymphocytes in a cohort of 46 testicular cancer patients. *B4GALT1* enhances immune cell crosstalk via glycosylation of surface molecules. A high expression level of *B4GALT1* in T-lymphocytes, but not in monocytes, was associated with a lower risk of relapse with a hazard ratio (HR) of 0.66 (95% confidence interval (CI) of HR: 0.45-0.97; $p = 0.02$) upon multivariate Cox regression analysis. Correspondingly, interleukin 10 (IL10), a cytokine released by cytotoxic T-cells, was likewise significantly elevated in T-lymphocytes of non-relapse GCC patients (HR: 0.3; 95% CI of HR: 0.14-0.65; $p = 0.002$). Our data indicate that glycosylation and activation of T-lymphocytes may play a pivotal role in disease control in GCC patients with primary progressive or relapsed disease.

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Introduction

Testicular cancer occurs in young to middle-aged male patients and can be cured even in metastatic disease, after progression or relapse. The 3-year overall survival (OS) probability of patients with progression or relapse after a first-line cisplatin-based chemotherapy regimen ranges between close to zero and 70% or more.[1]

Salvage treatment consists either of four cycles of conventional-dose chemotherapy (CDCT) or high-dose chemotherapy (HDCT) with autologous stem cell reinfusion (ASCR)[2] followed by residual tumor resection if needed. The score of the International Prognostic Factor Study Group (IPFSG) helps to predict relapse and survival according to tumor marker levels of alpha fetoprotein (AFP) and human chorionic gonadotropin (HCG) as well as clinical parameters such as primary tumor site, response to first-line treatment, progression-free time interval, presence of liver, bone and brain metastases and histology, namely seminoma and non-seminoma. The score ranges from -1 to 3 points, defining very low (-1 point), low (0 points), intermediate (1 point), high (2 points) and very high (3 points) risk categories in respect to progression free survival.[3]

As T-cell function is important for disease control in cancer patients[4,5] and little is known about mechanisms of disease control in germ cell-cancer (GCC) patients, we sought to retrospectively identify immunomodulatory properties in T-lymphocytes and monocytes derived from samples of stored leukapheresis products. All products had been obtained prior to HDCT by mobilization with chemotherapy plus granulocyte colony-stimulating factor (G-CSF) and frozen for subsequent ASCR. Lymphocytes and monocytes were isolated from thawed samples via flow cytometry based cell sorting.

We particularly focused on β-1,4-Galactosyltransferase-I (*B4GALT1)* gene expression levels which were reported to be highly relevant for disease control in stage I non-small-cell-lung cancer (NSCLC) patients.[6] *B4GALT1* is a type II membrane-bound glycoprotein, which exclusively transfers UDP-galactose to different acceptor sugar molecules and is of particular importance for the interaction and adhesion of immune cells,[7] either through binding to appropriate glycoside substrates on adjacent cell surfaces or modification of N-acetylglucosamine (GlcNAc)

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residues of surface molecules such as leucocyte integrins.[8,9] The mRNA exists in two isoforms.[10,11] In our study we focused on the longer transcript variant coding for a surface membrane protein. *B4GALT1* seems to be important for B-cell function and migration[12] and in B-cell malignancies.[13] Furthermore, *B4GALT1* may represent a novel biomarker in colorectal cancers that is regulated by DNA promotor methylation[14] and predicts adverse outcome in non-metastatic clear cell renal cell carcinoma.[15]

Because selectins and leucocyte integrins are modified by *B4GALT1* and play a substantial role in tumor control,[6] we additionally measured the protein levels of very late antigen 4 (VLA4; CD49 d), platelet endothelial cell adhesion molecule 1 (PECAM1; CD31) and chemokine receptor 4 (CXCR4; CD184) on peripheral lymphocytes and monocytes in GCC patient samples via flow cytometry but did not find an association with relapse-free survival in our patient cohort. Association of IL10 expression in T-lymphocytes with relapse-free survival and up-regulation of *B4GALT1* after lectin stimulation *in vitro* supports the notion that activated lymphocytes are involved in tumor surveillance in GCC.

