Cell adhesion receptors in lymphoma dissemination
Drillenburg (ook Lelijveld), P.

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Chapter 5

CD44 expression predicts disease outcome in localized large B-cell lymphomas

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submitted
Chapter 5

Abstract

Diffuse large B-cell non-Hodgkin's lymphomas (DLCL) form a heterogeneous group of tumors with diverse morphology, clinical features, treatment response and prognosis. The biological variables underlying this heterogeneity are unknown. In the present study, we explored the value of the lymphocyte homing receptor CD44, a putative determinant of lymphoma dissemination, in predicting prognosis in DLCL. Expression of the standard form of CD44 (CD44s) and of CD44 isoforms containing exon v6 (CD44v6) on tumor cells was assessed by immunohistochemistry in a cohort of 276 DLCL patients from a population based lymphoma registry. We observed that CD44s as well as CD44v6 expression correlated with tumor dissemination in patients with primary nodal DLCL. Importantly, in patients with localized nodal disease, CD44s was a strong prognosticator predicting tumor related death independent of the other parameters of the International Prognostic Index (IPI). Incorporation of CD44s in the IPI parameter “stage”, increased the prognostic value of this parameter in nodal DLCL. Our data identify CD44 as a biological prognosticator, which can be used to “fine-tune” the IPI for nodal DLCL.
Introduction

Diffuse large B-cell lymphomas (DLCL) constitute 30 to 40% of adult non-Hodgkin’s lymphomas and are biologically related to (activated) mature B cells. In the recently proposed Revised European American Lymphoma (REAL) classification these tumors are categorized as one single group, since previous attempts to subclassify them on morphological grounds, were largely irreproducible. However, there is consensus that DLCL represent a diverse group of neoplasms, with heterogeneous genetic background, clinical features, treatment response, and prognosis. Although prognosis in DLCL can be predicted on the basis of clinical characteristics, the genetic and molecular basis underlying the heterogeneity in disease aggressiveness and tumor progression, as well as in response to therapy remain to be elucidated.

Normal recirculating lymphocytes express cell-surface glycoproteins of the CD44 family. These molecules have a wide tissue distribution and function in several important biological processes including lymphocyte homing and activation, hematopoiesis, and tumor progression and metastasis. Due to extensive alternative splicing and posttranslational modification by N- and O-linked sugars as well as by glycosaminoglycan (GAG) side chains, CD44 is highly diverse. On lymphocytes, the short 80-90 kDa standard (hematopoietic) form of CD44 (CD44s) is most abundant, while larger variants predominate on some normal and neoplastic epithelial cells and are also expressed on activated lymphocytes and on aggressive lymphomas. Clinical studies in non-Hodgkin’s lymphomas have demonstrated that expression of CD44s epitopes is associated with disseminated disease and tumor related death. A causal role of CD44s in determining this unfavorable disease outcome was suggested by experiments in nude mice, showing an enhancing effect of CD44s on lymphoma growth and dissemination. More recently, CD44 splice variants containing exon v6 were shown to be expressed in a subgroup of high-grade lymphomas with poor prognosis. In rodents, homologues of these variants confer metastatic potential on carcinoma and lymphoma cell lines. Together, these data indicate that CD44s and CD44v6 are important mediators of lymphoma dissemination that could be of use as molecular markers to identify subgroups of high-risk DLCL patients. Here, we have explored this hypothesis by studying CD44s and CD44v6 expression in tumor tissue of patients with DLCL from a population-based NHL registry; the results indicate that CD44 can be considered as a major prognosticator in patients with localized DLCL.
Chapter 5

Materials and methods

Patients and material. Between 1981 and 1989 1168 patients with a histologically proven diagnosis of NHL were included in the population based non-Hodgkin’s lymphoma registry program of the Comprehensive Cancer Center West (CCCW) in The Netherlands. The region of the CCCW comprises 1.6 million inhabitants and 15 hospitals. Only newly diagnosed NHL were included. All tumors were reviewed by a panel of hematopathologists. Patients were staged according to the Ann Arbor classification with modifications for extranodal lymphoma. For adequate staging at least a chest radiograph and/or CT scan, a CT scan of the abdomen or lymphangiogram supplemented by isotope spleen and liver scan in the early years of registration, and a bone marrow biopsy had to be performed. In addition to clinical stage, the presence or absence of lymphoma at various anatomical sites was recorded. Patients were treated according to the preference of the local specialist in internal medicine, and this treatment was considered adequate for large cell NHL, when doxorubicin-containing polychemotherapy was administered with or without radiotherapy, whereas for stage I patients, radiotherapy alone was also considered as adequate. All tissue specimens were obtained prior to treatment. Clinical parameters as well as treatment modalities and outcome were recorded. The follow-up was annually updated up to 1996.

