Genomic Landscape and Clinical Features of Triple-Negative Myelofibrosis

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Background

Activation of the JAK/STAT signaling pathway is a hallmark feature of myelofibrosis (MF), and is driven by mutations in *JAK2*, *CALR*, and *MPL*. However, up to 10% of patients with MF lack mutations in these driver genes, termed “triple-negative MF” (1, 2).

Triple-negative MF portends a poor prognosis, with higher rates of leukemic transformation and shorter survival (1, 2, 3).

Very little literature is available regarding genomic drivers or pathogenic mechanisms of triple-negative MF.

Here, we present Next Generation Sequencing (~1700 gene assay) profiles, clinical features, and pathologic features of a cohort of triple-negative MF patients studied at the University of Michigan.

Objective

We aimed to further characterize the clinical and genomic features of patients with triple-negative MF.

Methods

We approached consecutive patients with MF undergoing evaluation in the University of Michigan heme/onc clinics, and they were consented per IRB approved protocol. We performed next generation sequencing (NGS) on peripheral blood or bone marrow samples at the University of Michigan using a CLIA-certified Laboratory Developed Test (LDT) designed to efficiently identify non-synonymous somatic point mutations in a panel of 1700+ genes with suggestive links to cancer. The assay includes 24,774 deeply sequenced capture targets that together cover all or part of 1,711 genes and spans approximately 3.8Mb of the human exome. Somatic insertions and deletions and copy number aberrations, as well as gene fusions and outlier gene expression, are reported (4). Clinical features were reported at the time of study enrollment, and followed longitudinally.

Results

We performed genomic sequencing on 97 consecutive patients with MF. Seven (7%) were found to lack classic driver mutations in *JAK2*, *CALR*, or *MPL*.

Clinical Features

Clinical characteristics of our 7 patients with triple-negative MF are described in **Table 1**.

Results (continued)

Clinical features were heterogeneous. Our patient population tended to present at a younger age than expected with MF, and were primarily female. Most patients (5/7) had high risk disease by DIPSS-plus score at diagnosis. Splenomegaly was present in 3 patients, absent in 3, and uncertain in 1 (remote h/o splenectomy). Most had anemia (6/7) or thrombocytopenia (5/7) at diagnosis. Pulmonary hypertension has developed in 2. Concurrent autoimmune conditions were present in 2 (ITP, RA). None have had a blast phase transformation to date.

Treatment history to date is also presented. Median duration of follow up is 14 months (range 3-62 months). One patient in the cohort has passed away to date. JAK inhibitors were included in management in 3/7; all 3 (100%) had an objective response by spleen size or symptom reduction. Response has been maintained for 15 months (ongoing), 10 months (ongoing), and 4 months (transplanted).

Table 1. Clinical Characteristics of Patients with Triple-Negative MF

Patient Characteristics (n=7)	
Median Age at Diagnosis (Range)	59 (49-73)
Male (%)	2 (28%)
DIPSS-Plus Risk Classification at Diagnosis (n)	
Low	0
Intermediate-1	1
Intermediate-2	1
High	5
Splenomegaly (n)	3
Leukemic Transformation to Date (n)	0
Pulmonary Hypertension to Date (n)	2
History of Autoimmune Condition (n)	2
Presence of Circulating Blasts (n) (Range)	5 (0-2%)
Anemia at Presentation (Hb<10 g/dL)	6
Thrombocytopenia at Presentation (<100 k/uL)	5
Median WBC at Presentation (Range)	5.5 (2.3-13.8)
Treatment History	
Number of Therapies to Date	
0	4
1	1
2	2
Treated with AlloHSCT to Date (n)	1
Treated with JAK Inhibitors to Date (n)	3
Response to JAK Inhibitors to Date (n, %)	
Response defined as >50% in spleen length by palpation or significant subjective symptom improvement	3 (100%)

Results (continued)

Pathologic Features

Pathologic features present at diagnosis in patients with triple-negative MF are described in **Table 2**.

Table 2. Pathologic Features in Triple-Negative MF

Pathologic Features (by subject, at diagnosis)			
Subject #	Marrow Cellularity	Fibrosis Grade (WHO 2016 Grading, 0-3 Scale)	Cytogenetics
1736	90%	2	Normal
1828	70%	2	Del(13q)
2261	50%	3	Inv(9).i(17q)
2262	90%	2-3	Del(13q)
2086	70%	2	Normal
2215	50%	3	Unknown
2178	95%	3	Normal

Genomic Landscape

Figure 1. Genomic Aberrations in Triple-Negative MF

Results (continued)

- The median number of aberrant genes identified was 1 (range 0-6), see **Figure 1**.
- Mutated genes included those involved in DNA damage repair (*TP53* in 2, *ATM* in 1, and *RAD51* in 1), epigenetic modifiers (*ASXL1* in 3, *DNMT3A* in 1, and splicing factors (*SRSF2* in 2, *U2AF1* in 1).
- 2 patients had germline mutations identified (*TP53* in 1, *ATM* in 1).
- One patient had no clonal marker identified by cytogenetic or molecular studies. That patient did not have any clear reactive etiology of marrow fibrosis.
- We also identified outlier expression of several genes without identifiable mutations including *MPL*, *GATA1*, and *ELANE*. Clinical and pathological implications of these gene expression abnormalities are yet unclear.

Discussion

- Triple-negative MF represents a small subset of MF with heterogeneous clinical features, but tends to present with high-risk disease features.
- The mutation burden in triple-negative MF is low, and includes genes commonly mutated in other myeloid malignancies. Recurring mutations include *ASXL1*, *SRSF2*, *TP52*, and *U2AF1*.
- Select patients may exist with no clonal markers yet no clear reactive etiologies for MF, in whom inflammatory and/or immune pathways may contribute.
- The pathophysiology and genomic drivers of triple-negative MF remain incompletely understood and warrant further study.

References

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Acknowledgments

Funding for this research was provided by the D. Dan and Betty Kahn Foundation (PI Talpaz).