Primary T Cells from Cutaneous T-cell Lymphoma Skin Explants Display an Exhausted Immune Checkpoint Profile

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Abstract

Cutaneous T-cell lymphoma (CTCL) develops from clonally expanded CD4+ T cells in a background of chronic inflammation. Although dendritic cells (DCs) stimulate T cells and are present in skin, cutaneous T cells in CTCL do not respond with effective antitumor immunity. We evaluated primary T-cell and DC emigrés from epidermal and dermal explant cultures of skin biopsies from CTCL patients (n = 37) and healthy donors (n = 5). Compared with healthy skin, CD4+ CTCL populations contained more T cells expressing PD-1, CTLA-4, and LAG-3. CD8+ CTCL populations contained more T cells expressing CTLA-4 and LAG-3. CTCL populations also contained more T cells expressing the inducible T-cell costimulator (ICOS), a marker of T-cell activation. DC emigrés from healthy or CTCL skin biopsies expressed PD-L1, indicating that maturation during migration resulted in PD-L1 expression irrespective of disease. Most T cells did not express PD-L1. Using skin samples from 49 additional CTCL patients for an unsupervised analysis of genome-wide mRNA expression profiles corroborated that advanced T3/T4-stage samples expressed more checkpoint inhibition mRNA compared with T1/T2 stage patients or healthy controls. Exhaustion of activated T cells is therefore a hallmark of both CD4+ and CD8+ T cells isolated from the lesional skin of patients with CTCL, with increasing expression as the disease progresses. These results justify identification of antigens driving T-cell exhaustion and the evaluation of immune checkpoint inhibition to reverse T-cell exhaustion earlier in the treatment of CTCL. Cancer Immunol Res; 6(8); 900–9. ©2018 AACR.

Introduction

Cutaneous T-cell lymphomas (CTCL) comprise a set of related malignancies characterized by the accumulation of neoplastic memory T cells in the skin in a background of chronic inflammation. The most common subtypes of CTCL are mycosis fungoides (MF) and Sézary syndrome (SS), the advanced stages of which have an estimated 5-year overall survival of 24% (1, 2). There is no cure, and patients frequently relapse, requiring repeated treatment courses for disease control.

The lack of a clear understanding of CTCL etiology has impeded therapeutic advances toward disease-free and overall survival. Although dendritic cell (DC) subsets populate all cutaneous and mucosal surfaces in both the steady state and inflammation (3), and irrespective of the presence of tumor-infiltrating CD8+ T cells in the CTCL lesions, clonally expanded malignant CD4+ T-cell clones persist in chronically inflamed skin. This observation suggests that chronic inflammation in the tumor microenvironment is ineffective or may even impair successful antitumor immunity mediated by the CD8+ T cells (4, 5).

The type and duration of signals encountered by naïve T cells at the time of antigen presentation by DCs determine T-cell activation and immune responsiveness. During initial antigen sensitization, T cells express the T-cell receptor (TCR) for DC-presented antigen along with costimulatory receptors that bind DC costimulatory ligands and cytokines, all of which in concert ensure the initiation of effective T-cell effector responses (6, 7). T cells in turn express compensatory immune checkpoint receptors, which dampen an otherwise unchecked immune response (8–10). In tumors that express self-differentiation antigens or during chronic viral infections, however, chronic or excessive T-cell stimulation can lead to overexpression of inhibitory checkpoint receptors. Ligand binding to these receptors, which include PD-1, LAG-3, TIM-3, CTLA-4, and others, can elicit a survival maneuver termed T-cell exhaustion,
whereby chronically overactivated T cells neither transition to memory T cells nor undergo apoptosis or deletion. Instead, these exhausted T cells enter a potentially reversible state of unresponsiveness toward pathogens or tumor antigens (11, 12). The functional consequences are impaired immune surveillance and unchecked viral persistence or tumor growth. Antibody-based drugs that block these checkpoint inhibitors can reverse T-cell exhaustion, a therapeutic approach that has revolutionized treatment of multiple cancers, especially in patients who have evidence of a prior immune response.

The rarity of CTCL and the lack of robust methods for isolating tumor cells directly from involved skin have limited translational investigations to optimize control or cure. Results from immunohistochemical analyses of T-cell immune checkpoint markers (13–16) have been inconclusive due to limitations imposed by the small number of distinguishable antibodies and fluorochromes available for simultaneous evaluation of checkpoint inhibition epitopes.

