


possible with the data from smaller CTCL cohorts, was mutual exclusivity of mutations and disrupted pathways in CTCL. Mutual exclusivity provides critical information about the genomic basis of cancer formation. Mutual exclusivity of mutations within the same pathway may indicate that disruption of that pathway alone is sufficient to trigger tumorigenesis. In addition, mutual exclusivity of mutations among different pathways could be the result of negative selection, as cancer cells could not survive with concurrent mutations in multiple pathways. Importantly, such mutual exclusivity among different pathways may signify subtypes of a tumor, each of which results from a distinct mechanism of tumorigenesis (Kim et al., 2017).

We took advantage of the combined dataset to study mutual exclusivity of mutations in CTCL. We found that mutations within the NFkB pathway genes PLCG1, CARD11, and TNFRSF1B were mutually exclusive in SS (Figure 1a). Mutations in the KIT gene were exclusive from the three NFkB pathway genes in all but two SS cases. KIT is known to activate PLCG1, which contributes to activation of the NFkB pathway, which may explain this finding. Furthermore, mutations in p53 were mutually exclusive from the three NFkB/KIT genes (Fisher P-value = 0.03). This exclusivity held true when an expanded set of 90 genes annotated as NFkB pathway genes were tested (KEGG ID:04064) (Fisher P-value = 0.05) (Supplementary Figure S2 online). Using these data we were able to classify CTCL by underlying pathogenic pathways. Nineteen percent of SS cases in the entire cohort had mutations in p53; the remaining nonmutant p53 cases could be further divided into NFkB/KIT-mutant (28% of cases) or NFkB/KIT-normal (53% of cases) (Figure 1a).

Because p53 is a major tumor suppressor, either a disrupting mutation or a copy loss may be sufficient to disrupt the normal p53 pathway and contribute to malignant phenotype in CTCL. Thus, we evaluated if disrupting mutations in p53 were mutually exclusive with p53 gene copy loss in SS. We and others observed p53 gene deletion on chromosome 17p to be a common finding in SS, which is detected in 40% of SS cases. However, nearly equal proportion of p53-mutant and nonmutant SS cases had one p53 copy loss (43% and 40%, respectively; Fisher P-value = 0.82). Thus, although p53 is the most frequently mutated gene in CTCL, dysregulation of this pathway is not sufficient to trigger cancer progression. These data support previous studies that
showed that \( p53 \) alteration alone was not associated with disease prognosis of SS (Gros et al., 2017). On the contrary, \( p53 \) gene status may affect overall survival in patients with advanced mycosis fungoides, suggesting differences in the pathophysiology of the progression of these two diseases (Wooler et al., 2016). To further evaluate this finding, we performed survival analysis on 39 SS cases with clinical data available. We found no significant difference in overall survival between SS cases with or without a \( p53 \) genomic mutation (Figure 1b). When both genomic mutation and gene copy loss were considered, no difference in overall survival was seen between \( p53 \)-altered and nonaltered SS cases (Figure 1c). Similarly, mutations in \( NFkB/KIT \) did not confer a significant difference in overall survival (Figure 1d). When SS cases were stratified by the total number of mutations, there was a difference in overall survival between the SS cases with the most mutations (poor prognosis) and those with the fewest mutations (better prognosis, Figure 1e).

In summary, we created an integrated genomic dataset in CTCL and successfully used this tool to show that there are mutual exclusive mutations affecting \( p53 \) or \( NFkB/KIT \) genes and pathways. Remarkably, the cases that did not carry \( p53 \) or \( NFkB/KIT \) abnormalities did not have any significantly mutated genes using the Poisson method, suggesting that other aberrant genomic features such as those implicated in transcription and epigenetic regulation may cooperate with or enhance the damaging effects of genetic mutations. Further prognostic studies utilizing clinicopathologic characteristics, survival data, and integrated genomic datasets expanded from this report may uncover these interactions. These findings have direct implications for future diagnosis and therapy design in CTCL through selective targeting of patient-specific mutations, because CTCL is a particularly heterogeneous malignancy and “one size” of therapy does not fit all patients. We believe that this dataset, as it continues to expand and offers more statistical power, would be a very useful tool in uncovering previously indiscernible relevant relationships, revealing affected pathways, and inferring possible genomic classification of CTCL.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
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