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High intratumoral Galectin-1 expression predicts adverse outcome in ALK⁻ ALCL and CD30⁺ PTCL-NOS

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Short running title: Gal-1 expression in PTCL

Key words: Peripheral T-cell lymphoma, Galectin-1, CD30, immunohistochemistry, prognosis

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**Conflict of interest**

The authors declare that they have no conflict of interest.
Abstract

Galectin-1 (Gal-1) has been associated with adverse prognosis in several cancers including lymphoma entities with CD30 expression. However, Gal-1 expression has not been systematically assessed in peripheral T-cell lymphomas (PTCL).

Specimens from 169 nodal PTCL were assessed for intratumoral Gal-1 expression by immunohistochemistry. Overall survival (OS) in groups exhibiting high and low Gal-1 expression was compared in the cohort and in a subset analysis of CD30-positive PTCL only. Gal-1 expression was also correlated with biomarkers of the tumor microenvironment.

No significant difference in OS based on Gal-1 expression was observed in the entire PTCL cohort. However, in the CD30-positive cohort, patients with high Gal-1 levels had significantly poorer outcome (5 years OS 10%, 95% confidence interval (CI): 1-36) than their low Gal-1 counterparts (5 years OS 48%, 95%CI: 30-64, P=0.021). In univariate analyses age≤60, non-elevated LDH, and performance score<2 correlated with superior survival but high Gal-1 expression significantly predicted adverse outcome at both univariate (HR 2.5, 95%CI: 1.1-5.7, P=0.026) and multivariate levels (HR 3.2, 95%CI: 1.2-8.5, P=0.017). Tumors with high Gal-1 had few cytotoxic T cells in the tumor microenvironment.

High intratumoral Gal-1 expression before therapeutic intervention correlates with adverse outcome in nodal CD30+, ALK− PTCL patients.
Introduction

Peripheral T-cell lymphoma (PTCL) is a heterogeneous group of clinically aggressive neoplasms, accounting for 10-12% of all lymphomas in Western countries\textsuperscript{1,2}. Among nodal subtypes angioimmunoblastic T-cell lymphoma (AITL), PTCL-not otherwise specified (PTCL-NOS) and anaplastic large cell lymphoma (ALCL) are the most frequent entities. ALCL is divided in ALK\textsuperscript{+} and ALK\textsuperscript{−} ALCL depending on whether they express the anaplastic large cell lymphoma kinase (ALK) fusion protein. Histomorphologically, ALK\textsuperscript{+} and ALK\textsuperscript{−} ALCLs have striking similarities, e.g. strong and uniform CD30-positivity by tumor cells and characteristic neoplastic hallmarks, although they differ in a number of clinical features. ALK\textsuperscript{−} ALCL patients are older and usually display a more aggressive clinical course and poorer response to chemotherapy in the majority of the cases\textsuperscript{3}. Recently, chromosomal rearrangements involving the DUSP22 and TP63 genes have been reported addressing a refined prognostic category\textsuperscript{4,5}; yet the distinction from the heterogeneous PTCL-NOS entity with CD30 expression is still challenging\textsuperscript{6,7}.

Most PTCLs respond poorly to classical treatments and exhibit a dismal prognosis. Hence, novel therapeutic approaches are needed and efforts are being made to discover new drugs. CD30 is a member of the tumor necrosis factor receptor superfamily\textsuperscript{8} and has emerged as a potential therapeutic target. CD30 is strongly and uniformly expressed by all ALCLs and to varying degrees in other PTCL subtypes\textsuperscript{9,10}.

Galectin-1 (Gal-1), a prototype member of the galectin family endogenous family of galectins, plays key roles in tumor progression, during haematopoiesis, modulation of acute and chronic inflammation and immune cell activation, differentiation and evasion in hae-
matological malignancies \textsuperscript{11,12}. Compared to healthy individuals, Gal-1 is overexpressed in various haematological cancers, including primary cutaneous T-cell lymphomas, B lymphoblastic leukaemia, chronic lymphocytic leukaemiae and Hodgkin lymphoma \textsuperscript{13–18}. Reed-Sternberg cells in Hodgkin lymphoma express high amounts of Gal-1 through mechanisms involving an activator protein-1 (AP-1)-dependent enhancer \textsuperscript{17}. Moreover, ALCL tumor cells co-express Gal-1 and its transcriptional regulator c-Jun, an important component of the AP-1 transcription factor \textsuperscript{13}.