Here we demonstrate for the first time that a high mRNA expression level of *B4GALT1* in peripheral blood T-lymphocytes derived from leukapheresis products correlates with a lower risk of relapse in patients with GCC, who had undergone HDCT followed by ASCR. To our knowledge, the prognostic impact of *B4GALT1* expression in T-lymphocytes in patients with GCC has not been reported so far.

Methods

Patients and sample preparation

Between May 1999 and January 2012, a total of 73 patients with progressive or relapsed GCC after cisplatin-based first-line chemotherapy were treated at the Department of Hematology, Oncology and Immunology, University Hospital Marburg with single or sequential HDCT and ASCR. Of 46/73 patients, aliquots of leukapheresis products were available and cell counts were sufficiently high for analysis. Among these patients, 27/46 had been treated within a multicenter, randomized phase III clinical trial.[16] Upon a patient's written informed consent leukapheresis products were collected after mobilization with chemotherapy and G-CSF, frozen in 10% dimethyl sulfoxide (Carl Roth GmbH, Karlsruhe, Germany) and stored in liquid nitrogen prior to be used as ASCR after HDCT. For quality assurance and research purpose several 1 ml samples of the leukapheresis products were stored separately. Subsequently, all patients received HDCT followed by ASCR. HDCT consisted of three cycles carboplatin (1,500 mg/m^2) and etoposide (1,500 mg/m^2) per cycle in 22/27 patients. Due to renal impairment 4/27 patients received three HDCT cycles at a reduced dose of carboplatin (1,200 mg/m^2) and etoposide (1,200 mg/m^2) per cycle as requested by the study protocol.[16] 1/27 patients was randomized to a single cycle of HDCT with carboplatin (2,200 mg/m^2), etoposide (1,800 mg/m^2) and cyclophosphamide (6,400 mg/m^2).[16] Outside of the study protocol, 15/46 patients were treated on an individual basis with paclitaxel (225 mg/m^2), cisplatin (100 mg/m^2), etoposide (750 mg/m^2) and ifosfamide (10 g/m^2).[17] Exact data about the chemotherapy dosages were unavailable for 4/46 patients. A single HDCT was used in 17/46 patients, two HDCT cycles were used in 2/46 patients, and three HDCT cycles were used in 27/46 patients.

For analysis, leukapheresis aliquots were thawed and immediately resuspended in 5 ml of sodium chloride solution (0.9%) with 5% human serum albumin (HSA) (CSL Behring, Marburg, Germany). All steps were performed on ice. Density centrifugation with Ficoll-Paque Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 4°C was performed in order to extract mononuclear cells (MNC). After washing with sodium chloride solution (0.9%) containing 5% HSA, cells were resuspended in 1 ml phosphate buffered saline (PBS). 100 μl of the cell suspension were used for RNA isolation from total MNC with peqGOLD TriFast® (peqlab, Erlangen, Germany) according to the manufacturer's instructions. MNC obtained from buffy coats of healthy donors were treated with 1 μg/ml Concanavalin A (Con A) in RPMI medium for 24 h at 37°C prior to cell sorting.

