MS. MARJAANA JENNI JASMIINA HÄYRINEN (Orcid ID : 0000-0002-6801-5271)

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Twist and Zeb1 expression identify Mycosis fungoides patients with low risk of disease progression

Marjaana J. Häyrinen[1,2*] and Pyry M. Uotila[1,2], Helka Sahi[3], Kirsi-Maria Haapasaari[4], Hanna-Riikka Teppo[4], Ylermi Soini[5], Maria Lapela[6], Kaija Vasala[7], Taina Turpeenniemi-Hujanen[1,2], Annamari Ranki[3], Milla E. L. Kuusisto[1,8] and Outi Kuittinen[1,2,9]

[1] Department of Oncology and Radiotherapy, Oulu University Hospital and University of Oulu, Oulu, 90220, Finland

[2] Medical Research Center, Oulu University Hospital and Unit of Cancer and Translational Medicine Research, University of Oulu, Oulu, 90220, Finland

[3] Department of Dermatology, Allergology and Venereology, University of Helsinki and Helsinki University Hospital, P.O. Box 160, 00029 HUS, Helsinki, Finland

[4] Cancer Research and Translational Medicine Research Unit, University of Oulu, Department of Pathology, Oulu University Hospital Oulu, 90220, Finland

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[5]Department of Clinical Pathology and Forensic Medicine, Cancer Center of Eastern Finland, Kuopio University Hospital and University of Eastern Finland, Kuopio, 70210, Finland

[6] Department of Oncology and Radiotherapy, Turku University Hospital and University of Turku, Turku, 20521, Finland

[7] Department of Oncology, Jyväskylä Central Hospital, Jyväskylä, 40620, Finland

[8] Department of Haematology, Oulu University Hospital and University of Oulu, Oulu, 90220, Finland

[9] Department of Oncology and Radiotherapy, University of Eastern Finland, Faculty of Health Medicine, Institute of Clinical Medicine, Kuopio University Hospital

***Corresponding author:** Peltolankaari 20 a 33, 90230 Oulu, Finland; Phone: +358504125521; Fax: +358 83153229; email: marjaana.hayrinen@student.oulu.fi

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Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma[1]. The clinical course is usually slow and the initial treatment is a skin-directed therapy[2,3]. When MF becomes refractory, systemic therapies are needed[2,3]. Disease progression is seen in

about 30% of MF patients but no undisputable markers are available to identify these patients[2,3]. Twist, Zeb1 and Slug are transcription factors (TFs) that induce epithelial-to-mesenchymal transition (EMT) and regulate metastasis and other detrimental steps in cancer progression[4]. Twist, a T-cell oncoprotein, is generally not expressed in mature lymphoid cells[5,6]. Zeb1 suppresses haematopoiesis and downregulates CD4 expression during T-cell maturation[7]. These TFs have received increasing interest in lymphoma pathogenesis as well. Previous studies have demonstrated increased Twist expression in advanced MF lesions, and deletions and mutations targeting the ZEB1 gene in MF[6-9]. Sézary syndrome patients with biallelic ZEB1 gene inactivation have a poor prognosis[10]. Our hypothesis is that immunohistochemical (IHC) detection of these TFs has potential prognostic value in MF.

In this study, the expression of Twist, Zeb1 and Slug proteins was studied in diagnostic MF biopsy specimens (n=39) using IHC (Fig. 1). The median patient age was 61 years (range 29–86) and the median follow-up time was 61 months (range 8–164). Depending on the clinical setting, patients received skin-directed therapies (topical corticosteroids, phototherapy using psoralens and ultraviolet-A irradiation, ultraviolet-B (UVB) or narrowband-UVB, or total skin electron beam therapy (TSEB)), systemic therapies (including bexarotene, prednisolone, methotrexate and interferon-α) or chemotherapy (including CHOP-type treatments)[2,3]. The IHC results were retrospectively compared with clinical parameters by statistical analysis. We chose two surrogate variables to indicate disease progression (clinician's decision): Initiation of systemic therapy or TSEB (time to next systemic treatment or TSEB; TTNST) or initiation of chemotherapy (time to next chemotherapy treatment; TTNCT). At ten years of follow-up, 78% of cases with high Twist expression in the diagnostic skin biopsy had received chemotherapy versus 27% of cases with low Twist expression (p=0.004) (Fig. 2a). At ten years of follow-up, 15% of cases with high Zeb1 expression required systemic therapy or TSEB versus 54% of cases with low Zeb1 expression (p=0.046) (Fig. 2b). The Twist-/Zeb+ variable identified patients who responded to, and did well with, skin-directed therapies alone. Conversely, 60% of

Twist+/Zeb- cases had disease progression within four years (p=0.044) (Fig. 2c). Slug expression had no significant associations.

