NPM1 mutation is associated with leukemia cutis in acute myeloid leukemia with monocytic features

Leukemia cutis (LC), the infiltration of the epidermis, dermis, or subcutis with leukemia cells, complicates 5-10% of cases of acute myeloid leukemia (AML) in adults and is considered a marker of poor prognosis.[1-3] While the association between AML with monocytic features and LC has been described, little is known about the association of other AML characteristics and LC.[2,4,5] Recently, a number of recurrent gene mutations have been described in AML; however, the association of these mutations and LC has not been systematically investigated.[6,7] Using amplicon-based next-generation sequencing (NGS) of a panel of recurrent, hematologic malignancy-associated mutations, we sought to determine the association between molecular markers and LC.

We identified 284 patients diagnosed with AML at the University of Pennsylvania (2001-2014) who had undergone targeted NGS analysis of 33 genes associated with hematologic malignancy;[8] of these, 23 are recurrently mutated in AML and were studied (listed in Table 2). These 284 cases were identified either from the Hematologic Malignancies Tissue Bank at the University of Pennsylvania (2001-2013), or from a pathology database of patients tested in a clinical context (after February 2013). Using a clinical database of acute leukemia patients (February 2011 - August 2014) who were evaluated for *NPM1* mutations by a targeted method, an additional 276 patients with known *NPM1* status were identified. Redundant cases were excluded.

All cases of AML were confirmed by a hematopathologists review of the diagnostic material. The presence of monocytic features was determined by a combination of morphology and immunophenotypic analysis, as well as cytochemistry, as appropriate. For each AML patient, a dermatopathology database was reviewed to identify cases of skin biopsy-proven LC at any time during the disease course. Independent dermatopathology review was obtained for indeterminate cases of LC; cases still classified as indeterminate after re-review were excluded from the analysis. Information regarding clinical and disease characteristics was determined by review of the medical records. Targeted NGS testing of 33 genes associated with hematologic malignancies (including *NPM1*) was conducted by the Center for Personalized Diagnostics at the University of Pennsylvania. Average read depth was 3000X, minimal depth was 250x, and reporting frequency cutoff for variants was 5%.[8] Mutations were classified into four categories: pathogenic, likely disease-associated, variant of uncertain significance (VUS), or likely benign based on review of publically available data; only pathogenic or likely disease-associated mutations were included in this analysis. Targeted *NPM1* analysis was performed in the Department of Pathology at the University of Pennsylvania. The targeted *NPM1* test consists of multiplex RT-PCR followed by detection on a liquid bead array. This assay allows for the simultaneous detection of the most common *NPM1* mutations in exon 12 (type A, B, and D). The analytical sensitivity of the assay is approximately 0.01%. The Institutional Review Board of the University of Pennsylvania approved this research.

Patient and clinical characteristics were summarized by descriptive statistics. Association between the presence of mutation and LC was assessed by the chi-square test or logistic regression, stratified by monocytic subtype when appropriate. Only genes with a mutation frequency ≥ 5% (n=24) were assessed for association with LC. All statistical tests were two-sided, with *P* values <.05 considered statistically significant. All analyses were conducted in STATA 12 (StataCorp, College Station, TX).

Table 1. Patient and disease characteristics.

Characteristic	Initial cohort (n=284) N (%)	*NPM1* cohort (n=560) N (%)
Median Age (Range)	59 (17-86)	62 (17-91)
Age < 60 years	143 (50%)	239 (43%)
Age ≥ 60 years	141 (50%)	321 (57%)
Sex		
Male	160 (56%)	325 (58%)
Female	124 (44%)	235 (42%)
Race		
White	208 (73%)	419 (75%)
Black	26 (9%)	54 (10%)
Asian	6 (2%)	8 (1%)
Unknown	44 (15%)	79 (14%)
White blood cell count, diagnosis		
Median (Range)	22.0 (0.4 – 387.7)	13.6 (0.3 – 543.7)
<100 K/ μL	230 (81%)	461 (82%)
≥100 K/ μL	54 (19%)	99 (18%)
Cytogenetic risk		
Favorable	33 (12%)	58 (10%)
Intermediate	169 (60%)	321 (57%)
Unfavorable	66 (23%)	134 (24%)
Unknown	16 (6%)	47 (8%)
Monocytic features		
Yes	107 (38%)	207 (37%)
No	162 (57%)	334 (60%)
Unknown	15 (5%)	19 (3%)
NPM1 Mutation		
Yes	83 (29%)	145 (26%)
No	201 (71%)	415 (74%)
Leukemia cutis		
Yes	27 (10%)	48 (9%)
No	257 (90%)	512 (91.4%)

