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Recurrent translocations involving the *IRF4* oncogene locus in peripheral T-cell lymphomas

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Abstract

Oncogenes involved in recurrent chromosomal translocations serve as diagnostic markers and therapeutic targets in hematopoietic tumors. In contrast to myeloid and B-cell neoplasms, translocations in peripheral T-cell lymphomas (PTCLs) are poorly understood. Here, we identified recurrent translocations involving the multiple myeloma oncogene-1/interferon regulatory factor-4 (*IRF4*) locus in PTCLs. *IRF4* translocations exist in myeloma and some B-cell lymphomas, but have not been reported previously in PTCLs. We studied 169 PTCLs using fluorescence *in situ* hybridization and identified 12 cases with *IRF4* translocations. Two cases with t(6;14)(p25;q11.2) had translocations between *IRF4* and the T-cell receptor-alpha (*TCRA*) locus. Both were cytotoxic PTCLs, unspecified (PTCL-Us) involving bone marrow and skin. Eight of the remaining ten cases were cutaneous ALCLs without *TCRA* rearrangements (57% of cutaneous ALCLs tested). These findings identified *IRF4* translocations as a novel recurrent genetic abnormality in PTCLs. Cytotoxic PTCL-Us involving bone marrow and skin and containing *IRF4/TCRA* translocations might represent a distinct clinicopathologic entity. Translocations involving *IRF4* but not *TCRA* appear to occur predominantly in cutaneous ALCLs. Detecting these translocations may be useful in lymphoma diagnosis. Further, due to its involvement in translocations, MUM1/IRF4 protein may play an important biologic role in some PTCLs, and might represent a possible therapeutic target.

Keywords

MUM1; IRF4; T-cell receptor-alpha; PTCL; ALCL; translocation; CD30; cutaneous lymphoma

Peripheral T-cell lymphomas (PTCLs) are malignant neoplasms of mature (peripheral) T lymphocytes, and represent approximately 10% of non-Hodgkin lymphomas.1 Despite treatment with conventional chemotherapy, the majority of patients with PTCLs die of their disease.2 New, targeted therapies might be aimed at genes involved in recurrent chromosomal translocations,3-5 but, in contrast to myeloid neoplasms and B-cell lymphomas,1 translocations in most PTCLs remain poorly understood. Identifying novel

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recurrent translocations in PTCLs also may help define specific disease entities and serve as the basis for clinical diagnostic testing, as they have in the case of chromosomal translocations involving *ALK* in ALK-positive anaplastic large-cell lymphomas (ALCLs).6 However, ALK-positive ALCLs represent only about 6% of PTCLs.2

In this report, we demonstrate for the first time the presence of translocations involving the multiple myeloma oncogene-1/interferon regulatory factor-4 (*IRF4*) locus on 6p25 in PTCLs. Based on the observation of a PTCL, unspecified (PTCL-U), with a karyotype including t(6;14)(p25;q11.2), we studied 169 PTCLs and found 12 with *IRF4* translocations (including the index case). Two had t(6;14)(p25;q11.2) translocations that fused the *IRF4* and T-cell receptor-alpha (*TCRA*) gene loci. Both were cytotoxic PTCLUs that presented in the bone marrow and rapidly developed skin lesions. Such tumors might represent a distinct clinicopathologic entity.

Eight cutaneous ALCLs (C-ALCLs) had translocations involving *IRF4* but not *TCRA* (57% of C-ALCLs tested). One systemic ALK-negative ALCL and an additional PTCL-U showed translocations involving *IRF4* but not *TCRA*. *IRF4* translocations were not detected in ALK-positive ALCLs. C-ALCL is an indolent disease that currently lacks specific pathologic features to distinguish it from skin involvement by systemic ALK-negative ALCL,7,8 which is aggressive and often fatal.1,2 Testing for *IRF4* translocations may have clinical diagnostic utility in C-ALCL. While molecular pathways associated with *ALK* translocations in ALK-positive ALCLs have been studied extensively,9 corresponding pathways in ALCLs lacking *ALK* translocations are poorly understood. MUM1/IRF4 protein could play a biologic role in PTCLs with *IRF4* translocations, since *IRF4* is oncogenic *in vitro*.10 Utilization of MUM1/IRF4 as a therapeutic target in patients with PTCL merits exploration.

Materials and methods

Cases

We studied specimens from 169 patients with PTCL diagnosed by WHO criteria.1 There were 104 males and 65 females (M:F ratio, 1.6:1), with a mean age of 58 years (range, 5−92 years). Cases included 23 angioimmunoblastic T-cell lymphomas (AITLs, 13%), 72 PTCL-Us (43%), 18 ALK-positive ALCLs (11%), 24 ALK-negative ALCLs (14%), 14 C-ALCLs (8%), and 18 other PTCLs (Table 1). The study was approved by the Institutional Review Board and the Biospecimens Committee of Mayo Clinic.

Fluorescence *In Situ* Hybridization (FISH)

IRF4 and *TCRA* FISH probes were developed as previously described.11-13 BAC clones (Table S1) were identified using the University of California Santa Cruz Genome Browser (http://www.genome.UCSC.edu) and ordered from ResGen™ Invitrogen (Carlsbad, CA). Since telomeric *IRF4* clones showed minimal cross-hybridization to 16p11, positive cases were confirmed using a second breakapart *IRF4* probe (probe #2, Table S1). BAC DNA was isolated using the Qiagen (Valencia, CA) Plasmid Maxi Kit and fluorescently labeled using SpectrumOrange-dUTP or SpectrumGreen-dUTP and the Abbott Molecular (Des Plaines, IL) Nick Translation Kit. Centromeric and telomeric BAC DNA was labeled with different

fluorophores for breakapart probes, and with the same fluorophore for dual-fusion (D-FISH) probes. Probe validation was conducted based on previously described 'familiarization' procedures.14 Specificity of hybridization was confirmed on metaphases from a splenic marginal zone lymphoma with *IRF4/IGH* fusion,13 a PTCL with a *TCRA* translocation,11 and normal samples. *TCRB* and *TCRG* probes were purchased from Dako (Carpinteria, CA). The upper limit of the normal range for each probe was determined using a 95% confidence interval as previously described.11,14 Upper limits of normal for *IRF4*, *TCRA*, *TCRB*, and *TCRG* were 6%, 9%, 5%, and 6%, respectively.

