OPEN

Citation: Blood Cancer Journal (2014) **4**, e253; doi:10.1038/bcj.2014.73

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LETTER TO THE EDITOR

Somatic rearrangement of the *TP63* gene preceding development of mycosis fungoides with aggressive clinical course

Blood Cancer Journal (2014) **4,** e253; doi:10.1038/bcj.2014.73; published online 17 October 2014

Cutaneous T-cell lymphomas (CTCLs) comprise a heterogeneous spectrum of T-cell neoplasms with widely varying clinical presentation, biologic behavior and overall outcome.[1,2] The most common CTCL is mycosis fungoides (MF), which can range from a localized, indolent process to an aggressive lymphoma with widespread cutaneous and extracutaneous involvement and large-cell transformation (MF-LCT). This biologic and clinical heterogeneity is a feature shared with other T-cell lymphomas, including systemic peripheral T-cell lymphomas (PTCLs). Also common to both CTCLs and PTCLs is a limited understanding of genetic mechanisms of pathogenesis and progression, the elucidation of which could facilitate classification, prognostication and individualized therapy. For example, accurate classification of CTCLs often requires careful clinical and pathological follow-up over time, and an enhanced understanding of CTCL genetics might allow earlier, more definitive classification. Furthermore, genetic biomarkers that identify patients at greatest risk of aggressive clinical behavior could allow initiation of earlier or more intensive treatment protocols that might lead to better outcomes. Finally, knowledge of CTCL genetics could improve biologic understanding of this group of diseases and facilitate the development of more specific, targeted therapies.

Although CTCLs and PTCLs are clinically distinct groups of diseases, genetic data have highlighted similarities that suggest biologic interconnectedness. For example, we have shown that chromosomal rearrangements of the *DUSP22/IRF4* locus on 6p25.3 are seen in about 30% of both primary cutaneous anaplastic large-cell lymphomas (cALCLs) and systemic ALK-negative ALCLs.[3,4] More recently, we identified recurrent rearrangements of *TP63* on 3q28 in PTCLs that were associated with poor clinical outcomes.[4,5] *TP63* encodes p63, a member of the p53 family of transcription factors. Interestingly, although our study was primarily focused on patients with systemic PTCLs, we also identified two patients with unusually aggressive cALCLs that had *TP63* rearrangements. Therefore, we undertook the current multi-institutional study to determine the frequency and clinical significance of *TP63* rearrangements in an independent series of CTCLs.

We reviewed CTCL biopsy specimens from 136 previously unreported patients (mean age, 60 years; age range, 14–96 years; male:female, 1.4:1). Classification followed World Health Organization criteria and is summarized in Table 1. All cases were evaluated for p63 protein expression by immunohistochemistry using the 4A4 clone as previously described,[5] defining positivity as nuclear staining in $\geqslant$30% of tumor cells. FISH using dual-fusion and/or breakapart *TP63* probes was performed as previously described[5] in all cases that were p63 positive by immunohistochemistry. The validity of using immunohistochemistry to select cases for FISH analysis is supported by our previous data, which showed that *TP63* fusion transcripts encoded the 4A4 epitope and that these immunohistochemical criteria identified all T-cell lymphomas with *TP63* rearrangements.[4,5] In the current study, we also performed *TP63* FISH in 21 additional p63-negative CTCLs and all lacked *TP63* rearrangements.

Immunohistochemistry for p63 was positive in 8 of 136 CTCLs tested (6%), including 5 cALCLs, 1 case of Sézary syndrome, and 2 cases of MF-LCT (Table 1). No positivity was seen in any other subtype, including MF without LCT. One case of MF-LCT had a *TP63* rearrangement, representing 1 of 14 (7%) of the cases of MF-LCT examined in this series. This case was a skin biopsy from the elbow of a 79-year-old female obtained in 2006 (Figure 1a). Morphologic examination revealed a dense dermal infiltrate of hyperchromatic tumor cells with $>$25% large cells, meeting criteria for MF-LCT (Figures 1b and c). The tumor cells showed strong nuclear positivity for p63 by immunohistochemistry (Figure 1d). FISH was positive in the majority of cells using both dual-fusion (Figure 1e) and breakapart probes (not shown), indicating the presence of *TBL1XR1/TP63* fusion corresponding to inv(3)(q26q28). Extra copies of the non-rearranged *TBL1XR1* and *TP63* genes also were present.