We initially identified 284 AML patients with extended mutation testing; the molecular profiling was completed at AML diagnosis in most patients (86%, n=243) with the remainder undergoing assessment after initiation of therapy (persistent disease or relapse) (Table 1). The median age was 59 years (range 17-86) with the majority of patients having intermediate cytogenetics (12% favorable, 59% intermediate, 23% unfavorable, and 6% unknown). The 3 most common mutations were *NPM1* (29%), *DNMT3A* (25%), and *FLT3*-ITD (23%). Biopsy-confirmed LC was present in 10% (n=27) of patients overall.

The presence of an *NPM1* mutation was associated with LC (OR: 2.9; 95% CI: 1.3-6.6, P=0.009), as 14 out of 27 cases of LC had an *NPM1* mutation (all the common exon 12 insertion). An association between *PTPN11* and LC was also detected (OR: 3.6; P=0.014); however, 4 of the 6 cases of LC in patients with a *PTPN11* mutation also had a concurrent *NPM1* mutation. No association was detected between LC and the presence of any other myeloid malignancy-associated mutations or functional class of mutations (Table 2). We further examined the association of *NPM1* mutation status and LC in an expanded cohort of patients with known *NPM1* mutant

status. Characteristics of the expanded cohort are similar to the initial cohort (Table 1). In this larger cohort, we confirmed the association of *NPM1* mutations with LC (OR: 2.7; *P*=0.001). In total, 46% of cases of AML with LC were *NPM1* mutant compared to 24% of AML cases not associated with LC (Table 3).

Since both *NPM1* and LC have been associated with AML with monocytic features, we further examined the association of NPM1 and LC accounting for monocytic subtype. AML with monocytic features was enriched for the presence of *NPM1* mutations compared to AML without monocytic features (36% *versus* 20%, *P*<0.0001). Among patients with monocytic AML, 14 out of 19 (74%) of those with LC had *NPM1* mutations compared to 61 out of 188 (32%) of those without LC, suggesting that the presence of mutated *NPM1* was significantly associated with the development of LC (OR: 5.8; *P*=0.001) in the monocytic subgroup. In contrast, among those with non-monocytic AML, 7 out of 21 (25%) of patients with LC were *NPM1* mutant compared to 59 out of 306 (19%) without LC, indicating no association between *NPM1* and LC in the non-monocytic subgroup (OR: 1.4; *P*=0.469). Interestingly, monocytic AML was not itself associated with LC in the NPM1 mutant (OR 1.9, *P*=0.185) or *NPM1* wild-type cohort (OR: 0.46; *P*=0.131).

The true incidence of LC has been difficult to define as reports have been limited by small numbers of patients with varying demographic characteristics. This study is among the largest single-center reviews of LC in AML. Biopsy-proven LC was found in 10% of patients in this large cohort, which appears to be higher than some previously reported series in adult AML patients. This suggests that LC is a more common complication of adult AML than is sometimes reported.[1-3] This higher incidence in our cohort may also be a reflection of the increased surveillance and clinician suspicion of the condition in our institution.

We identified a unique association between *NPM1* mutation status and the presence of LC among patients with AML with monocytic features. We initially identified this finding in a cohort of 284 patients who had undergone extended mutation testing, and subsequently confirmed the association in an expanded cohort of 560 AML patients with known *NPM1* mutant status. Interestingly, although we show an association between *NPM1* and LC, we did not confirm an independent association between monocytic features and LC.