Flow cytometric analysis

The remaining cells were pelleted and the following antibodies were added: CD3 (UCHT1) conjugated with V450, CD31 (M89D3) with Alexa Fluor® 488, CD184 (12G5) with Phycoerythrin or BD Multitest™ CD3/16/56/45/19 (all BD Biosciences, San José, USA), CD14 (TÜK4) with Peridinin Chlorophyll (PerCP) and CD49 d (MZ18-24A9) with Allophycocyanin (both Miltenyi Biotec, Bergisch Gladbach, Germany). The suspension was incubated for 30 min in the dark at 4°C and cells were suspended in 400 μl of PBS after washing with PBS. Cells and reagents were continuously kept on ice. 1 μl of Sytox Blue Dead Cell Stain® (Molecular Probes, Eugene, USA) was added immediately before measurement with a MoFlo® flow cytometer (Beckman Coulter, Brea, California, USA). For analysis and sorting, exclusion of debris was done with forward scatter versus side scatter gating (Fig. 1a). Employing CD14 versus CD3 gating, vital T-cells (left upper black gate in Fig. 1b) and vital monocytes (lower right black gate in Fig. 1b) were sorted into separate tubes containing cold peqGOLD Trifast® reagent (peqlab, Erlangen, Germany). RNA extraction was performed directly after sorting according to the manufacturer's instructions. RNA samples were stored at -80°C.

Real-time PCR

cDNA was obtained employing the Omniscript RT Kit (Qiagen, Hilden, Germany). RT-PCR for the target genes *B4GALT1* (primer sequences forward: 5'-tgaccataatgcgtacaggtg-3 ' and reverse: 5'-ccaaaatactgaacataaggtaggc-3') and *IL10* (forward: 5'-agaacctgaagaccctcaggc-3' and reverse: 5 '-ccacggccttgctcttgtt-3'; internal control gene: abl forward: 5 '-tcatgaaagagatcaaacacccta-3' and reverse 5'-catgaactcagtgatgatatagaacg-3') was performed in the CFX96 Real-time system® device (Bio-Rad, Munich, Germany) using the SYBR Green® PCR Master Mix assay (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Amplifications included one step at 95°C for 15 min, followed by 45 cycles of 94°C for 15 s, 59°C for 30 s and 72°C for 30 s for