We then examined the relationship of the *TP63* rearrangement to the development of LCT. The patient first sought medical attention for her skin disease in September 2001. At that time she reported a 6-month history of pruritic scaly papules and plaques involving multiple anatomic sites. We obtained her original skin biopsy from 2001, which had been interpreted as suggestive of evolving MF. The biopsy showed clusters of atypical small lymphocytes, without prominent large cells (Figures 1f and g). Interestingly, many of the dermal lymphocytes were positive by p63 immunohistochemistry (Figure 1h), a finding absent in other cases of MF without LCT examined in the current series (Table 1). FISH demonstrated cells with both the *TP63* rearrangement and extra copies of non-rearranged *TBL1XR1* and *TP63*, identical to the subsequent MF-LCT specimen. These findings indicate that *TP63* rearrangement and copy number abnormalities involving 3q occurred before the LCT.

The patient's prior medical history was significant for stage IB, grade 2 endometrial adenocarcinoma in 1999 for which she underwent total abdominal hysterectomy/bilateral salpingo-oophorectomy with pelvic and para-aortic lymphadenectomy. No metastatic carcinoma was identified in the lymph nodes. Because the origins of early cells leading to MF are poorly understood, we reexamined the lymphadenectomy specimen, which showed preservation of the nodal architecture and patent sinuses containing small lymphocytes (Figures 1j and k). Immunohistochemical staining performed retrospectively demonstrated CD3-positive T cells in and around the sinuses, some of which had nuclear irregularities and were positive for p63 (Figures 1l and m). FISH identified the presence of *TBL1XR1/TP63* fusion and extra copies of both genes in these areas (Figure 1n) but showed a normal FISH signal pattern in background reactive cells and in the

Table 1. Frequency of p63 protein expression and *TP63* rearrangement in cutaneous T-cell lymphomas

Cutaneous T-cell lymphoma subtype[a]	*Number positive/number tested (%)*[b]					
	Present study		*Blood 2012*[5]		*Total*	
	p63 protein expression	*TP63 rearrangement*	*p63 protein expression*	*TP63 rearrangement*	*p63 protein expression*	*TP63 rearrangement*
Mycosis fungoides without large-cell transformation	0/48 (0)[c]	0/48 (0)[c]	0/5 (0)	0/5 (0)	0/53 (0)[c]	0/53 (0)[c]
Mycosis fungoides with large-cell transformation	2/14 (14)	1/14 (7)	1/2 (50)	0/2 (0)	3/16 (19)	1/16 (6)
Sézary syndrome	1/6 (17)	0/6 (0)	0/0 (0)	0/0 (0)	1/6 (17)	0/6 (0)
Primary cutaneous anaplastic large-cell lymphoma	5/22 (23)	0/22 (0)	7/19 (37)	2/19 (11)	12/41 (29)	2/41 (5)
Lymphomatoid papulosis	0/32 (0)	0/32 (0)	0/0 (0)	0/0 (0)	0/32 (0)	0/32 (0)
Primary cutaneous peripheral T-cell lymphoma, not otherwise specified	0/7 (0)	0/7 (0)	0/0 (0)	0/0 (0)	0/7 (0)	0/7 (0)
Subcutaneous panniculitis-like T-cell lymphoma	0/4 (0)	0/4 (0)	Not tested	0/1 (0)	0/4 (0)	0/5 (0)
Extranodal NK/T-cell lymphoma, nasal type	0/2 (0)	0/2 (0)	0/0 (0)	0/0 (0)	0/2 (0)	0/2 (0)
Primary cutaneous CD4-positive small/medium T-cell lymphoma	0/1 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/1 (0)	0/1 (0)
Total	8/136 (6)	1/136 (1)	8/26 (31)	2/27 (7)	16/162 (10)	3/163 (2)

[a]Nine cases of reactive dermatosis also were tested in the present study and all were negative. [b]Protein expression was defined by nuclear staining in ⩾ 30% of tumor cells by immunohistochemistry; *TP63* rearrangement was considered absent if protein expression was absent and/or negative by FISH, as supported by previous studies.[4,5] [c]Without including the single case (Figures 1f–i) identified retrospectively in a previous biopsy from a patient with mycosis fungoides with large-cell transformation that demonstrated both p63 protein expression and *TP63* rearrangement.

primary endometrial adenocarcinoma (not shown). These results indicate that T lymphocytes with *TP63* rearrangement were present in the patient's lymph nodes about 18 months before the clinical development of cutaneous lesions and 2 years before the diagnosis of MF, and strongly suggest that the rearrangement was acquired somatically. In the two previously reported cALCLs with *TP63* rearrangements, no tissue preceding diagnosis was available for testing. In one of the patients, multiple biopsies were tested and all bore the *TP63* rearrangement, including the diagnostic specimen. In the other patient, only a single biopsy was available for testing. Thus, based on the limited available data, *TP63* rearrangement appears to be a rare, early event in CTCL. A combined summary of findings from this and our previous study are shown in Table 1.

