Prognostic significance of metallothionein in B-cell lymphomas

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Word counts: abstract: 197; references: 877; remaining manuscript: 3149 (excluding tables and legends).

Running title: Metallothionein in B-cell lymphoma.

Scientific heading: Neoplasia
Abstract

We have investigated metallothionein (MT) I and II mRNA and protein in B-cell lymphomas with particular reference to diffuse large B-cell lymphoma (DLBCL). The mRNA profiling was performed on Affymetrix arrays and showed upregulated MT mRNA in 15 of 48 DLBCL, including 12 of 23 ABC and 3 of 9 type-3 lesions. By contrast MT mRNA was low to undetectable in 16 GCB-type DLBCL. Only one of 15 patients with upregulated MT mRNA achieved a sustained remission, suggesting that upregulated MT mRNA constitutes a significant risk factor for treatment failure. This was confirmed in two independent series (Rosenwald et al. 2005; Monti et al. 2005), which showed significantly shorter 5-year survival in DLBCL with high vs. low MT-IIa levels. By immunohistology, MT was shown to be present in both macrophages and lymphoma cells. The proportion of MT-positive macrophages did not correlate with the survival. By contrast, in 115 DLBCL MT-labeling of > 20% lymphoma cells was associated with a significantly poorer 5-year survival, independent of the age, stage or International Prognostic Index. Taken together, it is suggested that both increased MT mRNA and MT protein expression by > 20% lymphoma cells constitute independent risk factors in DLBCL.

Key words: Metallothionein, B-cell lymphoma, prognosis, microarray, macrophages.
Introduction

The metallothioneins (MT) are low molecular weight (6-7 kD) non-enzymatic proteins consisting of 60-68 amino acids. Four subtypes are recognized (MTI-IV). Among these MT-I and MT-II are the major forms. They are encoded by genes located on the same chromosome (no. 16 in humans), they are regulated and expressed co-ordinately and their distribution and biological functions are analogous.\(^1\)\(^2\) Both proteins contain 2 globular, cystein rich domains, which can bind essential and toxic metals, such as zinc, copper, cadmium and mercury. A main function is to act as an intracellular zinc reservoir.\(^3\) Furthermore, there are indications that the MT are also involved in the protection against DNA damage, oxidative stress and apoptosis.\(^1\)\(^4\)\(^-\)\(^6\)

Several observations have indicated that altered MT expression is important for diseases in humans, including cancer which often upregulates MT compared to normal cells.\(^5\)\(^,\)\(^7\)\(^,\)\(^8\) Moreover, increased MT levels have been associated with high-grade histology and an aggressive behavior in several of types of cancer.\(^5\)\(^,\)\(^9\)\(^-\)\(^11\)

So far most studies of MT in malignant diseases have been performed in epithelial tumours. Very limited information is available about the expression of MT in lymphomas. In this study we have investigated 141 B-cell lymphomas for MT protein expression by immunohistology. Furthermore, MT mRNA levels were investigated by expression profiling in 48 DLBCL previously classified into three groups (GCB, ABC and type-3) on Affymetrix arrays. The results indicate that increased MT mRNA and protein may constitute an independent risk factor in DLBCL.
Material and methods:

Biopsies

Formalin fixed, paraffin embedded specimens of 131 B-cell lymphomas sampled during the period 1982-2004 were collected from the archives at the Department of Pathology, Rigshospitalet, University of Copenhagen. An additional 10 cases were kindly made available from the Department of Pathology, Odense University Hospital. Only specimens obtained at diagnosis, prior to treatment were included. All cases were reviewed by histology and immunohistology using standard panels of antibodies, i.e. CD20, CD79a, CD10, CD21, CD23, CD3, CD5, Bcl-2 and cyclin D1. The samples were then classified in accordance with the WHO (2001) and comprised 115 diffuse large B-cell lymphomas (DLBCL), 11 follicular lymphomas (FL), 2 mantle cell lymphomas (MCL), 7 small lymphocytic lymphomas (SLL) and 6 cases of multiple myeloma (MM). Of the DLBCL 86 were primary nodal and 29 were primary extranodal at presentation with involvement of the tonsil (8 cases), salivary glands (4 cases), thyroid (3 cases), testis (3 cases), bone (2 cases), nasal cavity (1 case), cervix uteri (1 case), liver (1 case), breast (1 case), kidney (1 case), pericardium (1 case), rhinopharynx (1 case) and skin (1 case).