We therefore used multicolor flow cytometry to analyze primary T-cell and DC émigrés in liquid culture medium (17, 18) from ~36-hour skin explant cultures of separated epidermis and dermis from lesional and healthy skin. These studies were complemented by unsupervised genome-wide mRNA sequencing, as well as multiplex immunohistochemistry, using checkpoint markers restricted to PD-1 and PD-L1 because of the limitations noted above. Our goal was to evaluate immune checkpoint expression as a marker of T-cell dysregulation across the continuum of CTCL disease stages in order to develop a rationale for identifying antigens responsible for chronic T-cell overstimulation and to guide use of checkpoint inhibitors earlier in the treatment of CTCL.

Materials and Methods

Study population and tissue samples

Lesional skin shave biopsies (approximately 1–1.5 cm) were obtained under local anesthesia from 37 CTCL outpatients (23 males and 14 females; median age 57 years; range, 24-86 years) seen at the Memorial Sloan Kettering Cancer Center (MSKCC) Multidisciplinary Cutaneous Lymphoma Clinic (Table 1). Additional skin from the same biopsy area was submitted for investigations to optimize control or cure. Results from immunohistochemical analyses of T-cell immune checkpoint markers (13–16) have been inconclusive due to limitations imposed by the small number of distinguishable antibodies and fluorochromes available for simultaneous evaluation of checkpoint inhibition epitopes.

We therefore used multicolor flow cytometry to analyze primary T-cell and DC émigrés in liquid culture medium (17, 18) from ~36-hour skin explant cultures of separated epidermis and dermis from lesional and healthy skin. These studies were complemented by unsupervised genome-wide mRNA sequencing, as well as multiplex immunohistochemistry, using checkpoint markers restricted to PD-1 and PD-L1 because of the limitations noted above. Our goal was to evaluate immune checkpoint expression as a marker of T-cell dysregulation across the continuum of CTCL disease stages in order to develop a rationale for identifying antigens responsible for chronic T-cell overstimulation and to guide use of checkpoint inhibitors earlier in the treatment of CTCL.

Table 1. Demographic characteristics of the CTCL patient population (n = 37) studied using T-cell and DC émigrés from skin explant cultures of epidermal and dermal lesional skin

| Age (years) | 57 (24-86) |
| Gender | Male | 23 |
| | Female | 14 |
| Race/ethnicity | Caucasian | 28 |
| | African American | 4 |
| | Hispanic | 3 |
| | Unknown | 2 |
| Clinical stage | IA | 9 |
| | IB | 11 |
| | IIA | 1 |
| | IIB | 1 |
| | IVA | 3 |
| | IVB | 5 |
| | Total | 37 |

required ≥1 skin tumor, and T4 necessitated generalized erythroderma. This staging also applied to the few patients with Sézary syndrome, defined by leukemic involvement of the cancerous T cells in addition to their presence in skin tumors. Thirty-seven patients provided biopsies in the following categories: clinical stage IA/IB, n = 20 (T1 + T2); stage IIA, n = 1 (T2); stage IIB, n = 7 (T3); stage IIIB, n = 3 (T4); stage IVA, n = 5 (T4); and stage IVB, n = 1 (T4). Five patients undergoing reconstructive plastic surgery after successful treatment of breast cancer provided healthy skin.

Forty-nine formalin-fixed, paraffin-embedded (FFPE), and histologically confirmed lesional skin biopsies from 45 distinct CTCL patients identified from the COH CTCL tissue bank underwent unsupervised genome-wide mRNA sequencing. Stages included T1 (n = 7), T2 (n = 16), T3 (n = 18), and T4 (n = 4). Three FFPE tissue samples of healthy skin from 3 patients undergoing reconstructive plastic surgery served as controls.

All patient tissue collection and research use adhered to protocols approved by the Institutional Review and Privacy Boards at MSKCC and COH, in accordance with the Declaration of Helsinki. All participants signed written informed consents.

Cell isolation and preparation

Fresh skin samples cut into small pieces were incubated for 30 minutes at 37°C in 5% CO2 in DMEM/F-12 (Stem Cell Technologies) with dispase II (1 IU/mL, Roche Diagnostics) to facilitate separation of the epidermis from the dermis. The epidermis was peeled away from the dermis, after which each was placed in separate suspension cultures (Costar) in RPMI 1640 medium with 10 mmol/L L-HEPES, 1% penicillin/streptomycin (Media Lab, MSKCC), 50 mmol/L L-glutamine (Cellgro), 50 μmol/L L-2-mercaptoethanol (Gibco, Life Technologies), and 10% heat-inactivated pooled healthy human serum (Atlanta Biologicals).
T-cell and DC phenotyping by flow cytometry