We propose that lesional expression of Twist and Zeb1 has potential prognostic value in MF. Immunohistochemical detection of these EMT-inducing TFs can delineate different prognostic groups among MF patients and could, for example, aid in selecting patients for allogenic stem cell transplantation. This is the first study to describe these associations between IHC-detected Zeb1 protein expression and the clinical course of MF. In line with previous findings[7-10], Zeb1 protein expression was associated with a favorable disease outcome. MF cases with high nuclear Zeb1 expression had longer TTNSTs and these lymphomas were efficiently kept in check with topical steroids and UV-therapy alone. Since Zeb1 and Twist immunostaining is a rapid, technically simple and easily reproducible method, it is well suited for routine clinical use and has potential for prognostication in MF. Our study had several limitations. The sample size was relatively small, and we used novel time variables that may be affected by physician- or patient-related factors. Genomic data was not available to be compared with the IHC results. However, responses to standard systemic therapies in MF are often short and relapses frequent. There is an unmet medical need to find more reliable prognostic markers and targeted therapies. In the future, molecular inhibitors of Twist or Zeb1 mimetics may be suitable options for targeted therapy of MF.

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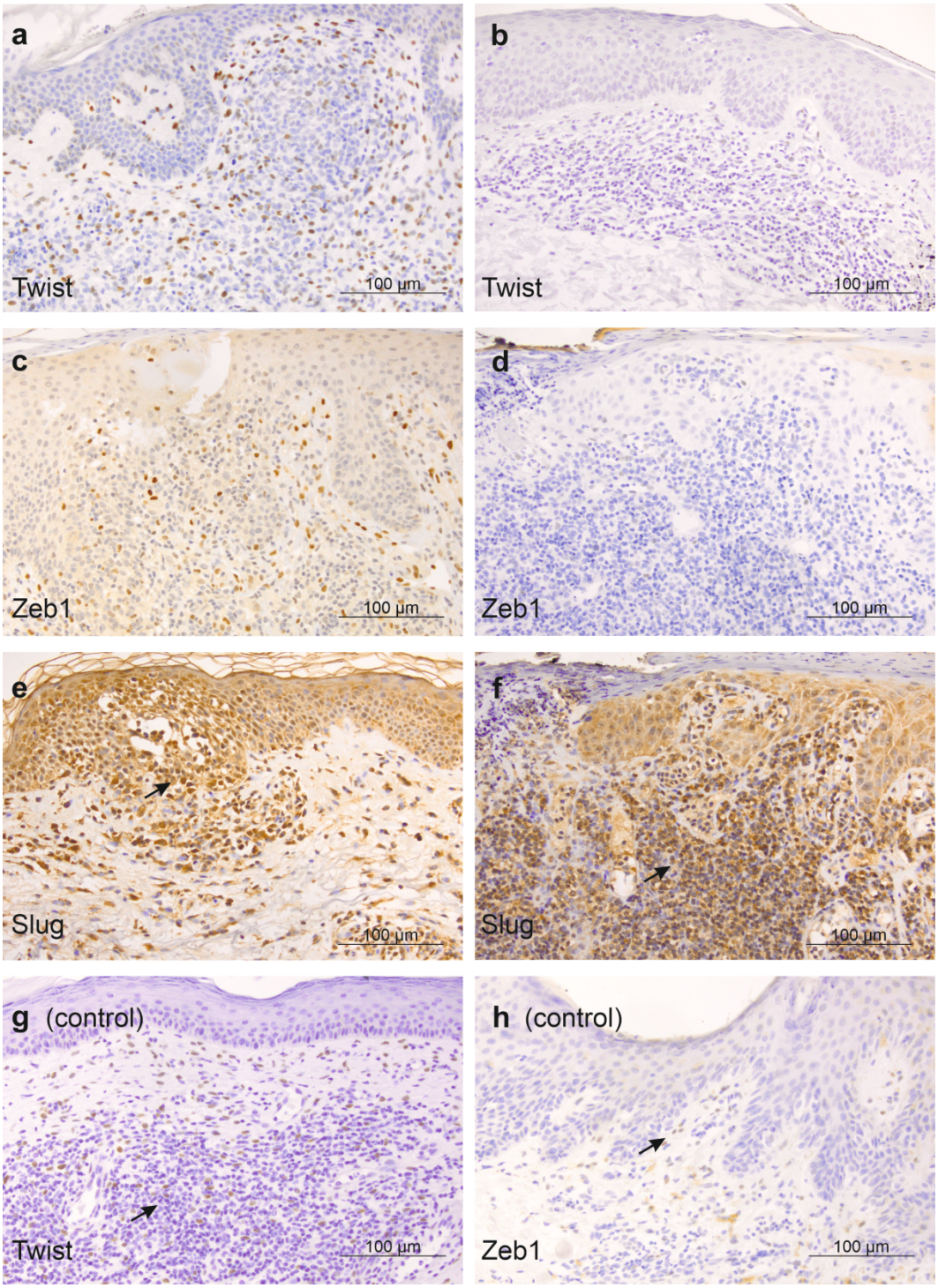

