
T-cell clonality assessment by next-generation sequencing improves detection sensitivity in mycosis fungoides

Kari E. Sufficool, MD,^a Christina M. Lockwood, PhD,^a Haley J. Abel, PhD,^a Ian S. Hagemann, MD, PhD,^a Jonathan A. Schumacher, MS,^b Todd W. Kelley, MD,^c and Eric J. Duncavage, MD^a
Saint Louis, Missouri, and Salt Lake City, Utah

Background: T-cell receptor (TCR) clonality assessment is a principal diagnostic test in the management of mycosis fungoides (MF). However, current polymerase chain reaction–based methods may produce ambiguous results, often because of low abundance of clonal T lymphocytes, resulting in weak clonal peaks that cannot be size-resolved by contemporary capillary electrophoresis (CE).

Objective: We sought to determine if next-generation sequencing (NGS)-based detection has increased sensitivity for T-cell clonality over CE-based detection in MF.

Methods: Clonality was determined by an NGS-based method in which the *TCR-γ* variable region was polymerase chain reaction amplified and the products sequenced to establish the identity of rearranged variable and joining regions.

Results: Of the 35 MF cases tested, 29 (85%) showed a clonal T-cell rearrangement by NGS, compared with 15 (44%) by standard CE detection. Three patients with MF had follow-up testing that showed identical, clonal TCR sequences in subsequent skin biopsy specimens.

Limitations: Clonal T-cell populations have been described in benign conditions; evidence of clonality alone, by any method, is not sufficient for diagnosis.

Conclusion: TCR clonality assessment by NGS has superior sensitivity compared with CE-based detection. Further, NGS enables tracking of specific clones across multiple time points for more accurate identification of recurrent MF. (J Am Acad Dermatol 2015;73:228-36.)

Key words: cutaneous T-cell lymphoma; molecular diagnostics; mycosis fungoides; next-generation sequencing; T-cell clonality; T-cell receptor rearrangement.

Mycosis fungoides (MF) is a cutaneous T-cell lymphoma (CTCL) that accounts for almost 50% of all CTCL, and its incidence in the United States appears to be increasing.¹⁻³ Classic MF commonly arises in late adulthood, has a male predominance, and shows an indolent course, progressing over years to decades.^{1,4-6} Early patch

lesions show a superficial lichenoid infiltrate of lymphocytes and histiocytes.⁷ However, a predominance of reactive lymphocytes and lack of cytologic atypia in initial biopsy specimens may resemble inflammatory skin diseases, confounding the diagnosis.⁸⁻¹⁰ Lesional cells are small to medium with cerebriform nuclei that colonize the basal layer

From the Washington University School of Medicine, Saint Louis^a; ARUP Laboratories, Salt Lake City^b; and University of Utah School of Medicine.^c

This project was funded by the Washington University Department of Pathology and Immunology.

Conflicts of interest: None declared.

Presented in poster format at the United States and Canadian Academy of Pathology Annual Meeting held in San Diego, CA on March 3, 2014.

Accepted for publication April 16, 2015.

Reprints not available from the authors.

Correspondence to: Eric J. Duncavage, MD, Department of Pathology and Immunology, Washington University School of Medicine, 660 S Euclid Ave, Campus Box 8118, Saint Louis, MO 63110. E-mail: EDuncavage@path.wustl.edu.

Published online June 3, 2015.

0190-9622

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<http://dx.doi.org/10.1016/j.jaad.2015.04.030>

of the epidermis often as haloed cells.⁶ In plaque stage, this epidermotropism is more pronounced, and intraepidermal collections of atypical cells (Pautrier microabscesses) are a characteristic feature, but are observed in a minority of cases.¹¹ Serial biopsy specimens are often needed to render a definitive diagnosis in early patch and plaque stages.^{12,13} In tumor stage, the infiltrate becomes more diffuse and tumor cells increase in both number and size.⁵

Immunophenotypically, the CD3⁺ T lymphocytes express a T-cell helper phenotype (CD4⁺) and an elevated CD4:CD8 ratio is often seen.¹⁴⁻¹⁸ These cells also express CD45RO.¹⁹ There is variable expression of pan T-cell markers CD2, CD5, and CD7; loss of CD2 and CD5 by T cells can be a helpful finding in diagnosing CTCL and supports a diagnosis of lymphoma.²⁰

T-cell receptor (TCR) gene rearrangement studies can be highly useful in the diagnosis and monitoring of MF,²¹ in that the specific antigen receptor on the cell surface serves as a marker of neoplastic clonal transformation.²² TCR molecules are formed as heterodimers composed of either α and β chains (majority of cases) or γ and δ chains. Each of these TCR chains is encoded by distinct DNA elements known as variable (V), joining (J), diversity (D), and constant (C) gene segments. *TCR- γ* gene rearrangement and expression is thought to occur earliest in development, followed closely by *TCR- δ* and *TCR- β* gene rearrangement.^{22,23}

As most T cells harbor α/β chains, we will focus on the details of this rearrangement. During development, the TCR- β chains first undergo a sequence of ordered recombination events: D β -to-J β recombination occurs first, followed by V β -to-D β J β rearrangements, and, lastly, all gene segments between the V β -D β -J β gene segments in the newly formed complex are deleted.²³ A primary transcript is then synthesized that incorporates the C domain gene (V β -D β -J β -C β). Once a productive β chain is achieved, the *TCR- α* gene undergoes rearrangement characterized by V α -J α recombination.²³ Finally, messenger RNA transcription splices out any intervening sequence and allows translation of

a full-length protein for the α/β TCR chain.²³ In the end, each T cell will have multiple rearranged TCR loci, not all of which are productive.

