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LETTER TO THE EDITOR

# Clinical relevance of galectin-1 in hematologic malignancies treated with non-myeloablative hemopoietic stem cell transplantation

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Non-myeloablative hemopoietic stem cell transplantation (NM-HSCT) is often the only curative treatment option for patients with hematologic malignancies.[1] However, the treatment is frequently complicated by significant morbidity and mortality, acute GvHD (aGvHD) and chronic GvHD (cGvHD) being among the major causes.[2]

The pathophysiology of aGvHD is complex and can be summarized as a three-step process including (i) activation of APCs, (ii) donor T-cell activation and (iii) target tissue destruction.[3] On the contrary, the pathophysiology of cGvHD is poorly understood, although abnormalities in B-cell and T-cell functions, including alterations in the function of Th1, Th2 and regulatory T (Tregs; CD4+CD25+FoxP3+) cells, have been hypothesized to result in a global loss of immune tolerance.[4]

Galectin-1 (Gal-1), an endogenous lectin with specificity for N-acetyllactosamine (LacNAc; Galβ1, 4GlcNAc)-containing glycoconjugates, has an important role in immune cell activation, differentiation and homeostasis.[5] Multiple experimental models have shown the role of Gal-1 in suppressing chronic inflammation, autoimmunity and preventing allograft rejection. Gal-1 therapy significantly enhanced host survival and ameliorated GvHD following allogeneic bone marrow transplantation in mice.[6] Common features of these mouse models include a loss of Th1 and Th17 cells, a pronounced skewing towards a Th2-type cytokine profile and a substantial increase in the frequency of apoptotic T cells.[5,7] Gal-1 favors the expansion of $FoxP3^+$ Treg cells and $FoxP3^-$ type-1 Treg (Tr1) cells,[8] further limiting the extent of T-cell-mediated immunologic response.

Despite comprehensive research over the past decades, little is known about the clinical impact of Gal-1 on outcome-related parameters and occurrence of aGvHD and cGvHD in patients treated with NM-HSCT. Here, we aimed to investigate the impact of Gal-1 serum levels measured before NM-HSCT on (i) the incidence and severity of aGvHD and cGvHD, (ii) PFS and overall survival (OS) and (iii) transplant-related mortality (TRM).

For this retrospective study, we used frozen serum samples and clinical data from 57 patients with hematologic malignancies treated with NM-HSCT at our institution between August 2011 and March 2014. Baseline patient characteristics are summarized in Table 1. Serum samples were collected on day 25 (range 9–42) before NM-HSCT. Patients who received chemo or irradiation therapy $< 14$ days before sample collection were excluded. The median time interval between latest chemotherapy and sample collection was 45 days (range 14–188). The median follow-up was 417 days (range 88–1040).

To compare Gal-1 levels between healthy individuals and patients, serum samples from 30 healthy blood donors were collected; 16 males aged 45–62 years (mean 54) and 14 females aged 46–66 years (mean 52).

The study was approved by the Regional Ethics Committee (1-10-72-541-12) and by the Danish Data Protection Agency (1-16-02-335-12).

Serum Gal-1 levels were assessed according to a standard time-resolved immunofluorometric assay protocol as described previously with a few alterations.[9] For full description, please see supplementary information.

Patient characteristics were compared using Student´s *t*-test and $\chi^2$ test. aGvHD was defined as a complex of symptoms occurring within 100 days after transplantation and graded according to international consensus criteria.[10] cGvHD was graded according to European Bone Marrow Transplantation criteria and considered limited if it presented only in the liver and/or in a localized area of the skin and/or cavum oris. If the cGvHD affected any other organ or there was generalized skin involvement, it was considered to be extensive. Associations between variables were assessed by Spearman´s correlation test and Mann–Whitney *U* test. For survival analysis related to Gal-1, the continuous value of Gal-1 was dichotomized at the median (4.81 ng/mL). Survival was estimated by the Kaplan–Meier method and compared using log-rank test. TRM and time to occurrence of cGvHD were both evaluated in a competing risk model employing a pseudo-value method. Risk estimates at fixed timepoints were calculated using the pseudo-value approach and expressed with 95% confidence intervals (CI). Multivariate analysis was performed using Cox proportional hazard model. Two-sided *P*-values $< 0.05$ were considered statistically significant. All statistical analyses were done using STATA software version 13.1 (STATA, TX, USA).