Migrated skin cells were stained with fluorochrome-conjugated monoclonal antibodies and analyzed on an LSR Fortessa (Becton Dickinson) flow cytometer. The following antibodies were used: anti-CD3 (BV650, Biolegend 317324), anti-CD8 (BV785, Biolegend 301046), anti-CD4 (PE-Texas Red, Life Technologies MHCD0417), anti-CD1a (PE-Cy5, eBioscience 25-9948), anti-ICOS (PE-Cy7, eBioscience 25-9948), anti-CD11c (APC, BD Pharmingen 555877), anti-CD14 (FITC, BD Pharmingen 555397; PE-Cy7, BD Pharmingen 557743), anti-CD14 (ITC, BD Pharmingen 555397; PE-Cy7, BD Pharmingen 557742; and BV785, Biolegend 301839), and anti-HLA-DR (ECD, BD Pharmingen 559877 and Alexa Fluor 700, BD Pharmingen 335795). T-cell numbers were sufficient to use nonreactive isotype-matched controls to determine gating of negative versus positive events. In contrast, too few DCs from these small shave biopsies were left for analysis with FlowJo 9.7.6 software (TreeStar). T-cell analyses included viable events that were positive for CD3 and either CD4 or CD8 to capture the respective T-cell subsets. Analyses of cutaneous DC crawlouts after live gating included the following antibodies: anti-CD3 (BV650, Biolegend 317324), anti-CD4 (PE-Texas Red, Becton Dickinson), anti-CD8 (BV785, Biolegend 301046), anti-CD1a (PE-Cy5, Biolegend 335795), anti-CD11b (FITC, BD Pharmingen 555877), dermal CD1a+ DCs (CD1a+, CD14neg, HLA-DRbright), dermal CD1a+ DCs (CD1a+, CD14neg, HLA-DRbright, CD11b+, CD11c+), and dermal CD14+ DCs (CD1a+neg, CD14+, HLA-DRbright, CD11b+, CD11c+; refs. 21, 22).

RNA sequencing, library preparation, and data analysis

Total RNA from FFPE skin from healthy controls and CTCL patients was extracted with the miRNeasy FFPE kit (Qiagen). Total RNA was RNA-depleted with the Ribo-Zero low input kit for Human/Mouse/Rat (Illumina) using 300 ng of starting material. RNAseq libraries were prepared from Ribo-Zero mRNA-enriched material using KAPA Stranded RNA-Seq Library Preparation Kit (Illumina Platforms; Kapa Biosystems) and 10 cycles of PCR amplification. Libraries were purified using AxyPrep Mag PCR Clean-up kit (Thermo Fisher Scientific). Each library was quantified using a Qubit fluorometer (Life Technologies), and the size distribution was assessed using the 2100 Bioanalyzer (Agilent Technologies). Sequencing was performed on an Illumina Hiseq 2500 (Illumina) instrument using the TrueSeq PE Cluster Kit V4-cBot-HS (Illumina) to generate 101 bp paired-end reads sequencing with v4 chemistry. Quality control of RNA-Seq reads used FastQC. These data sets are deposited in the Gene Expression Omnibus (GEO) repository under the accession number GSE113113.

Immunohistochemical evaluation of intact CTCL skin biopsies

Intact FFPE skin biopsies from the COH CTCL tissue bank were sectioned and subjected to standard immunohistochemical evaluation. Despite limitations imposed by antigen specificities of available reagents with nonoverlapping isotypes, secondary antibodies, and/or fluorochromes, we evaluated PD-1 and PD-L1 expression by CD4+ and CD8+ T cells.

Data and statistical analyses

The Wilcoxon rank sum test assessed the significance of differences in expression of T-cell exhaustion markers between healthy skin versus all samples from lesional CTCL skin (Table 2). The significance threshold was \( P = 0.05 \), and all

