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Immunophenotypic switch in cutaneous T-cell lymphoma: A series of three cases and review of the literature.

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Abstract

Primary cutaneous T-cell lymphoma (CTCL) comprises a heterogeneous group of neoplasms with variable clinical behavior. Immunophenotypic switch (IS) is a phenomenon that occurs during lymphoma progression and is defined by an alteration in the immunophenotypic expression of a tumor with retention of its genotypic signature. This has been well-recognized in hematopoietic neoplasms; however, it has been rarely reported in CTCL and its clinical implications are not well understood. We present the clinical, histopathologic, immunophenotypic, and genetic findings of three cases of CTCL that demonstrated IS post treatment with variable outcomes. We add our cases to the small number previously reported to increase awareness of this phenomenon and its diagnostic challenge.

Introduction

Immunophenotypic switch (IS) is defined as an alteration in the immunophenotypic expression of a tumor while retaining its genotypic signature and occurs during lymphoma progression.[1] It is a known phenomenon in hematologic malignancies,[2] but has rarely been reported in cutaneous T-cell lymphoma (CTCL)[1,3-14] and its clinical and biological significance is not well studied. IS poses a diagnostic challenge and may be associated with a worse prognosis.[1,3-14] Herein, we describe three cases of CTCL that demonstrate IS during the course of disease with variable presentations and outcomes.

Case reports

Case 1

A 78-year-old man presented with 3 years of erythematous, scaly plaques with alopecia on the eyebrows, back and arms (Figure 1A). Biopsy revealed folliculotropic and syringotropic MF (Figure 2A). T cells were positive for CD3, CD4, TCRβF1 and CD5 with significant loss of CD7, and a CD4:CD8 ratio of >10:1. CD20 marked a few scattered B cells. TIA-1, granzyme and TCR-δ were negative. The patient underwent treatment with narrow band UVB (nbUVB), bexarotene and topical steroids with good response.

After 18 months of therapy, the patient presented with an enlarging nodule on the right leg (Figure 1B). Biopsy revealed large cell transformation. The large cells were positive for CD3, CD7, TIA-1 and TCR-δ (Figure 2B) and negative for CD4, CD8, CD30, CD5, TCRβF1, granzyme, and EBV in situ hybridization (EBER). They also expressed CD20, CD79A, and Bcl-2, but were negative for PAX-5. A dual CD3/CD20 stain confirmed co-expression of both markers by T cells (Figure 2C). TCR γ and β gene rearrangement studies performed by polymerase chain reaction (PCR) showed a monoclonal T-cell population with identical base pair peaks in both the initial and subsequent biopsy (Figure 2D). Immunoglobulin heavy chain and kappa light chain gene rearrangement studies showed no monoclonal B-cell population.

The patient was diagnosed with MF with large cell transformation and IS. There was no systemic involvement on laboratory and imaging studies. In particular, peripheral blood flow cytometry showed no immunophenotypic evidence of aberrant T-cells. Radiation to the right leg had good initial response with flattening of the lesion to the level of his background skin; however, the patient relapsed 3 months later with regrowth of the leg nodule and a new adjacent subcutaneous nodule showing the same IS. He was treated with vorinostat for 2 months before it was discontinued due to disease progression. He was referred to oncology and was started on pralatrexate with favorable initial response.

Case 2

A 16-year-old Caucasian boy with Fitzpatrick skin type 3 to 4 had 4 years of hypopigmented to erythematous patches/plaques on his trunk and extremities, previously treated as eczema with topical steroids (Figure 3A). These lesions initially involved his neck and back and resolved with topical steroids, but began to spread to other sites and became more difficult to treat. He eventually developed nodules on his back and abdomen that rapidly enlarged with ulceration (Figure 3B). Three skin biopsies were performed over a one-week period. A biopsy from a hypopigmented patch on his thigh revealed atypical lymphoid cells tagging the dermal-epidermal junction consistent with CD8-positive MF, with weak co-expression of CD4 and without CD30 (Figure 4). An ulcerated nodule on the right mid back showed large lymphoid cells with pleomorphic nuclei that were strongly positive for CD30 and CD8 without significant expression of CD4 (Figure 5). An ulcerated nodule on the left abdomen revealed large atypical lymphoid cells underlying an ulcer with a background of smaller, epidermotropic T cells (Figure 6). The large cells were positive for CD30 and CD4 without expression of CD8. The smaller, epidermotropic population was positive for CD4 and weakly positive for CD8, and they lacked significant expression of CD30. TCR γ and β studies performed by PCR on all lesions demonstrated monoclonal T cells with identical base pair peaks. The patient was diagnosed with MF and an associated CD30-positive T-cell lymphoproliferative disorder (LPD). Given the patients four-year history of worsening, hypopigmented, erythematous patches and plaques, he was considered to have MF with IS demonstrating variable expression of CD4 and CD8. In addition, he meets the WHO criteria for LCT. [15] No systemic involvement was detected. He was

treated with nbUVB and topical steroids with resolution of his nodules and stable patch/plaque disease.