Clinical data, survival

The clinical records were reviewed in all of the DLBCL patients with particular reference to age, site of initial involvement, stage at diagnosis, response to treatment and survival. Information on the International Prognostic Index (IPI) could be retrieved in 74 of the cases. All patients were treated using anthracyclin based regimens (CHOP or CHOP-like), supplemented in six patients with additional antibody-therapy (e.g. Rituximab). Survival was calculated from the day of diagnosis until death or the date of last follow-up. Overall survival duration curves were plotted according to the Kaplan and Meier method. The log-rank test was used to assess the differences between the survival curves and nominal $P$-values were calculated. Group comparisons were done using two-sided, paired students T-test (equal variance assumed).

Immunohistology
Sections of formalin-fixed, paraffin-embedded biopsies were heated in a Milestone Micromed microwave oven in citrate buffer (pH: 6) for 15 min., incubated 60 minutes at room temperature with a 1:40 dilution of DAKO-MT (clone: E9) reactive with MT-I and MT-II in routinely processed histological samples and stained in the Techmate 500 Immunostainer, using the DAKO Envision K5007 as a secondary antibody. Since the results suggested that some of the MT-positive cells represented macrophages, samples were also stained using CD68 (PGM1) on adjacent sections.

As a preliminary approach to scoring, all specimens were screened by two authors (CBP, ER) to obtain an overall impression of the reactivity patterns. Furthermore, in 20 randomly selected DLBCL, the number of MT-positive tumour cells was counted in 10 representative areas at 400 times magnification. These results showed that four broad categories could be distinguished, i.e. no/occasional (< 5%); few (5-20%); moderate (20-50%); and many (> 50%) MT-positive lymphoma cells. In general, the intensity of the staining paralleled the number with weak to moderate staining reactions in cases with few MT-positive tumour cells and stronger reactions in cases with more MT-positive tumour cells. Similarly, for macrophages, 3 categories could be distinguished, i.e. few (+), moderate (++) and many (+++) MT-positive tumour-infiltrating macrophages. Hence for the scoring of the entire series, this semi-quantitative scoring system was adopted. All samples were scored independently by two authors (CBP, ER) and were in case of disagreement reviewed in consensus.

Controls were included in all experiments and consisted of autopsy specimens of normal liver and kidney. In these controls, strong staining was seen of both nuclei and cytoplasm in proximal tubuli and hepatocytes.

**mRNA expression profiling**

Frozen tissue kept at –80°C was available from 48 of the DLBCL and was subjected to expression profiling as described. Briefly, from each case, 5 micrograms of purified RNA was used to generate biotin-labeled antisense cRNA. After fragmentation at 94°C for 35 min., the labeled cRNA samples were hybridized for 16 hours to Affymetrix HG-U133A arrays (Affymetrix Inc.), washed and stained with phycoerythrin conjugated streptavidin (SAPE) and scanned in the Affymetrix GeneArray® 2500 scanner to generate fluorescent images. All of the above as described in the Affymetrix GeneChip® protocol. The image files (cel files) were
imported into the software package DNA-Chip Analyzer (C. Li and W.H. Wong (www.dchip.org)). The array files were normalized and expression values calculated as described. Samples were categorized as being GCB, ABC and type-3 by hierarchical clustering using a list of 78 classifier genes, as already published.