In the CCCW records 494 B-cell lymphomas were diagnosed according to the Kiel classification as diffuse centroblastic-centrocytic (diff CbCc), diffuse centroblastic (diff Cb), immunoblastic (lb) and mucosa-associated lymphoid tissue lymphomas of high malignancy (MALT high), compatible with large B-cell NHL as recently defined by the REAL-classification. From 310 patients, tissue blocks containing tumor were available and slides of paraffin embedded tumor tissue were made. From 276 patients, interpretable staining of tumor tissue for CD44s and CD44v6 was obtained. For 236 of these patients, all prognostic parameters of the International Prognostic Index (IPI) were available (table 1); demographics, and stage distribution of these 236 patients was not different than those of the total group of 494 B-cell lymphomas in the CCCW registry (data not shown).

According to our previous reports at presentation three patterns of lymphoma localization were defined: (1) “primary nodal” NHL with presentation in lymph node, Waldeyer’s ring or spleen (n=155); (2) “primary extranodal” NHL with presentation at other sites without or with regional lymph node involvement (n=91); (3) other NHL with disseminated disease at presentation and no possibility to determine the primary site (n=30); the latter were called “extensive”. Both in primary nodal and extranodal disease, bone marrow involvement was accepted in cases with a positive staging biopsy. All parameters of the IPI were available in these three subgroups for 137, 75 and 24 patients respectively.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>total n=276 (%)</th>
<th>nodal n=155 (%)</th>
<th>extranodal n=91 (%)</th>
<th>extensive n=30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>male/female</td>
<td>131/145</td>
<td>82/73</td>
<td>38/53</td>
<td>11/19</td>
</tr>
<tr>
<td>age (range)</td>
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<tr>
<td>age (mean)</td>
<td>65</td>
<td>64</td>
<td>67</td>
<td>65</td>
</tr>
</tbody>
</table>

Ann Arbor stage
- stage I: 88 (32), 39 (25), 49 (54), 0
- stage II: 73 (26), 53 (34), 20 (22), 0
- stage III: 38 (14), 38 (25), 0, 0
- stage IV: 77 (28), 25 (16), 22 (24), 30 (100)

IPI
- low: 97 (35), 57 (37), 40 (44), 0
- low/intermediate: 62 (23), 46 (30), 14 (15), 2 (7)
- high/intermediate: 39 (14), 23 (15), 10 (11), 6 (20)
- high: 38 (14), 11 (7), 11 (12), 16 (53)
- not evaluable: 40 (14), 18 (11), 16 (18), 6 (20)

Therapy
- no therapy and surgery only: 36 (13), 11 (7), 23 (25), 2 (7)
- radiotherapy only: 67 (25), 38 (25), 28 (31), 1 (3)
- chemotherapy and +/- radiotherapy
  - without doxorubicin: 48 (17), 24 (15), 11 (12), 13 (43)
  - containing doxorubicin: 125 (45), 82 (53), 29 (32), 14 (47)