In this study, we assessed expression of Gal-1 in nodal PTCL specimens using immunohistochemistry (IHC) and digital image analysis, and correlated these findings with clinical outcome of these patients and frequency of immune cells in the tumor microenvironment.
Methods and materials

Patients and samples

The establishment of a Danish cohort of 169 patients diagnosed with PTCL between 1984-2011 has been previously described\(^5\). Formalin-fixed, paraffin-embedded tissue specimens were retrieved from pre-therapeutic biopsies of previously untreated, newly diagnosed patients, aged 16 years or older, where pre- and post therapeutic clinical information were available. Two expert haematopathologists reviewed and reclassified the cases in accordance with the 2008 WHO classification\(^9\). Clinicopathological data were obtained from the population-based database of the Danish lymphoma registry\(^19\) and supplemented by medical records. Tissue microarrays (TMA) were constructed using a TMA master-01 (3DHISTECH Ltd., Budapest, Hungary) using four cores per case. Six cases (4%) were excluded due to inadequate material.

The study was approved by The Central Denmark Region Committees on Health Research Ethics (record no. 1-10-72-392-12) and the Danish Data Protection Agency (record no. 1-16-02-26-11). The project was conducted in accordance with the Helsinki Declaration.

Immunohistochemistry

IHC stains were performed on 4 μm tissue sections according to standard antibody-specific protocols, optimized in-house for use with the Ventana Benchmark automated staining system (Ventana Medical Systems, Tucson, AZ, USA). Primary rabbit polyclonal anti-Gal-1 antibody (dilution 1:5000) used for Gal-1 detection was generated in G.A.R.'s
laboratory as described\textsuperscript{15,20}. The remaining antibodies were: CD30 (clone Ber-H2; Dako), CD8 (clone SP57; Ventana), Ki-67 (clone SP6; Cell Marque), CD68 (clone KP1; Cell Marque), CD163 (clone EDHu-1; Serotec), FOXP3 (clone 236A/E7; Abcam). Positive and negative controls were included for each stain where relevant. Slides were counterstained with haematoxylin.

TMA slides were captured and digitalized at a magnification of x40 using the Hamamatsu Nanozoomer 2.0HT (Hamamatsu Photonics, Hamamatsu City, Japan). For Gal-1, Ki-67, FOXP3, CD68, and CD163 IHC, stained sections were digitally quantified using Visiopharm (Visiopharm Integrator System 4.0.3.0, Visiopharm, Hoersholm, Denmark). For quantification of Gal-1, CD68, and CD163, an Analysis Protocol Package was designed to detect stained area and total core area. Immunolocalized proteins were recorded as an area fraction (AF) \textit{i.e.} positive IHC signals normalized to total core area. For the nuclear staining of Ki-67 and FOXP3, results were quantified as a fraction of positive cell profiles \textit{i.e.} positive IHC nuclei normalized to all detectable nuclei in the cores. The Analysis Protocol Package was optimized to robustly discriminate different degrees of staining intensity and nonspecific background\textsuperscript{16–18}. CD8 and CD30 expression was evaluated separately to morphologically discriminate between tumor cells and other cells in the tumor microenvironment. A semi-quantitative estimate of the frequency of tumor-infiltrating CD8\textsuperscript{T} T cells was scored as high \textit{(i.e.} >10\% of reactive non-tumor cells) or low \textit{(i.e.} \leq 10\% of reactive non tumor cells) in each case by two independent observers. To ensure inclusion of non-ALCLs with high CD30-expression, cases were regarded as CD30-positive if $\geq$30\% of the tumor cells expressed CD30.
Statistical analysis