Paraffin tissue microarrays (TMAs) were constructed as described.3,11,12 In cases with insufficient tissue, whole-tissue sections were analyzed. B5- and formalin-fixed cases were included, since we have shown similar FISH results with both fixatives.15,16 FISH was performed as previously described.11 Sections were sequentially immersed in Citrisolve, Lugol solution, and sodium thiocyanate. Slides were microwaved for 5 minutes in citrate buffer, then digested in 0.4% pepsin solution at 37°C. Ten microliters of FISH reagent (7 μL LSI buffer and 3 μL probe) were placed on each slide and slides were cover-slipped, denatured, and incubated in a humidified chamber at 37°C for 12 hours. Slides were washed, counterstained with 4',6-diamidino-2-phenylindole dihydrochloride, and analyzed by a microscopist (ML) using a fluorescent microscope with appropriate filter sets. A minimum of 50 cells and a maximum of 200 cells were scored per case. A minimum of 20 abnormal cells were required for a sample to be considered abnormal. Some cases were non-informative due to hybridization failures. Positive cases detected on TMAs were confirmed on whole tissue sections. Scoring for *IRF4* in 4 translocated cases with areas showing confluent sheets of tumor cells areas revealed a mean of 71% positive cells (range, 55% −93%).

Immunohistochemistry (IHC)

Five-micron paraffin whole-tissue sections were immunostained using previously described techniques17 and antibodies18 to assist in disease classification. For MUM1/IRF4 immunostaining, whole-tissue or TMA sections were pretreated in 1 mM EDTA buffer at pH 8.0 for 30 min at 98°C (PT Module, Lab Vision, Fremont, CA), then stained for MUM1/IRF4 using a monoclonal mouse anti-human antibody (MUM1p, 1:50; Dako). Detection was with Dual Link Envision+/DAB+ (Dako). Scoring was performed in correlation with H&E and appropriate immunostains (e.g. CD20 and CD3). MUM1/IRF4 was considered positive when >30% of tumor cells demonstrated nuclear staining. Technical factors precluded scoring in rare cases. Diagnosis of C-ALCL required CD30 positivity in >75% of tumor cells, per WHO criteria.1 This cutoff also was used to define CD30 positivity in cases of PTCL-U.

Conventional Cytogenetics

Results of karyotype analysis prepared at the time of biopsy using previously described methods19 were reviewed retrospectively when available.

Results and discussion

We identified 12 PTCLs with *IRF4* translocations among 155 PTCLs with informative FISH results (8%; Tables 1,2). These included 3/64 PTCL-Us (5%), 1/23 ALK-negative ALCLs (4%), and 8/14 C-ALCLs (57%). *IRF4* translocations were not seen in ALK-positive ALCLs, AITLs, or other PTCL subtypes. MUM1/IRF4 protein was detected in the majority of ALCLs, regardless of type (Table 1). MUM1/IRF4 was positive in 72% of CD30-positive PTCL-Us and 13% of CD30-negative PTCL-Us, and was negative in other PTCL subtypes. All cases with *IRF4* translocations were positive for MUM1/IRF4 by IHC.

Two PTCL-Us with *IRF4* translocations by FISH had karyotypes with t(6;14)(p25;q11.2) (Cases 1 and 2, Table 2). Karyotypes were not performed in the other 10 PTCLs with *IRF4* translocations. Karyotypes of 35 PTCLs without *IRF4* translocations by FISH and 2 PTCLs in which *IRF4* FISH failed showed no anomalies of 6p25 (Table S2). The PTCL-Us with t(6;14)(p25;q11.2) had similar clinicopathologic features. Both presented in older adult males with mild cytopenias and without significant lymphadenopathy or hepatosplenomegaly. In both patients, imaging showed diffuse skeletal uptake and renal mass lesions (not biopsied). No tumor cells were seen in the peripheral blood. Both patients had extensive bone marrow infiltration by tumor with admixed plasma cells and reticulin fibrosis (Fig. 1 a,g). The cells were larger and more pleomorphic in Case 1 (Fig. 1 b,h). Both tumors were positive for CD3, beta-F1, TIA1, and MUM1/IRF4 (Fig. 1 c,d,e,i). They were negative for CD5, CD30, CD25, FoxP3 and EBV. Case 1 was positive for granzyme B and CD4, and partially positive for CD8. In both cases, the t(6;14)(p25;q11.2) corresponded to *IRF4/TCRA* fusion by D-FISH (Fig. 1 j,k). The patient in Case 1 received chemotherapy (Table 2) but developed progressive skin lesions and probable cerebrospinal fluid involvement. Despite additional therapy his skin lesions progressed (Fig. 1 f); he is alive with disease 4 months after presentation. The patient in Case 2 developed skin and soft tissue lesions during the course of evaluation (not biopsied). Chemotherapy was recommended but follow-up data are not available. We classified these cases as PTCL-Us. Adult T-cell leukemia/lymphoma (ATLL) can present with bone marrow and skin involvement, but ATLLs typically involve peripheral blood, are non-cytotoxic, and express CD25 and/or FoxP3.1,20 Serology for human T-cell leukemia virus type 1 (HTLV1) was not performed, but both patients were from a non-endemic region (Midwestern United States).