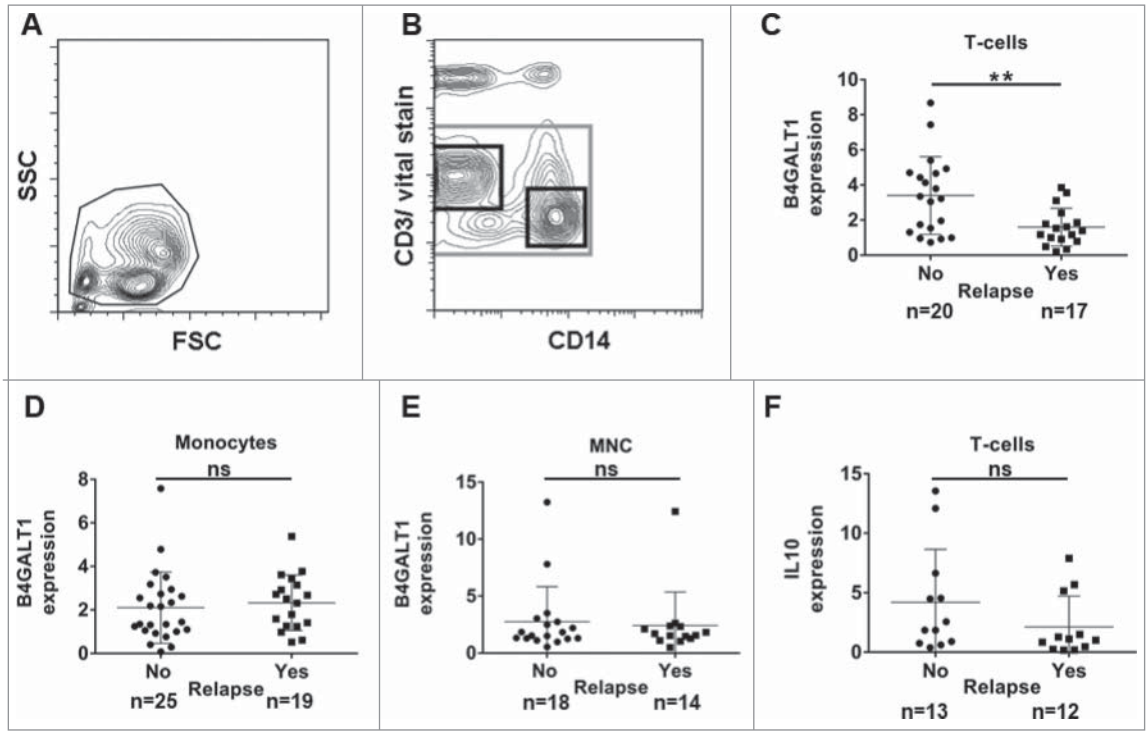

Figure 1. Gating strategy applied for cell sorting with further RT-PCR analysis of *B4GALT1* and *IL10* expression levels in compartments of MNC from leukapheresis products. (A) Forward scatter versus side scatter gating for exclusion of debris. (B) CD14 versus vital stain gating for the exclusion of dead cells (grey gate). CD14 versus CD3 plotting in order to identify CD3-positive T-cells (upper left black gate) and CD14-positive monocytes (lower right black gate). (C) *B4GALT1* levels in peripheral blood T-lymphocytes are significantly higher in patients with no relapse compared to those patients with relapse (Mann-Whitney-test, $p = 0.008$). Neither *B4GALT1* levels in (D) monocytes ($p = 0.251$), and (E) unsorted MNC samples ($p = 0.650$), nor (F) *IL10* expression levels in T-cells ($p = 0.178$) are significantly different upon Mann-Whitney-testing. Plots show mean and standard deviation, respectively. FSC: forward scatter; MNC: mononuclear cells; ns: not significant; SSC: side scatter.

B4GALT1 RT-PCR. For *IL10* RT-PCR, the annealing temperature was 60°C. For amplification of human interferon gamma (*IFNG*), the RT^{2}` qPCR Primer Assay for Human IFNG (Qiagen, Hilden, Germany) was used. Primer annealing was done at 58°C and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control gene (forward: 5 '-ctcctccacctttgacgctg-3 ' and reverse: 5 '-accaccctgttgctgtagcc-3 '). RT-PCR data were analyzed using a standard curve and the ΔΔCt-method. Gene specific signals were normalized to internal control signals from the same samples, respectively.

Statistical analyses

Means of the fluorescence signals and cell counts were calculated with FlowJo® 7.6.5 software (FlowJo LLC, Ashland, Oregon, USA) and graphs were created with GraphPad Prism® 5.02 software (GraphPad software Inc., San Diego, California, USA).

In a first step prognostic relevance was investigated with univariate Cox proportional hazard models for each biomarker. As for some biomarkers the distributions of the observed values were positively skewed, their values were log-transformed. Additionally, each marker was dichotomized at the median and Kaplan-Meier curves were created for the two groups with low and high marker values. The curves were compared using the log-rank test. In a second step, multivariate Cox proportional hazard models with covariates international prognostic factor score and number of transplantations were fitted for each biomarker significant in univariate analysis (Table 1). All these analyses were performed using the statistical analysis software R (www.r-project.org, version 3.2.3). Differences with a probability of $p \leq 0.05$ were considered statistically significant and indicated with a*.

Results and discussion

To the current state of knowledge immunomodulatory treatment approaches are not applied in GCC but at least little success has been reported with checkpoint inhibitor treatment in platinum refractory disease state.[18] In order to address the issue of immunosurveillance, patients were retrospectively grouped according to their remission status after HDCT. Among all patients, 27/46 (58.7%) remained relapse-free after HDCT. The median follow-up period in this patient cohort was 90 months (range 15 to 168). The remaining 19/46 patients (41.3%) suffered from relapses after HDCT (median 6 months, range 1 to 22).