Given the multiple roles of Gal-1 as a novel immune checkpoint inhibitor[11] and regulator of vascular-signaling programs,[12] and the critical role of immune and vascular components in the pathogenesis of GvHD and associated complications,[13] we explored here the clinical relevance of Gal-1 in hematologic malignancies treated with NM-HSCT. We found that circulating levels of Gal-1 were lower in the patient cohort as compared to healthy donors. The median Gal-1 serum level in healthy blood donors aged 45–66 years was 11.93 ng/mL (range 3.20–400). Median Gal-1 level in healthy men was 11.21 ng/mL (range 3.20–400) vs 15.85 ng/mL (range 3.85–400) in healthy women, $P = 0.382$. The median Gal-1 serum levels in the whole patient cohort was 4.81 ng/mL (range 0.89–400) (Figure 1a). Differences in Gal-1 levels between healthy individuals and patients could be explained, at least in part, by the patients' recent exposure to chemotherapy resulting in ongoing immunosuppression and altered secretion of Gal-1. Notably, Gal-1 concentrations did not correlate with total leukocyte, neutrophil or lymphocyte count recorded at the time of sample collection. An association between Gal-1 levels and pretransplant disease status (CR vs non-CR) was not observed either, $P = 0.369$. In addition, our data confirm that Gal-1 levels do not correlate with engraftment of donor hemopoietic cells, which is in line with the observations made in murine model of GvHD,[6] where Gal-1 treatment did not affect engraftment. The median time to engraftment was 19 days (range 8–27) in patients with low Gal-1 serum levels vs 21 days (range 11–24) in patients with high Gal-1 serum levels, $P = 0.299$.

**Table 1.** Patient characteristics and Gal-1 serum levels

| *Characteristics* | *Low Gal-1* | *High Gal-1* | P-*value* |
|---|---|---|---|
| Number of Patients | 28 | 29 | |
| Patient age, mean (range) | 58 (33–69) | 56 (17–68) | 0.634 |
| *Patient gender* | | | |
| Male | 21 | 14 | 0.038 |
| Female | 7 | 15 | |
| Gal-1 median (range) | 3.1 (0.9–4.5) | 11.9 (4.8–400) | — |
| *Donor age, years* | | | |
| $<40$ | 15 | 14 | 0.689 |
| $>40$ | 13 | 15 | |
| *Patient/donor sex* | | | |
| Patient male/donor female | 3 | 7 | 0.183 |
| Other | 25 | 22 | |
| *Patient/donor CMV status* | | | |
| Negative/negative | 9 | 4 | 0.045 |
| Negative/positive | 2 | 5 | |
| Positive/negative | 12 | 7 | |
| Positive/positive | 5 | 13 | |
| *Donor type* | | | |
| HLA-matched sibling | 9 | 10 | 0.851 |
| HLA-matched unrelated | 19 | 19 | |
| *Donor match* | | | |
| 10/10 | 23 | 27 | 0.208 |
| 9/10 | 5 | 2 | |
| *Type of disease* | | | |
| AML | 7 | 13 | 0.178 |
| Myelodysplastic syndrome | 6 | 3 | |
| Myelofibrosis | 3 | 0 | |
| CLL | 4 | 3 | |
| Non-Hodgkin's lymphoma | 5 | 3 | |
| Hodgkin's lymphoma | 0 | 2 | |
| Others | 3 | 5 | |
| *Disease status* | | | |
| CR | 17 | 21 | 0.349 |
| Non-CR | 11 | 8 | |
| *Disease risk for relapse* | | | |
| Low | 7 | 4 | 0.055 |
| Standard | 9 | 18 | |
| High | 12 | 6* | |
| *Conditioning regimen* | | | |
| Flu 90 mg/m$^2$ +TBI 2 Gy | 18 | 24 | 0.113 |
| Flu 90 mg/m$^2$+TBI 2 $\times$ 2 Gy | 10 | 5 | |
| *Stem cell source* | | | |
| Peripheral blood | 28 | 29 | 1.00 |
| *Post-Tx immunosuppression* | | | |
| CNI+MMF | 28 | 29 | 1.00 |