Immunohistochemistry. Tissues were fixed in buffered formalin and routinely embedded in paraffin. Sections of 5μm were cut and put on APES-coated slides. After deparaffinization in xylene and rehydration in alcohol endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide in methanol for 20 minutes. Prior to staining with monoclonal antibody (mAb) VFF18 (see below), the tissue sections were incubated in a microwave oven at 100°C for 10 minutes in citrate buffer (0.01 M, pH=6). The slides were washed in PBS, pre-incubated for 15 minutes with 10% normal goat serum, and then incubated for 1 hour with the primary mAb. The primary monoclonal antibodies used were Hermes-3 against an epitope on the constant part of CD44 (kindly provided by Dr S. Jalkanen, Turku, Finland), and VFF18 (Bender Co. Vienna, Austria) against the epitope QWFGRWHEGYRQT on exon CD44v6. After incubation with the primary mAb, the sections were incubated with biotinylated rabbit anti-mouse antibodies (DAKO, Glostrup, Denmark) in PBS containing 10% normal human serum, and with streptavidin-biotin peroxidase complex (DAKO) each for 30 minutes. HRP activity was detected by incubation in 1 mg/ml 3,3-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, U.S.A.) and 0.015% H2O2 in 50 mM Tris-HCL (pH=7.6). Slides were counterstained with Mayer's hematoxylin. Normal tonsil tissue was used as a
positive control in each staining procedure. Furthermore, within the tumors, non-neoplastic lymphocytes and macrophages expressing high levels of CD44s were always present and served as internal standard and control. To facilitate identification of the neoplastic lymphoid cells, serial sections were routinely stained for CD20 (CD20, DAKO) and CD3 (CD3, DAKO). On the basis of the estimated percentage of positively staining tumor cells, CD44s and CD44v6 expression was scored as follows: 0 (negative/low) = less than 10% positive tumor cells; 1 (intermediate) 10-50% tumor cells positive; 2 (high) more than 50% positive tumor cells. This scoring system is based on our experience from prior studies\textsuperscript{24,26}. Intensity of the stainings was also estimated, but subdivision of the groups based on staining intensity proved of no additional prognostic value (data not shown). All slides were read by two independent observers (P. Drillenburg and S.T. Pals); discrepancies were solved by consensus. During the whole procedure, the observers were blinded for clinical data/disease outcome.

**Statistical analysis.** The correlation of CD44s and CD44v6 with biological and clinical parameters was estimated with the Spearman correlation coefficient. Survival curves were plotted following the Kaplan-Meier method and tested for statistical significance using the log-rank test. Overall survival (OS) was calculated from the date of diagnosis until death (all causes) or last follow-up. For patients who reached complete remission (CR), disease free survival was estimated from the date of CR until first relapse or last contact, if disease free. For the multivariate analysis and calculation of the hazard ratios and its confidence intervals Cox proportional hazard model was used. In Cox regression, the forward stepwise selection method was used, the results were controlled with backward stepwise selection.

**Results**

**CD44s and CD44v6 expression in diffuse large B-cell lymphoma (DLCL).**  
CD44s expression in DLCL was highly variable, ranging from negative to strongly positive, which confirms a previous study from our laboratory\textsuperscript{24}. Intermediate or high expression of CD44s was present in 13% and 63% of the tumors, respectively; 24% of the tumors were CD44s negative. Unlike CD44s, CD44v6 was only expressed in a minority of the tumors: whereas 84% showed no detectable staining for CD44v6, intermediate or high expression was present in 10% and 6% of the tumors, respectively.

Subclassification of DLCL on the basis of morphology (Kiel-classification) or primary tumor localization did not reveal significant differences in distribution of CD44s or CD44v6 between the groups (data not shown).
CD44 in large B-cell lymphoma

**Correlation of CD44s and CD44v6 with clinical risk factors.**

In view of the strong evidence for a role of CD44 in lymphocyte homing and lymphoma dissemination, we analyzed the relation between CD44 expression and tumor dissemination in our study group. In the total group (n=276), no correlation between CD44s expression and tumor dissemination was found (p=0.20). However, in the tumors with a primary nodal localization (n=155), CD44s expression significantly correlated to clinical stage (Fig. 1A) (p=0.003) and, as an alternative measure of dissemination, to the total number of anatomical sites involved by tumor (Fig. 1B) (p=0.018). In extranodal lymphomas (n=91), CD44s did not correlate with tumor dissemination (p=0.21). Also, CD44v6 expression showed a significant correlation to tumor stage in nodal, but not in extranodal tumors (p=0.04). Bone marrow involvement did not correlate with expression of CD44s or CD44v6 (p=0.28 and p=0.72 respectively). Table 2 shows the relation between CD44 expression and the clinical parameters of the International Prognostic Index (IPI). With the exception of the parameter “stage”, neither of the IPI parameters correlated to CD44 expression.

**CD44 predicts prognosis in localized DLCL.**

We next explored the prognostic value of CD44s and CD44v6. For the whole cohort
Table 2. Expression of CD44 related to the clinical parameters of the International Prognostic Index (n=236).

