The handle http://hdl.handle.net/1887/39089 holds various files of this Leiden University dissertation.

**Author:** Cetinozman, F.
**Title:** PD-1 Expression in primary cutaneous lymphoma
**Issue Date:** 2016-04-20
SUMMARY AND DISCUSSION
SUMMARY AND DISCUSSION

The studies presented in this thesis are focused on the expression of the molecule programmed death-1 (PD-1) in cutaneous lymphomas. The main goal was to determine the presence and distribution pattern of this molecule in different types of primary cutaneous T-cell lymphomas (CTCL) and cutaneous B-cell lymphomas (CBCL) in order to find out if PD-1 might be a useful biomarker to improve diagnosis, and possibly, to enable better classification of provisionally classified cutaneous lymphoma entities. In one chapter two additional markers were investigated, namely thymocyte selection-associated high mobility group box protein (TOX) and C-MYC. In this final chapter, the results described in this thesis are summarized and compared with the data described in the literature and conclusions are drawn.

PD-1 EXPRESSION AS DIAGNOSTIC MARKER FOR PRIMARY CUTANEOUS CD4+ SMALL/MEDIUM-SIZED PLEOMORPHIC T-CELL LYMPHOMA AND CUTANEOUS PSEUDO-T-CELL LYMPHOMA

In recent cutaneous lymphoma classifications [World Health Organization (WHO)-European Organization for Research and Treatment of Cancer 2005; WHO 2008], primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma (PCSM-TCL) is a cutaneous T-cell lymphoma (CTCL) that has been included as a provisional entity.1;2 Histologically, these lymphomas show nodular-to-diffuse infiltrates with a predominance of CD3+, CD4+, CD8–, CD30– small to medium-sized pleomorphic T cells and a small proportion (<30%) of large CD4+ pleomorphic T cells. In most cases there is a considerable admixture with small reactive CD8+ T cells and CD20+ B cells, including some blast cells, histiocytes, and in some cases plasma cells and eosinophils. Clinically, these PCSM-TCL characteristically present with a solitary plaque or tumor that is generally localized on the face or the upper trunk, and uncommonly with multiple papules, plaques, or tumors.1;3;4 In particular, patients presenting with a solitary skin lesion have an excellent prognosis.5 The clinical, histological and immunophenotypical features of these PCSM-TCL are strikingly similar to those described previously in so-called pseudo-T cell lymphomas.6;7 Demonstration of a T-cell clone and loss of pan-T-cell antigens are often used as diagnostic criteria for PCSM-TCL. However, using the Biomed-2 protocol, clonal TCR gene rearrangements are now found in most cases classified as pseudo-T-cell lymphoma and loss of pan-T-cell antigens is not observed in PCSM-TCL presenting with a solitary lesion (Chapter 2). Therefore, the relationship between PCSM-TCL and pseudo-T-cell lymphomas is still a matter of debate. More important, these PCSM-TCL/pseudo-T-cell lymphomas should be differentiated histologically from
other types of CTCL, in particular cutaneous peripheral T-cell lymphoma, not otherwise specified (PTCL–NOS) and tumor stage mycosis fungoides (MF).

In 2009, Rodriguez-Pinilla et al. reported that the large atypical CD4+ T cells in PCSM-TCL express PD-1, BCL6, and CXCL13. In Chapter 2 we investigated expression of PD-1 and other TFH markers in skin biopsies of 26 cases classified as either PCSM-TCL or pseudo-T-cell lymphoma and skin biopsies obtained from patients with MF and a cutaneous PTCL–NOS. The primary goal of this study was to determine whether PD-1 could serve as a useful marker to differentiate between PCSM-TCL and these other types of CTCL. In all 26 PCSM-TCL/pseudo-T-cell lymphoma cases the medium-sized to large atypical T cells consistently expressed PD-1, which is in agreement with observations of more recent studies. In addition, serial sections showed that CXCL13 generally stained 50–75% of the PD-1+ cells, and BCL6 25–50% of the PD-1+ cells, suggesting that these tumor cells might have been originated from TFH cells. In contrast, PD-1 expression was found in only 2 of 16 PTCL–NOS and in only 2 of 21 cases of MF. Although not a specific marker, the presence of scattered or small clusters of medium-sized atypical CD4+ T cells expressing PD-1 and many admixed small reactive CD8+ T cells, CD20+ B cells and histiocytes, strongly suggests a diagnosis of PCSM-TCL/pseudo-T-cell lymphoma. This characteristic pattern was found in both cases classified initially as pseudo-T-cell lymphoma and cases classified as PCSM-TCL. Therefore, we and others feel that there is no reason to classify PCSM-TCL and pseudo-T-cell lymphoma separately. There is increasing doubt if such cases should be considered as a genuine malignant lymphoma and, small- to medium-sized pleomorphic T-cell nodules of undetermined significance and cutaneous CD4+ small-medium T-cell lymphoproliferation have been suggested as unifying terms for this condition (Beltraminelli et al., and Chapter 2).

