Table II. Cases of human males exposed to spironolactone in utero

Case no.	Maximum daily dose, mg	Results	Reference
1	400	Normal genitals at birth and puberty	Groves et al[7]
2	400	Normal genitals at birth	Groves et al[7]
3	200	Normal genitals at birth	Neerhof et al[8]
4	50	Normal genitals at birth	de Arriba et al[9]
5	25	Normal genitals at birth	Rigo et al[10]

identified 5 males exposed to spironolactone in utero without genital feminization, retrospective examination of patient databases may help us identify more cases. Additional human data showing an absence of feminization at acne doses would have a profound impact on how we counsel patients.

Given the limited number of animal studies and human cases in this study, there are insufficient data to safely argue that spironolactone does not have the potential to cause feminization of male offspring. However, with more data, this recommendation could change.

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High-throughput sequencing of the T-cell receptor β chain gene distinguishes 2 subgroups of cutaneous T-cell lymphoma

To the Editor: Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of T-cell neoplasms of the skin. Mycosis fungoides is an indolent form, and the leukemic variant, Sézary syndrome, portends a much worse prognosis. In current practice, the blood tumor burden is determined by morphologic assessment and enumeration of peripheral blood lymphocytes or quantification of $CD4^+CD26^-$ and $CD4^+CD7^-$ cells by flow cytometry.[1] These methods readily identify patients with advanced blood involvement but can be ambiguous in patients with earlier stage disease. Here, we used T-cell receptor (TCR) Vβ CDR3 high-throughput sequencing (HTS)

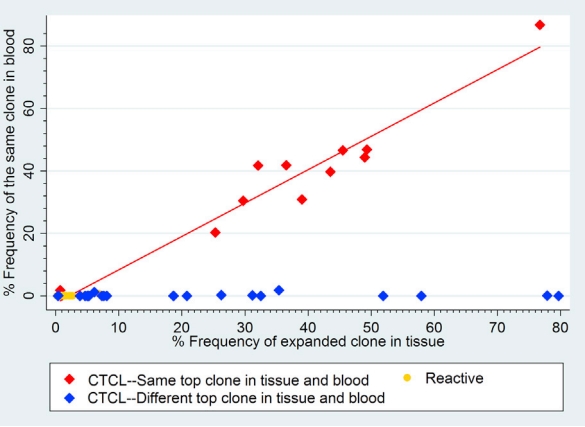

Fig 1. Frequency of top skin clones in paired skin and blood samples from CTCL patients and patients with reactive skin disease. Pearson $R^2 = 0.92$, $P < .0001$. For CTCL patients with >1 expanded skin clone, both clones are plotted. Seven patients have the same top clone in tissue and blood, 4 of whom have 2 expanded clones, accounting for the 11 *red diamonds*. Sixteen patients have different top clones in tissue and blood, 6 of whom have 2 expanded clones, and they are represented by the 22 *blue diamonds*. *CTCL*, Cutaneous T-cell lymphoma.

to quantitate the blood tumor burden in matched skin and blood samples from CTCL and reactive control patients.

We identified 46 patients who underwent skin biopsy and simultaneous peripheral blood assessment, 23 with histologically and clinically evident CTCL and 23 with reactive conditions. TCR-β HTS (ImmunoSEQ, Adaptive Biotechologies, Seattle, WA) was performed on matching skin and blood samples, and clone frequency was analyzed (Supplementary Appendix; available at http://www.jaad.org). Seven patients with definitive CTCL had identical top frequency skin and blood clones (1.8%-86.8% in blood), with a direct linear relationship between the frequency of the top clone in the skin and frequency of that clone in the blood. No correlation was seen between the frequency of the top clones in the remaining CTCL patients, who had minimal blood involvement (0%-1.8%), despite significant skin clone involvement of up to 79.4% (Fig 1). None of the 23 patients with reactive disease had substantial blood trafficking by the top clone found in the skin, with detectable circulation (0.0012%-0.71%) of the top skin clone found in 16 reactive patients. Clinical and flow cytometry findings indicated that CTCL patients with the same top clone in blood and skin were more likely to have attributes associated with a worse prognosis than those with different top clones (Table I).

Others have reported that identification of the same dominant clone in skin and blood is an independent prognostic factor for disease progression, disease-specific survival, and overall survival in CTCL.[2,3] In these studies, TCR-γ or TCR-β PCR or Southern blotting was used for clone identification. Given the sensitivity of TCR-β HTS, we believe this method is useful in identifying the implicated skin clone in the blood. Recently, Masson et al reported that the tumor clone frequency in lesional skin determined by TCR-β HTS is an independent prognostic factor in patients with mycosis fungoides but not Sézary syndrome[4]; however, the relationship between tumor clone frequency of matched blood and skin samples was not explored. We examined this relationship and found that CTCL patients belong to 2 different prognostic groups with different propensities for blood involvement. Our study suggests that increasing blood tumor burden is not simply a manifestation of increasing clonal tumor burden in the skin but that biologic differences underlie the clinical heterogeneity in CTCL.[5] Recognizing the different subtypes of CTCL early in the disease might be useful in making treatment decisions and facilitate accurate enrollment into clinical trials.