For validation, data from two independent studies of 240 newly diagnosed DLBCL analyzed on the Lymphochip platform and 176 newly diagnosed DLBCL with available Affymetrix HU133 A+B data sets were downloaded from the Internet (Rosenwald et al.; http://llmpp.nih.gov/DLBCL/ and Monti et al.; http://www.broad.mit.edu/cgi-bin/cancer/publications/pub_paper.cgi?mode=view&paper_id=102) together with the relevant clinical information. Using this approach data on MT-IIa mRNA levels and survival could be retrieved and analyzed from 238 patients in the Rosenwald study and from 130 patients in the study by Monti et al.

**Ethics**

This study was approved by the local ethical committee (journal no. 01-226/02) and the Danish Data Protection Agency (journal no. 2002 111129A). Informed consent was provided according to the Declaration of Helsinki.
Results

MT mRNA expression in DLBCL

mRNA extracted from frozen tissues from 48 of the DLBCL was examined by global expression profiling on Affymetrix arrays. Similar to other reports,18-20 the tumours could be grouped into 3 categories depending upon the differential expression of 78 genes as described in our previously developed classifier model, i.e. GCB (n=16), ABC (n=23) and type-3 (n=9).15 A further analysis of other genes differentially expressed in these groups showed that the genes encoding mRNA MT-IIa and the different isoforms of MT-I (e.g. MT-IL,G,H,X,G,F,E,K) were upregulated (above mean) in 12 of 23 of the ABC tumours and in 3 of 9 type-3 tumours, but were low to undetectable in the 16 GCB lesions (see Fig. 1 A+B). A correlation with the treatment outcome showed that only one of 15 patients with upregulated MT mRNA achieved a sustained complete remission (CR). The remaining either showed only a temporary response (n=4) or had refractory disease (n=10). Conversely, in the 33 patients with low MT mRNA levels, 17 achieved a sustained CR, 9 achieved a temporary CR but relapsed, and 7 had refractory disease. These data suggested that upregulated MT mRNA may identify a particular subgroup of ABC and type-3 DLBCL with a poor response to conventional chemotherapy and a potentially aggressive behavior.

To address this possibility in more detail, the survival of patients with high vs. low MT mRNA was investigated both in this series and in another two, independent data sets (Rosenwald et al.16 and Monti et al.17). In our series of 48 patients, a tendency was seen towards an inferior survival in patients with high MT mRNA compared to those with low MT mRNA (Fig. 2A). However, this difference did not reach statistical significance, presumably due to the relatively small number of patients. By contrast, among 238 patients downloaded from the publication of Rosenwald et al.,18 those with high (above mean) MT-IIa mRNA showed a significantly poorer survival than those with lower MT-IIa levels (Fig. 2B). A similar difference (p=0.05) was observed when data from 130 patients from the study by Monti et al.17 were analyzed (data not shown).
MT protein expression

To characterize MT expression at the protein level, an extended series of pre-treatment, diagnostic biopsies from 141 B-cell lymphomas sampled during the period 1982-2004 were examined by immunohistology using anti-MT antibody (E9) reactive with MT-I and MT-II in formalin fixed specimens. This series included the 48 DLBCL described above as well as samples from an additional 67 DLBCL. Furthermore, specimens from 20 small B-cell lymphomas (see Material and Methods) and 6 multiple myelomas were investigated.

In all the B-cell lymphomas, the antibody stained a population of CD68-positive, dendritic cells, representing tumour infiltrating macrophages (Fig. 3). MT labeling of the macrophages was prominent in the cytoplasm, but the nuclei were also weakly stained in most cases. The number of MT-positive tumour infiltrating macrophages was most abundant in DLBCL (Table 1), but had no impact on the 5-year survival (Fig. 4).