We applied the IPFSG score to our patient cohort as follows: 2/46 patients underwent HDCT primarily and could not be grouped according to the IPFSG score. Score calculation was also not possible for 4/46 patients because of missing data. 26 of the remaining 40 patients received HDCT at first relapse or upon progression. 5/40 patients (12.5%) with a score of 0 were classified as “good” and 10/40 (25%) with a score of 1 were classified as “intermediate”. 11/40 patients with a score of 2 (high risk) and 3 (very high risk) points were combined in one group and their prognosis was

Table 1. Difference in relapse-free survival was calculated with the Cox regression model.

	Univariate Cox-Models		Multivariate Cox-Model for B4GALT1 T-cells		Multivariate Cox-Model for IL10 T-cells	
Covariate	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
B4GALT1 T-cells	0.62 (0.42,0.91)	0.02	0.66 (0.45,0.97)	0.03		
log IL10 T-cells	0.62 (0.39,1)	0.05			0.3 (0.14,0.65)	0.002
NoTx				0.21		0.008
1			reference		reference	
2			3.02 (0.3,30.87)		0.22 (0.02,2.35)	
3			0.51 (0.17,1.47)		0.04 (0.01,0.3)	
IPFSG score class				0.53		0.24
good			reference		reference	
intermediate			2.54 (0.26,24.74)		5.9 (0.42,83.77)	
poor			3.25 (0.4,26.15)		7.43 (0.71,77.44)	
Further PCR measurements						
log BaGALT1 Mono	1 (0.99,1.01)	0.77				
log B4GALT1 MNC	1.02 (0.98,1.05)	0.37				
log IL10 Mono	0.93 (0.76,1.16)	0.54				
Cytometric measurements						
log CD31 T-cells	1.27 (0.84,1.93)	0.26				
log CD49 d T-cells	1 (0.99,1.01)	0.93				
log CD184 T-cells	1[1,1]	0.43				
log Cd31 Mono	0.45 (0.25,0.79)	0.01				
log Cd49 d Mono	0.97 (0.58,1.63)	0.92				
log CD184 Mono	0.6 (0.23,1.61)	0.31				

In the multivariate analysis *B4GALT1* mRNA expression levels in T-lymphocytes had a significant influence on the RFS ($p = 0.03$). The IPFSG score and the number of transplantations did not influence survival in our patient cohort. For *IL10* mRNA expression levels in T-lymphocytes the multivariate analysis also showed a significant impact on the RFS ($p = 0.002$).

CI: confidence interval of the hazard ratio; HR: hazard ratio; IPFSG: International Prognostic Factor Study Group; Tx: transplantation.

classified as "poor". In addition, all patients who received HDCT after second or subsequent relapse (14/40) were also classified as "poor" apart from the score calculation, resulting in 25/40 patients in this group (62.5%).[19] Patient data are depicted in detail in Table 2. Analysis of distributions of T-lymphocytes and monocytes were performed by flow cytometry and revealed no differences in the compartments between the two patient groups with or without relapse after HDCT (Fig. 1a and b and Fig. S1a and b). We also determined the expression levels of surface molecules

Table 2. Patients' characteristics.