Case 3

A 63-year-old man presented with 3 years of progressive, annular, ulcerated, erythematous to violaceous plaques on the trunk and extremities. He also described night sweats, shortness of breath and was noted to have an enlarged right axillary lymph node measuring 1.7 cm. Biopsy of a left hip plaque demonstrated a predominantly dermal infiltrate of atypical lymphoid cells positive for CD3, CD4, and CD5 with limited epidermotropism and a CD4:CD8 >10:1 (Figure 7). Atypical T cells showed somewhat diminished expression of CD3 and a subset of cells was positive for TCRβF1. They were negative for CD2, CD7, CD8 and TCR δ. The patient was started on triamcinolone 0.1% cream, but showed no improvement of his cutaneous lesions, and he was referred to our institution. A nodule on his left anterior shoulder was biopsied 3 weeks later and showed diffuse dermal and subcutaneous atypical lymphoid infiltrate with epidermotropism. By IHC, the atypical cells were positive for CD3 (dim), CD4, CD8 (weak), CD5 and TIA-1. They did not express CD2, CD7, CD30, granzyme, CD56, TCRβF1, TCR δ, or EBER by in situ hybridization. A retrospective review revealed the initial biopsy to be positive for TIA-1 and negative for granzyme (Figure 5). PCR-based TCR γ and β gene rearrangement studies showed a monoclonal T-cell population with identical base pair peaks in the two biopsies. Flow cytometry showed 26% of CD4-positive T cells with partial loss of both CD26 and CD7, suggestive of peripheral blood involvement by CTCL. A positron emission tomography/computed tomography scan showed diffuse nodal involvement; however, a lymph node biopsy was not performed. A bone marrow biopsy was negative for involvement by

lymphoma. His LDH was elevated to 304 IU/L. Our patient was diagnosed with an unusual and difficult to classify CTCL, possibly representing an irregular primary cutaneous CD8-positive aggressive, epidermotropic, cytotoxic T-cell lymphoma with an unconventional immunophenotype, prolonged clinical course, and IS; cases of this lymphoma have been previously described with variant immunophenotypes.[16] Due to the unique clinical and immunophenotypic presentation, a peripheral T-cell lymphoma, not otherwise specified was additionally considered in the differential diagnosis. The patient completed 6 cycles of cyclophosphamide, doxorubicin, vincristine and prednisone, which was complicated by neutropenic fever and pneumonia. He eventually succumbed to his disease. Because the patient's lymphoma immunophenotype changed over time and with disease progression showing gain of CD8 and loss of TCRβF1, but retained its genotypic signature, this case was considered IS.

Discussion

We reviewed PubMed for all reported cases of IS in skin biopsies of CTCL and identified 25 cases with detailed clinical and pathologic information. We add 3 new cases. The age, sex, clinical presentation, IS and disease outcome for all reported cases are summarized in Table 1. The ages range from 2-92 years old with a mean age of 57. The male: female ratio is 1.8:1. MF is the most common type of CTCL to undergo IS, occurring in 15 of the 28 cases. PCGD TCL constitutes the second most common CTCL subtype in which IS was described in 9 of 28 cases. IS was seen in all stages of MF ranging from stage IA to Sèzary syndrome. Many cases with IS were treated with multiple agents before the switch was documented on relapse. The most common IS reported in MF (13/15 cases) is a switch from a CD4-positive/CD8-negative profile

without cytotoxic proteins to a CD4-negative/CD8-positive or CD4-negative/CD8-negative profile with expression of the cytotoxic markers TIA-1 and granzyme B.[1,3-13] 18 of 28 patients expired. The average progression time from diagnosis of IS to death is 22 months with a range of (1-76 months).[1,3-14] Notably, Merrill et al. described 20 patients with a gamma delta phenotype and noted that CD8 expression appeared to change over time in 2 patients and CD30 changed over time in 4 patients, but detailed clinicopathologic information is not available for these cases.[14] Similarly, Vermeer et al. described gain of cytotoxic marker expression with disease progression in MF, including 5 patients who may meet criteria for IS, but detailed demographic, immunophenotypic and genetic information was not provided.[13]