MT expression by the lymphoma cells was more restricted. As shown in Table 2 significant numbers of MT-positive tumour cells (> 20%) was observed in 21 of 115 of the DLBCL. In the remaining B-cell lymphomas, no or only occasional MT-positive tumour cells were identified. Labeling of the lymphoma cells was mainly nuclear, although cytoplasmic reactivity was also present in some cases (Fig. 5). No significant differences with respect to age, stage or IPI at diagnosis were observed between DLBCL with MT-positive tumour cells above or below 20% (Table 3). However, the survival at 5 years was significant poorer in the former category (Fig. 6), suggesting that > 20% MT-positive lymphoma cells constitutes an independent risk factor in DLBCL. Small, reactive, tumour infiltrating lymphocytes were MT-negative (Fig. 5).

Correlation between the number of MT-positive tumour cells and macrophages in individual DLBCL is summarized in Table 4. As shown no specific correlation was identified. For example, among 18 DLBCL with >50% MT-positive tumour cells, 6 contained only few MT-positive macrophages, 6 contained an intermediate number, and 6 contained many MT-positive macrophages. Similarly, among 25 cases with many MT-positive macrophages, only 7 contained >20% positive lymphoma cells. Hence it is less likely that phagocytosis of MT produced in lymphoma cells contributed significantly to the observed MT-staining of macrophages.
MT mRNA vs. protein expression

MT-II and the several different isoforms of MT-I are co-ordinately expressed under a number of conditions.1,23 This was also seen in our study, as illustrated in Fig. 1A. For comparison between the MT mRNA and protein expression levels, we focused on MT-II. The results for 48 DLBCL in which samples were available for both immunohistology and expression profiling on Affymetrix arrays are illustrated in Figs. 7A and B and showed significantly increased MT mRNA (mean: 4253) in lymphomas with abundant (+++ numbers of MT-positive macrophages as compared to those with few (+) or moderate (++ numbers (mean: 1598), p=0.001. A similar correlation was seen between the MT mRNA expression level and the number of MT-positive tumour cells detected by immunohistology, i.e. mean: 2737 in DLBCL with > 20% positive tumour cells vs. mean: 1814 in DLBCL with <20% positive tumour cells, p=0.04. These data indicated that the MT mRNA level determined by expression profiling in crude extracts of DLBCL correlated with and was contributed to by MT produced in both lymphoma cells and macrophages.
Discussion

The MT are single chain 6-7 kD proteins with a high content of cysteine, sulfur and metal. Four subtypes are recognized in humans, MT-I and MT-II, which are widespread, and MT-III and MT-IV, which are more restricted in distribution. All the MT contain 2 metal binding domains, a C-terminal $\alpha$ and a N-terminal $\beta$ domain. Both are rich in cysteins, which can bind essential and toxic metals. The MT genes are located on chromosome 16 in humans and can be activated by a variety of stimuli, including metal ions, oxidative stress, cytokines, glucocorticoids, growth factors and hypoxia.$^{2,8,21,22}$ The MT have established roles in metal homeostasis and in the protection against reactive oxygen species. Furthermore, there are indications that the MT are involved in cell proliferation, in the protections against DNA damage, in the prevention of apoptosis and in the acquisition of resistance to a variety of commonly used cancer chemotherapies.$^{8,21-24}$ Accordingly, it is conceivable that altered MT expression may play a role for neoplastic disorders.

Expression of the MT have been studied in considerable detail in human carcinomas, and these studies have shown that the MT are often upregulated in malignant compared to normal cells. Furthermore it has been shown, that upregulated MT associates with high-grade histology and an aggressive behavior in many types of carcinoma.$^5$ By contrast, very limited information is available about MT expression in lymphoma. Thus to data, only 2 previous reports of a total of 12 lymphomas of different subtypes are available.$^7,25$