<table>
<thead>
<tr>
<th></th>
<th>CD44s(%)</th>
<th>CD44v6(%)</th>
<th></th>
<th>CD44s(%)</th>
<th>CD44v6(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>-/ -/ +/+</td>
<td>p</td>
<td>n</td>
<td>-/ -/ +/+</td>
</tr>
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<td><strong>age classes</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>≤60 years</td>
<td>80</td>
<td>24</td>
<td>76</td>
<td>NS</td>
<td>81</td>
</tr>
<tr>
<td>&gt;60</td>
<td>156</td>
<td>22</td>
<td>78</td>
<td>NS</td>
<td>85</td>
</tr>
<tr>
<td><strong>LDH</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Normal</td>
<td>114</td>
<td>21</td>
<td>79</td>
<td>NS</td>
<td>83</td>
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<tr>
<td>Abnormal</td>
<td>122</td>
<td>24</td>
<td>76</td>
<td>NS</td>
<td>84</td>
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<td><strong>Karnofsky</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&gt;80%</td>
<td>174</td>
<td>23</td>
<td>77</td>
<td>NS</td>
<td>85</td>
</tr>
<tr>
<td>&lt;80%</td>
<td>62</td>
<td>21</td>
<td>79</td>
<td>NS</td>
<td>79</td>
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<td><strong>Ann Arbor Stage</strong></td>
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<td><strong>Extranodal sites</strong></td>
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<td>&gt;1</td>
<td>39</td>
<td>26</td>
<td>74</td>
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<td>87</td>
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* -(negative/low)= less than 10% positive tumor cells; +/+ +(intermediate/high)= more than 10% positive

of patients (n=276), CD44s and CD44v6 did not predict overall survival (p=0.17 and 0.96, respectively). Interestingly, however, in patients with localized (stage I) disease (n=88), CD44s proved to be a highly significant unfavorable prognosticator (p=0.0076) (Fig. 2A). No statistically significant prognostic value was present in stage II or IV disease (Fig. 2B,C) although there was a similar trend in stage II patients. The stage III patient group was too small to be analyzed.

Although a strong prognostic effect of CD44s was evident in the whole stage I group, this was largely accounted for by the effect of CD44s in the patient subgroup (n=39) with a primary nodal lymphoma (p=0.0014) (Fig. 2D). If stage I extranodal lymphomas were analyzed separately, CD44s had no significant prognostic value (p=0.79). Hence, CD44s was a strong prognosticator in patients with localized nodal DLCL. In a multivariate comparison with the clinical parameters of the International Prognostic Index (IPI), CD44s was the major prognosticator in this patient group (RR= 5.07; 95% CI: 1.12-22.90). These data were also found for the 26 patients with nodal stage I disease who were treated with doxorubicin-containing polychemotherapy with or without radiotherapy, or radiotherapy alone (Fig.3A).
Figure 2. CD44s predicts outcome in patients with localized DLCL. Overall survival for patients with CD44s positive (1+ and 2+) and negative (-) DLCL subdivided according to Ann Arbor stage. A) stage I, whole study group; B) stage II, whole study group; C) stage IV, whole study group; D) stage I; primarily nodal lymphomas. The stage III group was too small to be analyzed.

It is important to note that the proportion of patients who received doxorubicin-containing chemotherapy and/or radiotherapy did not differ significantly between the CD44s positive and negative groups (table 3). Also the percentage of patients who entered complete
remission was similar (table 3). However, the relapse rate for CD44s positive lymphomas (58%) was significantly increased in comparison to the negative lymphomas (10%, p=0.018) (table 3). Of the relapses 86% took place outside the region originally involved by tumor. In line with the high relapse rate in CD44s positive patients, CD44s expression was also an unfavorable prognosticator for disease free survival (p=0.03) (Fig. 3B). The number of patients with a relapse was too small to analyse the impact of treatment on relapse rates in CD44s positive and negative cases.

Table 3. CD44s expression in stage I, nodal DLCL in relation to therapy, complete remission and relapse in ‘adequately’ treated patients.

<table>
<thead>
<tr>
<th></th>
<th>chemotherapy&amp;</th>
<th>radiotherapy</th>
<th>complete</th>
<th>relapse</th>
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<tr>
<td></td>
<td>+/- radiotherapy</td>
<td>only</td>
<td>remission</td>
<td>rate</td>
</tr>
<tr>
<td></td>
<td>n=9</td>
<td>n=17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>-</td>
<td>11</td>
<td>45</td>
<td>NS</td>
<td>55</td>
</tr>
<tr>
<td>CD44s*</td>
<td>15</td>
<td>27</td>
<td>NS</td>
<td>73</td>
</tr>
<tr>
<td>+/-++</td>
<td></td>
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</tbody>
</table>

& doxorubicin-containing polychemotherapy
* -(negative/low)= less than 10% positive tumor cells; +/-++(intermediate/high)= more than 10% positive.