TCR sequences are effectively unique to an individual T lymphocyte because of the immense diversity created through V(D)J recombination.^{22,24}

Thus, clonal expansions of T cells will result in overrepresentation of a sequence compared with the background sequence diversity. The resulting TCR gene rearrangement is a unique “fingerprint” for a particular T cell.^{22,24} It bears mentioning that the evidence of TCR clonality is not entirely synonymous for malignancy and may be seen in reactive or autoimmune conditions.

The most widely used method for detection of TCR rearrangements in the clinical laboratory is polymerase chain reaction (PCR) coupled with capillary electrophoresis (CE). Although any TCR chain (α , β , γ , or δ) could, in principle, be used for clonality assessment, the *TCR- γ* locus is preferred over other loci because it is rearranged early in T-cell development both in α/β and γ/δ T cells, can be PCR amplified using widely available primers sequences,^{25,26} and is retained even if the protein is not expressed on mature T lymphocytes.²⁷ The γ gene structure is also simpler as it has 12 variable segments; therefore, fewer primer sets are needed to detect most of the possible gene rearrangements. Current CE methodology relies on 3 main steps: (1) extraction of genomic DNA from the skin specimen; (2) multiplex PCR amplification using standard primer sets; and (3) detection by electrophoresis to separate PCR products according to relative differences in amplicon size.²⁷ Although quantitative rules are not strictly followed, clonality is generally defined as the existence of peaks with greater than 2-fold signal intensity above the gaussian background (Fig 1).²⁸ Although simple in concept, CE-based detection has several shortcomings. First, interpretation is subjective, especially in cases where multiple clonal peaks are present (biclonal or oligoclonal). Second, because CE only measures PCR product size as a surrogate for TCR sequence, it is possible that a monoclonal peak represents a mixture of different, nonclonal sequences that share the same size, leading to a false-positive result.²⁹

CAPSULE SUMMARY

- Mycosis fungoides often poses a diagnostic challenge by morphology, requiring correlation with T-cell clonality assessment.
- Next-generation sequencing-based methods for T-cell clonality have superior diagnostic sensitivity compared with capillary electrophoresis-based methods in mycosis fungoides.
- Next-generation sequencing-based methods also allow the tracking of specific clones when monitoring for disease recurrence.

Abbreviations used:

CE:	capillary electrophoresis
CTCL:	cutaneous T-cell lymphoma
MF:	mycosis fungoides
NGS:	next-generation sequencing
PCR:	polymerase chain reaction
TCR:	T-cell receptor

Third, for recurrent cases of MF, it is often difficult to compare clonal products between primary diagnostic samples and recurrences because of technical differences in the assay, including CE run-to-run variability, especially if testing is performed in different clinical laboratories.

In contrast, next-generation sequencing (NGS)-based methods have the advantage of identifying the exact clonal sequence and its relative abundance in a given sample (Fig 2). Several studies have shown that NGS is capable of identifying TCR rearrangement from formalin-fixed skin tissues.^{29,30} Further, such assays can be used to monitor disease recurrence (Fig 3).³¹ In the current study, we sought to determine if NGS-based TCR-clonality detection methods had increased sensitivity over CE-based detection in MF.

METHODS

Approval for this study was granted by the Washington University School of Medicine Institutional Review Board (October 2011; no. 2011-02311). A 10-year archival review of DNA samples extracted from formalin-fixed, paraffin-embedded skin tissue at the Barnes-Jewish Hospital Molecular Diagnostics Laboratory, Saint Louis, MO, yielded 34 cases with morphologic features of MF diagnosed by standard histologic and immunohistochemical assessment (Fig 4). Specifically, these consecutive skin cases had DNA previously sent to our clinical molecular laboratory for TCR clonality assessment and had at least 400 ng remaining for study; all cases had a morphologically confirmed diagnoses of MF rendered by subspecialty board-certified dermatopathologist. Of the 34 cases, 15 were clonal by CE and 19 were polyclonal/oligoclonal by CE based on clinical TCR clonality studies reported by a CAP-/CLIA-certified laboratory using BIOMED-2 TCR- γ primers.²⁶ The clinical characteristics of the 2 cohorts are outlined in Table I.