Differences between groups were determined with Fisher’s exact test for categorical values and Wilcoxon’s rank sum test for continues variables. Treatment outcome was described by overall survival (OS), defined as the time interval from diagnosis to last follow-up or death from any cause. For survival analysis related to Gal-1 expression, two groups were compared, i.e. the quartile of patients with highest expression values versus the lower three quartiles taken as one group. This cut off point was chosen in concordance with previous reports. Survival fractions were estimated using the Kaplan-Meier method and differences between groups were evaluated with the log-rank test. A Cox proportional multivariable model was used to assess possible association of Gal-1 with overall survival, while adjusting for parameters with significant levels below 0.1 at the univariate level. The proportional hazards assumption was checked with Schoenfeld residuals. \(P\)-values below 0.05 were considered to be statistical significant. All statistical analyses were performed using STATA IC 11 (StataCorp, College Station, TX, USA).
Results

Association of Gal-1 expression with demographics and clinical outcome of PTCL-NOS, AITL, ALK⁻ ALCL and ALK⁺ ALCL patients

Pre-therapeutic tumor tissue samples from a total of 163 patients (84 PTCL-NOS, 28 AITL, 37 ALK⁻ ALCL, and 14 ALK⁺ ALCL) were evaluated for Gal-1 expression. Gal-1 was highly expressed in all the PTCL subtypes analysed with a median AF of 70% (range 9-98%). The Gal-1 AF cut-off was 90% according to the definition mentioned in the Method section. ALCL had significantly higher Gal-1 expression compared with PTCL-NOS and AITL (P <0.001).

Figure 1 shows the flowchart of the study. The clinico-pathological features of the PTCL cohort have been previously described. In brief, classic risk factors were in line with other reports with a median age of 59 years (range: 17-92 years) and a male to female ratio of 1.6. Based on Gal-1 expression, the cohort was categorized into two groups. Figure 2A-B shows representative images of high and low intratumoral Gal-1 expression. Clinico-pathologic features were evenly distributed between the groups (data not shown). No difference in OS was found between specimens categorized into those exhibiting high or low Gal-1 expression in the PTCL cohort when taken as a whole (Figure 3A).
Association of Gal-1 expression with demographics and clinical outcome in ALK\(^+\) ALCL and CD30\(^+\) PTCL-NOS patients

Due to a more favourable outcome and few events, ALK\(^+\) ALCL patients were excluded from the CD30\(^+\) cohort resulting in a final ALK\(^-\) ALCL and CD30\(^+\) PTCL-NOS cohort of 43 cases (37 ALK\(^-\) ALCL and 6 PTCL-NOS), (Figure 1). Table 1 outlines the demographic features between the patients with high Gal-1 expression and low Gal-1 expression. No differences in classical risk factors were present. The median age was 60 years (range 17-89 years), with a male to female ratio of 2.6. The majority of the patients (79%) received curatively intended combination chemotherapy consisting of CHOP or CHOP-like regimens.

At the univariate level, high Gal-1 expression, age >60, elevated LDH at diagnosis, and performance score \(\geq 2\) correlated with lower survival (Table 2). High intratumoral Gal-1 expression was associated with an adverse clinical outcome (5 year OS 10%, 95% CI: 1-36) compared with low Gal-1 expression (5 year OS 48%, 95% CI: 30-64), HR 2.5 (95% CI: 1.1-5.7, \(P=0.021\)) (Figure 3B). In a multivariate analysis, high Gal-1 expression retained its adverse prognostic impact, HR 3.2 (95% CI: 1.2-8.5, \(P=0.017\)) (Table 3).

Correlation of Gal-1 expression with intratumoral cytotoxic T lymphocytes

Given the broad immunosuppressive effect of Gal-1 in tumor microenvironments\(^{23}\), we further explored whether Gal-1 expression was associated with the frequency of infiltrating immune cells. Interestingly, all PTCL cases with high Gal-1 expression exhibited low frequency of tumor-infiltrating CD8\(^+\) T lymphocytes. A trend toward a positive correlation
between high Gal-1 expression and tumor cell proliferation, as determined by Ki-67 staining, was observed, although this association was not statistically significant (data not shown). In the tumor tissue analysed assessed by digital image analysis, we did not find correlations between Gal-1 expression and the percentage of total intratumoral macrophages (stained for CD68 and CD163) or regulatory T cells (determined by FOXP3 expression) (Figure 2C-F). Thus, high Gal-1 expression in PTCL tumors is associated with a lower frequency of CD8+ T lymphocytes.
Discussion

In this study, we report an adverse prognostic impact of a pre-therapeutic high expression of tumoral Gal-1 in a cohort of ALK- ALCL and CD30⁺ PTCL-NOS patients. However, no difference in overall survival between patients with low and high Gal-1 expression was found when analysing the entire PTCL cohort, i.e. including patients diagnosed with PTCL-NOS, ALK⁻ ALCL, ALK⁺ ALCL, and AITL.