In an attempt to investigate whether MT have implications in lymphoma, we have examined MT mRNA and protein expression in mature B-cell lymphomas with particular reference to diffuse large B-cell lymphoma (DLBCL), which constitutes the most frequent subtype of B-cell lymphoma in Western countries. Although classified as one disease the REAL and WHO classifications,$^{26}$ several more recent investigations have indicated that DLBCL is likely to encompass more than one biological entity. Thus is has been shown that different clinical subtypes can be recognized by phenotypic and genotypic investigations at the protein, mRNA and DNA level.$^{27-29}$ For example, it has been shown in this and other laboratories that at the least three subgroups with different outcomes can be distinguished depending upon the mRNA expression profiles. These include DLBCL with a germinal center B-cell (GCB)-like mRNA signature, DLBCL with an activated B-cell (ABC)-like signature and an intermediate, so-called type-3 category. Whereas the GCB-like DLBCL
generally shows an indolent course, the behavior of the ABC and type-3 lesions is more aggressive.\textsuperscript{15,18,19,30-32}

Here we show that mRNA encoding MT-I and MT-II is differentially upregulated in half of DLBCL with an ABC signature and in one third of type-3 tumours, but is low to undetectable in DLBCL with a GCB signature. Furthermore, it is shown that upregulated MT-I and II mRNA in DLBCL correlates with treatment failure and a short survival. This was evident both in this series and in another two independent data sets published by Rosenwald et al. (2002)\textsuperscript{16} and Monti et al (2005).\textsuperscript{17} Since the genes encoding MT-I and II are not contained in either our or other previously reported classifier lists for distinction between ABC and GCB DLBCL,\textsuperscript{15} they are clearly not merely markers of ABC vs. GCB tumours, but rather represent additional markers for a particular subgroup of DLBCL with a poor response to conventional chemotherapy and a short survival. This was supported by the results obtained by immunohistology, which showed that MT protein expression by the lymphoma cells in an extended series of 115 DLBCL, 20 small B-cell lymphomas and 6 multiple myelomas was confined to the DLBCL. Furthermore, in the DLBCL the presence of > 20% MT-positive lymphoma cells was a significant risk factor associated with a short survival, independent of the age, stage and IPI.

Very few previous reports have specifically addressed the MT mRNA and protein levels in lymphomas, and it is presently not clear how the MT influence the behavior of DLBCL at the molecular level. One obvious possibility is that enhanced MT can confer resistance to conventional chemotherapies commonly used in treating DLBCL, e.g. anthracyclins and cyclophosphamide.\textsuperscript{23,24} Indeed, studies have shown that MT can inhibit anthracyclin induced mitochondrial cytochrome c release and caspase-3 activation,\textsuperscript{33,34} and can mediate intracellular sequestration of cyclophosphamide.\textsuperscript{35,36} Thus it is possible that upregulated MT mRNA and protein may identify a particular subgroup of DLBCL, which could benefit from more intensive therapy and/or from other treatment modalities than conventional CHOP or CHOP-like regimens. Potentially interesting options in this respect are electron-affinic compounds such as Motexafin gadolinium, which increases oxidative stress, enhances expression of metal response element-binding transcription factor-1 regulated genes, including MT, and can induced cell cycle arrest and apoptosis in B-cell lines in vitro under appropriate conditions.\textsuperscript{37} Since cells may be more susceptible if already subjected to oxidative stress, both
the MT<sub>high</sub> DLBCL described in this report and the recently identified so-called OxPhos consensus cluster of DLBCL, characterized by increased levels of several genes associated with oxidative phosphorylation,<sup>17</sup> could constitute interesting targets for future investigations in clinical trials.

In a previous study it has been suggested that MT may constitute a “malignancy marker” in B-cell lymphomas based on the observation that tumour infiltrating lymphocytes in 11 gastro-intestinal tract lymphomas were MT-negative, unlike the lymphoma cells which were positive to varying extents.<sup>25</sup> The results from this investigation support the view that tumour-infiltrating lymphocytes in B-cell lymphomas are generally MT-negative. However, it is also shown that MT protein in these conditions is not confined to the lymphoma cells, but that a proportion of tumour infiltrating macrophages are often MT-positive, especially in DLBCL. Since no specific correlation was identified between the number of MT-positive macrophages and lymphoma cells in this series, the observed MT-staining of macrophages presumably did not result from phagocytosis, but rather reflected endogenous synthesis. In keeping with this assumption are the observations that MT can be induced in monocytes under in vitro conditions and that MT-positive macrophages are also recruited to mice brain tissue subjected to cryoinjury.<sup>38,39</sup>