DNA aliquots from the above 34 cases were subsequently investigated via NGS-based clonality assessment. The TCR- γ locus was first PCR amplified using consensus primers that targeted V γ 2 to V γ 11 and JP1, JP, J1, JP2, and J2 in a single multiplex reaction.^{25,30} Each PCR reaction contained 100 ng of

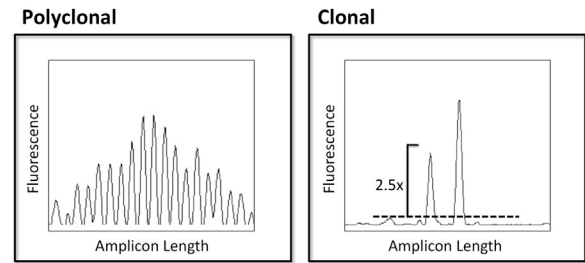


Fig 1. Capillary electrophoresis (CE) relies on relative differences in polymerase chain reaction product size. In CE-based T-cell receptor (TCR) clonality testing the distribution of amplicon lengths is used to infer clonality. Normal, polyclonal T cells consist of a mixture of rearranged TCRs of various sizes that approximate a gaussian distribution (*left*). Clonal TCR rearrangements can be detected when the distribution of TCR amplicons is restricted, showing only 1 or 2 peaks that are generally twice the amplitude of the background (*right*).

genomic DNA, 1 \times HF buffer (New England Biolabs, Ipswich, MA), 0.2 mmol/L of dNTPs (New England Biolabs), 0.6 μ mol/L of each primer (Integrated DNA Technologies, Coralville, IA) (Supplemental Table I, available at <http://www.jaad.org>), and 2 U of Phusion HS DNA polymerase (New England Biolabs) in a final volume of 20 μ L. Specimens were amplified on an Applied Biosystems (Foster City, CA) 9700 thermal cycler using the following cycling conditions: 98 $^{\circ}$ C (30 seconds) followed by 30 cycles of 98 $^{\circ}$ C (10 seconds), 60 $^{\circ}$ C (30 seconds), and 72 $^{\circ}$ C (10 seconds plus 1 additional second per cycle). Quadruplicate PCR reactions were pooled and column-purified using the MiniElute PCR purification kit (Qiagen Inc, Valencia, CA), following the manufacturer's instructions, with a final elution volume of 30 μ L. Concentrations were determined using a Nanodrop spectrophotometer (Thermo Scientific).

PCR products were made into sequencing libraries (Illumina Inc, San Diego, CA) by ligation of indexed adapters. Purified libraries were mixed in equimolar ratios and directly sequenced in pools of 10 to 12 cases on MiSeq (Illumina Inc) using 2 \times 150 base pair paired-end reads. To offset possible redundant sequences occurring early in the read cycle that may interfere with instrument phasing, 20% (by mass) PhiX DNA was added to the pooled libraries. Merged contigs (contig, a set of overlapping DNA sequences) were created based on the overlapping areas in each pair using the EA utilities fastq-join function with default parameters.³¹ Contigs were then counted to determine the most frequent sequence using the FASTX toolkit collapse function.³² To account for sequencing and PCR errors, TCR sequences with fewer than 10 supporting