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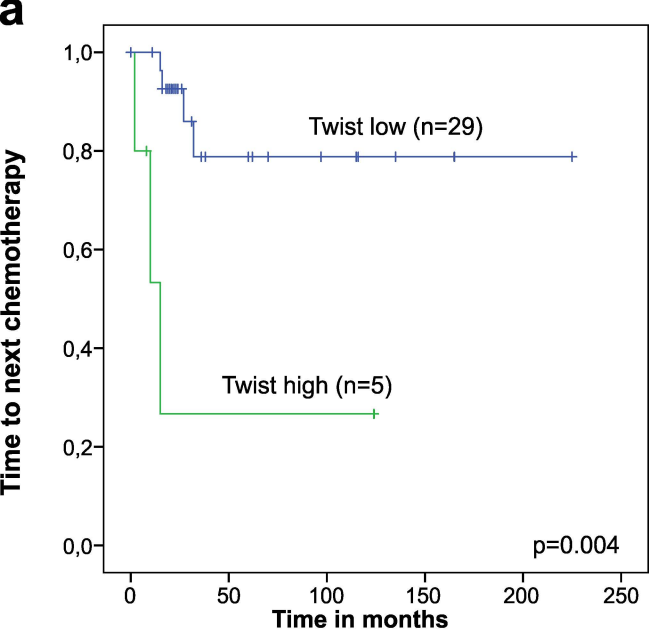
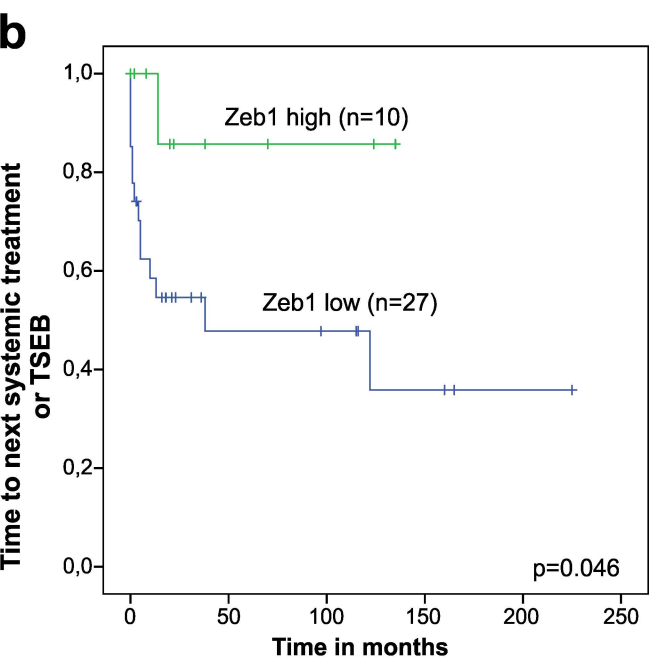
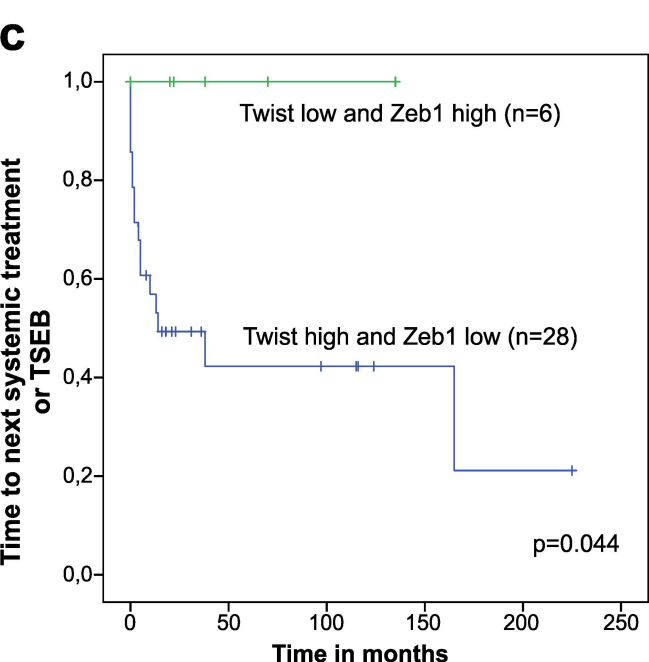

a
Time to next chemotherapy
1,0
0,8
0,6
0,4
0,2
0,0
Twist low (n=29)
Twist high (n=5)
p=0.004
0
50
100
150
200
250
Time in months


b
Time to next systemic treatment or TSEB
1,0
0,8
0,6
0,4
0,2
0,0
Zeb1 high (n=10)
Zeb1 low (n=27)
p=0.046
0
50
100
150
200
250
Time in months


c
Time to next systemic treatment or TSEB
1,0
0,8
0,6
0,4
0,2
0,0
Twist low and Zeb1 high (n=6)
Twist high and Zeb1 low (n=28)
p=0.044
0
50
100
150
200
250
Time in months