The functional implications of the MT-positive tumour infiltrating macrophages seen in this study and the stimuli, which induce them is not known. Interestingly, in this report the proportion of MT-positive macrophages in DLBCL did not seem to influence the survival although they correlated with and presumably contributed to the MT mRNA levels measured in crude extracts. Hence, from a clinical perspective, it is recommended that MT measurements of crude extracts be supplemented with immunohistological assessments, which permit distinction between MT-positive lymphoma cells and macrophages.

In conclusion, it is suggested that upregulated MT-I and MT-II mRNA and increased MT-I and MT-II protein expression by the lymphoma cells constitute independent risk factors in DLBCL. How the MT influence the behavior of DLBCL at the molecular level is unknown. However, it is tempting to assume that increased proliferation, decreased apoptosis and increased acquisition of resistance to chemotherapy may be implicated. These issues will be important to address in future investigations.
Acknowledgements

This work was supported by grants from HS Research Foundation, the Novo Nordisk Foundation, the Danish Foundation for Cancer Research, the Toyota Foundation, the Danish Medical Research Council, the Gangsted Foundation and the Svend Andersen Foundation.
Reference List


Legends

Fig 1
(A) MT-I and II mRNA expression in 48 DLBCL previously classified as GCB (n=16), ABC (n=23) and type-3 (n=9) by expression profiling on Affymetrix arrays. For MT-I the expression of 7 different isoforms represented on the Affymetrix 133A GeneChip is illustrated.
C = Complete remission, r = recurrent disease, R = Refractory disease.
(B) MT-II mRNA expression values (arbitrary units) in the 3 different subtypes of DLBCL (GCB, ABC, type-3). CI = confidence intervals.

Fig 2
Survival in DLBCL with high vs. low MT mRNA in this series (A) and the series from the Rosenwald study (B).17

Fig 3
Adjacent sections of a DLBCL stained for MT-I and II (A, DAKO-MT x 600) and CD68 (B, PGM1 x 600) showing numerous MT-positive stromal cells with a dendritic morphology (A), similar to the pattern with CD68 (B). In C and D, DLBCL with few (C) and abundant (D) numbers of MT-positive tumour infiltrating macrophages is shown (DAKO-MT x 400). In these cases only very occasional tumour cells (asterisks in C) are stained with a weak nuclear reactivity.

Fig 4
Survival in 115 DLBCL in relation to the proportion of MT-positive tumour infiltrating macrophages. No significant differences were seen either between the 3 groups few, moderate, many (A) or between few vs. moderate/many (B) or few/moderate vs. many MT-positive macrophages (C).

Fig 5
A DLBCL with strong MT staining of a majority of the tumour cells in both nuclei and cytoplasm (A, DAKO-MT x 200 and B, DAKO-MT x 600). Note that small reactive lymphocytes (arrow) are MT negative.

Fig 6
Survival in 115 DLBCL in relation to the proportion of MT-positive lymphoma cells. No difference was seen between tumours with no/occasional vs. few (5-20%) MT-positive tumour cells (A). By contrast, a significantly poorer survival was seen in patients with above 20% MT-positive tumour cells compared to those with below 20% MT-positive tumour cells (B).

Fig 7
MT-II mRNA expression levels in 48 DLBCL related to the number of MT-positive tumour cells (A) and macrophages (B) determined by immunohistology. As shown, increased MT mRNA in crude extracts correlated both with the no. of MT-positive tumour cells and macrophages.
Table 1: MT expression by macrophages in different types of B-cell lymphoma.