IRF4 translocations were detected in 8/14 C-ALCLs tested (57%). All initial diagnostic biopsies were reviewed. Clinical or pathologic features of lymphomatoid papulosis were not observed. None of the patients had a history of mycosis fungoides (MF) or dermatitis suggestive of clinical MF. Four patients developed nodal disease 1 to 126 months after diagnosis (Table 2). The patient with a 1-month interval between cutaneous and nodal disease (Case 7) had multiple skin nodules and local adenopathy. Staging was otherwise negative, suggesting C-ALCL with locoregional spread; however, it is possible the disease originated in the lymph node. Cases 3 and 8 showed different histology in cutaneous and nodal specimens. The skin showed mostly medium-sized tumor cells with admixed histiocytes in the background (Fig. 2 a), and occasional perivascular “hallmark” cells, reminiscent of the small-cell variant of systemic ALCL.21 The subsequent lymph node

biopsies showed sheets of large “hallmark” cells (Fig. 2 b). Histologic progression associated with nodal involvement also was seen in a C-ALCL without *IRF4* translocation (not shown). The remaining C-ALCLs with *IRF4* translocations had typical histologic features (Fig. 2 c-e). CD30 and MUM1/IRF4 were positive (Fig. 2 f,g). FISH showed *IRF4* translocations (Fig. 2 h); *TCRA, TCRB,* and *TCRG* were negative (not shown). C-ALCLs with and without *IRF4* translocations showed similar clinicopathologic features (Table 3). TIA1 positivity was somewhat less common in *IRF4*-translocated cases than in untranslocated cases and in C-ALCLs in the literature.22-24 Only one untranslocated case developed nodal disease.

We identified two additional PTCLs with translocations of *IRF4* but not *TCRA*. Case 11 was a PTCL-U in the pleura of a 73 year-old female who also had multiple lung nodules. The tumor cells were medium to large and pleomorphic. “Hallmark” cells were not seen. Staging was negative. Case 12 was an ALK-negative ALCL in a 79 year-old male with generalized lymphadenopathy but without cutaneous disease. Lymph node biopsy showed sheets of “hallmark” cells (fig. 2 i). Both cases were positive for CD30 and MUM1/IRF4 and were non-cytotoxic. FISH for *TCRB* and *TCRG* was negative in Case 12; hybridization failed in Case 11. Follow-up is not available.

C-ALCL is an indolent, cutaneous disease with an excellent prognosis,2,25,26 while systemic ALK-negative ALCLs have a poor prognosis and ALK-positive ALCLs have an intermediate prognosis.25,27 Comparative genomic hybridization and gene expression profiling show molecular differences among these three ALCL subtypes,28-30 but have not established the biologic basis for the disparate clinical behaviors. Unlike ALK-positive ALCLs,1,6,9 recurrent translocations have not been reported previously in C-ALCLs or systemic ALK-negative ALCLs. The finding of *IRF4* translocations in a subset of these cases (but not in ALK-positive ALCLs) may provide new clues to understanding the biology of these diseases.

MUM1/IRF4 is a transcription factor expressed in normal plasma cells, some B cells, and activated T cells.31 *IRF4* is translocated in some multiple myelomas and B-cell lymphomas and causes MUM1/IRF4 overexpression.10,13,32-34 Rearrangements involving 6p have been reported in T-cell neoplasms,35-37 but the involved gene(s) were not identified and *IRF4* translocations have not been previously reported. Translocations involving *TCR* loci in T-cell neoplasms upregulate oncogene expression on the partner chromosome, but are uncommon in PTCLs other than T-cell prolymphocytic leukemia.11,38-40 As in previous series,31,41-43 we found that MUM1/IRF4 expression in PTCLs tended to parallel CD30 expression. The PTCL-Us with *IRF4/TCRA* were the only CD30-negative PTCLs with *IRF4* translocations; thus, the MUM1/IRF4 expression in these PTCL-Us likely resulted from the translocations (rather than CD30-associated activation pathways). This study does not address whether *IRF4/TCRA* is lymphomagenic, but the marked similarity between the two cases with *IRF4/TCRA* suggest the translocation may have biologic significance and that such cases might represent a distinct clinicopathologic entity.

Since most CD30-positive PTCLs express MUM1/IRF4 protein regardless of *IRF4* translocation status, the biologic significance of *IRF4* translocations not involving *TCRA* in

CD30-positive PTCLs is unclear, and merits further study. *IRF4*-translocated cases might have higher MUM1/IRF4 expression than non-translocated cases, but limitations of IHC precluded quantitative comparison. Alternatively, the *IRF4* breakpoint might lead to altered MUM1/IRF4 protein function, or might affect expression of another gene near *IRF4*. Finally, the biologic consequences of *IRF4* translocations might result from altered expression of the non-*TCRA* partner gene(s). Among B-cell lymphomas, for example, high expression levels of known *BCL6* partner genes have been reported in cases with *BCL6* translocations involving non-*IgH* partners.44

Testing for *IRF4* translocations may be useful in the diagnosis of C-ALCL, which can be morphologically and immunophenotypically indistinguishable from systemic ALK-negative ALCL with secondary skin involvement.1,7,8 Since rare PTCL-Us and systemic ALK-negative ALCLs showed *IRF4* translocations, the specificity of *IRF4* translocations for C-ALCL in skin biopsies requires further study; identifying the *IRF4* partner gene(s) may be helpful in this regard. Lymphomatoid papulosis and transformed MF also should be evaluated for *IRF4* translocations, since these disorders express CD30 and MUM1/IRF443,45 but were not included in the present study. Finally, *IRF4* translocations might be prognostic in C-ALCL, since lymph node involvement was more common in translocated C-ALCLs than in untranslocated C-ALCLs or in previous series.26