Our study is limited by its retrospective nature – not all patients were prospectively monitored, particularly from a dermatologic perspective, and therefore our data likely represents an under-ascertainment of LC. It is also possible that patients known to have AML with monocytic features may have been preferentially evaluated for LC. This partiality, however, would not be expected to bias our finding of an association between *NPM1* mutant status and LC within the monocytic AML cohort. Our analysis is additionally limited by the use of a discrete gene panel, as some genes recurrently mutated in AML are challenging to identify using NGS techniques and therefore not included (e.g., CEBPA, partial tandem duplication of MLL). Additionally, mutation assessment was not always conducted contemporaneously with LC diagnosis. While this may have led to the misclassification of LC with regard to *NPM1* status in a small number of cases, we expect this impact to be limited given the reported high rate of stability (>90%) of *NPM1* status at diagnosis and relapse.[9] A further limitation of our study is the lack of response and survival data in this cohort.

Table 2. AML-Associated Somatic Mutations and Leukemia Cutis (n=284).

Gene	N (%)	OR	*P*
NPM1	83 (29%)	2.9	0.009
Tumor suppressor	45 (16%)	0.4	0.222
TP53	25 (9%)	0.8	0.788
WT1	16 (6%)	–	–
PHF6	4 (1%)	–	–
DNA methylation	138 (49%)	1.6	0.247
DNMT3A	71 (25%)	1.9	0.134
TET2	43 (15%)	0.4	0.252
IDH1	30 (11%)	1.5	0.453
IDH2	34 (12%)	1.3	0.633
Activated signaling	156 (55%)	2.1	0.096
FLT3-ITD	66 (23%)	1.2	0.728
FLT3-TKD	18 (6%)	2.0	0.293
PTPN11	25 (9%)	3.6	0.014
*JAK2**	8 (7%)	1.4	0.757
KIT	11 (4%)	–	–
NRAS	36 (13%)	0.5	0.395
KRAS	12 (4%)	–	–
CBL	6 (2%)	–	–
GNAS	2 (1%)	–	–
PTEN	2 (1%)	–	–
*BRAF**	1 (1%)	–	–
Chromatin modifiers	24 (8%)	–	–
ASXL1	22 (8%)	–	–
EZH2	2 (1%)	–	–
RNA splicing	12 (4%)	–	–
SF3B1	12 (4%)	–	–
Transcription factors	29 (10%)	0.7	0.615
RUNX1	24 (8%)	0.9	0.838
ETV6	6 (2%)	–	–

*Full gene panel: myeloid neoplasm associated: NPM1, TP53, WT1, PHF6, DNMT3A, TET2, IDH1, IDH2, FLT3-ITD, FLT3-TKD, PTPN11, JAK2, KIT, NRAS, KRAS, CBL, GNAS, PTEN, BRAF, ASXL1, EZH2, SF3B1, RUNX1, ETV6; Other hematologic malignancy associated genes (data not shown): ATM, CDKN2A, DDX3X, FBXW7, KLHL6, MAPK1, MYD88, NOTCH1, XPO1, ZMYM3. *Data available only in 117 patients.*

Table 3. AML with *NPM1* mutation is associated with leukemia cutis.

	LC present	LC absent	*P*
	Total cohort (n=560)		0.001
NPM1 mutant	22 (46%)	123 (24%)	
NPM1 wild-type	26 (54%)	389 (76%)	
	Monocytic cohort (n=207)		<0.0001
NPM1 mutant	14 (74%)	61 (32%)	
NPM1 wild-type	5 (26%)	127 (68%)	
	Non-Monocytic cohort (n=334)		0.467
NPM1 mutant	59 (19%)	7 (25%)	
NPM1 wild-type	247 (81%)	21 (75%)	

NPM1-mutant AML in the absence of *FLT3*-ITD mutation is reported to have a favorable association in both younger and older patients, while the presence of LC has been associated with unfavorable outcome.[2,10-12] The implication of having both of these prognostic features is unknown and should be the subject of further investigation.

The cellular mechanisms through which *NPM1* mutations might alter leukemic myeloblasts homing to the skin require further study. Regardless of mechanism, our data support the World Health Organization's provisional classification of *NPM1*-mutated AML as a distinct biological entity. We note that an association between *NPM1* mutation and myeloid sarcoma has formerly been described, supporting the unique biology of *NPM1*-mutated AML.[13] In summary, our data suggests that the previously described association between AML with monocytic features and LC may largely be explained by an association between *NPM1* and LC.

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