In our case series, two patients had the most common IS seen in MF, from a CD4-positive/CD8-negative profile to a CD4-negative/CD8-positive or CD4-negative/CD8-negative profile. One of our patients had co-expression of a B-cell marker (CD20) and a T-cell marker (CD3) on malignant T cells that was confirmed with a double stain (CD3/CD20). CD20 positive T-cell lymphoma is a rare entity that appears to be correlated with disease progression, poor outcome and resistance to anti-CD20 therapy.[14] One patient presented with a very unusual case of a cytotoxic CD4-positive/CD8-negative CTCL that underwent an IS to a CD4-positive/CD8-positive lymphoma. Notably, two patients experienced an IS with loss of TCR βF1 expression; one patient gained expression of TCR δ associated with large cell transformation (Case 1), while another became TCR silent (Case 3). TCR δ expression has been associated with a worse prognosis in mycoses fungoides,[17] and shifts from TCR βF1 to TCR δ were described in large cell transformation.[18] A null TCR phenotype, as observed in Case 3, was described in CD30+ anaplastic T cell lymphoma and confers a more aggressive phenotype due to dysregulation of T cell activation and survival.[19] Two of our patients received several lines of therapy before an IS

occurred. Treatments included topical steroids, nbUVB, bexarotene, and radiation therapy. One patient expired from the disease, one patient had stable disease and one patient had progressive disease.

The underlying mechanism by which IS occurs is still unclear. Agbay et al. proposed post-therapy antigenic stimulation and clonal selection as a possible explanation.[3] As many cases of IS occur post therapy, it is possible that treatment might be contributing to this shift. Therapy alters the microenvironment around the tumor and creates different antigenic stimuli contributing to the immunophenotypic instability of the tumor.[3] Emergence of a resistant CD30-negative clone is a known event post administration of anti-CD30 therapy for CD30+ cutaneous lymphomas. [20,21] A similar phenomenon was described in B-cell lymphoma with loss of CD20 expression after the use of rituximab.[22] Histone deacetylase inhibitors have been demonstrated to decrease CCR4 expression in T-cell lymphomas and confer resistance to mogamulizumab.[23] Thus therapy can contribute to clonal selection by creating a tumor with a different phenotype than the initial one. Marks et al. raise the theory of T cell immaturity.[1] Immature thymic T cells (CD3-positive/CD4-positive/CD8-positive) can be the predominant clone in CTCL and differentiate to a CD4-positive or a CD8-positive phenotype upon different stimulation from therapy.[1] Following this theory, the IS to a CD4-negative/CD8-negative profile and the coexpression of B and T-cell markers may derive from a multipotent premature T-cell clone that carries a worse prognosis. Finally, for cases with change from strong to weak expression, it is difficult to absolutely exclude a technical artifact.

CTCL has been reported to have a stable aberrant T-cell phenotype that can be followed in peripheral blood.[16] However, Novelli et al. found that nearly a quarter of Sèzary syndrome patients show a change in immunophenotype over time by peripheral blood flow cytometry,

including loss or gain of CD26, CD7, and/or CD2.[17] Whether IS in CTCL is a rare or an under-recognized entity is yet to be determined. Nonetheless, recognizing IS is essential from a diagnostic standpoint and may carry prognostic value. Eighteen of 28 reviewed CTCL patients with IS succumbed to their disease. Three patients with MF and IS from CD4-positive to CD4-positive/CD8-positive profile presented with the very rare complication of ocular involvement and succumbed to their disease.[4,12] While none of our patients had ocular disease, it seems reasonable to closely follow patients for occult involvement of other organ systems.

In summary, IS is a diagnostically challenging and possibly an under-recognized phenomenon. This study expands and further refines the concept of IS, highlighting the diagnostic complexity and serving to alert clinicians to its possible prognostic implications.

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Table Legend:

Table 1: Demographics, diagnosis, stage, summary immunohistochemical profiles and clinical outcomes of reported patients with IS.