As all patients had an uneventful follow-up and that a skin relapse was only observed in a few cases, we suggest these cases should not be treated aggressively and no staging procedures should be performed. PCSM-TCL cases that do not meet the criteria described above are rare and should be fully staged. PCSM-TCL cases with rapidly growing multiple bulky tumors, a low percentage of admixed CD8+ T cells, and/or a high proliferative fraction, have been described to be at risk to develop progressive disease, but this study awaits further confirmation.

Recently, Battisella and colleagues described five patients with a CTCL, who presented with papules, nodules and/or infiltrated plaques not preceded by chronic patches or plaques, excluding MF. Except for one patient with a solitary infiltrated plaque, who responded well to radiotherapy, the other cases were relatively resistant to current therapies for CTCL. Histologically, these cases showed either a non-epidermotropic subepidermal band-like or a diffuse dense dermal lymphoid infiltrate, containing about 50% CD3+ T cells and 50% CD20+ B cells. The infiltrates contained a subset of atypical medium to large CD4+ T-cells, which expressed TFH
markers PD-1, BCL6, CXCL-13, CD10 or ICOS. Therefore, the authors suggested the term primary cutaneous TFH lymphomas for their cases. However, the relationship between these TFH-expressing CTCL and PCSM-TCL is at present unclear.

**DIFFERENTIAL EXPRESSION OF PD-1 BY NEOPLASTIC CELLS IN SS AND MF**

In our initial study described in Chapter 2, PD-1 expression was found in only a small proportion of MF cases (2 out of 21 cases), which contrasted with the results of earlier studies.\(^{12-14}\) Moreover, our results in MF also contrast with the high expression of PD-1 reported in skin and peripheral blood of patients with SS.\(^{13,15}\) This prompted us to investigate PD-1 expression in a large number of skin biopsies obtained from patients with SS and patients with MF, including cases with patch/plaque, tumor and erythrodermic stage of diseases and cases with a CD4\(^+\)CD8\(^-\), CD4\(^-\)CD8\(^+\) and CD4\(^-\)CD8\(^-\) T-cell phenotype.

The results presented in Chapter 3 showed that PD-1 was expressed by more than 50% of the neoplastic T cells in the skin of the majority (89%) of patients with SS. The association of PD-1 with Sézary cells is very strong. Even if a cut-off level of 75% was applied, still 81% of the SS patients were PD-1 positive. In line with our observation, Wada and colleagues found PD-1 expression by Sézary cells in 73% of skin biopsies of SS patients.\(^{13}\) In addition, Samimi and colleagues found increased PD-1 expression in the circulating neoplastic T cells in SS, but not in CD4\(^+\) peripheral blood T cells from patients with MF or healthy controls.\(^{15}\)

Our data revealed that 25% to 50% of the PD-1\(^+\) neoplastic cells in SS co-expressed CXCL13 and BCL6, so it may be questioned whether Sézary cells originate from TFH cells and/or have functional characteristics of TFH cells. Picchio et al.\(^{16}\) found high expression of CXCL13 in skin lesions, lymph nodes, and peripheral blood of most patients with SS (over 80%), but only in a minority of patients with MF, which nicely parallels our observations. A derivation of Sézary cells from TFH cells seems unlikely, as the presence of B cells is uncommon in SS skin specimens and no relationship between PD-1 expression and the number of admixed B cells or plasma cells was found. Apparently, the simultaneous expression of TFH-cell markers PD-1, CXCL13, and BCL6 by a particular T cell does not necessarily mean that this cell is a TFH cell.