Because *IRF4* overexpression is oncogenic *in vitro*,10 and because most PTCLs lack good treatment options, MUM1/IRF4 might represent a therapeutic target in patients with PTCL. MUM1/IRF4 expression in PTCLs recently was linked to expression of PRDM1, another regulatory protein expressed in some PTCLs.46 PTCL cell lines treated *in vitro* with the proteasome inhibitor bortezomib down-regulated MUM1/IRF4, an effect dependent on NF-κB inhibition and associated with PRDM1 down-regulation. Bortezomib is effective in the treatment of myelomas,47 which express MUM1/IRF4, and occasional clinical responses have been reported in PTCL patients.48,49 Further studies should examine whether MUM1/IRF4 positivity, and particularly the presence of *IRF4* translocations, might select PTCLs likely to respond to proteasome inhibition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Jaffe, ES.; Harris, NL.; Stein, H.; Vardiman, J. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press; Lyon, France: 2001.

2. Savage KJ, Chhanabhai M, Gascoyne RD, Connors JM. Characterization of peripheral T-cell lymphomas in a single North American institution by the WHO classification. Ann Oncol. 2004; 15:1467–1475. [PubMed: 15367405]

3. Feldman AL, Sun DX, Law ME, Novak AJ, Attygalle AD, Thorland EC, et al. Overexpression of Syk tyrosine kinase in peripheral T-cell lymphomas. Leukemia. 2008; 22:1139–1143. [PubMed: 18401419]

4. Streubel B, Vinatzer U, Willheim M, Raderer M, Chott A. Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. Leukemia. 2006; 20:313–318. [PubMed: 16341044]

5. Li R, Morris SW. Development of anaplastic lymphoma kinase (ALK) small-molecule inhibitors for cancer therapy. Med Res Rev. 2008; 28:372–412. [PubMed: 17694547]

6. Jaffe ES. Anaplastic large cell lymphoma: the shifting sands of diagnostic hematopathology. Mod Pathol. 2001; 14:219–228. [PubMed: 11266530]

7. Lae ME, Ahmed I, Macon WR. Clusterin is widely expressed in systemic anaplastic large cell lymphoma but fails to differentiate primary from secondary cutaneous anaplastic large cell lymphoma. Am J Clin Pathol. 2002; 118:773–779. [PubMed: 12428799]

8. Droc C, Cualing HD, Kadin ME. Need for an improved molecular/genetic classification for CD30+ lymphomas involving the skin. Cancer Control. 2007; 14:124–132. [PubMed: 17387297]

9. Amin HM, Lai R. Pathobiology of ALK+ anaplastic large-cell lymphoma. Blood. 2007; 110:2259–2267. [PubMed: 17519389]

10. Iida S, Rao PH, Butler M, Corradini P, Boccadoro M, Klein B, et al. Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. Nat Genet. 1997; 17:226–230. [PubMed: 9326949]

11. Feldman AL, Law M, Grogg KL, Thorland EC, Fink S, Kurtin PJ, et al. Incidence of TCR and TCL1 Gene Translocations and Isochromosome 7q in Peripheral T-Cell Lymphomas Using Fluorescence In Situ Hybridization. Am J Clin Pathol. 2008; 130:178–185. [PubMed: 18628085]

12. Remstein ED, Dogan A, Einerson RR, Paternoster SF, Fink SR, Law M, et al. The incidence and anatomic site specificity of chromosomal translocations in primary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in North America. Am J Surg Pathol. 2006; 30:1546–1553. [PubMed: 17122510]

13. Remstein ED, Law M, Mollejo M, Piris MA, Kurtin PJ, Dogan A. The prevalence of IG translocations and 7q32 deletions in splenic marginal zone lymphoma. Leukemia. 2008; 22:1268–1272. [PubMed: 17989713]

14. Wiktor AE, Van Dyke DL, Stupca PJ, Ketterling RP, Thorland EC, Shearer BM, et al. Preclinical validation of fluorescence in situ hybridization assays for clinical practice. Genet Med. 2006; 8:16–23. [PubMed: 16418595]

15. Paternoster SF, Brockman SR, McClure RF, Remstein ED, Kurtin PJ, Dewald GW. A new method to extract nuclei from paraffin-embedded tissue to study lymphomas using interphase fluorescence in situ hybridization. Am J Pathol. 2002; 160:1967–1972. [PubMed: 12057901]

16. Einerson RR, Kurtin PJ, Dayharsh GA, Kimlinger TK, Remstein ED. FISH is superior to PCR in detecting t(14;18)(q32;q21)-IgH/bcl-2 in follicular lymphoma using paraffin-embedded tissue samples. Am J Clin Pathol. 2005; 124:421–429. [PubMed: 16191511]

17. Kurtin PJ, Hobday KS, Ziesmer S, Caron BL. Demonstration of distinct antigenic profiles of small B-cell lymphomas by paraffin section immunohistochemistry. Am J Clin Pathol. 1999; 112:319–329. [PubMed: 10478136]

18. Roden AC, Macon WR, Keeney GL, Myers JL, Feldman AL, Dogan A. Seroma-associated primary anaplastic large-cell lymphoma adjacent to breast implants: an indolent T-cell lymphoproliferative disorder. Mod Pathol. 2008; 21:455–463. [PubMed: 18223553]

19. Barch, M.; Knutsen, T.; Spubeck, J., editors. The AGT Cytogenetics Laboratory Manual. Lippincott-Raven; Philadelphia, PA: 1997.