Incorporation of CD44 in the IPI parameter “stage” improves its prognostic power in nodal lymphoma.

In view of the evidence that CD44s is instrumental in mediating lymphoma dissemination, we adopted the working hypothesis that the adverse prognostic effect of CD44s in clinically localized tumors might result from occult dissemination. If so, CD44s might be used as a tool to predict outcome. As the International Prognostic Index is currently widely used to predict prognosis in DLCL, we addressed this hypothesis by exploring whether the prognostic power of the IPI parameter “stage” might be improved by including CD44s as an additional parameter in primary nodal lymphomas. Hence, we replaced the parameter Ann Arbor stage >II disease by Ann Arbor stage >II disease or positivity for CD44s. This redefined parameter (“stage/CD44s”) was compared in multivariate analysis with the conventional parameters of the IPI, i.e., age >60; elevated LDH; Karnofsky performance status <80 and Ann Arbor stage >II. Upon regression analysis (table 4), the parameter “stage/CD44s” (and not the conventional IPI parameter “stage”) was selected, indicating that inclusion of CD44s increases the prognostic power of the IPI parameter “stage”. As is shown in figure 4, incorporation of CD44s into the IPI parameter “stage” clearly improves the separation between the risk groups (Fig. 4A versus 4B).
CD44 in large B-cell lymphoma

Figure 3. Cumulative survival for adequately treated patients with CD44s positive (1+ and 2+) and negative (-) stage I, nodal DLCL. A) overall survival (OS); B) disease free survival (DFS).

Discussion

The heterogeneity in outcomes in aggressive non-Hodgkin’s lymphoma has prompted the development of the IPI, a clinical prognostic factor model, which identifies patients with different likelihoods of being cured of their disease. However, there is consensus that the clinical prognostic features incorporated in the IPI are, in large part, surrogate variables, with a remote relation to the biological variables determining disease heterogeneity. Identification of these unknown biological variables is of great importance since they may improve prognostic factor models and, in addition, serve as targets for therapy in specific subsets of patients. In our present study, we demonstrate that CD44s, a biological determinant of lymphoma dissemination, predicts outcome in patients with localized DLCL and that the IPI parameter “stage” can be improved by incorporating CD44s as an additional parameter.

Previous studies from our own and other laboratories have related CD44 expression to tumor dissemination in non-Hodgkin’s lymphoma. This association is confirmed in our present population based study group: In patients presenting with a primary nodal DLCL lymphoma, but not in those with extranodal disease, both CD44s and CD44v6 were correlated...
Table 4. Factors Independently Prognostic of Overall Survival in nodal DLCL (n=137)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>p value</th>
<th>step of selection</th>
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</thead>
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</table>

* see text for definition

with tumor spread (Fig. 1). We currently have no explanation for the differential effect of CD44 in nodal versus extranodal lymphoma. It should be noted, however, that nodal and extranodal lymphomas show important genetic, functional and phenotypic differences suggesting that they represent distinct biological entities 2,27,38,39. These differences include differential expression of adhesion/homing receptors other than CD44, for example of L-selectin, which may co-determine tumor spread 40.

The key finding of our present study is that CD44s is a strong independent prognosticator in localized nodal DLCL (Fig. 2). Whereas patients with localized (stage I) disease and a CD44s negative tumor have a favorable prognosis, the prognosis of those with CD44s positive tumors is poor, even at stage I. The observation that CD44s expression is both correlated with lymphoma dissemination in the entire group and predictive of relapse of disease in patients with localized disease, matches with studies indicating an important physiological role of CD44s in lymphocyte trafficking. In this trafficking process, CD44s mediates lymphocyte binding to high endothelial venules 8,10,34, lymphocyte rolling 41,42, and migration to inflammatory sites 43,44. Presumably, CD44s expression in localized DLCL reflects a high propensity to disseminate and/or the presence of occult dissemination, a scenario which is supported by the fact that CD44s promotes the dissemination of human lymphoma cells xenografted into nude mice 15. A similar scenario has also been proposed to explain the prognostic value of CD44 splice variants containing exon v6 in colorectal and breast cancer 36,45. It should be noted, however, that other biological functions of CD44,
specifically its recently described role in counteracting apoptosis, may also contribute to the less aggressive biological behavior of CD44 negative tumors.

