Molecular Analysis in the Pacritinib Dose-Finding PAC203 Study in Patients with Myelofibrosis Refractory or Intolerant to Ruxolitinib

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BACKGROUND

Myelofibrosis (MF) After Ruxolitinib Discontinuation

- Survival after ruxolitinib discontinuation is poor, particularly for patients with thrombocytopenia (median <1 year) and for patients who acquired new mutations on ruxolitinib (6 months).[1]

Molecular Risk in Myelofibrosis

- Mutation type and number has prognostic significance in patients with MF
- Patients with ≥3 total mutations have shorter survival, decreased spleen volume response (SVR) with ruxolitinib, and shorter time to treatment discontinuation.[2]
  - Prior cohorts suggest that the frequency of ruxolitinib-treated patients harboring ≥3 non-driver mutations is 8.7%.[3]

Pacritinib in Myelofibrosis

- Pacritinib is an oral JAK2/IRAK1 inhibitor[4] that has demonstrated clinical efficacy in two Phase 3 MF trials (PERSIST-1 and PERSIST-2)[5,6] and in PAC203, a dose-finding trial in patients who failed to benefit from or were intolerant of ruxolitinib.[7]
  - These studies included patients with severe thrombocytopenia.
- The mutational landscape of the PAC203 "post-ruxolitinib" patient population has not been well characterized.

Study Objectives

- To describe the mutational landscape of MF patients after failure of ruxolitinib therapy, including those with severe thrombocytopenia, and to correlate mutational findings with baseline patient characteristics and clinical outcomes (including ≥35% spleen volume reduction and development of grade 3/4 cytopenias.

METHODS

- Baseline mutational analysis was performed on patients enrolled on PAC203.
- Mutational data was obtained in 110 (of 164 recruited; 161 treated) patients using an ISO accredited Illumina TruSeq Custom Amplicon Panel, including 32-gene mutation hotspots and exons (~36,000 bp, 287 amplicons): *ASXL1, ATRX, DNMT3A, EZH2, TET2, CEBPA, ETV6, NPM1, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, TP53, U2AF1, WT1, ZRSR2, CBL, CBLB, CBLC, CSF3R, FLT3, HRAS, JAK2, KIT, KRAS, MPL, NRAS, PDGFRA, PTEN, IDH1, IDH2.*
- Pathogenic variants were reported at a variant allele frequency (VAF) of ≥1%
- *CALR* mutation screening was carried out independently.

RESULTS

- PAC203 patients with available molecular data had a high incidence of anemia and severe thrombocytopenia (platelet counts <50 x 10^9/L, **Table 1**).

Table 1: Baseline Patient Characteristics (Subset with DNA Sequencing Available)

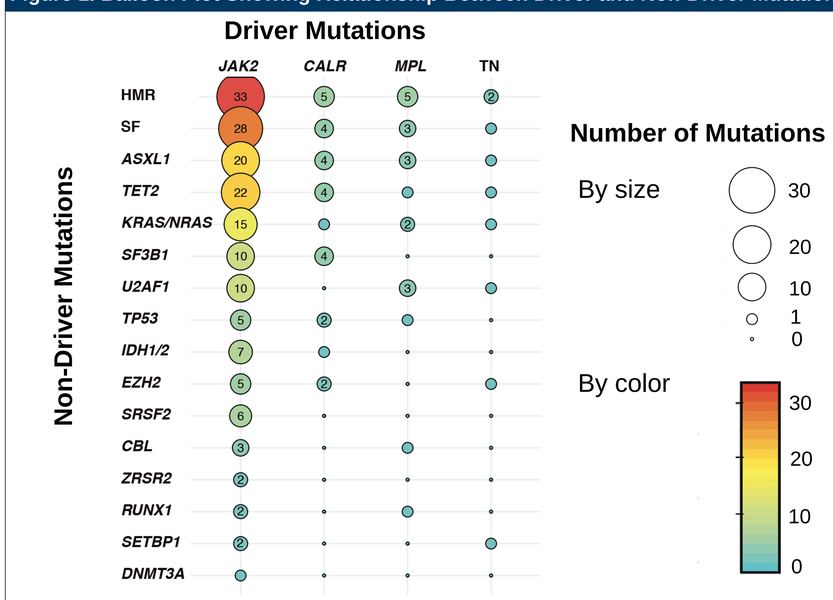
Characteristic	Patients (N=110)
Follow-up time (median, range)	163 (28-476) days
Age (median, range)	67.5 (37-87) years
Primary MF (%)	56.4%
Platelet count (median, range)	63 (13 – 910) x 10^9/L
Platelet count <50 x 10^9/L (%)	38.3%
Hemoglobin <10 g/dL (%)	65.1%