Patients	Age (years)	Sex	Disease (stage)	Initial phenotype	Phenotypic Switch	TCR gene rearrangement	Clinical course
Bitar et al. (current series, case 1)	78	M	MF (IB)	CD4+/CD8-CD7-/ TCRβ+/ CD20-	CD4-/CD8-CD7+/TIA1+/ TCRδ +/ CD20+	Identical TCR clone	Progressive disease
Bitar et al. (current series, case 2)	16	M	MF (IB) and CD30+ T-cell LPD	MF with CD4+/CD8 weak, separate lesion CD8+/CD4 weak	CD4+/CD8-/ CD30+ T-cell LPD, separate lesion CD8+/CD4-CD30+ T-cell LPD	Identical TCR clone	Stable disease
Bitar et al. (current series, case 3)	63	M	Cytotoxic T-cell lymphoma, possibly representing PCAgETCL	CD2-/ CD3+/ CD4+/CD7-/CD8-/TIA1+/ granzyme B-	CD2-/C3+/ CD4+/CD7-/CD8+/ TIA1+/ granzyme B-	Identical TCR clone	Death of disease

Tschetter et al.	66	M	MF (uk)	CD3+/CD4+/ CD20+	CD3+/CD20-	Identical TCR clone	Death of disease
Marks et al.	92	F	MF (IIB)	CD4+/CD8- TIA1-/ granzyme B-/ TCRδ -	CD4-/CD8- TIA1+/ granzyme B+/ TCRδ +	Identical TCR clone	Death of disease
Marks et al.	58	F	MF (IIB)	CD4+/CD8-/ granzyme B-	CD4- /CD8-/ granzyme B+	Identical TCR clone	Death of disease
Braue et al.	54	F	MF (IA)	CD4 +/CD8-	CD4-/ CD8+	Not performed	Death of disease
Braue et al.	69	F	MF (IA)	CD4+/ CD8-	CD4-/CD8+	Not performed	Death of disease
Agbay et al.	24	M	PCGD TCL	CD30-/ TCRβ +	CD30+/ TCRβ + (weak)	Not performed	Death of disease
Agbay et al.	52	F	PCGD TCL	CD7-/CD30-(weak)/CD8+/CD56+	CD7+/CD30 (strong)/ CD8+/CD56-	Identical TCR clone	Remission
Agbay et al.	50	M	PCGD TCL	CD30-/CD56+	CD30+/ CD56-	Not performed	Remission
Agbay et al.	2	M	PCGD TCL	CD5 + (weak)	CD 5 + (strong)	Identical TCR clone	Remission

Agbay et al.	87	F	PCGD TCL	CD4-/TCRβ-/ TIA1-	CD4+/ TCRβ+/ TIA1+	Identical TCR clone	Death of disease
Agbay et al.	70	F	PCGD TCL	CD30+/ CD56+ (weak)	CD 30+ (weak)/ CD56 +	Identical TCR clone	Death of disease
Agbay et al.	79	F	PCGD TCL	TIA1+	TIA1	Not performed	Death of disease
Agbay et al..	39	M	PCGD TCL	TCRδ +	TCRδ + (weak)	Not performed	Death of disease
Agbay et al.	59	M	PCGD TCL	CD7+ (weak)	CD7+	Not performed	Active disease
Aung et al.	67	M	PTCL, NOS	CD4+/CD8−	CD4+ (rare)/ CD8+/TIA1+/ granzyme B+	Identical TCR clone	Death of disease
Aung et al.	54	F	MF (uk)	CD4+/ CD8−	CD4-/CD8+/ granzyme B+	Identical TCR clone	Remission
Aung et al.	41	M	PTCL, NOS	CD4-/CD8+	CD4+/CD8-	Not performed	Death of disease
Al-Ibraheemi et al.	40	F	PTCL, NOS	CD4-/CD8+/ TIA1+	CD4+/CD8-/ TIA1-	Not performed	Remission
Endo et al.	46	M	MF (uk)	CD4+/ CD8−	CD4-/CD8+/ granzyme B+	Identical TCR clone	Stable disease