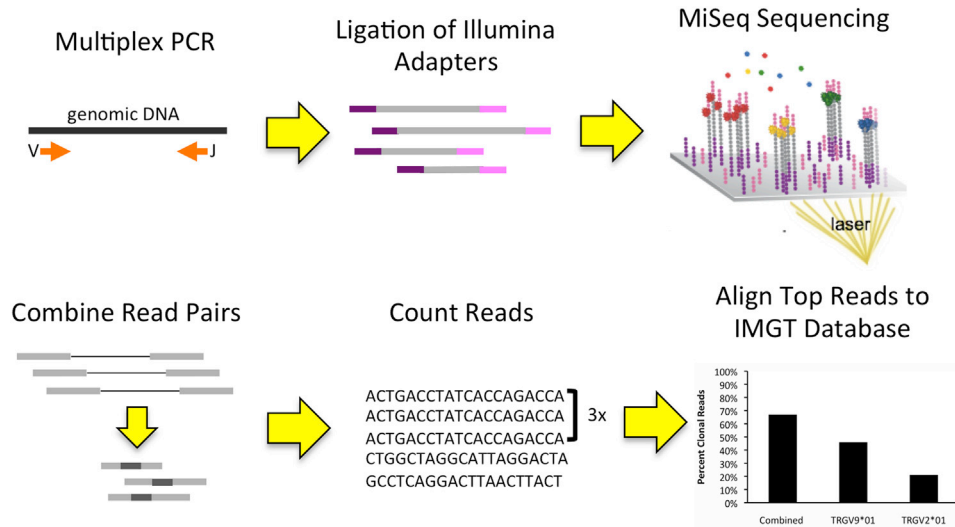


Fig 2. Next-generation sequencing (NGS)-based T-cell clonality testing. Rather than relying on the size of T-cell receptor (TCR) region amplicons as a surrogate for TCR sequence, NGS-based detection methods sequence V and J regions and compare the distribution of unique sequences. In our analysis method DNA (*black*) was first amplified using a common set of publicly available TCR V and J region primers (*orange*). Amplicons (*gray*) were then purified and made into libraries (Illumina Inc, San Diego, CA) by ligation of sequencer motifs (*pink*) and sample specific indexes (*purple*), allowing samples to be pooled and sequenced in multiplex. Pooled libraries were then sequenced on a MiSeq (Illumina Inc) instrument using 2 × 150 base pair reads. Paired end reads were then collapsed into contigs based on the overlap between the read pairs (*dark gray*). Resulting unique sequences were counted to determine the relative abundance of top-50 most common V region sequences relative to the total number of sequences. The top-50 sequences were aligned to the IMGT database to determine the identity of V and J region sequences. Similar to capillary electrophoresis-based methods, if the most frequent 2 TCR sequences accounted for over 5% of the total reads the case was called “clonal.” PCR, Polymerase chain reaction.

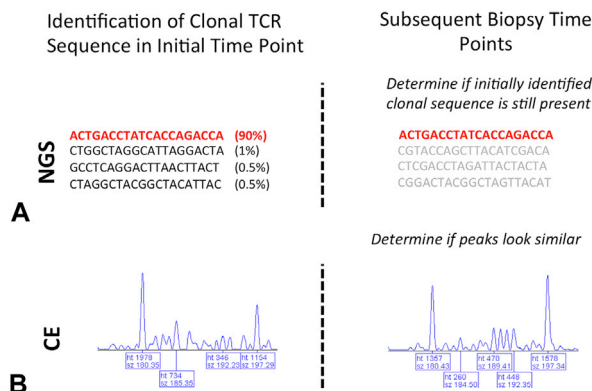


Fig 3. Recurrence detection by next-generation sequencing (NGS). **A**, For cases with multiple biopsy time points, the identity of the clonal T-cell receptor (TCR) sequence was first established on the initial biopsy specimen using the NGS-based method. **B**, Subsequent follow-up biopsy specimens were then analyzed to determine if the same clonal sequence could be identified. In contrast, standard capillary electrophoresis (CE)-based methods cannot definitively establish a clonal relationship between specimens taken at multiple time points as the assay only measures the size of the amplicon and not the actual amplicon sequence. HT, Height; sz, size.

contigs were discarded. The top-50 most common sequences in each case were aligned to the ImmunoGenetics/IMGT database³³ to identify the V and J regions and assess rearrangement productivity. Because each T cell will have 2 rearranged TCR-γ loci, of which up to 1 may be productive, the 2 most common sequences (ie, TCR VJ regions) were analyzed. Clonal cases were defined as those in which the 2 most common TCR VJ regions accounted for greater than 5% of total high-quality reads. This threshold for clonality is comparable with the traditional 2-fold greater signal in CE-based methods compared with that identified in nonclonal dermatoses.²⁵

RESULTS

An average of 886,111 paired end reads (range 461 000-4 800 000) were obtained per case, of which an average of 732,695 were deemed high-quality and represented an average of 5347 unique TCR sequences. Depth of coverage was nearly 2 million reads per case or 500 000 contigs. Of these thousands of unique sequences, the combination of the 2 most

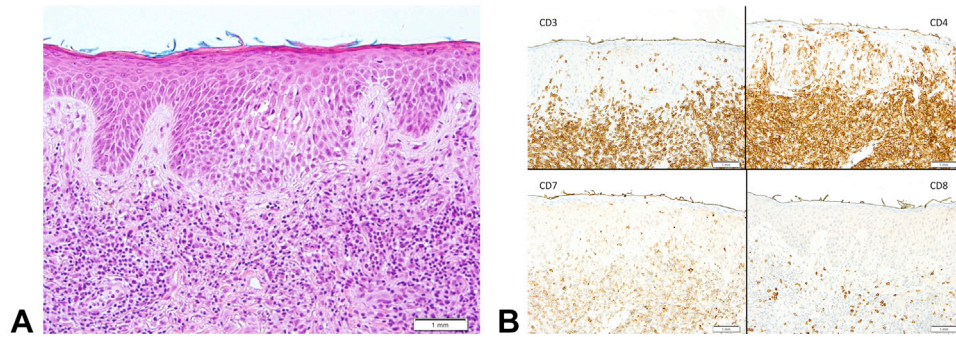


Fig 4. Mycosis fungoides. **A**, Representative skin biopsy specimen characterized by a lymphocytic infiltrate composed of small to medium atypical cerebriform cells demonstrating epidermotropism. Haloed cells are notable within the epidermis. (Hematoxylin-eosin stain; original magnification: $\times 20$.) **B**, Lymphocytes were immunoreactive for CD2, CD3, and CD5, with reduced CD7 positivity. As CD4 also stains Langerhans cells in the epidermis, there are more CD4⁺ cells than CD3⁺ cells in the epidermis. Because of this, it is important to compare CD3 and CD8 when examining the epidermal compartment. The CD3⁺CD8⁻ cells likely correspond to CD4⁺ T cells. CD4 expression was greater than that of CD8. (Immunohistochemistry, original magnification: $\times 20$.)