Consistent with results in chapter 2, the results in Chapter 3 show that PD-1 expression by >50% of the neoplastic T cells was found in a minority (only 13%) of the MF cases. We did not observe differences in PD-1 expression between the patch/plaque, tumor and erythrodermic cases. In addition, no differences between the CD4\(^+\)CD8\(^-\), CD4\(^-\)CD8\(^+\) and CD4\(^-\)CD8\(^-\) T-cell phenotypes was found. These
SUMMARY AND DISCUSSION

Results conflict with other studies on PD-1 expression in MF, showing variable (27–100%) PD-1 expression by the neoplastic T cells in MF specimens. Despite six studies have been published on expression of PD-1 in MF (Table 1), no consensus is reached on this topic. The diversity of these observations may be explained by differences in cut-off levels of PD-1 positivity, antibodies and staining protocols (Table 1). Interestingly, 1 out of 8 E-MF cases showed PD-1 expression compared to 24 out of 27 cases of SS, which contributes to the debate on the relationship between both conditions.

RELATIONSHIP BETWEEN MF AND SS

The relationship between SS and MF is still a matter of debate as extensively discussed in Chapter 3. On the one hand, there is a general belief that SS is a leukemic phase or leukemic variant of MF because of the morphological atypical cells with cerebriform nuclei) and phenotypical (CD3⁺CD4⁺CD8⁻ T cells) similarities between both conditions, which resulted in the concept of CTCL. In addition, patients with MF may sometimes present with erythroderma and/or develop peripheral blood involvement during follow-up, while rare patients with SS

Table 1. Expression of PD-1 in mycosis fungoides: comparison of the literature

<table>
<thead>
<tr>
<th>Total samples</th>
<th>PD-1 antibody (supplier)</th>
<th>Cut-off level for positivity</th>
<th>Number of positive cases</th>
<th>Percentage of positive cases</th>
<th>CD10 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roncador et al. (2007)</td>
<td>Mouse monoclonal antibody; clone NAT105 (in-house generated)</td>
<td>Not indicated</td>
<td>5</td>
<td>56%</td>
<td>ND*</td>
</tr>
<tr>
<td>Wada et al. (2011)</td>
<td>Goat polyclonal antibody AF1086 (R&amp;D Systems)</td>
<td>&gt; 30%</td>
<td>15</td>
<td>50%</td>
<td>ND</td>
</tr>
<tr>
<td>Kantekure et al. (2011)</td>
<td>Mouse monoclonal antibody (Abcam); clone not specified (either NAT105 or J116)</td>
<td>&gt; 25% &gt; 50%</td>
<td>16 7</td>
<td>62% 27%</td>
<td>ND</td>
</tr>
<tr>
<td>Cetinozman et al. (2012)</td>
<td>Goat polyclonal antibody AF1086 (R&amp;D Systems)</td>
<td>&gt; 10% &gt; 50%</td>
<td>12 8</td>
<td>20% 13%</td>
<td>negative</td>
</tr>
<tr>
<td>Park et al. (2014)</td>
<td>Mouse monoclonal antibody clone NAT105 (Cell Marque)</td>
<td>&gt; 10%</td>
<td>21</td>
<td>84%</td>
<td>negative</td>
</tr>
<tr>
<td>Bosisio and Cerroni (2015)</td>
<td>Mouse monoclonal antibody (Abcam); clone not specified (either NAT105 or J116)</td>
<td>&gt; 10% &gt; 50%</td>
<td>13–17 9–16</td>
<td>62–85% 43–76%</td>
<td>1 biopsy &gt;50%</td>
</tr>
</tbody>
</table>

*Abbreviations: ND, not determined; MF, mycosis fungoides
†30 biopsies obtained from 26 patients with MF
‡32 biopsies obtained from 17 patients with MF
may develop skin tumors as in MF. On the other hand, SS and MF show obvious differences in clinical presentation and course, there are histologic, phenotypic, and genetic differences between both conditions. The list of differences between SS and MF is still growing (summarized in Table 2) and multiple authors have suggested that SS and MF should be considered as distinct entities arising from different functional T-cell subsets. The almost consistent expression of PD-1 and CXCL13 in SS (Chapter 3 and Picchio et al.) and the uncommon expression of these markers in MF provide additional support for the view that SS and MF should be considered as distinct lymphomas.