<table>
<thead>
<tr>
<th>Cell subset</th>
<th>Marker</th>
<th>Control Median (Range)</th>
<th>Control P</th>
<th>CTCL Median (Range)</th>
<th>CTCL P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ Epidermis</td>
<td>PD-1</td>
<td>21.80 (2.86–60.00)</td>
<td>77.90 (30.7–98.30)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>CD8+ Epidermis</td>
<td>PD-1</td>
<td>33.30 (21.04–79.40)</td>
<td>72.60 (11.8–96.9)</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>CD8+ Dermis</td>
<td>PD-1</td>
<td>49.50 (35.20–70.40)</td>
<td>66.90 (7.41–96.40)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>PD-1</td>
<td>33.30 (21.04–79.40)</td>
<td>72.60 (11.8–96.9)</td>
<td>0.031</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: \( P \) values from the Wilcoxon rank sum test reflect comparison of the percentage of cells from healthy versus diseased skin expressing each marker. \( P \) values in bold are statistically significant.
tests were two-sided. All analyses of percentages obtained from flow cytometric data were plotted and calculated using Prism 6 software (GraphPad). The Mann–Whitney U test compared the significance of differences in the mean fluorescent intensity (MFI) of PD-L1 expressed by DC subtypes isolated from healthy versus lesional CTCL skin.

The RNA-Seq reads were aligned to hg19 genome assembly using TopHat2 with default settings, and gene expression levels of Refseq genes were counted with customized R scripts. The expression data were normalized using the trimmed mean of M values (TMM) method, implemented in the Bioconductor package “edgeR” to obtain counts per million (CPM) and then scaled by gene length in kb. The normalized expression values were log2 transformed with offset of 1 and filtered to retain 6,418 genes with log2 expression value scaled by gene length in kb. The normalized expression values (TMM) method, implemented in the Bioconductor of Refseq genes were counted with customized R scripts. The using Tophat2 with default settings, and gene expression levels (MFI) of PD-L1 expressed by DC subtypes isolated from healthy skin (Fig. 1B; Table 2). Expression of CTLA-4, ICOS, LAG-3, PD-1, and LAG-3, PD-1, or PD-L1 for all stages of CTCL, compared with healthy skin, we performed a supervised analysis following the expected phenotypes for gating by IFNG, FOXP3, and IL10. Five gene clusters were identified with gene ontology (GO) annotation analysis based on the basis of their related functions. Cluster 1 highlights genes related to inflammatory response, T-cell activation, and inhibition/exhaustion, with a different gene expression profile observed in advanced-stage MF/SS (T3/T4) samples compared with those of early-stage (T1/T2) samples. RNAseq analysis of these archival tissues used total RNA isolated from skin sections. Clusters 2 to 5 therefore reflect other components of the cutaneous microenvironment: (2) epidermis and keratinocyte development, (3) extracellular matrix regulation, (4) neutrophil degranulation; negative regulation of transcription, and (5) innate immune response. All of these clusters displayed differentially expressed profiles for T1/T2 stage compared with advanced T3/T4 stage.

To elucidate whether MF/SS display an exhaustion-related marker expression profile from cluster 1 different from that of healthy skin, we performed a supervised analysis following the previously mentioned criteria (Fig. 3B). Immune checkpoint and inflammatory gene expression profiling [PDCD1 (PD-1), CD274 (PD-L1), HAVCR2 (TIM3), CTLA4, LG3, ICOS, IFNG, FOXP3, and IL10] were performed. Clustering analysis divided CTCL skin samples into two groups based on low/decreased versus increased/high expression of gene sets associated with immune checkpoints and inflammation. Advanced stages (T3/T4) displayed higher gene expression for immune checkpoints and inflammation than did T1/T2 stage patients or healthy controls. In addition, the expression profiles corroborated our phenotypic analyses of immune checkpoint receptors by flow cytometry. For mRNA transcripts of IFNG, FOXP3, and IL10, higher expression correlated with advanced T3/T4 stage disease (23).
Immune checkpoint expression by CD3+CD4+ and CD3+CD8+ T cells. 

A, Percentage of CD3+CD4+ epidermal cells stained with the indicated immune checkpoint markers. 

B, Percentage of CD3+CD4+ dermal cells stained with the indicated immune checkpoint markers. 

C, Percentage of CD3+CD8+ epidermal cells stained with the indicated immune checkpoint markers. 

D, Percentage of CD3+CD8+ dermal cells stained with the indicated immune checkpoint markers. 

C, controls; T1, patches/plaques <10% of BSA; T2, patches/plaques >10% of BSA; T3, >1 skin tumor; and T4, erythroderma. Diamonds indicate the mean value; boxes span the 25th–75th percentile (interquartile range, IQR); whiskers indicate the highest/lowest data point within 1.5 IQR from the box. Any remaining data points are shown as circles. The Kruskal–Wallis test determined the indicated P values, which compare healthy control skin versus all tumor stages combined. There were too few samples from patients with T3 or T4 disease to discern true differences between tumor stages. See also Table 2. 