Okada et al.	56	M	MF (IA)	CD4+/CD8−/ granzyme B−	CD4−/CD8+/ granzyme B-	Identical TCR clone	Death of disease
Kreuter et al.	72	M	Sezary syndrome	CD4+/CD8−	CD4-/CD8+	Identical TCR clone	Death of disease
Vargas Nevado et al.	84	M	MF (IIB)	CD4+/CD8-/CD30+/ TIA1−/ granzyme B−/ CD56−	CD4-/CD8-/CD30-/ TIA1+/ granzyme B+/ CD56 -	Identical TCR clone	Death of disease
Johnson et al.	77	M	MF (IIB)	CD4+/CD8−/ TIA1+/ granzyme B+	CD4-/CD8+/ TIA1+/ granzyme B+	Identical TCR clone	Death of disease
Lois et al.	82	M	MF (uk)	CD4+/CD8−	CD8+/CD4 –/coexpression of CD3/CD79A	Not performed	Death of disease
Nikolova et al.	81 y.o	M	MF (uk)	CD4+/CD8-	CD4-/CD8+	Identical TCR clone	Unknown|

PCAgETCL: primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma, PCGD TCL: primary cutaneous gamma delta T-cell lymphoma, (+): positive, PTCL, NOS: peripheral T-cell lymphoma, not otherwise specified, TCR: T-cell receptor, uk: unknown.

Figure Legends:

Figure 1: Mycosis fungoides with large cell transformation and immunophenotype switch (Case 1). A. The patient initially presented with erythematous patches with associated alopecia, compatible with folliculotropic mycosis fungoides. **B.** 18 months later, he presented with a right lower leg nodule that showed large cell transformation and immunophenotypic switch.

Figure 2: Mycosis fungoides with large cell transformation and immunophenotype switch (Case 1). A. H&E section from the initial biopsy of the left eyebrow shows a prominent atypical lymphocytic infiltrate around the hair follicles and eccrine glands (original magnification x 40) that are TCR β positive and TCR δ negative (inset x100). **B.** The subsequent biopsy from the left leg demonstrates a diffuse atypical lymphoid infiltrate consistent with large cell transformation (original magnification x 40, H&E) that is positive for TCR δ and negative for TCR β (inset, x100). **C.** The atypical lymphoid cells with large cell transformation are positive for dual CD3/C20 stain (x600, brown=CD3 and red=CD20). **D.** TCR γ Gene rearrangement studies reveal a monoclonal T-cell population with identical base pair peaks in both biopsies (186 and 190 in TCR γ, 261 for Vb - Jb2 in TCR β).

Figure 3: Mycosis fungoides with large cell transformation and immunophenotypic switch (Case 2). A. Hypopigmented to erythematous patches and plaques were initially treated as eczema. **B.** The patient developed rapidly-enlarging, ulcerated, nodules.

Figure 4: Mycosis fungoides. Skin biopsy from a hypopigmented patch on the thigh (Case 2). A. H&E section shows a prominent atypical lymphocytic infiltrate at the dermoepidermal junction with epidermotropism (original magnification x 100). **B.** CD4 weakly highlights

atypical lymphoid cells (original magnification x 100). **C.** The atypical lymphoid cells are CD8-positive (original magnification x 100). **D.** CD30 is negative (original magnification x 100).

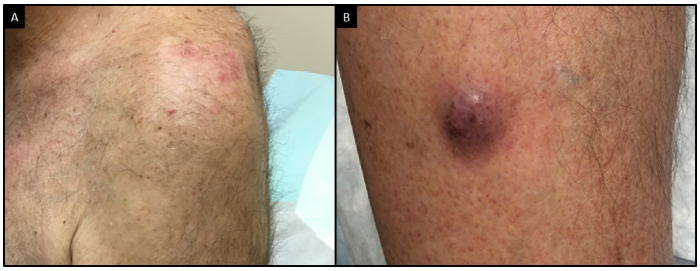
Figure 5: Mycosis fungoides with large cell transformation. Nodule from the back (Case 2). **A.** An H&E section from the back shows an ulceration with underlying prominent large atypical lymphocytic infiltrate (original magnification x 200) **B.** The atypical lymphoid cells do not show significant expression of CD4 (original magnification x 200) **C.** CD8 highlights the atypical lymphoid cells (original magnification x 200) **D.** CD30 also marks the large atypical lymphoid cells (original magnification x 200).

Figure 6: Mycosis fungoides with large cell transformation. Nodule from the abdomen (Case 2). A. H&E section from the abdomen shows an ulceration with underlying prominent large atypical lymphoid infiltrate and adjacent smaller lymphocytes at the dermoepidermal junction (original magnification x 100). **B.** CD4 stain strongly highlights both populations of atypical lymphoid cells (original magnification x 100). **C.** The large atypical lymphoid cells are negative for CD8, while the adjacent smaller, epidermotropic lymphoid cells are weakly positive for CD8 (original magnification x 100). **D.** CD30 highlights the large atypical lymphoid cells (original magnification x 100).