PTCL tumors are aggressive lymphomas showing a modest response to polychemotherapy, frequent relapses and a poor overall survival. PTCL encompasses more than 25 different heterogeneous entities, which are distinguished by morphology and immunophenotypic analyses, genetics and clinical features. According to the latest WHO classification, ALK⁻ ALCL is now considered a distinct entity. Although high Gal-1 expression was also found among ALK⁺ ALCL, we excluded the ALK⁺ ALCL cases from our subset analysis of CD30⁺ PTCLs due to their a priori favourable outcome compared with other PTCL subtypes including ALK⁻ ALCL and PTCL-NOS. In this regard, ALK⁺ ALCL tumors have a unique tumor biology not shared with that of PTCL-NOS and ALK⁻ ALCL.

The remaining cases in our CD30⁺, ALK⁻ cohort were of PTCL-NOS origin. A diagnostic grey zone exists between CD30⁺ PTCL-NOS and ALK⁻ ALCL. CD30 positivity is pathognomonic for a diagnosis of ALCL. However, to some extent more than 50% of PTCL-NOS patients also express CD30. Indeed, Bisig et al showed a substantial overlap of the molecular signatures and protein expression levels between CD30⁺ PTCL-NOS and ALK⁻ ALCL. Although the number of CD30⁺ patients in our study was limited, they represent an interesting sub-
group since CD30-targeted therapy is a promising approach in PTCL patients. The CD30 antibody-drug conjugate, Brentuximab Vedotin (BV), has shown an important effect in relapsed ALCL with objective response rates of 86%\(^{29}\) and has now been approved for these patients in the USA and Europe. Recently, the final results of the randomized, multicentric phase-III clinical trial (ECHELON-2) comparing BV+CHP with CHOP in the first-line treatment of CD30\(^+\) PTCLs showed superior OS and progression free survival for the directly CD30-targeted treatment arm\(^ {30}\). Whether BV or other upfront treatment may overcome the negative prognostic effect of high Gal-1 in CD30\(^+\) PTCL cannot be assessed from our data. In this regard, our study has limitations, e.g. including its retrospective design, a long time period of patient inclusion, and the relatively small number in sub-analyses limiting the statistical power. Particularly, in the high Gal-1 group, we acknowledge that few patients may impact the outcome analyses despite balanced clinical features between the two groups. Digital pathology was used as an attempt to standardize the quantification of the expression levels between different specimens and to rule out possible inter-observer variations.

Galectins and its specific glycosylated ligands have emerged as novel regulatory checkpoints during tumor progression, being implicated in cell proliferation, apoptosis, immune escape and angiogenesis\(^{12,31}\). Studies comparing malignant and healthy tissues have shown altered expression of Gal-1, a prototype member of this family, in a broad range of solid tumors\(^ {32}\) and lymphomas\(^ {12-14}\). In PTCL, strong expression of Gal-1 has been observed in 18 out of 19 ALCL patients\(^ {13}\). Our study confirms a very high Gal-1 expression in ALCL, and CD30\(^+\) PTCL-NOS. Interestingly, increased Gal-1 expression has been associa-
ed with poor prognosis and tumor metastasis in most tumor types analysed\textsuperscript{18,32}. Indeed, Gal-1-specific inhibitors and antibodies have been developed\textsuperscript{33,34} and a clinical phase I trial testing the efficacy of the Gal-1 inhibitor OTX008 to patients with advanced solid tumors is ongoing\textsuperscript{35}.

The interaction between malignant cells and the surrounding tumor microenvironment is central in the development and progression of haematological malignancies\textsuperscript{36,37}. Interestingly, we found high Gal-1 expression that correlated with a lower frequency of intratumoral cytotoxic T lymphocytes in PTCL. This is in line with other malignancies, where high expression of this lectin correlated with diminished percentage of intratumoral and peritumoral T lymphocytes\textsuperscript{38,39}.

