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Increased expression of \textit{PLS3} correlates with better outcome in Sézary syndrome

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Abbreviations:

SS = Sézary syndrome

OS = overall survival

MF = mycosis fungoides

WHO = World Health Organization

EORTC = European Organization for Research and Treatment of Cancer

DSS = disease-specific survival
To the editor:

**Increased expression of PLS3 correlates with better outcome in Sézary syndrome**

Patients with Sézary syndrome (SS), a rare erythrodermic and leukemic form of cutaneous T cell lymphoma, have a poor prognosis with a 5-year overall survival (OS) of 20-42% and a median OS between 2.5 and 5 years (Bernengo et al., 1998; Diamandidou et al., 1999; Kim et al., 2003; Agar et al., 2010; Talpur et al., 2012; Kubica et al., 2012).

Prognostic factors associated with a worse survival reported in SS include advanced age (Diamandidou et al., 1999; Kim et al., 2003; Foulec et al., 2003; Agar et al., 2010; Talpur et al., 2012; Kubica et al., 2012), short duration of skin lesions before diagnosis of SS (Foulec et al., 2003), previous history of mycosis fungoides (MF) (Bernengo et al., 1998; Kubica et al., 2012), elevated serum lactate dehydrogenase levels (Bernengo et al., 1998; Diamandidou et al., 1999; Foulec et al., 2003; Agar et al., 2010; Talpur et al., 2012; Kubica et al., 2012), (the degree of) nodal involvement (Diamandidou et al., 1999; Kim et al., 2003), and factors reflecting blood tumor burden, such as increased leukocyte counts (Bernengo et al., 1998; Vidulich et al., 2009; Talpur et al., 2012) or high Sézary cell counts (Bernengo et al., 1998).

However, the results of these studies are not consistent, which may be due to different diagnostic criteria of SS, such as inclusion of patients without a T-cell clone in the peripheral blood, and analysis of mixed populations of patients with SS and erythrodermic MF.

Recently we investigated the diagnostic significance of a large number of immunophenotypic and molecular biomarkers for SS in a group of Sézary patients (Boonk et al., 2016) that fulfilled the diagnostic criteria of the World Health Organization - European Organization for Research
and Treatment of Cancer (WHO-EORTC) classification (Willemze et al., 2005). None of these patients had a history of MF. Molecular biomarkers diagnostic for SS were copy number alterations in \textit{MYC} (gain) and/or \textit{MNT} (loss), increased expression of \textit{DNM3}, \textit{TWIST1}, \textit{EPHA4} and \textit{PLS3} and decreased expression of \textit{STAT4}.

Here we investigated the prognostic significance of these molecular biomarkers and previously reported clinical prognostic markers using the same cohort of Sézary patients. Between September 2009 and October 2013 sixty-four SS patients from six EORTC centers, including Helsinki (Finland), London (England), Leiden (Netherlands), Mannheim (Germany), Turin (Italy) and Paris (France) were included and followed until 30 January 2015. At inclusion of the study, clinical variables (gender, age at diagnosis, duration of skin lesions before diagnosis SS, lymph node involvement, leukocyte count, absolute CD4 count and Sézary cell count) were recorded and peripheral blood samples were collected for copy number variation and gene expression qPCR analysis, as described previously (Boonk et al., 2016). Lymph node involvement was defined by presence of enlarged lymph nodes of 1.5 cm or larger in the longest transverse diameter on computed tomography scan or histologically confirmed lymph node involvement.

Aberrant gene expression in the SS samples was compared to samples from patients with erythrodermic inflammatory dermatoses (EID) and healthy controls. ROC curve analysis was used to determine fixed cut-off thresholds for each individual gene expression qPCR assay with a specificity of 100% and an accuracy above 0.80. An one-tailed Mann-Whitney test was applied to test for significant differential expression between the SS and EID samples. \textit{P}-values below 0.05 were regarded as statistically significant. The results of aberrant expression of genes \textit{DNM3},
Twist1, EPHA4, PLS3 and STAT4 were included in the statistical analysis. A more detailed method section including these thresholds is added in the supplementary material.