Table I. Clinical characteristics

	MF with positive TCR rearrangement by CE n = 15	MF with negative TCR rearrangement by CE n = 19
Mean age, y (range)	57.2 (37-76)	57.8 (49-74)
Gender		
Male	8/15 (53%)	6/19 (32%)
Female	7/15 (47%)	11/19 (68%)
Race		
Caucasian	11/15 (73%)	11/19 (58%)
African American	4/15 (27%)	8/19 (42%)
Location of lesion		
Trunk	12	7
Upper extremity	1	6
Lower extremity	1	2
Head and neck	1	4
Histologic stage		
Patch	4 (27%)	6 (32%)
Plaque	8 (53%)	8 (42%)
Tumor	3 (20%)	5 (26%)

CE, Capillary electrophoresis; MF, mycosis fungoides; TCR, T-cell receptor.

common TCR sequences in each case represented a mean of 28% (range 0.8%-80.3%) of all high-quality reads. *TCR- γ* V γ 10 was the most frequently used V region in 14 of 34 cases (41%) (Supplemental Table II, available at <http://www.jaad.org>). With a clonality cutoff of 5% by NGS (ie, 5% of the total *TCR- γ* reads coming from the 2 most common sequences), 13 of 15 (86%) CE clonal cases (as determined in a CAP/CLIA laboratory) were deemed clonal by NGS, and 16 of 19 (84%) CE polyclonal/oligoclonal cases

(as determined in a CAP/CLIA laboratory) were deemed clonal by NGS. There were 2 cases in which CE was positive for TCR rearrangement and NGS was negative. In both cases, the most common reads were 2.5% or 2.4% of total reads, suggesting that a cutoff lower than 5% may increase sensitivity. Using histology as a gold standard, the overall sensitivity was 85% for TCR analysis by NGS (29 of 34 cases, 95% CI 0.70-0.94) and 44% for TCR analysis by conventional CE (15 of 34 cases, 95% CI 0.29-0.60). It is unclear what the specificity of this method is as we did not sequence reactive T-cell conditions. Case examples are highlighted in Fig 5.

Of positive cases by the NGS method, 3 patients had subsequent biopsy specimens for comparison, ranging from 1 to 4 years apart, showing identical *TCR- γ* sequences by NGS (Fig 6). The first patient initially presented with an arm lesion harboring a T-cell receptor gamma, variable region (TRGV)3*01 rearrangement (clonal reads = 9.63%) that was detectable 1 year later within a new abdominal lesion (clonal reads = 5.57%). Patient 2 had a facial lesion with *TCR- γ* variable region 10*01 (V10*01) rearrangement (clonal reads = 74.0%), which was subsequently identified 2 years later in a new thigh lesion (clonal reads = 38.7%). Review of serial biopsy specimens from a third patient, 4 years apart, showed detectable TRGV10*01 rearrangements from 2 separate sites on the upper extremity (clonal reads = 64.1% and 40.9%, respectively). For all 3 cases, the percent clonal reads were higher in initial skin biopsy specimens than in the follow-up skin samples.