		Relapse		
	[ALL] *N = 46*	No *N = 27*	Yes *N = 19*	*N*
Patients				
Follow-up time months (median)	29	90	6	*44*
[min; max]	[1; 168]	[15; 168]	[1; 22]	
IPFSG Score Class:				*40*
Good (0)	5 (12.5%)	4 (10.0%)	1 (2.5%)	
Intermediate[1]	10 (25.0%)	6 (15.0%)	4 (10.0%)	
Poor[2-3] and 2nd relapse	25 (62.5%)	13 (32.5%)	12 (30.0%)	
No. of HDCT/SCT				*46*
1	17 (37.0%)	7 (25.9%)	10 (52.6%)	
2	2 (4.3%)	1 (3.7%)	1 (5.3%)	
3	27 (58.7%)	19 (70.4%)	8 (42.1%)	
Gene expression levels				
B4GALT1_T-cells	2.57 ± 1.98	3.40 ± 2.22	1.60 ± 1.08	*37*
log.IL10_T-cells	0.58 ± 1.40	1.08 ± 1.36	0.04 ± 1.30	*25*
log.B4GALT1_Mono	0.61 ± 0.99	0.42 ± 0.95	0.86 ± 1.01	*44*
log.IL10_Mono	3.02 ± 1.41	3.02 ± 1.40	3.02 ± 1.47	*38*
log.B4GALT1_MNC	0.64 ± 0.72	0.68 ± 0.75	0.58 ± 0.70	*32*
Protein expression levels				
log.CD31_T-cells	4.36 ± 0.89	4.33 ± 0.59	4.39 ± 1.21	*46*
log.CD49 d_T-cells	3.65 ± 0.50	3.68 ± 0.50	3.61 ± 0.51	*46*
log.CD184_T-cells	3.91 ± 1.02	3.81 ± 1.24	4.05 ± 0.58	*46*
log.CD31_Mono	6.39 ± 0.58	6.33 ± 0.44	6.46 ± 0.73	*46*
log.CD49 d_Mono	4.26 ± 0.43	4.20 ± 0.46	4.33 ± 0.38	*46*
log.CD184_Mono	5.68 ± 1.06	5.54 ± 1.33	5.89 ± 0.44	*46*

Number of samples analyzed, median follow-up period, IPFSG scores, number of HDCT cycles, gene and protein expression levels are depicted. The patients were divided into groups with and without relapse. Score calculation was not possible for 6/46 patients: For 4/46 patients no follow-up data were available, 2/46 patients underwent primary HDCT.

HDCT: high-dose chemotherapy; IPFSG: International Prognostic Factor Study Group; SCT: stem cell transplantation.

related to cell adhesion on both T-cells and monocytes, but did not find any difference in the expression levels of PECAM-1, VLA-4 and CXCR4 between the patient groups with and without relapse after HDCT, as depicted in Fig. S2. Measuring the basal expression level of *B4GALT1* in different peripheral blood lymphocyte populations from healthy donors, we show that B-lymphocytes exhibit higher basal *B4GALT1* transcript levels compared to T-lymphocytes or NKT-cells (Fig. S3a). Furthermore, granulocytes responded to G-CSF treatment with an induced expression of *B4GALT1 in vitro* but lymphocyte subpopulations did not exhibit *B4GALT1* alteration after treatment with G-CSF *in vivo* or *in vitro* (Fig. S4). After separation of T-lymphocytes and monocytes from MNC by flow cytometry based cell sorting (Fig. 1a and b) we performed RT-PCR in order to determine *B4GALT1* mRNA expression levels in those cell-subsets in our cohorts of GCC patients. Within the T-cell population the expression level of *B4GALT1* was found to be significantly higher ($p = 0.008$) in the group of patients without relapse after HDCT compared to the group of patients who experienced a further relapse after HDCT (Fig. 1c) upon Mann Whitney testing. Comparison of non-relapse versus relapse patients by univariate Cox regression analyses showed a hazard ratio (HR) for the risk of relapse of 0.62 (95% confidence interval (CI) of HR: 0.42-0.91, $p = 0.02$) (Table 1). However, we could not find a significant difference in *B4GALT1* expression levels within the monocyte population (Fig. 1d and Table 1) or total MNC (Fig. 1e and Table 1). Thus, only *B4GALT1* mRNA expression in T-lymphocytes was significantly associated with durable disease remission.