20. Roncador G, Garcia JF, Maestre L, Lucas E, Menarguez J, Ohshima K, et al. FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. Leukemia. 2005; 19:2247–2253. [PubMed: 16193085]

21. Benharroch D, Meguerian-Bedoyan Z, Lamant L, Amin C, Brugieres L, Terrier-Lacombe MJ, et al. ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood. 1998; 91:2076–2084. [PubMed: 9490693]
22. Boulland ML, Wechsler J, Bagot M, Pulford K, Kanavaros P, Gaulard P. Primary CD30-positive cutaneous T-cell lymphomas and lymphomatoid papulosis frequently express cytotoxic proteins. Histopathology. 2000; 36:136–144. [PubMed: 10672058]
23. Kummer JA, Vermeer MH, Dukers D, Meijer CJ, Willemze R. Most primary cutaneous CD30-positive lymphoproliferative disorders have a CD4-positive cytotoxic T-cell phenotype. J Invest Dermatol. 1997; 109:636–640. [PubMed: 9347791]
24. Felgar RE, Salhany KE, Macon WR, Pietra GG, Kinney MC. The expression of TIA-1+ cytolytic-type granules and other cytolytic lymphocyte-associated markers in CD30+ anaplastic large cell lymphomas (ALCL): correlation with morphology, immunophenotype, ultrastructure, and clinical features. Hum Pathol. 1999; 30:228–236. [PubMed: 10029454]
25. Savage KJ, Harris NL, Vose JM, Ullrich F, Jaffe ES, Connors JM, et al. ALK-anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008; 111:5496–5504. [PubMed: 18385450]
26. Bekkenk MW, Geelen FA, van Voorst Vader PC, Heule F, Geerts ML, van Vloten WA, et al. Primary and secondary cutaneous CD30(+) lymphoproliferative disorders: a report from the Dutch Cutaneous Lymphoma Group on the long-term follow-up data of 219 patients and guidelines for diagnosis and treatment. Blood. 2000; 95:3653–3661. [PubMed: 10845893]
27. ten Berge RL, de Bruin PC, Oudejans JJ, Ossenkoppele GJ, van der Valk P, Meijer CJ. ALK-negative anaplastic large-cell lymphoma demonstrates similar poor prognosis to peripheral T-cell lymphoma, unspecified. Histopathology. 2003; 43:462–469. [PubMed: 14636272]
28. Salaverria I, Bea S, Lopez-Guillermo A, Lespinet V, Pinyol M, Burkhardt B, et al. Genomic profiling reveals different genetic aberrations in systemic ALK-positive and ALK-negative anaplastic large cell lymphomas. Br J Haematol. 2008; 140:516–526. [PubMed: 18275429]
29. Mao X, Orchard G, Lillington DM, Russell-Jones R, Young BD, Whittaker S. Genetic alterations in primary cutaneous CD30+ anaplastic large cell lymphoma. Genes Chromosomes Cancer. 2003; 37:176–185. [PubMed: 12696066]
30. Thompson MA, Stumph J, Henrickson SE, Rosenwald A, Wang Q, Olson S, et al. Differential gene expression in anaplastic lymphoma kinase-positive and anaplastic lymphoma kinase-negative anaplastic large cell lymphomas. Hum Pathol. 2005; 36:494–504. [PubMed: 15948116]
31. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, Gambacorta M, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood. 2000; 95:2084–2092. [PubMed: 10706878]
32. Chesi M, Kuehl WM, Bergsagel PL. Recurrent immunoglobulin gene translocations identify distinct molecular subtypes of myeloma. Ann Oncol. 2000; 11(Suppl 1):131–135. [PubMed: 10707795]
33. Tamura A, Miura I, Iida S, Yokota S, Horiike S, Nishida K, et al. Interphase detection of immunoglobulin heavy chain gene translocations with specific oncogene loci in 173 patients with B-cell lymphoma. Cancer Genet Cytogenet. 2001; 129:1–9. [PubMed: 11520558]
34. Michaux L, Wlodarska I, Rack K, Stul M, Criel A, Maerevoet M, et al. Translocation t(1;6)(p35.3;p25.2): a new recurrent aberration in ”unmutated” B-CLL. Leukemia. 2005; 19:77–82. [PubMed: 15510210]
35. Mecucci C, Michaux JL, Tricot G, Louwagie A, van den Berghe H. Rearrangements of the short arm of chromosome No. 6 in T-cell lymphomas. Leuk Res. 1985; 9:1139–1148. [PubMed: 3877852]
36. Ott G, Katzenberger T, Siebert R, DeCoteau JF, Fletcher JA, Knoll JH, et al. Chromosomal abnormalities in nodal and extranodal CD30+ anaplastic large cell lymphomas: infrequent detection of the t(2;5) in extranodal lymphomas. Genes Chromosomes Cancer. 1998; 22:114–121. [PubMed: 9598798]
37. Mitelman F, Johansson B, Mertens F. Mitelman Database of Chromosome Aberrations in Cancer. 2007http://cgap.nci.nih.gov/Chromosomes/Mitelman

38. Pekarsky Y, Hallas C, Isobe M, Russo G, Croce CM. Abnormalities at 14q32.1 in T cell malignancies involve two oncogenes. Proc Natl Acad Sci U S A. 1999; 96:2949–2951. [PubMed: 10077617]

39. Almire C, Bertrand P, Ruminy P, Maingonnat C, Wlodarska I, Martin-Subero JI, et al. PVRL2 is translocated to the TRA@ locus in t(14;19)(q11;q13)-positive peripheral T-cell lymphomas. Genes Chromosomes Cancer. 2007; 46:1011–1018. [PubMed: 17696193]

40. Leich E, Haralambieva E, Zettl A, Chott A, Rudiger T, Holler S, et al. Tissue microarray-based screening for chromosomal breakpoints affecting the T-cell receptor gene loci in mature T-cell lymphomas. J Pathol. 2007; 213:99–105. [PubMed: 17582237]