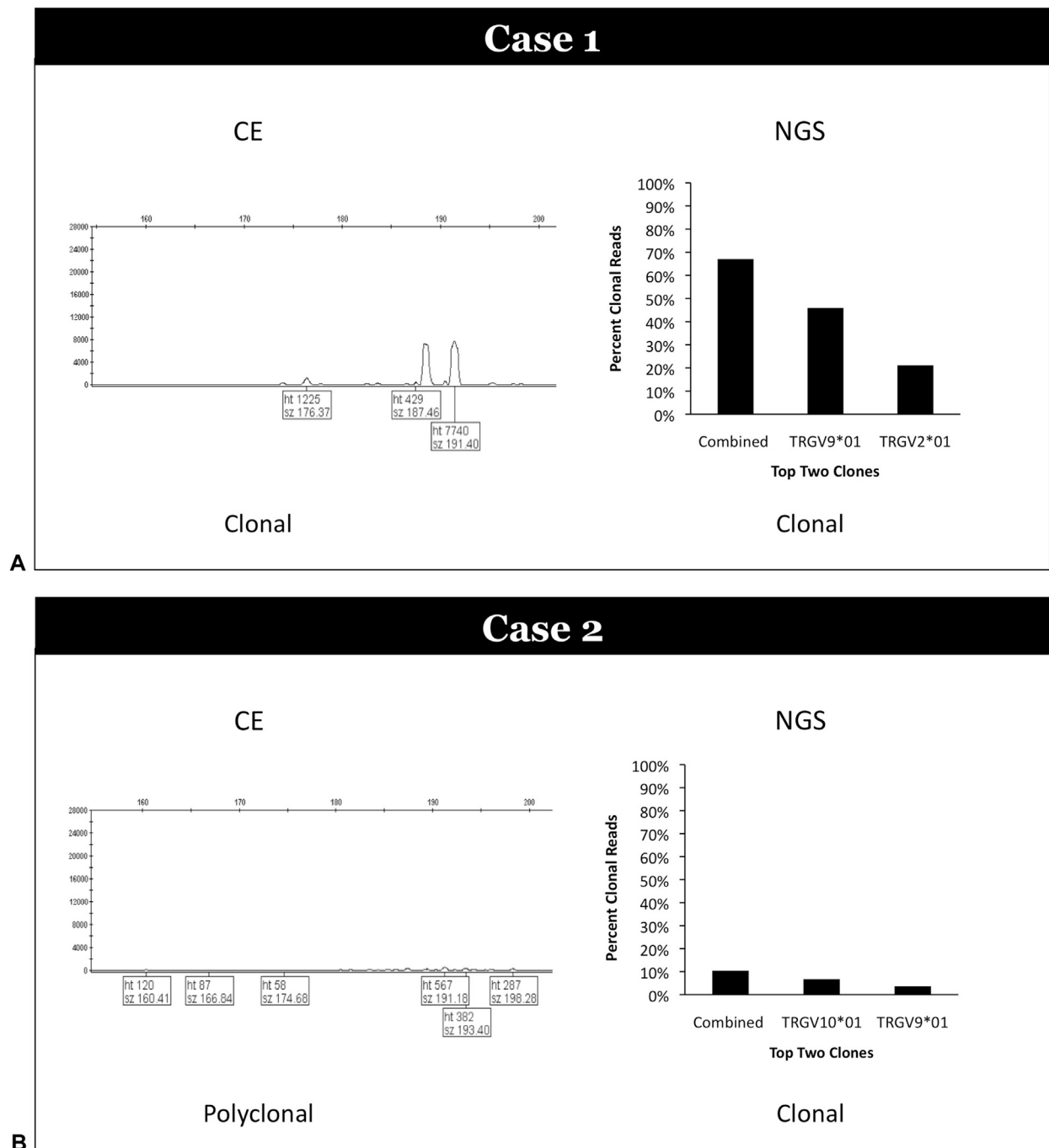


Fig 5. Case examples. Comparison of capillary electrophoresis (CE)- versus next-generation sequencing (NGS)-based T-cell receptor- γ results in a case deemed clonal by both CE and NGS (A) and a case deemed polyclonal/oligoclonal by CE but clonal by NGS (B). HT, Height; sz, size; TRGV, T-cell receptor gamma, variable region.

To determine if there was a correlation between clonal V region identity and site or stage (patch, plaque, or tumor) we performed a Fisher exact test. There was no apparent correlation between stage and V region ($P = .34$) and no apparent correlation between V region and site.

Limitations

The current investigation is a retrospective cohort study with limited sample size of DNA aliquots from the molecular laboratory archives. In addition, there are limitations inherent to T-cell clonality assessment: (1) tissue sampling is critically important

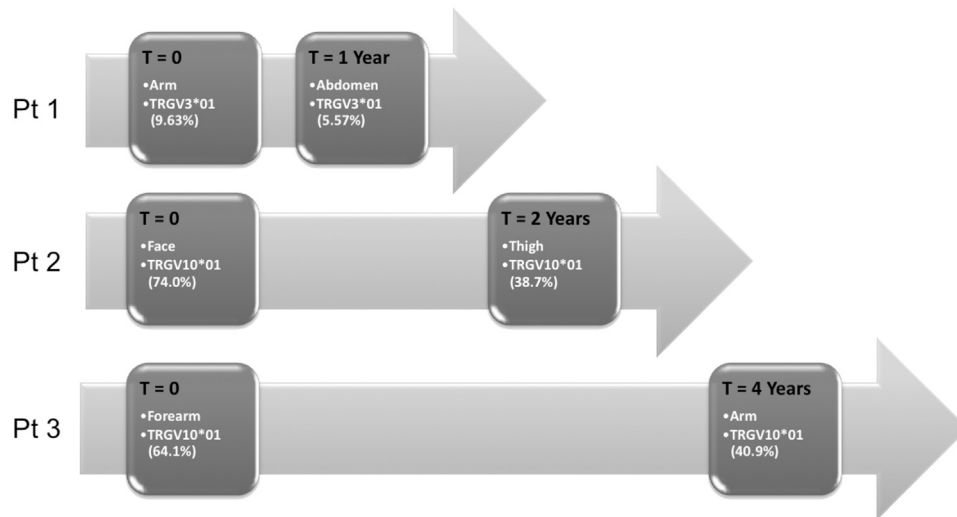


Fig 6. Next-generation sequencing (NGS)-based T-cell clonality testing is able to detect identical T-cell receptor (TCR) rearrangements in serial skin biopsy specimens. Of clonal cases, 3 patients (*Pt*) had follow-up skin sampling, ranging from 1 to 4 years apart, showing identical TCR clones. The percent clonal reads are noted in parentheses. Detection across multiple time points highlights a potential role for NGS-based TCR methods in minimal residual disease testing. *TRGV*, T-cell receptor gamma, variable region.