In order to substantiate our hypothesis that activated peripheral T-cells may be involved in disease control of GCC, we performed lectin stimulation of MNC with Concanavalin A (Con A) *in vitro* and found up-regulation of *B4GALT1* mRNA particularly in the CD4+ T-lymphocyte fraction (Fig. S3b). Furthermore, we determined the mRNA expression levels of *IL10* as a marker for T-cell activation via qPCR analysis in our samples. Upon univariate Cox regression analysis we found *IL10* to be significantly higher expressed in T-lymphocytes from leukapheresis products of patients without relapse after HDCT in comparison to the relapsed group (HR: 0.62; 95% CI of HR: 0.39-1.0; $p = 0.05$) (Table 1). No difference in *IL10* expression was detectable in monocytes or total MNC between patients with or without relapse after HDCT (Table 1). A subgroup of patients was also evaluated for

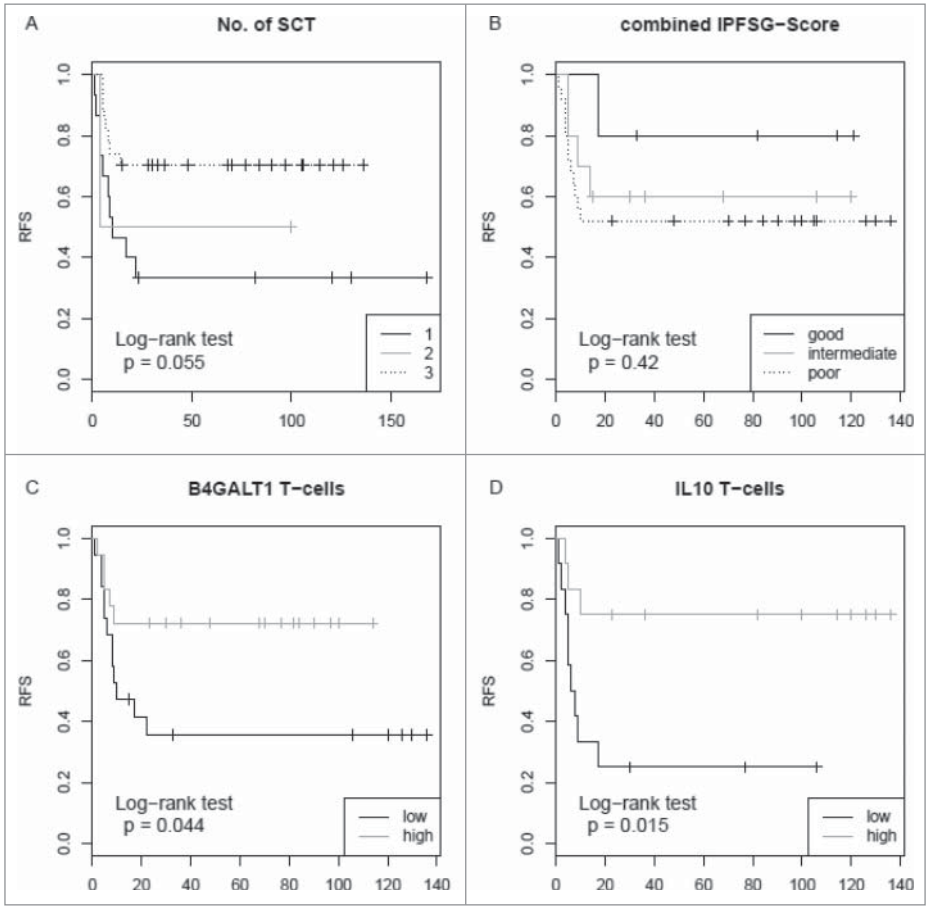

Figure 2. Relapse-free survival rates in 46 relapsed germ cell cancer patients with respect to the number of HDCT and ASCR, the international prognostic factor score, *B4GALT1* and IL10 expression levels. (A) RFS is not significantly different in patients with one (black line), two (grey line) or three (dotted line) SCT cycles (log-rank test, p = 0.055). (B) Analysis of patients' RFS according to the IPFSG categories. Patients with a good prognosis score (i.e. 0; black line) were compared to those with an intermediate (i.e. 1; grey line) and poor prognosis score (i.e. 2–3 and 2nd relapse; dotted line). RFS is not significantly different between these groups (log-rank test, $p = 0.420$). (C) RFS is significantly higher in patients with high T-cell *B4GALT1* mRNA expression levels (grey line) compared to those with low levels (black line) (log-rank test, $p = 0.044$). High and low refer to values above and below the median value, respectively. (D) RFS is significantly higher in patients with high *IL10* mRNA expression levels (grey line) compared to those with low levels (black line) (log-rank test, $p = 0.015$). IPFSG: International Prognostic Factor Study Group; RFS: relapse-free survival; SCT: stem cell transplantation.