It has been proposed that Gal-1 contributes to tumor-immune privilege by reducing the survival viability of CD4\textsuperscript{+} and CD8\textsuperscript{+} T-cell subsets\textsuperscript{40} and/or promoting the expansion of FOXP3\textsuperscript{+} T regulatory (Treg) cells\textsuperscript{41}, thus providing a rational explanation for the reduced number of effector T cells in the tumor microenvironment. Although we observed a correlation of between Gal-1 expression with and CD8\textsuperscript{+} T cells, we could find no significant association between Gal-1 and Tregs in the PTCL microenvironment. Moreover, although we previously demonstrated a correlation between the number of tumor-associated macrophages and adverse outcome in ALK\textsuperscript{+} ALCL\textsuperscript{22}, this study did not reveal an apparent association of Gal-1 expression with these cells.

In conclusion, in this study we report that high expression of intratumoral Gal-1, which correlated with poor prognosis and low frequency of intratumoral CD8\textsuperscript{+} T lymphocytes in a subgroup of ALK\textsuperscript{+} ALCL and CD30\textsuperscript{+} PTCL-NOS patients. These findings should be validated in
larger independent patient cohorts in order to better understand the underlying biology, assess the potential use of Gal-1 as a prognostic marker and understand its relevance in patients with PTCL.
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35. Wdowiak K, Francuz T, Gallego-Colon E, et al. Galectin targeted therapy in oncology:


Table 1: Demographics and pre-therapeutic clinical features of the ALK+ ALCL and 30+ PTCL-NOS patient cohort categorized according to Gal-1 expression.

<table>
<thead>
<tr>
<th>Feature</th>
<th>No. of patients (%)</th>
<th>Low Gal-1 expression (n=33)</th>
<th>High Gal-1 expression (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>60</td>
<td>61</td>
<td>51</td>
<td>NS</td>
</tr>
<tr>
<td>Range</td>
<td>17-89</td>
<td>28-89</td>
<td>17-77</td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>21 (49)</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>22 (51)</td>
<td>19</td>
<td>3</td>
<td></td>
</tr>
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<td>Sex</td>
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<td>31 (72)</td>
<td>25</td>
<td>6</td>
<td>NS</td>
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<tr>
<td>Female</td>
<td>12 (28)</td>
<td>8</td>
<td>4</td>
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<td>PS (WHO)</td>
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<td>0-1</td>
<td>30 (71)</td>
<td>25</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>≥2</td>
<td>12 (29)</td>
<td>7</td>
<td>5</td>
<td></td>
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<td>LDH above UNL</td>
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<td>16 (40)</td>
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<td>6</td>
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<td>19</td>
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<td>23 (53)</td>
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<td>5</td>
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<td>ALK- ALCL</td>
<td>37 (86)</td>
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<td>9</td>
<td>NS</td>
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<tr>
<td>PTCL-NOS</td>
<td>6 (12)</td>
<td>5</td>
<td>1</td>
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<td>Chemotherapy regimen</td>
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<td></td>
</tr>
<tr>
<td>CHOP/CHOP-like</td>
<td>34 (79)</td>
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<td>7</td>
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<tr>
<td>Other</td>
<td>6 (14)</td>
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<tr>
<td>No chemotherapy</td>
<td>3 (7)</td>
<td>1</td>
<td>2</td>
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PS: performance score; CS: clinical stage; LDH: lactate dehydrogenase; UNL: upper normal limit; IPI: international prognostic index; NS: not statistically significant (using Fishers’s exact test); *IPI was not evaluated in six patients due to missing value in at least one of the IPI-factors.
Table 2: Univariate analysis for survival in the ALK⁺ ALCL and 30⁺ PTCL-NOS patient cohort (n=43).