### Table 2. Differences between Sézary syndrome and mycosis fungoides

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sézary syndrome</th>
<th>Mycosis fungoides</th>
</tr>
</thead>
<tbody>
<tr>
<td>clinical presentation</td>
<td>erythroderma</td>
<td>patches, plaques, tumor</td>
</tr>
<tr>
<td>skin histology</td>
<td>mainly perivascular</td>
<td>epidermotropic (basal layer)</td>
</tr>
<tr>
<td>blood involvement</td>
<td>by definition</td>
<td>rare</td>
</tr>
<tr>
<td>lymph node histology</td>
<td>monotonous Sézary cells</td>
<td>dermatopathic lymphadenopathy + blast cell</td>
</tr>
<tr>
<td>CD4/CD8 phenotype</td>
<td>consistently CD4⁺CD8⁻ T-cell phenotype</td>
<td>may also present with CD4⁺CD8⁺ or CD4⁺CD8⁻ phenotype</td>
</tr>
<tr>
<td>cell of origin</td>
<td>central memory T cell</td>
<td>effector memory T cell</td>
</tr>
<tr>
<td>loss pan T cell markers</td>
<td>rare (even in patients with advanced disease)</td>
<td>frequent (in particular in advanced stage of disease)</td>
</tr>
<tr>
<td>CD2, CD3, CD5</td>
<td>&lt; 5% of patients</td>
<td>&gt; 50% of patients</td>
</tr>
<tr>
<td>PD-1 expression</td>
<td>&gt; 90% of patients</td>
<td>&lt; 15% of patients</td>
</tr>
<tr>
<td>(cut-off &gt; 50% positivity)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DIFFERENTIAL DIAGNOSIS BETWEEN SÉZARY SYNDROME AND ERYTHRODERMIC INFLAMMATORY DERMATOSES**

Clinical and histological differentiation between SS and erythrodermic inflammatory dermatoses (EID) is one of the most challenging issues in dermatopathology. We found (Chapter 3) that PD-1 is expressed by Sézary cells in the vast majority of SS, representing the prototype of erythrodermic CTCL. We wondered whether PD-1 would be a biomarker to distinguish SS from EID histologically. In contrast to the almost consistent expression of PD-1 in SS (92% of cases), our immunohistochemical study (Chapter 4) of skin biopsies from EID patients revealed that PD-1 was...
expressed by more than 50% infiltrating dermal CD3+ T cells in only 4 out of 30 cases (13%). While in SS PD-1 is mainly present by CD4+ neoplastic T cells, in EID PD-1 is predominantly expressed by CD8+ reactive dermal and epidermal T cells (Table 3). Although partial loss of CD7 expression can commonly be observed in both SS and EID, expression of CD7 by ≤ 20% of the infiltrating T cells was only found in SS. Our results suggest that expression of PD-1 by more than 50% of CD4+ T cells and expression of CD7 by less than 20% of the skin-infiltrating T cells may be considered as valuable adjuncts in the differentiation between SS and EID.

Using the same cohort as in Chapter 4, in Chapter 5 we investigated the diagnostic significance of two other markers TOX and C-MYC. Increased TOX expression by malignant CD4+ T cells has been found in MF and SS, but information on TOX expression in EID is lacking. In addition, C-MYC positivity has been demonstrated in a considerable number of infiltrating lymphoid cells in MF and SS, while there is no information on C-MYC expression in EID.27-31 We demonstrated that TOX was strongly expressed by more than 50% of the neoplastic T cells in 13 of 15 SS patients (87%), while all EID cases showed weak nuclear staining (Table 3), showing TOX positivity between 11% and 50% of the T cells. Our results are consistent with recent studies also showing strong nuclear staining for TOX by atypical CD4+ T cells in skin28,31 and blood30 of SS patients compared to benign inflammatory dermatoses and healthy controls. No significant difference in C-MYC expression between SS and EID was found. In both groups C-MYC was expressed by 11-25% of the skin-infiltrating T cells in about half of the cases. Kanavaros et
al. and Goswami et al. reported similar percentages of C-MYC positivity in SS patients.\textsuperscript{32,33} Strong expression of TOX, but not C-MYC, can be another useful adjunct in the differentiation between SS and EID.