Abbreviations: CNI = calcineurin inhibitors; Gal-1 = galectin-1; MMF = mycophenolate mofetil.

Of the 57 transplanted patients, 24 experienced aGvHD (Gal-1 low, $n=10$; Gal-1 high, $n=14$), with 7 presenting grade I aGvHD and 17 grade II (Gal-1 low, $n=7$; Gal-1 high, $n=10$). Grade III and IV aGvHD were not observed. The incidence of aGvHD in patients with low vs high Gal-1 levels was 36% (95% CI: 21–56) and 48% (95% CI: 32–68; $P=0.575$), respectively. No relationship between Gal-1 levels and severity grade of aGvHD was observed. These results are in line with observations made in a mouse liver transplant model, where blockade of the Gal-1 pathway by a neutralizing anti-Gal-1 monoclonal Ab did not affect survival of liver allografts, suggesting that endogenous Gal-1 does not participate in the spontaneous tolerance at least in this model.[14] On the other hand, administration of recombinant Gal-1 prolonged survival of renal and liver allografts, and ameliorated GvHD in experimental mouse models.[6,14] Additional studies are needed to further explore the role of Gal-1 in aGvHD.

In our study, we demonstrated increased risk of cGvHD in patients with low Gal-1 levels supporting the hypothesis that Gal-1 has an important regulatory role in the development of cGvHD. Of the 56 patients alive at day 100, 25 patients experienced cGvHD (Gal-1 low, $n=10$; Gal-1 high, $n=15$), of which 14 limited (Gal-1 low, $n=5$; Gal-1 high, $n=9$) and 11 extensive cGvHD (Gal-1 low, $n=5$; Gal-1 high, $n=6$). After 12 months, the incidence of cGvHD in patients with low vs high Gal-1 levels was 47% (95% CI: 26–69) and 25% (95% CI: 5–45), respectively. The corresponding values after 15 months were 57% (95% CI: 36–79) and 34% (95% CI: 10–57), respectively, $P=0.052$ (Figure 1b). When tested in a multivariable model *vis-a-vis* combined donor features and age $>60$ years, Gal-1 level retained independent predictive influence on the risk of developing cGvHD (HR 0.33, 95% CI = 0.12–0.92).

However, higher incidence of cGvHD did not have a significant impact on PFS and OS. PFS at 100 days, 12 and 24 months after transplantation was 96% (95% CI: 77–99), 92% (95% CI: 70–98) and 92% (95% CI: 70–98) in patients with low Gal-1 levels vs 93% (95% CI: 75–98), 85% (95% CI: 65–94) and 80% (95% CI: 58–91) in patients with high Gal-1 levels ($P=0.319$). OS at 100 days, 12 and 24 years after transplantation was 96% (95% CI: 77–99), 73% (95% CI: 49–87) and 73% (95% CI: 49–87) in patients with low Gal-1 vs 100%, 88% (95% CI: 67–96) and 79% (95% CI: 50–92) in patients with high Gal-1 levels, $P=0.259$. Predefined risk factors of clinical interest such as host-, disease- and donor-related parameters, were analyzed for their associations with cGvHD, PFS and OS. None of these had a statistically significant impact on the selected end points.