<table>
<thead>
<tr>
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<th>+</th>
<th>++</th>
<th>+++</th>
<th>n</th>
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<td>DLBCL</td>
<td>52</td>
<td>37</td>
<td>26</td>
<td>115</td>
</tr>
<tr>
<td>FL, grades I+II</td>
<td>8</td>
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<td>0</td>
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<td>FL, grade III</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td>MCL</td>
<td>2</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>MM</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>SLL</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
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</table>

DLBCL= Diffuse Large B-cell Lymphoma; FL= Follicular Lymphoma; MCL= Mantle cell Lymphoma; MM= Multiple Myeloma; SLL= Small Lymphocytic Lymphoma; + = few; ++ = moderate; +++ = abundant numbers of MT-positive macrophages.
Table 2: MT expression in tumour cells from different types of B-cell lymphoma.

<table>
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<tr>
<th>DIAGNOSIS</th>
<th>MT-positive tumour cells</th>
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<tr>
<td></td>
<td>&lt;5</td>
<td>5-20%</td>
<td>20-50%</td>
<td>&gt;50%</td>
<td>n</td>
</tr>
<tr>
<td>DLBCL</td>
<td>72</td>
<td>22</td>
<td>3</td>
<td>18</td>
<td>n=115</td>
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<td>FL, grades I+II</td>
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<td>0</td>
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<tr>
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<td>3</td>
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<tr>
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<tr>
<td>SLL</td>
<td>7</td>
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<td>n=7</td>
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</table>

DLBCL = Diffuse Large B-cell Lymphoma; FL = Follicular Lymphoma; MCL = Mantle cell Lymphoma; MM = Multiple Myeloma; SLL = Small Lymphocytic Lymphoma
Table 3: Clinical data in DLBCL with low vs. high MT-I+II expression in the tumour cells.

<table>
<thead>
<tr>
<th></th>
<th>Low expression &lt;20% (n = 97)</th>
<th>High expression &gt;20% (n = 18)</th>
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<tr>
<td>Age</td>
<td>64,2</td>
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<tr>
<td>Stage</td>
<td>2,58 (n=73)</td>
<td>2,65 (n=17)</td>
</tr>
<tr>
<td>IPI</td>
<td>2,31 (n=59)</td>
<td>2,40 (n=15)</td>
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<tr>
<td>Survival (5-year overall)</td>
<td>40,7%</td>
<td>12,0%</td>
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Table 4: MT expression in tumour cells vs. macrophage in 115 DLBCL.

<table>
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<tr>
<th>Macrophage</th>
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<td>+</td>
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<tr>
<td>+++</td>
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<td>6</td>
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<tr>
<td>Total</td>
<td>72</td>
<td>22</td>
<td>3</td>
<td>18</td>
</tr>
</tbody>
</table>
Fig. 1

A

B

Array expression values

Mean and 95% CI
Fig. 2

A

- MT high
- MT low

Percent survival

Time in month

n=33
n=15

P=0.08

B

- MT low
- MT high

Percent survival

Time in month

n=119
n=119

P=0.01
Fig. 3

A
MTJ+Il

B
CD86

C

D
Fig. 4

A

Percent survival

Time in month

n=37

n=52

n=26

Curve comparison:
Log rank P-value: 0.31

B

C

Percent survival

Time in month

p=0.20

p=0.83
Fig. 5

A

B
Fig. 6

A

B

Percent survival

Time in month

n=72
n=22
n=3
n=18

Percent survival

Time in month

n=94
n=21

p=0.0043
Fig. 7

A

Array expression values

mean and 95% CI

P = 0.04

MT < 20%  MT > 20%

B

Array expression values

Mean and 95% CI

+ + + vs + + +

P = 0.001

MT staining
Prognostic significance of metallothionein in B-cell lymphomas

Christian Bjorn Poulsen, Rehannah Borup, Niels Borregaard, Finn Cilius Nielsen, Michael Boe Moller and Elisabeth Ralfkiaer