Figure 7: Cutaneous T-cell Lymphoma and immunophenotypic switch (Case 3): A. Initial biopsy of the left hip plaque shows a dermal infiltrate of atypical, epidermotropic lymphoid cells (original magnification x100). Inset shows CD3 (original magnification x100). **B.** Atypical lymphoid cells are negative for CD8 (original magnification x100). **C.** They are positive for

CD4 (original magnification x100). **D.** They also express TIA-1 (original magnification x100), but are negative for TCRδ (inset, original magnification x100). **E.** Subsequent biopsy from a nodule on the left anterior shoulder shows an epidermotropic T-cell infiltrate. (original magnification x100). Inset shows CD3 (original magnification x100).

F. Atypical lymphoid cells in the deep dermis on subsequent biopsy are weakly positive for CD8 (original magnification x200). **G.** They are positive CD4 (original magnification x200). **H.** They are also positive for TIA-1(original magnification x200).



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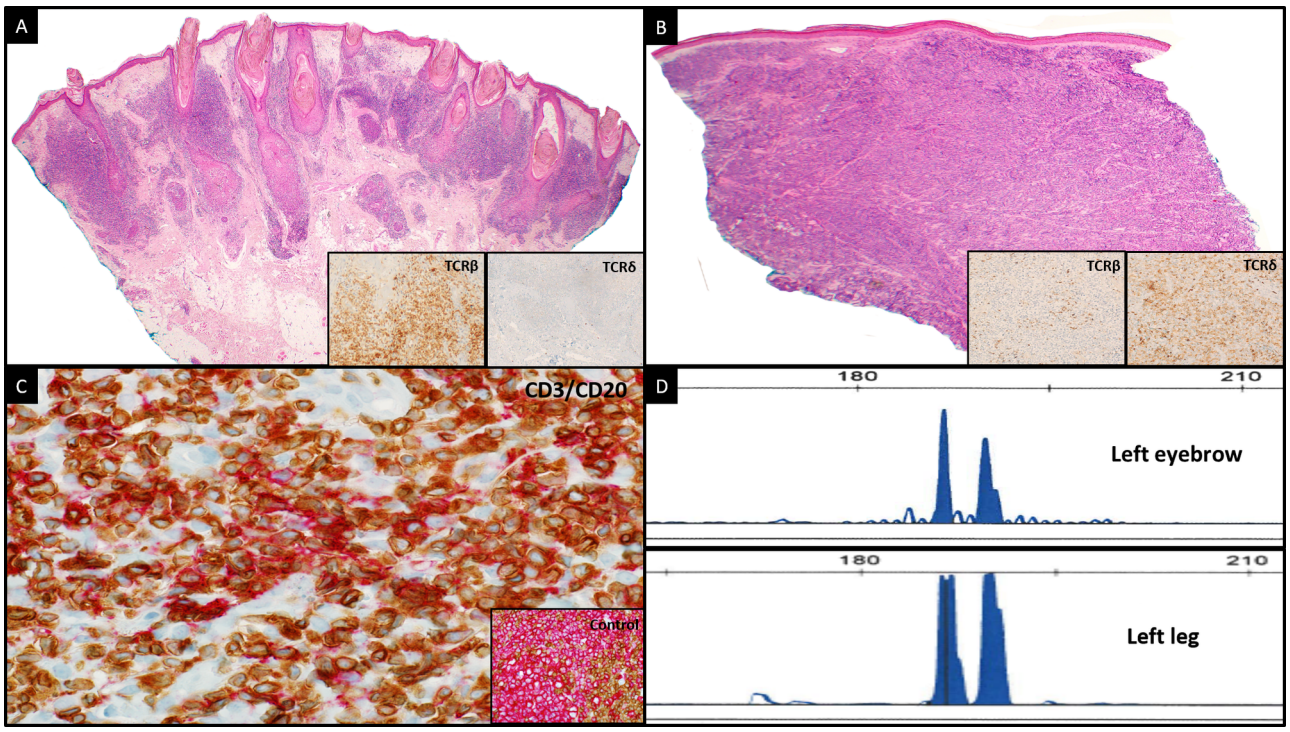