Of the 57 patients, 5 died of transplant-related causes. All of them belonged to the cohort with low Gal-1 levels. The cumulative incidence of TRM at 100 days and 12 and 24 months after transplantation was 4% (95% CI: 0–11), 22% (95% CI: 5–40) and 22% (95% CI: 5–40) in patients with low Gal-1 levels, whereas no TRM events were observed in patients with high Gal-1 levels, $P=0.016$ (Figure 1c). Because of the paucity of events in general and the absence of TRM events in the high Gal-1 level cohort in particular, a multivariate analysis was not performed for the TRM end point.

In our study, we demonstrated increased risk of chronic, but not aGvHD in patients with low Gal-1 levels, suggesting a possible role of Gal-1 in the development of cGvHD. TRM was only observed in patients with low Gal-1 levels and could be related to the increased incidence of cGvHD in these patients. Gal-1 has emerged as an immunoregulatory protein that suppress T-cell responses by specifically deleting Th1 and Th17 cells[7] or by promoting tolerogenic dendritic cells.[15] Because Gal-1 is mainly synthesized by stromal cells, fibroblasts and endothelial cells and released to the circulation, it is likely that host Gal-1 may prevent aGVHD and cGVHD by specifically dampening grafted T cells and impairing the activity of APCs.

In conclusion, we suggest that Gal-1 may have an immunoregulatory role in the development of GvHD and control immune reconstitution following NM-HSCT preventing development of cGvHD and TRM. Prospective studies are warranted to confirm the present results and to further explore the role of Gal-1 in the NM-HSCT setting.


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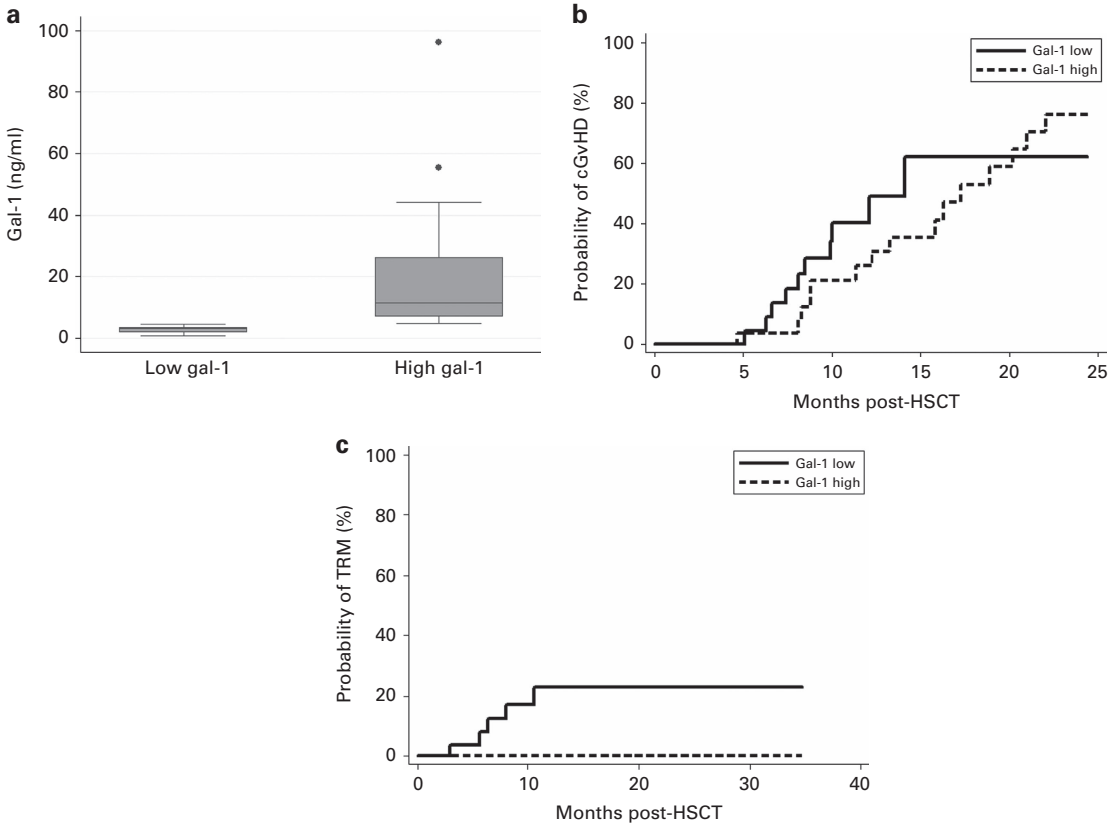