Representative data from 3 independent experiments are shown.
Multicolor immunohistochemistry of CTCL lesions

Immunohistochemistry (IHC) of intact FFPE sections (Supplementary Fig. S2) revealed the expected epidermotropic and dense dermal atypical lymphoid infiltrate interspersed with morphologic histiocyte-type cells that include some inflammatory DCs. The atypical lymphocytes were CD3+CD4+/CD8/CD7. Multicolor IHC of a representative patient with plaques (T2) and another patient with tumor lesion (T3) demonstrated colocalization of PD-1 with CD3, CD4, and CD8 expression (Figs. 4 and 5). As the atypical CTCL lymphocytes lacked CD8, the observed CD8+ T cells, by exclusion, must represent infiltrating and potentially reactive CTLs expressing the inhibitory PD-1 epitope. Infiltrates in tumor lesions (T3) expressed more PD-1 and PD-L1 than did infiltrates in plaque (T2) disease (200× magnification; insets represent 400× magnification of small regions of multiplex images).

Discussion

This study reports the immunophenotype of primary T cells and DC subsets isolated directly from 1 to 1.5 cm² shave biopsies of lesional skin from CTCL patients and compared with the...
immunophenotypes of T-cell and DC emigrés from healthy skin. Given the epidermotrophic nature of CTCL, skin explant cultures of separated epidermal and dermal sheets (17, 18) permitted characterization of T-cell and DC emigrés from each skin layer for phenotypic and molecular analyses. We evaluated patients according to tumor staging (T1, T2, T3, and T4), but the few samples from patients with the most advanced disease necessitated immunophenotypic comparisons of healthy skin versus skin from all CTCL stages combined. In contrast, gene expression profiling demonstrated positive correlations between increased checkpoint inhibitor expression and higher disease stage.

Other investigators have used skin explant cultures of epidermal sheets and dermis (17, 18). Here we characterized primary immune cells of CTCL in lesional skin. Our approach avoids problems caused by poor enzymatic digestion of the cutaneous extracellular matrix, long-term culture, or use of cell lines derived from malignant, epidermotrophic CD4+ T cells. Our skin explant cultures require mechanical manipulation, which activates DC subtypes as emigrés into the culture medium. We used 10% pooled normal human serum and limited exposure to approximately 36 hours, thus avoiding exposure to fetal calf serum or cytokines and bystander activation of DCs that could stimulate T cells against xenogeneic peptides. The DC and T-cell crawlouts we analyzed remained close to their in situ state, thus facilitating phenotypic characterization by flow cytometry and better phenotypic characterization than feasible with use of immunohistochemistry of tissue sections. Because the cell populations stained with monoclonal antibodies to immune checkpoint

Figure 3.
Unsupervised clustering heat map of genome-wide mRNA expression profiles, using skin samples from 49 MF/SS patients and 3 healthy individuals. A, The unsupervised hierarchical clustering analysis identified a distinctive mRNA expression pattern for early-stage MF (T1/T2) compared with advanced-stage MF/SS (T3/T4) samples, and both groups are different from healthy skin. Data shown are log-transformed expression values relative to the mean expression level across all samples of 6,418 genes (see Materials and Methods for details). Genes in rows and samples in columns were both hierarchically clustered, while samples were color coded by sample type. The following sample categories were assigned by color: healthy control (light blue), T1 stage (blue), T2 stage (black), T3 stage (ochre), and T4 stage (yellow). The GO analysis identified five clusters. The GO annotation for each cluster of genes shows their related functions and were associated with (i) an immune/inflammatory response (T-cell activation and dysregulation/exhaustion) signature, (ii) epidermis and keratinocyte development, (iii) extracellular matrix and cell migration regulation, (iv) neutrophil degranulation and negative regulation of transcription, and (v) innate immune response. B, To elucidate whether MF/SS display a distinctive exhaustion-related marker expression profile compared with healthy skin, we performed a supervised analysis following the previously mentioned criteria. Immune checkpoint and inflammatory gene expression profiling [PDCD1 (PD-1), CD274 (PD-L1), HAVCR2 (TIM3), CTLA4, LAG3, ICOS, IFNG, FOXP3, and IL10] was performed. Clustering analysis divided CTCL skin samples into two groups based on decreased/inactivated and increased/highly activated expression of gene sets associated with immune checkpoint inhibition. Advanced stages (T3/T4) displayed higher expression of immune checkpoint and inflammation genes than did T1/T2 stage patients or healthy controls. One independent experiment was performed with 45 CTCL patients and three healthy controls.
markers are compared against isotype-matched (or unstained, in the case of DCs) controls, determining positive expression is straightforward and avoids the challenges of interpreting immuno histochemical staining of tissue (15).