41. Tsuboi K, Iida S, Inagaki H, Kato M, Hayami Y, Hanamura I, et al. MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. Leukemia. 2000; 14:449–456. [PubMed: 10720141]

42. Natkunam Y, Warnke RA, Montgomery K, Falini B, van De Rijn M. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. Mod Pathol. 2001; 14:686–694. [PubMed: 11455001]

43. Wasco MJ, Fullen D, Su L, Ma L. The expression of MUM1 in cutaneous T-cell lymphoproliferative disorders. Hum Pathol. 2008; 39:557–563. [PubMed: 18234282]

44. Iqbal J, Greiner TC, Patel K, Dave BJ, Smith L, Ji J, et al. Distinctive patterns of BCL6 molecular alterations and their functional consequences in different subgroups of diffuse large B-cell lymphoma. Leukemia. 2007; 21:2332–2343. [PubMed: 17625604]

45. Kempf W, Kutzner H, Cozzio A, Sander CA, Pfaltz MC, Muller B, et al. MUM1 expression in cutaneous CD30+ lymphoproliferative disorders: a valuable tool for the distinction between lymphomatoid papulosis and primary cutaneous anaplastic large-cell lymphoma. Br J Dermatol. 2008; 158:1280–1287. [PubMed: 18410414]

46. Zhao WL, Liu YY, Zhang QL, Wang L, Leboeuf C, Zhang YW, et al. PRDM1 is involved in chemoresistance of T-cell lymphoma and down-regulated by the proteasome inhibitor. Blood. 2008; 111:3867–3871. [PubMed: 18235046]

47. Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008; 111:2516–2520. [PubMed: 17975015]

48. Zinzani PL, Musuraca G, Tani M, Stefoni V, Marchi E, Fina M, et al. Phase II trial of proteasome inhibitor bortezomib in patients with relapsed or refractory cutaneous T-cell lymphoma. J Clin Oncol. 2007; 25:4293–4297. [PubMed: 17709797]

49. Orlowski RZ, Voorhees PM, Garcia RA, Hall MD, Kudrik FJ, Allred T, et al. Phase 1 trial of the proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with advanced hematologic malignancies. Blood. 2005; 105:3058–3065. [PubMed: 15626743]

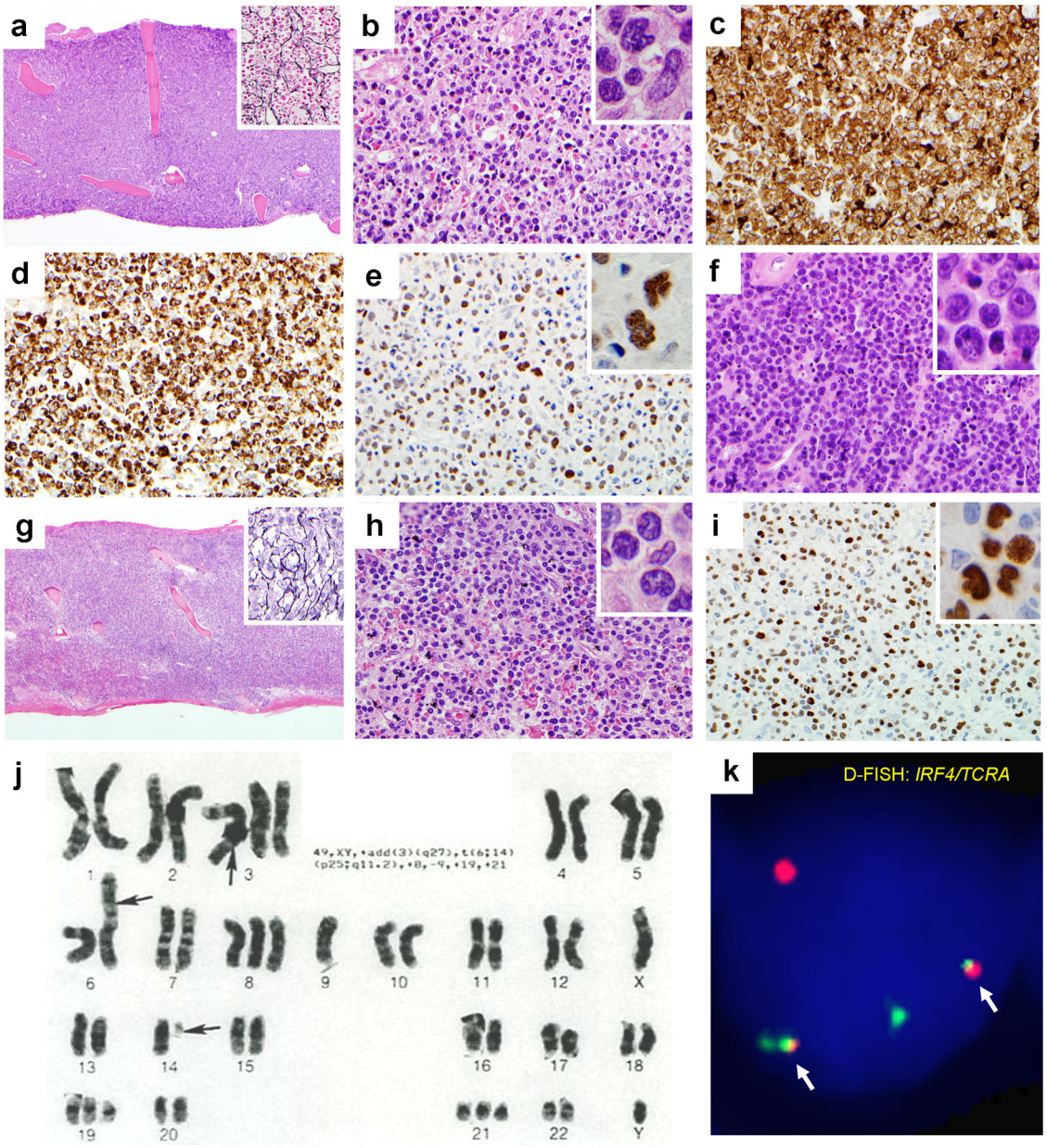

Figure 1.