in the initial step of DNA extraction, as bias may be introduced if the area submitted from the paraffin-embedded skin is not representative of the disease process; (2) PCR bias may occur based on the size and guanine and cytosine content of the *TCR-γ* sequences in the sample; and (3) CE-based TCR assays are subject to interpretation because clonality is based on relative peak size. Although molecular studies support histologic diagnoses, the astute dermatologist should be mindful to interpret findings within the context of available clinical, histologic, and immunophenotypic data.

DISCUSSION

MF is a diagnostic challenge, with the currently available CE method for determining TCR clonality only offering 44% sensitivity in our study, when compared with the gold standard of histologic diagnosis. We investigated the use of a NGS-based detection method in improving the detection of TCR clonality, and thus of MF. The current study recognizes that benign/reactive dermatoses have been shown to harbor a low level of clonal lymphocytes, and therefore, a conservative threshold (>5%) for positive clones was established at twice this baseline (2.5%) to reduce the number of potential false-positive results.²⁵ An average of 5347 unique TCR sequences were detected in each case; the 2 most frequent TCR sequences in MF cases represented a mean of 28% of all reads (range 0.8%-80.3%). Using the proposed cutoff of greater

than 5% of TCR sequence clonality,²⁵ NGS has a sensitivity of 88% (95% CI 0.70-0.94) when compared with the gold standard of histologic diagnosis. We conclude that NGS-based detection methods for TCR clonality are a useful adjunct to histologic diagnosis, and are an improvement over current CE-based detection methods. NGS not only improves initial diagnosis of TCR clonality and MF, but may improve the detection of disease recurrence as well. Once a specific clonal TCR sequence is identified in the diagnostic specimen, it may be used as a tumor marker to track disease progression or monitor for minimal residual disease.

Our results are comparable with other studies that have investigated NGS technology in the detection of and monitoring for CTCLs. NGS-based methods have been shown by other investigators to be able to detect clonal TCR rearrangements in formalin-fixed, paraffin-embedded biopsy specimens.²⁹ Moreover, the detection of identical clones from 2 different body sites has been shown to be quite specific for MF.³⁵ In hematopoietic malignancies (eg, acute lymphoblastic leukemia), others have demonstrated the use of NGS-based assays as highly sensitive measurements of disease burden.³⁴ Overall, TCR rearrangement in the assessment of minimal residual disease piques considerable interest; future studies are needed to elucidate its clinical use in this setting.

NGS-based methods can be easily incorporated into the daily practice of the dermatologist/dermatopathologist as they can be performed on

formalin-fixed, paraffin-embedded clinical skin tissue after routine histologic and immunohistochemical examination. NGS technology offers many exciting clinical applications: (1) as NGS determines the specific fingerprint of the TCR clone, it is more sensitive than conventional CE-based methods and could offer improved MF diagnosis while patients are in early patch and plaque stages; (2) once a clone is identified, the laboratory could query for the specific DNA sequence in subsequent skin biopsy specimens to identify low levels of disease that may have been missed by conventional methods; and (3) although further studies are needed, NGS may eventually guide targeted therapies based on detected molecular signatures.

Conclusion

NGS-based TCR analysis is a clinically relevant assay that has the potential to expand diagnostic accuracy, improve monitoring of disease recurrence, and enhance minimal residual disease testing in MF.

We would like to thank Jessica Hoisington-Lopez from the Center for Genome Sciences and Systems Biology in St. Louis, MO for her sequencing expertise.

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Supplemental Table I. Polymerase chain reaction primers used

J γ P1/2:	AAG TTA CTA TGA GCY TAG TCC CTT
J γ P:	AAG CTT TGT TCC GGG ACC AAA TAC
J γ 1/2:	TAC CTG TGA CAA CAA GTG TTG TTC
V γ 11:	GGC TCA AGA TTG CTC AGG TGG
V γ 10:	AAT CCG CAG CTC GAC GCA GCA
V γ 9:	ACG GCA CTG TCA GAA AGG AAT C
V γ 3:	CCG CAA GGG ATG TGT TGG AAT CA
V γ 2:	ACT CCA GGG TTG TGT TGG AAT CA

Supplemental Table II. T-cell receptor clonality results by capillary electrophoresis compared with next-generation sequencing