**Figure 1.** (**a**) Serum Gal-1 levels in the patient cohort ('low' vs 'high'). (**b**) Cumulative incidence of cGvHD in patients with 'low' vs 'high' Gal-1 levels. (**c**) Cumulative incidence of TRM in patients with 'low' vs 'high' Gal-1 levels. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

IP and FdA designed and coordinated the study, performed statistical analysis and wrote the manuscript; MV and PK assisted/advised in the performance of statistical analyses; ML, BH and RH performed and evaluated Gal-1 analysis; BSS collected and provided serum samples; BN advised during the design of the study and GAR critically reviewed the manuscript. All authors reviewed the manuscript and approved its final version.

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## REFERENCES

1 Ljungman P, Bregni M, Brune M, Cornelissen J, de Witte T, Dini G *et al.* Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. *Bone Marrow Transplant* 2010; **45**: 219–234.
2 Sorror ML, Sandmaier BM, Storer BE, Franke GN, Laport GG, Chauncey TR *et al.* Long-term outcomes among older patients following nonmyeloablative conditioning and allogeneic hematopoietic cell transplantation for advanced hematologic malignancies. *JAMA* 2011; **306**: 1874–1883.
3 Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet* 2009; **373**: 1550–1561.
4 Linhares YP, Pavletic S, Gale RP. Chronic GVHD: where are we? where do we want to be? will immunomodulatory drugs help? *Bone Marrow Transplant* 2013; **48**: 203–209.
5 Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol* 2009; **9**: 338–352.
6 Baum LG, Blackall DP, Arias-Magallano S, Nanigian D, Uh SY, Browne JM *et al.* Amelioration of graft versus host disease by galectin-1. *Clin Immunol* 2003; **109**: 295–307.
7 Toscano MA, Bianco GA, Ilarregui JM, Croci DO, Correale J, Hernandez JD *et al.* Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. *Nat Immunol* 2007; **8**: 825–834.
8 Dalotto-Moreno T, Croci DO, Cerliani JP, Martinez-Allo VC, Dergan-Dylon S, Mendez-Huergo SP *et al.* Targeting galectin-1 overcomes breast cancer-

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associated immunosuppression and prevents metastatic disease. *Cancer Res* 2013; **73**: 1107–1117.
9 Krassas GE, Pontikides N, Kaltsas T, Dumas A, Frystyk J, Chen JW *et al.* Free and total insulin-like growth factor (IGF)-I, -II, and IGF binding protein-1, -2, and -3 serum levels in patients with active thyroid eye disease. *J Clin Endocrinol Metab* 2003; **88**: 132–135.
10 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
11 Rabinovich GA, Croci DO. Regulatory circuits mediated by lectin-glycan interactions in autoimmunity and cancer. *Immunity* 2012; **36**: 322–335.
12 Croci DO, Cerliani JP, Pinto NA, Morosi LG, Rabinovich GA. Regulatory role of glycans in the control of hypoxia-driven angiogenesis and sensitivity to anti-angiogenic treatment. *Glycobiology* 2014; **24**: 1283–1290.
13 Henden AS, Hill GR. Cytokines in graft-versus-host disease. *J Immunol* 2015; **194**: 4604–4612.
14 Xu G, Tu W, Xu C. Immunological tolerance induced by galectin-1 in rat allogeneic renal transplantation. *Int Immunopharmacol* 2010; **10**: 643–647.
15 Ilarregui JM, Croci DO, Bianco GA, Toscano MA, Salatino M, Vermeulen ME *et al.* Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. *Nat Immunol* 2009; **10**: 981–991.

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