As part of the p53 family, p63 may have an important role in modulating p53 signaling pathways. p63 has two main classes of isoforms, TAp63 and ΔNp63, which differ in the structure of the N-terminal domain. TAp63 has an N-terminal transactivation domain with tumor suppressor activity, whereas ΔNp63 contains a truncated N-terminal domain that has been proposed to confer oncogenic properties.[6,7] *TP63* rearrangements encode fusion proteins homologous to ΔNp63, and although the function of p63 fusion proteins has not been reported, mechanisms implicated in the oncogenic properties of ΔNp63 include sequestration of transactivation domain-containing p53 family proteins, direct transcriptional regulatory activity, and modulation of stem cells.[8,9]

The clinical behavior of MF-LCT is more aggressive compared with that of MF without LCT. For example, one study reported a median survival of 37 months in MF-LCT compared with 163 months in MF.[10] Two previously reported cALCLs with *TP63* rearrangement[5] and the MF-LCT reported here demonstrated aggressive clinical behavior. The molecular mechanisms that lead to LCT are not completely understood, although LCT has been associated with tumor aneuploidy and immunophenotypic alterations.[11–13] In our case, the finding that *TP63* rearrangement preceded LCT raises the possibility that screening otherwise conventional MF by p63 immunohistochemistry followed by *TP63* FISH in positive cases might identify patients at increased risk of LCT. This would be in keeping with the prognostic utility of *TP63* rearrangements in other T-cell lymphomas. Specifically, we recently showed that although systemic ALK-negative ALCL had a 5-year overall survival (OS) rate of 52%, FISH identified a subgroup with *TP63* rearrangements and 5-year OS of only 17%.[4] However, given the rarity of *TP63* rearrangements in CTCLs, a larger study would be necessary to determine the predictive utility of this approach for cutaneous disease.

Of particular interest was the finding that *TP63* rearrangement was present in T cells before the clinical diagnosis of MF, raising the possibility that this rearrangement is an initiating event in T-cell lymphomagenesis. Analogously, *BCL2* rearrangements have been identified in cells obtained from healthy patients and higher frequency of these cells has been associated with significantly increased risk of developing follicular lymphoma.[14] Investigation of subsequent events that accompany development of MF and subsequent LCT in *TP63*-rearranged T cells may contribute substantially to understanding CTCL pathogenesis.

In summary, *TP63* rearrangements are rare, early events in CTCL and to date have been reported only in patients with aggressive clinical behavior. Further studies are merited to determine the role of clinical testing for p63 expression and/or *TP63* rearrangements in CTCL and to investigate strategies to target these rearrangements therapeutically.[15]