Case	Age, y	Sex	Race	Location	Histologic stage	Clonal/total reads	CE result	NGS result	Clonal 1 ID	Clonal 2 ID
1	59	M	Caucasian	Thigh	Patch	38.7%	Clonal	Clonal	TRGV10*01 or TRGV10*02	TRGV9*01
2	61	F	Caucasian	Flank	Plaque	67.5%	Clonal	Clonal	TRGV2*02	TRGV8*01
3*	62	F	Caucasian	Back	Tumor	2.5%	Clonal	Polyclonal/oligoclonal	TRGV2*02	TRGV2*02
4	61	M	African American	Chest	Tumor	33.6%	Clonal	Clonal	TRGV10*01 or TRGV10*02	TRGV9*01
5	37	F	African American	Back	Plaque	11.6%	Clonal	Clonal	TRGV2*02	TRGV2*01
6	37	F	African American	Back	Plaque	11.6%	Clonal	Clonal	TRGV2*02	TRGV2*01
7*	48	M	Caucasian	Abdomen	Patch	2.4%	Clonal	Polyclonal/oligoclonal	TRGV8*01	TRGV4*01
8	62	M	Caucasian	Chest	Plaque	24.0%	Clonal	Clonal	TRGV10*01 or TRGV10*02	TRGV9*01
9	62	M	Caucasian	Chest	Patch	41.8%	Clonal	Clonal	TRGV10*01 or TRGV10*02	TRGV2*01
10	56	F	African American	Chest	Tumor	67.0%	Clonal	Clonal	TRGV9*01	TRGV2*01
11	67	M	Caucasian	Forearm	Plaque	45.0%	Clonal	Clonal	TRGV9*01	TRGV9*01
12	66	F	Caucasian	Buttock	Patch	24.1%	Clonal	Clonal	TRGV8*01	TRGV2*02
13	47	F	Caucasian	Breast	Plaque	43.3%	Clonal	Clonal	TRGV11*01	TRGV9*01
14	76	M	Caucasian	Back	Plaque	38.0%	Clonal	Clonal	TRGV8*01	TRGV3*01
15	57	M	Caucasian	Face	Plaque	74.0%	Clonal	Clonal	TRGV10*01 or TRGV10*02	TRGV9*01
16	55	M	African American	Back	Patch	10.4%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV9*01
17	58	F	Caucasian	Scalp	Patch	8.4%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV10*01 or TRGV10*02
18	49	F	Caucasian	Abdomen	Plaque	15.6%	Polyclonal/oligoclonal	Clonal	TRGV8*01	TRGV3*01
19	57	F	African American	Arm	Tumor	44.3%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV2*02
20	74	F	Caucasian	Face	Tumor	40.9%	Polyclonal/oligoclonal	Clonal	TRGV9*01	TRGV9*01
21*	56	F	African American	Face	Tumor	0.8%	Polyclonal/oligoclonal	Polyclonal/oligoclonal	TRGV10*01 or TRGV10*02	TRGV10*01 or TRGV10*02
22	74	F	Caucasian	Back	Plaque	10.1%	Polyclonal/oligoclonal	Clonal	TRGV4*01	TRGV8*01
23	72	M	Caucasian	Flank	Patch	9.6%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV10*01 or TRGV10*02
24	62	M	Caucasian	Forearm	Plaque	27.0%	Polyclonal/oligoclonal	Clonal	TRGV9*01	TRGV2*02
25	62	M	Caucasian	Arm	Patch	21.0%	Polyclonal/oligoclonal	Clonal	TRGV9*02	TRGV3*01
26	74	M	Caucasian	Lower extremity	Patch	6.9%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV10*01 or TRGV10*02
27*	74	F	African American	Arm	Patch	1.0%	Polyclonal/oligoclonal	Polyclonal/oligoclonal	TRGV4*02	TRGV9*01
28	57	F	African American	Face	Plaque	9.6%	Polyclonal/oligoclonal	Clonal	TRGV3*01	TRGV3*01 or TRGV5*01
29	57	F	African American	Abdomen	Plaque	5.6%	Polyclonal/oligoclonal	Clonal	TRGV3*01	TRGV3*01 or TRGV5*01
30	52	F	African American	Forearm	Tumor	64.1%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV2*02
31	52	F	African American	Hand	Tumor	80.3%	Polyclonal/oligoclonal	Clonal	TRGV10*01 or TRGV10*02	TRGV10*01 or TRGV10*02
32	63	F	Caucasian	Abdomen	Plaque	29.8%	Polyclonal/oligoclonal	Clonal	TRGV4*02	TRGV8*01
33	63	F	Caucasian	Abdomen	Plaque	37.4%	Polyclonal/oligoclonal	Clonal	TRGV4*02	TRGV8*01
34*	62	M	Caucasian	Lower extremity	Plaque	0.8%	Polyclonal/oligoclonal	Polyclonal/oligoclonal	TRGV10*01 or TRGV10*02	TRGV10*01 or TRGV10*02

T-cell receptor variable identifications are provided for each of the 34 cases included in the analysis.

CE, Capillary electrophoresis; F, female; M, male; NGS, next-generation sequencing.

*Cases were not called clonal by NGS.