Survival was calculated with the Kaplan-Meier method from the date of diagnosis until the patient’s death or date of last follow-up. The median follow-up time after diagnosis was 45 months (range, 1-129 months). Twenty seven patients died during follow-up, including 21 SS related deaths. The disease-specific survival (DSS) after 1, 2, 3 and 5 years was 89%, 82%, 76% and 59%, respectively, and OS was 86%, 79%, 72% and 49%, respectively.

Univariate analysis of parameters with possible prognostic significance for DSS and OS was performed using Cox proportional hazards regression analysis and parameters that were significant at the 0.1 level were included in a multivariate analysis model. P-values below 0.05 were regarded as statistically significant.

Both in univariate and multivariate analysis upregulation of PLS3 was associated with a significantly better outcome for DSS and OS (multivariate $P = 0.006$ and $P = 0.002$, respectively). Patients with upregulation of PLS3 had a median survival of 71 months (range, 9-129) compared to only 33 months (range, 1-72) in SS patients with normal expression of PLS3 (Figure 1). Upregulation of DNM3 and Twist1 were associated with a better OS in univariate analysis ($P = 0.008$ and $P = 0.043$, respectively), but not in multivariate analysis ($P = 0.658$ and $P = 0.342$, respectively). Gain of MYC, loss of MNT, upregulation of EPHA4 and downregulation of STAT4 showed no association with DSS and OS (Table 1).

Of the clinical parameters both univariate and multivariate analysis showed that leukocyte count was a significant prognostic factor for DSS and OS (multivariate $P = 0.005$ and $P = 0.005$, respectively), while gender, age, duration of skin lesions before diagnosis, lymph node involvement, absolute CD4 count and Sézary cell count were not (Table 1).
PLS3 (T-plastin) is an actin-binding protein that is expressed in all normal cells of solid tissues that have a replicative role, but is normally not expressed in T cells (Lin et al., 1999). Expression of PLS3 has been described as a specific marker of Sézary cells (Nebozhyn et al., 2006; Jones et al., 2012). Studies investigating the mechanism underlying dysregulation of PLS3 expression in SS cells found no evidence for PLS3 mutations within coding or promoter regions, but demonstrated significant hypomethylation of CpG dinucleotides 95-99 within the PLS3 CpG island which was restricted to the PLS3+ cells (Jones et al., 2012). Reanalysis of recently published DNA methylation profiles (van Doorn et al., 2016) from 9 Sézary patients and 4 healthy controls included in this study confirmed this correlation between DNA methylation and PLS3 expression (data not shown). A recent study found that constitutive PLS3 expression was associated with apoptotic resistance to etoposide and suggested a role for cell survival in SS (Begue et al., 2012). How T-plastin expression is linked to a better outcome in Sézary patients is not known and should be the subject of further study.

Although for a rare disease as SS the number of included patients is relatively high, a limitation of this study is a small sample size yielding wide confidence intervals and these observations should be confirmed in an independent study.

In conclusion, we show that upregulation of PLS3 is associated with a favorable disease outcome in patients with SS and that increased leukocyte count is a significant adverse prognostic factor for survival.
Conflict of interest: M.F. received travel grants from TEVA.

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References


Table:

**Table 1.** Results of univariate and multivariate analysis for variables at Sézary syndrome diagnosis. Parameters significant at the 0.1 level were included in multivariate analysis.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Median survival (months)</th>
<th>Univariate analysis DSS HR (95% CI)</th>
<th>P-value</th>
<th>Multivariate analysis DSS HR (95% CI)</th>
<th>P-value</th>
<th>Univariate analysis OS HR (95% CI)</th>
<th>P-value</th>
<th>Multivariate analysis OS HR (95% CI)</th>
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Figure legend:

**Figure 1.** Survival curves. Disease-specific survival (a) and overall survival curve (b) according the groups with and without upregulation of *PLS3.*