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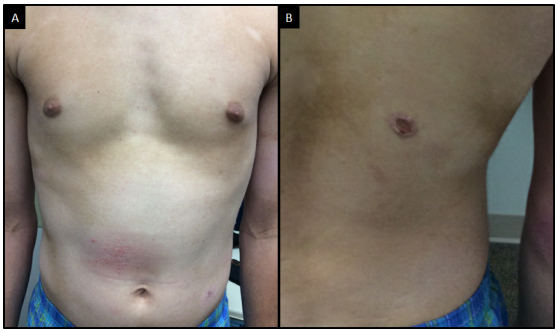

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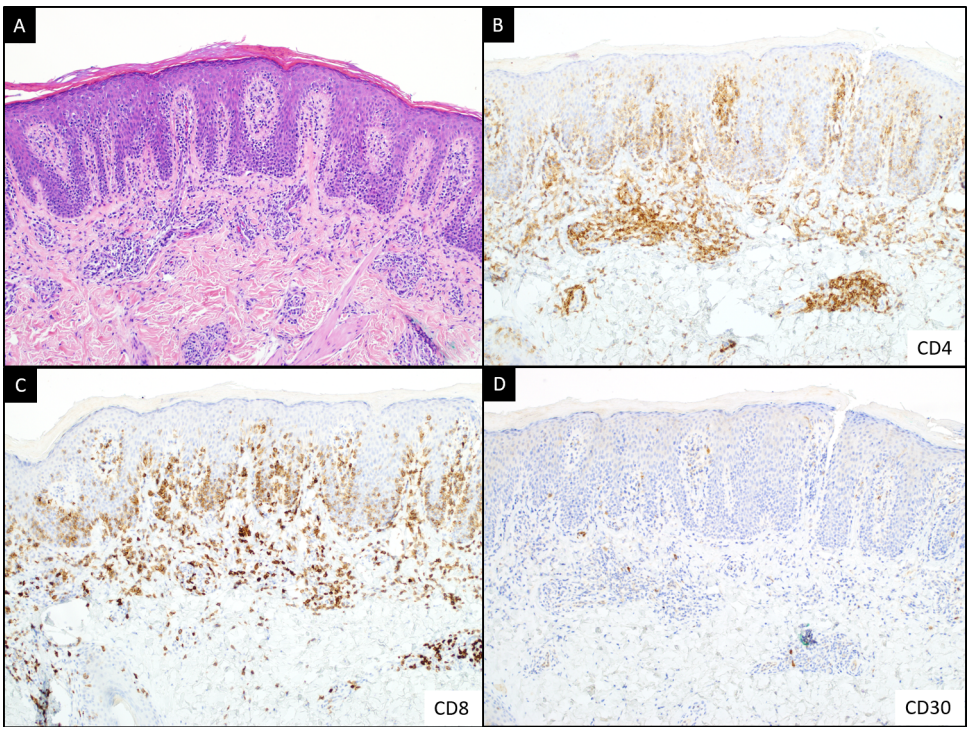

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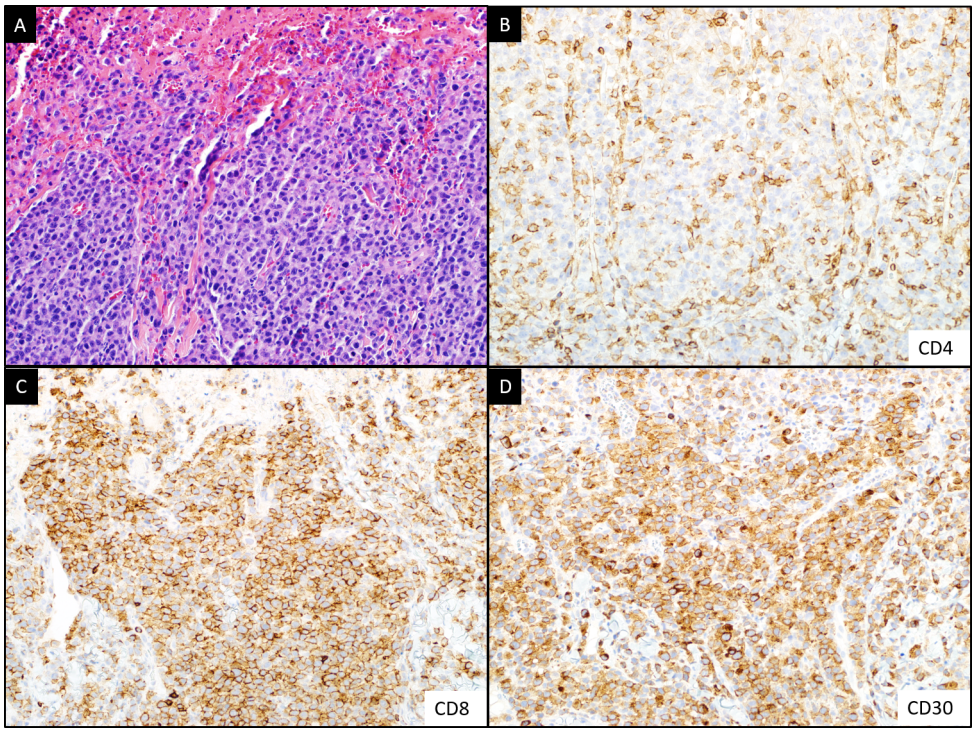