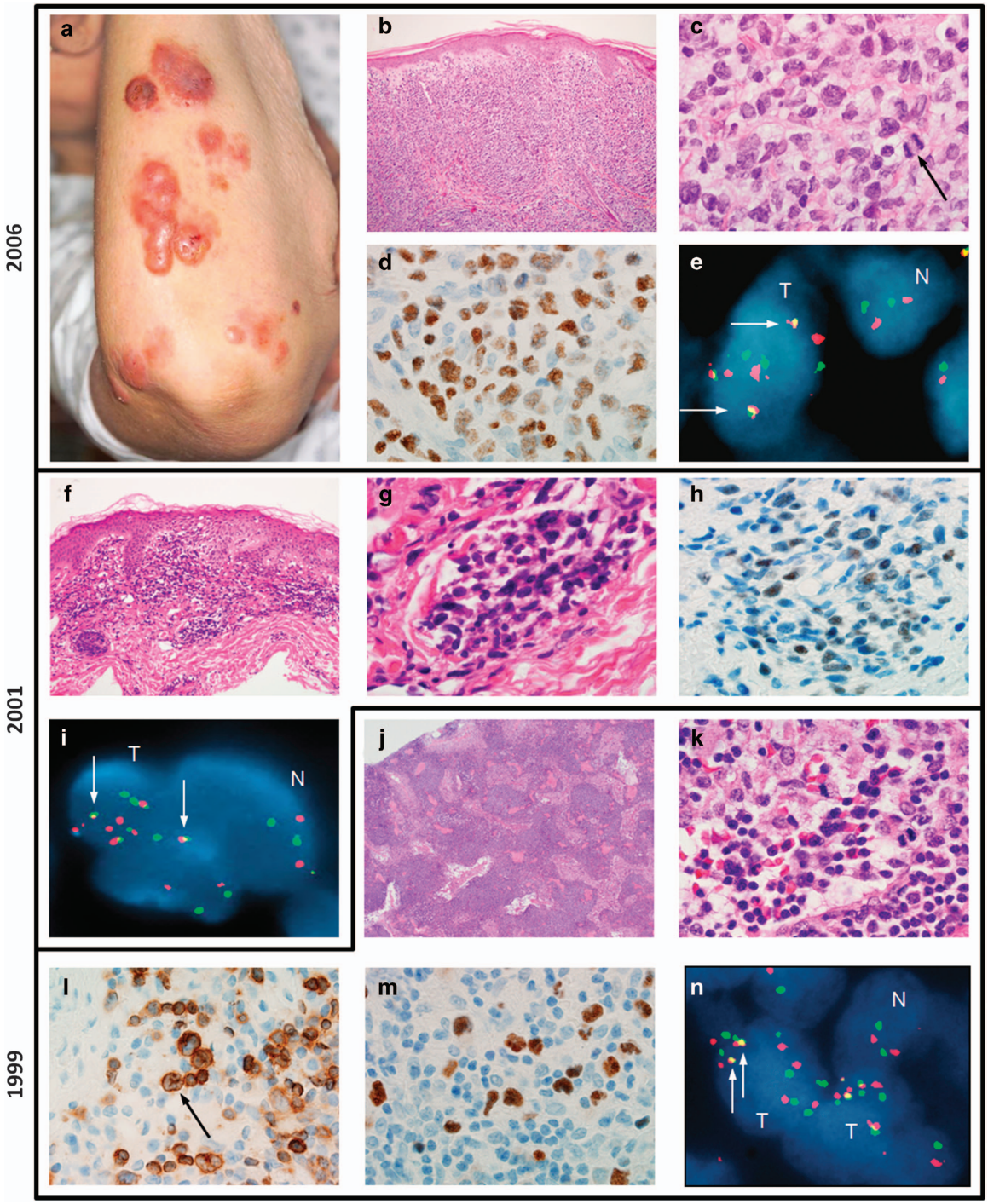

Figure 1. MF with *TP63* rearrangement. (**a**) Nodules and plaques on the upper extremity of a 79-year-old female with a history of MF. This photograph was taken in 2006, on the date of the biopsy included in the current series. (**b**) At low magnification (×100), a hematoxylin and eosin (H&E)-stained section of the biopsy showed an extensive, vaguely nodular lymphocytic infiltrate in the dermis. (**c**) At higher magnification (×1000), most of the cells were large, transformed lymphocytes, and mitotic figures were readily identified (arrow). These findings supported a diagnosis of MF with large-cell transformation. (**d**) Immunohistochemistry for p63 performed retrospectively as a part of the current study showed strong positivity in tumor cell nuclei. (**e**) Dual-fusion FISH demonstrated abnormal fusion signals (arrows), corresponding to *TBL1XR1/TP63* fusion in tumor cell nuclei (T; ×600). Extra non-rearranged copies of both *TBL1XR1* (green) and *TP63* (red) also were observed. FISH also demonstrated nuclei with a normal signal pattern (N), showing two non-rearranged copies each of *TBL1XR1* and *TP63*. (**f**) Review of a skin biopsy at the time of initial presentation in 2001 showed clusters of lymphocytes in the upper dermis with focal epidermal exocytosis (×200). (**g**) At higher magnification, the lymphocytes were mostly small and had irregular, hyperchromatic nuclei. (**h**) Scattered nuclei within the clusters of lymphocytes were positive for p63 by immunohistochemistry. (**i**) Dual-fusion FISH showed *TBL1XR1/TP63* fusion and extra non-rearranged copies of *TBL1XR1* and *TP63*, similar to the 2006 biopsy. (**j**) Retrospective review of pelvic lymph nodes obtained at the time of hysterectomy for endometrial carcinoma showed normal nodal architecture and expanded sinuses containing histiocytes and lymphocytes (×40). (**k**) At higher magnification, scattered medium-sized lymphocytes with irregular, hyperchromatic nuclei were observed. (**l**) Immunohistochemistry for CD3 performed retrospectively highlighted these atypical, sometimes cerebriform cells (arrow). (**m**) The atypical cells also were positive for p63. (**n**) Dual-fusion FISH showed *TBL1XR1/TP63* fusion and extra non-rearranged copies of *TBL1XR1* and *TP63*, similar to both subsequent biopsies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Supported by the Center for Individualized Medicine and the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; and by Award Numbers R01 CA177734 (ALF) and P50 CA97274 (University of Iowa/Mayo Clinic Lymphoma SPORE), National Cancer Institute. ALF is a Damon Runyon Clinical Investigator supported by the Damon Runyon Cancer Research Foundation (CI-48-09).

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