RESULTS

Molecular Landscape: MF Post-Ruxolitinib

- The most common driver mutation was *JAK2* (77.3%), whereas 1.8% had no driver mutation ("triple negative"), as shown in **Figure 1**.
- *CALR* mutation, associated with better prognosis in MF[8,9], was relatively rare in this population (12.7%) compared to other previously described MF populations.[9]
- Non-MPN driver mutations (NDM) were present in 76.4% of patients; 43.6% had ≥2 NDM and 18.1% had ≥3 NDM.
  - *ASXL1* (25.5%) and *TET2* (24.5%) were most prevalent, consistent with prior reports.[10]
  - Splicing factor (SF) mutations, present in 32.3%, were mutually exclusive (*SF3B1*, n=14; *U2AF1*, n=14; *SRSF2*, n=6; *ZRSR2*, n=2).

Figure 1. Balloon Plot Showing Relationship Between Driver and Non-Driver Mutations



HMR, high molecular risk; **SF**, splicing factors; **TN**, triple negative

- High molecular risk mutations were detected in 41% of patients (HMR: *IDH1/2, EZH2, ASXL1, SRSF2, U2AF1*Q157).[11]
- *TP53* mutations, associated with poor prognosis and leukemic transformation[12] were found in 7.3% of patients.
- RAS mutations *(K/NRAS),* associated with poor survival in MF[13], were found at a higher frequency (17.3%) than reported in prior MF cohorts.
  - Patients with a RAS mutation (vs. wild type) were more likely to be HMR (68.4% vs. 35.6%, P=.007).
  - RAS mutations and *TP53* mutations were mutually exclusive.

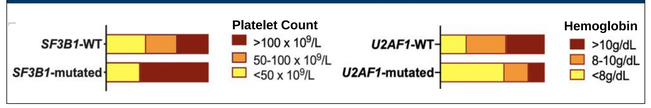
Longitudinal Outcomes

- Of patients with Week 24 molecular analysis, 13.2% (5/38) acquired at least 1 new mutation. Allele frequency was <5% in all cases.
  - *ASXL1* (n=3), *TET2* (n=1), *TP53* (n=1), *CBL* (n=1), *PHF6* (N=1)
- A statistically significant association was not observed between baseline mutation status and efficacy outcomes or treatment discontinuation.

Association Between Mutations and Disease Characteristics

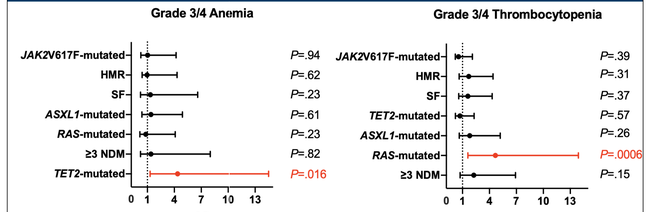
- Splicing factor mutations were more common in PMF (45.6%) than PPV- or PET-MF (3.7%, 33.3%; P=.002).
- *SF3B1* mutation was associated with higher platelet count (P=.022), whereas *U2AF1* mutation was associated with lower hemoglobin level (P=.026, **Figure 2**).

Figure 2. Mutations Associated with Cytopenias



- Patients with *TET2* mutation were more likely to have grade 3/4 anemia on study (OR 4.4 [95% CI 1.3-14.8], **Figure 3**).
- Patients with RAS pathway mutations were more likely to have grade 3/4 thrombocytopenia on study (OR 4.7 [95% CI 1.6-13.9], **Figure 3**).

Figure 3. High Grade Cytopenia Events by Mutation Group



HMR, high molecular risk; **NDM**, non-driver mutation; **OR**, odds ratio; **SF**, splicing factor

CONCLUSIONS

- The PAC203 cohort is molecularly high risk
  - High incidence of HMR, *TP53*, and RAS mutations
  - High mutational burden[3]
  - Low incidence of *CALR* mutations
- The molecular characteristics of the PAC203 cohort may impact overall response on this study
  - Lack of association between mutations and response rate on PAC203 noted but significant inferences cannot be made due to low event rate
  - Novel associations between mutation profiles and hematologic parameters and events were identified

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