Peripheral T-cell lymphomas, unspecified (PTCL-Us) with *IRF4/TCRA* translocations involving bone marrow and skin. (**a**) Diffuse infiltration of bone marrow (75% involvement) in a 67 year-old male (Case 1; H&E, x 4); reticulin fibrosis is present (inset, x 40). (**b**) Medium to large tumor cells with plasma cells in the background (H&E, x 40; inset, x 100). Tumor cells are positive for (**c**) CD3, (**d**) TIA1, (**e**) and MUM1/IRF4 (x 40; inset, x 100). (**f**) Skin biopsy from the same patient 4 mos later. Compared to the bone marrow, the tumor cells are more uniformly large and pleomorphic, and are accompanied by apoptotic debris without admixed plasma cells (H&E, x 40; inset, x 100). (**g**) Diffuse infiltration of bone marrow (40% involvement) in a 71 year-old male (Case 2; H&E, x 4; reticulin, inset, x 40). (**h**) Mostly medium-sized tumor cells with plasma cells in the background (H&E, x 40;

inset, x 100). (**i**) Tumor cells are positive for MUM1/IRF4 (x 40; inset, x 100). (**j**) Karyotype shows t(6;14)(p25;q11.2). (**k**) Dual-fusion fluorescence *in situ* hybridization (D-FISH) shows *IRF4/TCRA* fusion signals (arrows).

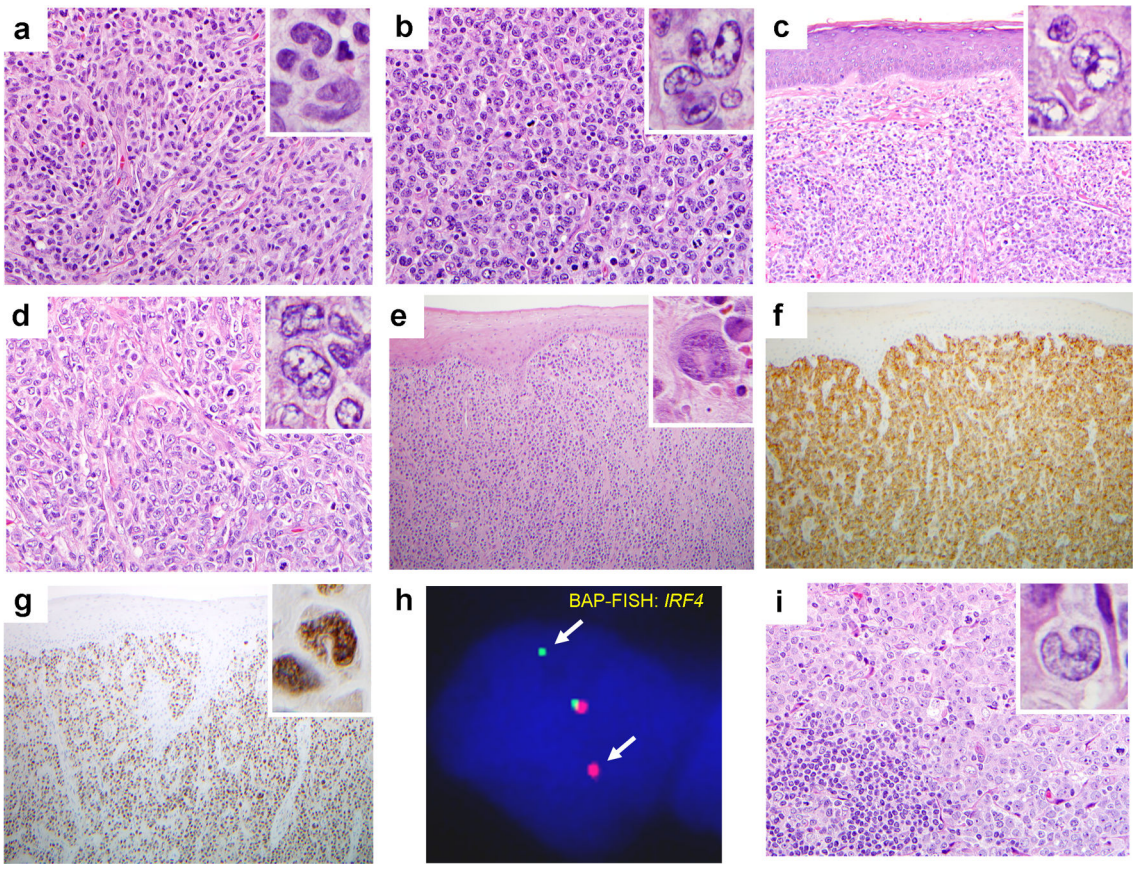

Figure 2.

Anaplastic large-cell lymphomas (ALCLs) with *IRF4* translocations. (**a**) Primary cutaneous ALCL (C-ALCL), 48 year-old female (case 3). Medium to large tumor cells with admixed histiocytes (H&E, x 40; inset, x 100). (**b**) Lymph node involvement, same patient, 10 years later. Confluent sheets of large "hallmark" cells (H&E, x 40; inset, x 100). (**c**) C-ALCL, 67 year-old male (case 4; H&E, x 20; inset, x 100). (**d**) Lymph node involvement, same patient, 7 mos later (H&E, x 40; inset, x 100). Both biopsies show sheets of "hallmark" cells. (**e**) C-ALCL, 89 year-old female (case 5), showing positivity for (**f**) CD30 and (**g**) MUM1/IRF4 (x 10; insets, x 100). (**h**) Breakapart fluorescence *in situ* hybridization (BAP-FISH) shows separation of red and green signals flanking the *IRF4* gene locus (arrows). (**i**) Systemic ALK-negative ALCL, cervical lymph node, 79 year-old male (case 12). Large "hallmark" cells surround a residual reactive follicle (lower left; H&E, x 40; inset, x 100).