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Table I. Comparison of clinical and flow cytometric features of CTCL cases with the same or different top circulating clone

Features	Same top clone	Different top clone	*P* value	High frequency in skin*	Low frequency in skin*	*P* value
Flow cytometric features						
CD4:CD8 ratio	4.91	2.94	.094	3.76	2.74	.71
$CD4^+CD7^-$, %	**31.6**	**6.37**	**.0066**	15.3	9.6	.39
$CD4^+CD26^-$, %	**43.7**	**7.5**	**.0004**	20.3	12	.60
Clinical features						
Men	4/7 (57)	12/16 (75)	.63	11/18 (61)	5/5 (100)	.272
Age, y	67.86	58.25	.20	60.83	62.4	.94
Stage III/IV, late stage	**6/7**	**3/16**	**.005**	6/18 (33)	3/5 (60)	.343
LDH, U/L	**275**	**188**	**.0091**	223.1	190.6	.13
LDH, >200 U/L	**6/7**	**3/15**	**.007**	8/17 (47)	1/5 (20)	.36
Systemic therapy	6/7	6/16	.069	9/18 (50)	3/5 (60)	1.0
Phototherapy	6/7	10/16	.37	12/18 (67)	4/5 (80)	1.00
Total skin electron beam therapy	0/7	3/16	.53	2/18 (11)	1/5 (20)	.54

Cases are grouped by the presence of the same top clone in both blood and skin and by frequency of the top clone in skin. Values are n/total (%) unless stated otherwise. Statistically significant differences are bolded. Statistical analysis was performed using the rank-sum test and Fisher's exact test (see Supplementary Appendix; available at http://www.jaad.org). Having the same top clone in blood and skin correlates with adverse risk features, whereas increased frequency of the top clone in the skin does not.

CTCL, Cutaneous T-cell lymphoma; *LDH*, lactate dehydrogenase.

*The clone frequency of those in the top 25th percentile was 5%; low frequency was considered <5%.

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Histologic predictors of invasion in partially biopsied lentigo maligna melanoma

To the Editor: Because of its location and frequent large size, the initial diagnosis of LM (lentigo maligna) or LMM (lentigo maligna melanoma) is usually made by an initial partial biopsy rather than excisional biopsy, which, in LMM cases, could be performed in an area without an invasive component. In this study, we aimed to determine the frequency of patients with a final diagnosis of LMM undetected on the partial biopsy and find out pathologic predictors of invasion in LM biopsies.

We selected 118 consecutive cases from 115 patients with a biopsy-proven LM or LMM treated with Mohs or slow Mohs surgery during October 1999-May 2013. The partial biopsy slides of LM cases were compared by 2 dermatopathologists blinded to the final diagnosis (LM or LMM).

Of 100 cases with an initial diagnosis of LM after analysis of partial biopsy, 20% were upstaged to LMM after analysis of debulking specimens. LMM cases diagnosed after partial biopsy specimen analysis had a similar mean Breslow Index to those not diagnosed after partial biopsy (0.53 ± 0.37 mm vs 0.47 ± 0.34 mm, $P = .60$). The sensitivity of the partial biopsy in detecting LMM was 47% (95% confidence interval 31%-64%) and the negative likelihood ratio 0.53 (95% confidence interval 0.39-0.71).

Clinical criteria (age, sex, size, type [primary or recurrent]) were not significantly different between cases of LM and LMM initially diagnosed as LM. However, the multivariate logistic regression analysis revealed that a pagetoid spread of tumor cells and moderate-to-strong dermal inflammation on partial biopsy were significantly and independently associated with the final diagnosis of LMM (Table I).

SUPPLEMENTARY APPENDIX: T-CELL RECEPTOR β CHAIN SEQUENCING

All TCR sequencing data reported on in this manuscript are available from the Dryad data repository: https://doi.org/10.5061/dryad.h54d699.

For each sample, genomic DNA was extracted, then T-cell receptor (TCR) β CDR3 regions from mature T cells were amplified and sequenced using the immunoSEQ assay (Adaptive Biotechnologies, Seattle, WA); immunoSEQ assays are for research use only and not for use in diagnostic procedures. Multiplexed primers targeting all V and J gene segments were used to amplify rearranged VDJ segments for high throughput sequencing. Synthetic templates mimicking natural VDJ rearrangements were used to measure and correct amplification bias in the multiplex PCR.[S1] Standard ImmunoSEQ bioinformatics processing was applied to generate raw TCR repertoire data, targeting a minimum of 10 sequence reads per TCR molecule of input. After correcting primary sequence errors and collapsing sequences to quantitate each clone using a clustering algorithm,[S2] CDR3 segments were annotated according to the International ImMunoGeneTics collaboration.[S3,S4] V, D, and J gene segments contributing to each rearrangement were identified, and rearrangements were classified as nonproductive if nontemplated insertions or deletions produced frame-shifts or premature stop codons.

STATISTICAL ANALYSIS

The skewness-kurtosis (Jarque-Bera) test of normality was used for all continuous variables. The normality assumption was not met in all groups of any continuous variable. Therefore, differences in continuous variables between 2 groups was determined by using a nonparametric rank-sum (Wilcoxon-Mann-Whitney) test. The differences in dichotomous variables between 2 groups were determined using Fisher's exact test. All measured variables were determined a priori, and all the results are presented. All *P* values are 2-tailed and reported without adjustment for multiple comparisons. Statistical analyses were performed using Stata version 14.2.

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