Although the pattern of immune checkpoint epitope expression of epidermal T cells approximated that of dermal T cells, the disease-associated increase was not as pronounced in the dermis. This finding may reflect a mixture of malignant and nonmalignant cells in the dermal compartment, but it is most compatible with the epidermotropic nature of CTCL. The gene expression data corroborate the association between increased expression of checkpoint inhibitors and more advanced tumor stage.

External antigens in the epidermis may also increase expression of the exhausted phenotype. Studies have not yet established a causal link, however, between the exhausted phenotype we report here and any specific antigen(s) driving chronic overstimulation, clonal expansion, and malignant transformation (24). Newer molecular methods, including high-throughput sequencing modalities, are revolutionizing our understanding of the microbiome and its relationship with disease and clinical treatment outcomes (25, 26). Such approaches may clarify why CTCL lesions have a high number of exhausted T cells, given that the skin has typical numbers of potent antigen-presenting and immunostimulatory DCs. The process of exhaustion may also involve increasing mutational load, as reported for CTCL, characterized by somatic mutations in genes involved in T-cell signaling, activation, and apoptosis, as well as chromatin remodeling and responses to DNA damage (27). Increasing mutational load generates multiple tumor neoantigens, which can in turn exacerbate T-cell exhaustion, rendering any CD8+ antitumor CTLs ineffective without the addition of immune checkpoint blockade.

A limitation to progress in CTCL is the rarity of the disorder. Obtaining skin biopsies of CTCL lesions from outpatients is feasible only under local anesthesia and yields only small specimens. Although the flow cytometry–based assessment of primary T-cell and DC crawlouts permits concurrent analysis of multiple markers, the limited cell numbers derived from the skin biopsies in this study precluded complementary functional assays of T-cell responsiveness. Another challenge is that apart from atypical morphology and the usual loss of CD7, there is no pathognomonic epitope that can distinguish malignant CD4+ T cells from benign CD4+ T cells in the infiltrate. Single-cell RNA sequencing focused on TCR α/β VDJ rearrangements with additional phenotypic markers may provide a strategy to identify clonal T cells in conjunction with phenotypic investigations (28–31). Nevertheless, our immunophenotypic and gene expression profile findings represent advances in understanding the pathophysiology of this uncommon lymphoma. These results provide a rationale to identify culprit antigens in an altered cutaneous microbiome driving T-cell exhaustion and to evaluate immune checkpoint inhibition to reverse T-cell exhaustion earlier in the treatment of this disease. We anticipate that this work will therefore facilitate the mechanistic discovery and development of more effective therapies for CTCL.

Figure 4.
Histologic and immunophenotypic features of CTCL plaque, with 6-color multiplex immunohistochemistry for immune checkpoint expression. Multiplex images performed on skin sections of MF lesion from a representative patient with plaques (T2) are shown. PD-1 colocalizes with CD3, CD4, and CD8 expression. PD-1 and PD-L1 expressions do not overlap (200× magnification; insets, 400× magnification of small regions of multiplex images). Representative data from 3 independent experiments are shown.
Disclosure of Potential Conflicts of Interest

S. Horwitz reports receiving commercial research funding from Celgene, Millennium/Takeda, Seattle Genetics, Forty-Seven, Kyowa-Hakka-Kirin, Infinity/Verastem, ADCT Therapeutics, Aileron Therapeutics, and Trillium, and is a consultant/advisory board member for Millennium/Takeda, Kyowa-Hakka-Kirin, Seattle Genetics, Forty-Seven, Mundipharma, Aileron, ADCT, Portola, and Innate Pharma. A. Moskowitz reports receiving commercial research funding from Bristol-Myers Squibb, Merck, Seattle Genetics, and Incyte; has received speakers bureau honoraria from Seattle Genetics; and is a consultant/advisory board member for Seattle Genetics. No potential conflicts of interest were disclosed by the other authors.

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Writing, review, and/or revision of the manuscript: C. Querfeld, P.L. Myksowski, S.A. Curran, D.A. Goldman, X. Wu, S.H. Kil, S. Sharma, K.J. Finn, S. Horwitz, A. Moskowitz, S.T. Rosen, J.W. Young
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Querfeld, S. Leung, S. Sharma, K.J. Finn, J.W. Young
Study supervision: C. Querfeld, A.C. Halpern, J.W. Young

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References

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