number of immunological pathways and processes, for example, by identifying antigen presentation and T-cell activation and/or differentiation. Our results additionally underscore contributions from the JAK-STAT signaling and the costimulatory pathway. These particular processes are capable of being modulated with available therapeutics, which we are currently studying within the context of clinical trials in AA, using JAK inhibitors and abatacept, respectively. Furthermore, these genes could be useful in our development of a biomarker panel, allowing us to prioritize transcriptional changes that occur over particular disease trajectories and/or during a therapeutic response. Finally, evidence obtained in this study can be integrated with results from next-generation sequencing, providing a framework for the interpretation of variants harbored by patients and laying a foundation for precision medicine in AA.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1038/JID.2015.402.

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Expression of Sézary Biomarkers in the Blood of Patients with Erythrodermic Mycosis Fungoides

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TO THE EDITOR

Sézary syndrome (SS) and erythrodermic mycosis fungoides (EMF) are part of a broader spectrum of erythrodermic cutaneous T-cell lymphoma. The revised ISCL/EORTC staging identifies three types of blood involvement: B0 (circulating Sézary cells [CSC] $\leq$ 5%), B1 (CSC $>$ 5% but $<$1,000/mm^3), and B2 (CSC $>$ 1,000/mm^3) (Olsen et al., 2007). Only B2 are recognized as SS, whereas B0 and B1 are considered as EMF. EMF is defined as erythroderma with papules and plaques greater than 80% of the body surface area associated with suggestive histology of cutaneous T-cell lymphoma in accordance with Olsen et al. However, patients with EMF must not meet SS criteria, namely a CSC count $>$ 1,000/mm^3, a CD4/CD8 ratio $\geq$ 10, a CD4$^+$CD7$^-$ lymphocyte population $\geq$ 40%, or a CD4$^+$CD26$^-$ population $\geq$ 30% (Olsen et al., 2007; Willemze et al., 2005). Several molecular markers have been specifically identified in the blood of patients with SS, including CD158k/KIR3DL2, the transcription factor Twist, T-plastin (PLS3), and CD335/NKp46 (Bensussan et al., 2011; Bouaziz et al., 2010; Michel et al., 2013; Nebozhyn et al., 2006; Tang et al., 2010; Van Doorn et al., 2004). Of interest, our previous results clearly evidenced that their combination allows diagnosis of 100% of untreated patients with SS (Michel et al., 2013).

Herein, this combination of blood biomarkers was assessed in all the patients with EMF diagnosed from three French dermatological centers during the last decade to determine whether EMF covers a set of various stages. To this aim, we compared patients with EMF with the cohort of patients with SS (n = 81, mean age 69.5 years [22–88 years]) and of healthy donors (n = 12, mean age 33.2 years [23–49 years]) previously published (Michel et al., 2013). The local ethics committees approved the study and all the subjects gave written informed consent. Thirty-one patients were included (20 males, 11 women), with a median age of 64.2 years (30–85 years) at diagnosis: 13 patients were B0 and 18 patients were B1, with a mean ± SD CSC absolute count of

Abbreviations: CSC, circulating Sézary cells; CT, cycle threshold; EMF, erythrodermic mycosis fungoides; SS, Sézary syndrome

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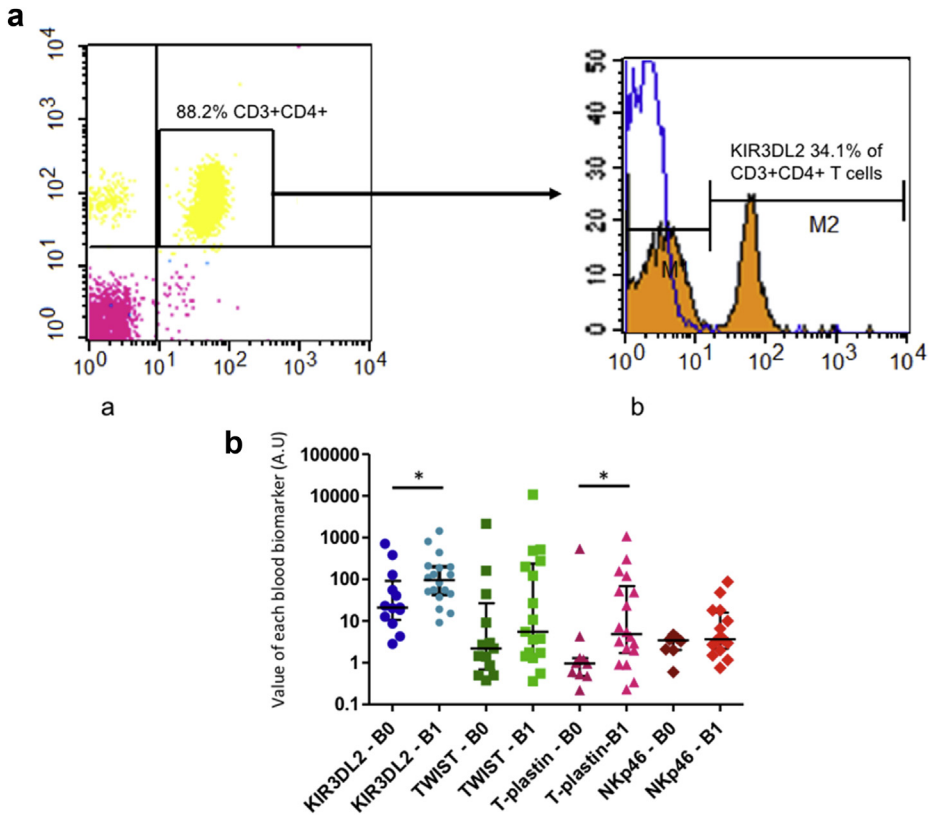