In view of the strong evidence that CD44 is instrumental in mediating lymphoma dissemination, we argued that CD44s might be used as an additional prognostic parameter in patients with localized disease. As the IPI is currently widely accepted as a major tool for the identification of "high" or "low" risk in DLCL, this was analyzed by including CD44s in the IPI parameter "stage". Indeed, we observed that the IPI parameter "stage" can be improved by including all CD44s positive patients in the 'high risk for stage' group. In a multivariate comparison with the other IPI parameters, the modified parameter "stage/CD44s" incorporating CD44s, and not the conventional IPI parameter "stage", was selected (table 4). Furthermore, by using this modified staging parameter a superior separation of the distinct IPI risk groups was obtained (Fig. 4A versus 4B).

We considered the possibility that more effective treatment of CD44s negative lymphomas might explain the favorable outcome, however, there were no differences in therapy between the CD44s negative and positive groups (table 3). The percentage of patients entering a complete remission was also identical in the CD44s positive and negative groups, by contrast, relapses were much more common in patients with CD44s positive tumors (table 3). As the vast majority of these relapses occurred outside the anatomical area of initial tumor

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![Figure 4. Incorporation of CD44s into the IPI (see text) improves the separation between the risk groups for nodal DLCL A) conventional IPI risk groups; B) IPI risk groups in which the staging parameter has been modified by incorporating CD44s. L denotes low risk, LI low intermediate risk, HI high intermediate risk and H high risk.](image-url)
involvement, this high relapse rate in CD44s positive tumors is consistent with a role of CD44s in (occult) lymphoma dissemination. Analysis of these patients with a relapse for the impact of therapy was not possible due to the small number of patients. Since recent data indicate that the most adequate therapy for stage I patients is a combination of 3 cycles of polychemotherapy and radiotherapy, instead of radio- or chemotherapy alone, it will be very interesting to see whether the impact of CD44s holds in a prospective study including this novel therapeutic modalities. Meanwhile, our current study clearly shows that stage I DLCL patients with a CD44 negative tumor have an excellent prognosis.

Our current findings extend earlier studies from our own and Jalkanen’s laboratory. We previously reported that B-DLCL with low CD44s expression have a favorable prognosis. In the current much larger study, this finding is confirmed and, moreover, the prognostic effect of CD44s is shown to be independent of other IPI parameters. Jalkanen and colleagues reported CD44s to have independent prognostic value, however, their study and our present study differ at important points. Whereas our present study focuses on a single clinicopathological entity, the study group of Jalkanen was heterogeneous, containing lymphomas of different subtype and grade. Furthermore, in their study from the pre-IPI era, not all clinical parameters of IPI were included in the analysis and, instead of overall survival rates, survival rates corrected for intercurrent death and death of unknown cause, were used as end point. Although these differences make a direct comparison between the two studies difficult, both studies underscore the prognostic value of CD44s.

In our present study CD44v6 expression was not a significant prognosticator. In contrast to our data, Stauder and colleagues reported CD44v6 to be a better prognosticator than CD44s. This discrepancy might be due to differences in the composition of the study groups. Unlike in our study group, which contains only B-DLCL, the ‘high-grade’ B-NHL group analyzed by Stauder et al. consists of a number of distinct, unrelated, clinicopathological entities including Burkitt’s lymphoma, precursor B-cell lymphoma, and DLCL. In view of their distinctive character, Burkitt’s lymphoma and precursor B-cell lymphoma were not included in the international non-Hodgkin’s lymphoma prognostic factor project. As CD44 is known to be differentially expressed at various stages of B-cell differentiation and in different lymphoma subtypes, lumping of different entities may greatly influence, and potentially confound, the results.

In conclusion, this population based study identifies CD44s as a strong risk factor in B-DLCL patients with localized nodal disease and shows that incorporation of CD44s into the IPI improves its prognostic value. Unlike the other parameters of the IPI, which have a remote relation to the biological variable determining disease heterogeneity, CD44s most likely represents a true biological prognosticator (co)determining lymphoma dissemination.
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