Table 1

IRF4 Translocations and MUM1/IRF4 Protein Expression in Peripheral T-cell Lymphomas

	FISH		Immunohistochemistry	
Diagnosis	**# pos.***	**%**	**# pos.***	**%**
Angioimmunoblastic T-cell lymphoma	0/19	0	0/23	0
PTCL, unspecified	3/64	5	20/72	28
(CD30-positive)	*(1/17)*	*(6)*	*(13/18)*	*(72)*
(CD30-negative)	*(2/47)*	*(4)*	*(7/54)*	*(13)*
Anaplastic large cell lymphoma, ALK-positive	0/18	0	16/17	94
Anaplastic large cell lymphoma, ALK-negative	1/23	4	20/22	91
Cutaneous anaplastic large cell lymphoma	8/14	57	13/14	93
T-cell large granular lymphocyte leukemia	0/4	0	0/4	0
Hepatosplenic T-cell lymphoma	0/3	0	0/3	0
Subcutaneous panniculitis-like T-cell lymphoma	-	-	0/1	0
Enteropathy-associated T-cell lymphoma	0/2	0	0/2	0
Extranodal NK/T-cell lymphoma, nasal type	0/8	0	0/8	0
Total	**12/155**	**8**	**69/166**	**42**

* Includes informative cases only, of 169 total cases tested.

Table 2

Peripheral T-cell lymphomas with *IRF4* Translocations

Case	Age/Sex	Diagnosis	Site	Time from diagnosis (mos)	Cytotoxic phenotype*	*IRF4* FISH	*IRF4/TCRA* Fusion	Karyotype	Treatment	Follow-up
1	67/M	PTCL-U	BM	-	YES	-	-	48−49,XY,+3[5],+5,der(6)t(6;14)(p25;q11.2), add(7)(p11.2),−14,+16,+16[5],−20,−22[4], +1−2mar[cp6]/95−98,idemx2,+3−8mar[2]	CHOP, ICE, ITMTX	alive, progressive cutaneous disease, 4 mos
			skin	2	YES	-	-	-		
			skin	4	-	POS	POS	-		
2	71/M	PTCL-U	BM	-	YES	POS	POS	49,XY,+add(3)(q27),t(6;14)(p25;q11.2), +8,−9,+19,+21[†]	-	-
3	48/F	Cutaneous ALCL	skin	-	-	-	-	-	PUVA, IFNα-2A, CHOP	alive, in remission, 154 mos
			skin	1	-	-	-	-		
			skin	83	YES	POS	NEG	-		
			LN	126	-	POS	-	-		
			LN	134	-	POS	NEG	-		
4	67/M	Cutaneous ALCL	skin	-	-	-	-	-	-	alive, LN involvement, 7 mos
			LN	7	NO	POS	NEG	-		
5	89/F	Cutaneous ALCL	skin	-	NO	POS	NEG	-	XRT, CHOP	died, progressive cutaneous disease, no autopsy, 4 mos
6	65/M	Cutaneous ALCL	skin	-	YES	POS	NEG	-	CHOP	alive, skin recurrence, 27 mos
7	52/M	Cutaneous ALCL	skin	-	-	POS	NEG	-	CHOP	died, unrelated cause, no autopsy, 9 mos
			LN	1	NO	-	-	-		
8	74/M	Cutaneous ALCL	skin	-	NO	-	-	-	-	alive, LN involvement, 34 mos
			LN	34	NO	POS	NEG	-		
9	35/M	Cutaneous ALCL	skin	-	NO	POS	NEG	-	-	-
10	50/M	Cutaneous ALCL	skin	-	NO	POS	NEG	-	-	-
11	73/F	PTCL-U	pleura	-	NO	POS	NEG	-	-	-
12	79/M	ALK-negative ALCL	LN	-	NO	POS	NEG	-	-	-

FISH, fluorescence *in situ* hybridization; BM, bone marrow; LN, lymph node; PTCL-U, peripheral T-cell lymphoma, unspecified; ALCL, anaplastic large-cell lymphoma; PUVA, psoralen UVA photochemotherapy; IFNα-2A, interferon alpha-2A; CHOP, cyclophosphamide +hydroxydoxorubicin+oncovin+prednisone; ICE, ifosfamide+carboplatin+etoposide; ITMTX, intrathecal methotrexate; XRT, radiotherapy

*Positive for TIA-1 by immunohistochemistry.

† FISH for *BCL6* at 3q27 was normal (data not shown).

Table 3

Clinicopathologic Features of Primary Cutaneous Anaplastic Large Cell Lymphomas With and Without *IRF4* Translocations

	***IRF4* Translocation**	
	Present	***Absent***
n (%)	8 (57)	6 (43)
M:F	6:2	3:3
Age, mean (range), y	60 (35−89)	64 (12−92)
Immunophenotype (%)		
CD30	8/8 (100)	6/6 (100)
CD3	6/8 (75)	2/6 (33)
CD4	5/8 (63)	4/6 (67)
CD8	0/8 (0)	1/6 (17)
ALK	0/8 (0)	0/6 (0)
TIA1	2/8 (25)	3/6 (50)
MUM1/IRF4	8/8 (100)	5/6 (83)
Subsequent extracutaneous disease	4	1
Follow-up, mean (range), mos	29 (0−154)	30 (0−76)