In conclusion, in order to make a proper distinction between SS and EID a combination of the following criteria seems to be relevant (Table 3): presence of clonality of the T cells in blood/skin, the CD4/CD8 ratio of infiltrating T cells in dermis and epidermis and in blood, the degree of epidermotropism, positivity of PD-1 and/or TOX by skin-infiltrating T cells and presence of loss of CD7 expression.

### PD-1 EXPRESSION IN CUTANEOUS B-CELL LYMPHOMAS

Several studies investigated PD-1 expression in nodal B-cell non-Hodgkin lymphomas (B-NHLs). Except for small lymphocytic lymphoma and rare cases of nodal follicular B-cell lymphoma and diffuse large B-cell lymphoma, PD-1 is generally not present on neoplastic cells in B-NHLs.\textsuperscript{12,34,35} However, PD-1 is detectable on variable amounts of tumor infiltrating lymphocytes, in particular in follicular lymphoma.\textsuperscript{35-39} In most studies increased numbers of PD-1\(^+\) T cells in these nodal follicular lymphomas are associated with a significantly improved survival.\textsuperscript{35-38} As described in Chapter 6, we studied PD-1 expression in primary cutaneous B-cell lymphomas and found that this marker was absent on neoplastic B cells, but was present on variable amounts of reactive T cells. PD-1\(^+\) T cells were significantly more numerous in primary cutaneous follicle center lymphoma (PCFCL) than in primary cutaneous marginal zone lymphoma (PCMZL) and primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBCL–LT). In both PCFCL and PCMZL, as well as in pseudo B-cell lymphomas, clusters of PD-1\(^+\) T cells, part of which were also CXCL13\(^+\), formed rosettes around large B cells, similar to TFH cells in reactive lymph nodes. We also showed that in PCFCL these PD-1\(^+\) T cells were not only located within germinal centers, but also outside germinal centers. These results were consistent with recent studies in CBCL.\textsuperscript{40,41}

### CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The results of the studies presented in this thesis demonstrated that PD-1 can serve as a useful tool in the diagnosis of CTCL in particular in PCSM-TCL/pseudo T-cell lymphomas and SS. However, it should be recognized that PD-1 is not specific marker, as the neoplastic T cells in some cases of CTCL (such as MF and PTCL–NOS) and reactive T cells in both benign and malign skin diseases might also express
PD-1. The presence of scattered or clusters of PD-1 on T cells in combination with many admixed CD8+ T cells, B cells, macrophages is highly suggestive of PCSM-TCL/ pseudo T-cell lymphomas and facilitates differentiation from other types of CTCL, such as tumor stage MF or PTCL–NOS. PD-1 and TOX are strongly expressed in SS (90%) and are useful adjuncts to differentiate SS from benign erythroderma.

Apart from its diagnostic significance, PD-1 and PD-L1 expression in CTCL might also have therapeutic potential. The PD-1/PD-L1 pathway has emerged as an important mechanism by which tumors escape from the antitumor immune response. PD-1 is expressed by tumor-infiltrating lymphocytes in many different types of tumors, while high PD-L1 expression has been reported in many human cancers. PD-L1 on the tumors binds to PD-1 on the tumor-infiltrating T lymphocytes causing reduction of the effector function and killing capacity of these T cells and consequently facilitating immune evasion of the tumors. Ligation of PD-1 by PD-L1 can be obstructed with blocking monoclonal antibodies directed either to PD-1 or to PD-L1 thereby restoring the antitumor immunity. Recent clinical trials with antibodies targeting the PD-1/PD-L1 pathway in patients with cancer demonstrated promising antitumor responses. It was demonstrated that PD-1/PD-L1 blockade is most effective in tumors with high PD-L1 expression, and it is proposed that tumor-associated PD-L1 expression can be used as biomarker for selecting patients who will have the best survival benefit upon PD-1/PD-L1-pathway inhibitor therapy.

Considering the importance of the PD-1/PD-L1 pathway, the question arises whether this pathway can also serve as a potential target in patients with SS and MF. Kantekure et al. showed strong expression of PD-L1 by neoplastic large transformed T cells in MF. Whether the neoplastic T cells in SS — in addition to PD-1 — also express PD-L1 is at present unknown. Clinical trials investigating the efficacy of an anti-PD-1 monoclonal antibody in patients with relapsed or refractory MF and SS have just started.

REFERENCES


42. Ahmadzadeh M, Johnson LA, Heemskerk B et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 2009;114:1537-1544.