expression of interferon gamma (*IFNG*) in CD4+ and CD8+ cells. Although up-regulation of *IFNG* in lymphocytes of healthy donors was observed after *in vitro* Con A stimulation we found no difference in *IFNG* expression between the relapsed patients and those with persistent remission (Fig. S5).

For further corroboration we performed a multivariate Cox regression analysis with regard to the number of cycles of high-dose chemotherapy in single or sequential HDCT and to the prognostic score of good versus poor prognosis.[20] Among patients with a high *B4GALT1* expression in T-lymphocytes the HR for relapse-free survival (RFS) was 0.66 (95% CI of HR: 0.45-0.97; $p = 0.03$). *IL10* expression in T-lymphocytes also proved to be an independent prognostic parameter as calculated by the multivariate Cox model with an HR of 0.3 (95% CI of HR: 0.14-0.65; $p = 0.002$). The corresponding graphs for log-rank testing are depicted in Fig. 2. Applying the prognostic score to our cohort resulted in an improved RFS between the good, intermediate or poor prognosis groups, but this did not reach statistical significance, possibly due to low patient numbers.

Next, we sorted lymphocyte subpopulations from a second independent cohort of 19 GCC patients, dividing CD4+ and CD8+ cells. As in healthy individuals we observed a twofold higher *B4GALT1* gene expression in B-lymphocytes compared to T-lymphocytes (data not shown) and found a difference of *B4GALT1* expression levels between relapse and non-relapse GCC patients in CD8+ cells but not in CD4+ cells (Fig. S6a and b). A trend toward better survival of the group with high *B4GALT1* expression in CD8+ cells was observed also in this second patient cohort, but log-rank testing did not give statistical significance for RFS ($p = 0.13$) or overall survival ($p = 0.08$), possibly due to the relatively small number of samples (Fig. S6c and d).

Thus, our data clearly support the notion that *B4GALT1* expression levels in T-lymphocytes denote a novel biomarker for the prognosis of GCC patients undergoing salvage HDCT and that activated T-lymphocytes may be involved in tumor control in such patients.

Immunohistochemistry (IHC) staining revealed moderate infiltration of *B4GALT1* expressing T-cells in the tumor tissue. Teratocarcinoma cells expressed more *B4GALT1* protein than cells of pure seminomas (Fig. S7 and Table S1). Given the up-regulation of epidermal growth factor receptor (EGFR) signaling pathways in GCC[21] and *B4GALT1* mediated inhibition of EGFR signaling in hepatocellular carcinoma,[22] it could be assumed hypothetically that *B4GALT1* expression renders lymphocytes potent to exert immunosurveillance, perhaps via interference with EGFR dimerization or phosphorylation.

We conclude that *B4GALT1* expression levels in T-lymphocytes from leukapheresis products of patients with GCC scheduled for salvage HDCT and ASCR seem to be relevant for tumor control and may represent a novel candidate biomarker in such patients. As our study was performed retrospectively and in relapse patients scheduled for salvage HDCT, who had already undergone chemotherapy, expression of *B4GALT1* should be evaluated prospectively and may include also patients at initial diagnosis, especially in order to see if the preceding chemotherapy and stimulation with G-CSF have altered the *B4GALT1* expression levels. Moreover, strategies of T-cell modulation particularly in patients with low *B4GALT1* expression levels in T-lymphocytes may be worthwhile to be studied in further clinical trials.

Disclosure of potential conflicts of interest

The authors declare no competing interests.

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