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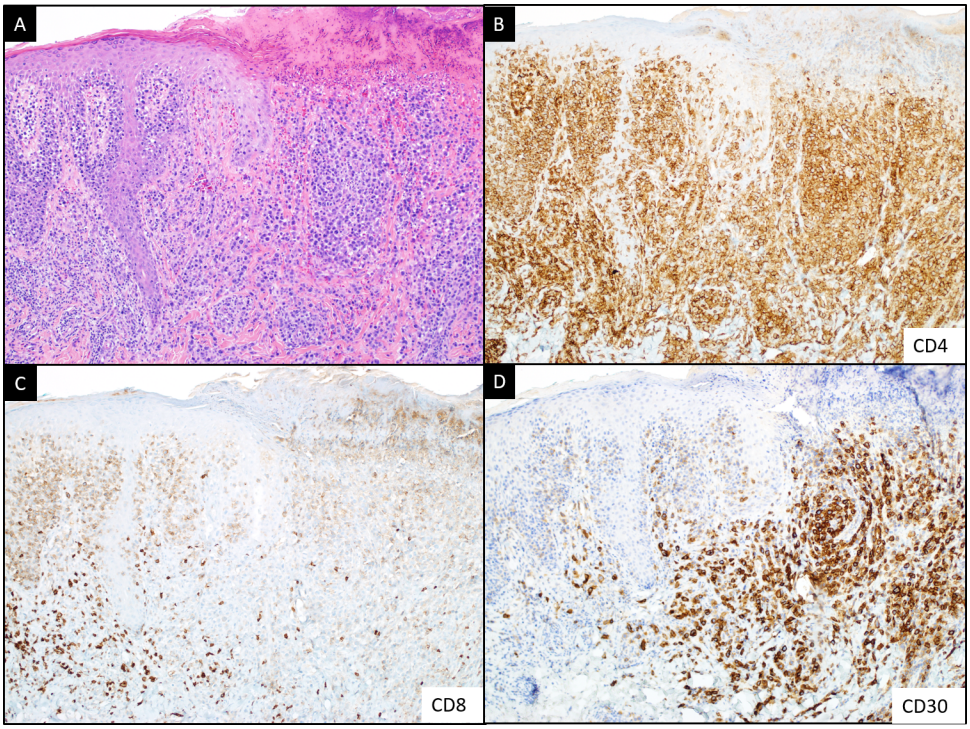
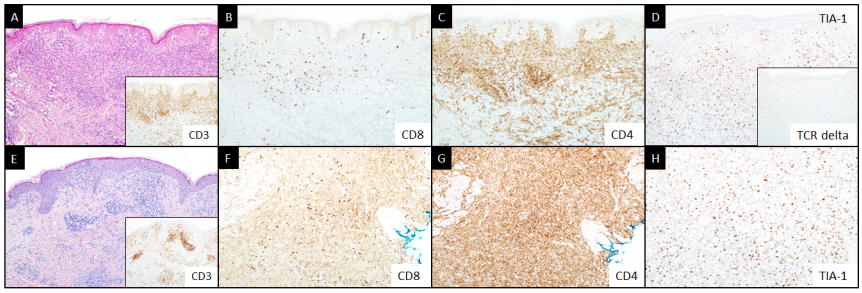


CUP_14026_Figure 6.tif



CUP_14026_Figure 7m2.tif