<table>
<thead>
<tr>
<th>Feature</th>
<th>No. of patients (%)</th>
<th>5 years OS (%)</th>
<th>HR</th>
<th>95% CI</th>
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<td>Age (years)</td>
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<td>Male</td>
<td>31 (72)</td>
<td>33.3</td>
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<td>0.31-1.66</td>
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<td>Female</td>
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<td>40.9</td>
<td>0.72</td>
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<td>0-1</td>
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<td>45.6</td>
<td>1</td>
<td>0.95-4.95</td>
<td>0.064</td>
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<tr>
<td>≥2</td>
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<td>25.0</td>
<td>2.17</td>
<td></td>
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</tr>
<tr>
<td>LDH above UNL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (40)</td>
<td>52.9</td>
<td>1</td>
<td>1.40-7.16</td>
<td>0.005</td>
</tr>
<tr>
<td>No</td>
<td>16 (60)</td>
<td>18.8</td>
<td>3.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS (Ann Arbor stage)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>22 (51)</td>
<td>48.7</td>
<td>1</td>
<td>0.76-3.63</td>
<td>0.201</td>
</tr>
<tr>
<td>III-IV</td>
<td>21 (49)</td>
<td>28.6</td>
<td>1.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of extranodal sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>35 (81)</td>
<td>45.0</td>
<td>1</td>
<td>0.88-5.00</td>
<td>0.097</td>
</tr>
<tr>
<td>≥2</td>
<td>8 (19)</td>
<td>12.5</td>
<td>2.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B symptoms</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>20 (47)</td>
<td>35.0</td>
<td>1</td>
<td>0.30-1.42</td>
<td>0.281</td>
</tr>
<tr>
<td>No</td>
<td>23 (53)</td>
<td>41.9</td>
<td>0.65</td>
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<tr>
<td>Gal-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>33 (77)</td>
<td>47.7</td>
<td>1</td>
<td>1.12-5.68</td>
<td>0.026</td>
</tr>
<tr>
<td>High</td>
<td>10 (23)</td>
<td>10.0</td>
<td>2.52</td>
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</tr>
</tbody>
</table>

OS, overall survival; HR, Hazard ratio; CI, confidence interval; PS, performance score; LDH, lactate dehydrogenase; UNL, upper normal level; CS, clinical stage.
**Table 3:** Multivariate analysis including Gal-1 expression.

<table>
<thead>
<tr>
<th>Feature</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;60 yrs</td>
<td>3.38</td>
<td>1.30-8.76</td>
<td>0.012</td>
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<td>PS (WHO) ≥2</td>
<td>1.27</td>
<td>0.46-3.51</td>
<td>0.650</td>
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<td>LDH above UNL</td>
<td>2.24</td>
<td>0.82-6.09</td>
<td>0.115</td>
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<tr>
<td>High Gal-1</td>
<td>3.23</td>
<td>1.23-8.46</td>
<td>0.017</td>
</tr>
</tbody>
</table>

HR, Hazard ratio; CI, confidence interval; PS, performance score; LDH, lactate dehydrogenase; UNL, upper normal level.
Figure legends

Figure 1:
Definition of the ALK− ALCL and CD30+ PTCL-NOS cohort after exclusion of CD30− and ALK+ specimens.

Figure 2:
Representative immunohistochemical staining of specimens showing high intratumoral Gal-1 expression (A), low intratumoral Gal-1 expression (B), low CD8 expression (C), high CD8 expression (D), high Ki-67 expression (E), low Ki-67 expression (F), FOXP3 (G), and CD30 (H) in PTCL patients. All magnifications are 40x.

Figure 3A and B:
Overall survival of the PTCL patient cohort (A) and the ALK− ALCL and CD30+ PTCL-NOS patient cohort (B).
Figure 1

PTCL cohort (n=163)
- PTCL-NOS (n=84)
-AITL (n=28)
-ALK- ALCL (n=37)
-ALK+ ALCL (n=14)

Excluded (n=106)
- CD30-negative samples

CD30+ cohort (n=57)
- PTCL-NOS (n=6)
-ALK- ALCL (n=37)
-ALK+ ALCL (n=14)

Excluded (n=14)
- ALK+ ALCL

CD30+, ALK- PTCL cohort (n=43)
- PTCL-NOS (n=6)
-ALK- ALCL (n=37)
Figure 2

A) High Gal-1

B) Low Gal-1

C) Low CD8

D) High CD8

E) High Ki-67

F) Low Ki-67

G) FOXP3

H) CD30