Figure 1. Flow cytometry analysis and blood biomarker qPCR values in EMF patients. (**a**) Flow cytometry analysis of $CD3^+CD4^+KIR3DL2^+$ cells in one representative patient with EMF at stage B1. Percentage of $CD3^+CD4^+$ T cells was ascertained among fresh peripheral blood mononuclear cell gate (left), and then KIR3DL2 expression was evaluated on the $CD3^+CD4^+$ T cells by immunofluorescence staining with a monoclonal phycoerythrin–conjugated KIR3DL2 antibody (Miltenyi) (right). The blue line (empty histogram) indicates staining with a control isotype monoclonal irrelevant antibody. (**b**) Individual values of each blood biomarker in B0 and B1 patients. All samples were run in duplicate. The single data points in (**b**) represent the mean value of the duplicate for each blood biomarker for each individual patient. The bars represent median values, and the error bars are the interquartile range. Mann-Whitney test: $*P < 0.05$. EMF, erythrodermic mycosis fungoides.

Table 1. Percentages of blood biomarker positivity in B0 (n = 13) and B1 EMF (n = 18), as compared with B2 (Sézary syndrome) patients (n = 81) and healthy donors (n = 12), using determination of the positive arbitrary threshold value obtained by q-RT-PCR at 95% significance: 25, 10, 5, and 5 for KIR3DL2, Twist, T-plastin, and NKp46, respectively

Biomarker positivity	KIR3DL2 % (number of positive patients/total)	Twist % (number of positive patients/total)	T-plastin % (number of positive patients/total)	NKp46 % (number of positive patients/total)	Any blood biomarker % (number of positive patients/total)
B0	23 (3/13)	15 (2/13)	8 (1/13)	0 (0/13)	31 (4/13)
B1	78 (14/18)	33 (6/18)	44 (8/18)	18 (3/17)	78 (14/18)
B2 = SS	84 (68/81)	91 (73/81)	87 (70/81)	28 (23/81)	100 (81/81)
Healthy donors	0	0	0	0	0

When biomarker expression was positive expression, the mean ± SD (min-max) mRNA expression levels of biomarkers for KIR3DL2, Twist, T-plastin, and NKp46 were 300 ± 403 (45–1,444), 2,057 ± 4,256 (11.4–10,738), 223 ± 357 (7.1–1,075), and 14.2 ± 7 (6.6–18.1), respectively, in B1 patients and 408 ± 298 (129–714), 1,155 ± 1,406 (161–2,149), 537, and no NKp46 positivity, respectively, in B0 patients.

Abbreviations: EMF, erythrodermic mycosis fungoides; SS, Sézary syndrome.

$247/mm^3 \pm 100$. In 11 patients, EMF was inaugural of the disease and blood analysis was done right at the beginning of the disease before any treatment. In the remaining 20 patients, there was first a localized MF, which evolved within a median of 3.5 years (0.2–20 years). The latter had already received treatments before biomarker analysis (skin-directed therapies only n = 12, systemic therapy n = 8).

All nine patients with EMF who were investigated for CD26 and CD7 expression had less than 30% $CD4^+CD26^-$ and 40% $CD4^+CD7^-$ T cells. Biomarker gene expression was evaluated by quantitative PCR (qRT-PCR, SYBR green technology, ABI PRISM7300 Real-time PCR System) on $CD4^+$ T cells isolated by positive magnetic-activated cell sorting (Miltenyi, Bergisch Gladbach,