Evidence of NF-κB pathway activation in patients with advanced, high molecular risk myelofibrosis

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INTRODUCTION

- Patients with myelofibrosis who discontinue treatment with the JAK1/2 inhibitor ruxolitinib have a poor prognosis that is often associated with advanced phases of disease and severe cytopenias.
- PAC203, a Phase 2 dose-finding study in patients with symptomatic myelofibrosis who were intolerant of or resistant to ruxolitinib[1], represents an opportune cohort for analyzing the association between high molecular risk (HMR) mutations and disease phenotype.
- While these patients are more likely to have high molecular risk genomic markers, biological drivers of disease in this advanced population are not well characterized. Pro-inflammatory cytokines have been shown to be elevated in MF[2] but no associations with mutation profiles have been identified.
- Patients had advanced disease at study entry, with profound cytopenias and high mutational burden[3].
- Here, we analyzed the interaction between high-risk mutations and cytokine profiles of patients treated in PAC203.

OBJECTIVE

- To study the interaction between high-risk mutations and cytokine profiles of patients treated in the PAC203 study.

METHODS

- Trial entry cytokine and mutation data were available in 108 (of total 164 recruited; 161 treated) patients.
- Using the Myriad RBM platform, a microsphere-based immuno-multiplexing technology, 47 cytokines were assessed.
- Mutation profiles were determined using an ISO accredited Illumina TruSeq Custom Amplicon Panel, including 32 gene mutation hotspots and exons (~36,000 bp, 287 amplicons). *CALR* mutation screening was carried out independently. Accepted coverage was achievement of a depth of ≥100 reads per base in ≥95% of targeted bases.
- The initial analysis assessed possible relationships between individual plasma cytokine levels and somatic gene mutation and clinical demographic data.
- An unsupervised approach was then used applying hierarchical agglomerative clustering to identify related sets of cytokines.
- Cluster scores (based on the median overall cytokine concentration within each cluster for each patient) were correlated with clinical/genomic data.

RESULTS

Clinical and molecular patient characteristics

- The PAC203 cohort has a high prevalence of severe thrombocytopenia and anemia **(Table 1).**
- The mutation profile of patients included for this analysis had a mutation profile enriched for high molecular risk (HMR) mutations (*IDH1/2, SRSF2, ASXL1, EZH2, U2AF1*Q157[4]) as previously described[3] (**Table 1**).
- In addition, splicing factor mutations (SF; *SF3B1, U2AF1, ZRSR2, SRSF2*) were detected in 35% of patients **(Table 1).**
- RAS-pathway activating mutations (*KRAS/NRAS/CBL*) mutations were present at a higher frequency than reported to date in any other MF cohorts[5,6] **(Table 1).**

Table 1. Patient Demographics

Demographics	Patients (n=108)
Follow-up time (median, range in days)	210 (27 – 526)
Age (median, range in years)	68 (37 - 87)
Primary MF (n,%)	61 (56.5)
Platelet count x 10^9/L (median, range)	64 (13 – 910)
Platelet count <50 x 10^9/L (n,%)	41 (38.3)
Hemoglobin level <10g/dL (n,%)	69 (64.5)
JAK-STAT signalling driver mutations (n,%)	
JAK2V617F	84 (77.8)
CALR	14 (12.9)
MPL	8 (7.4)
Triple negative	2 (1.9)
Addition somatic driver mutations (n,%)	
≥3 additional somatic driver mutations	21 (19.4)
High molecular risk	44 (40.7)
Splicing factor	35 (32.4)
RAS-pathway activating	23 (21.3)
ASXL1	29 (26.9)
TET2	26 (24.1)
TP53	7 (6.5)

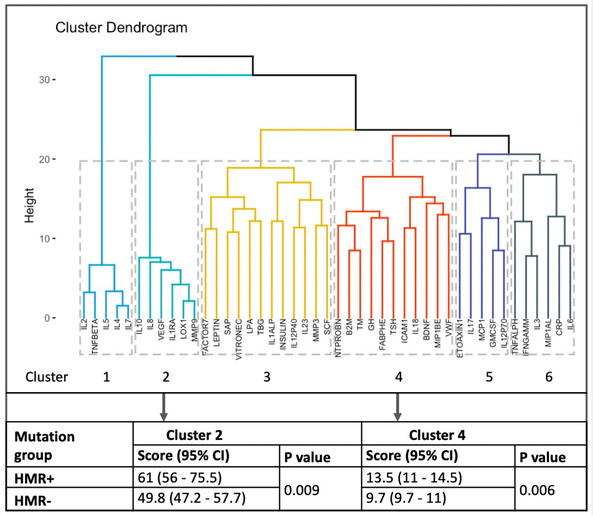
Cytokine analysis

- Unsupervised agglomerative clustering to identify sets of related cytokines identified 6 clusters (**Figure 1**).
- Clusters 2, 4, 5 and 6 are enriched for pro-inflammatory markers, with cluster 2 representing a transcriptional cluster regulated by the NF-κB pathway (interleukin-8 [IL-8], IL-10, IL-1 receptor alpha, vascular endothelial growth factor [VEGF]).

Cytokine-mutation correlations

- Elevations in clusters 2 and 4 were associated with presence of HMR mutations (HMR+).
  - Cluster 2 in particular represents a set of cytokines regulated by NF-κB
- Within cluster 2, IL-8 levels were most strongly associated with HMR status (HMR+, 40.5 pg/ml vs. HMR-, 24.5 pg/ml, *P*<0.0001).

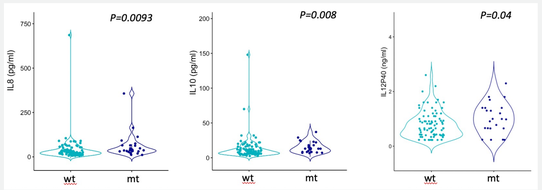
Figure 1. Cluster dendrogram of cytokine levels with cluster scores 2 and 4 highlighted in the table for high molecular risk positive (HMR+) and negative (HMR-) patients.



Mutation group	Cluster 2 Score (95% CI)	P value	Cluster 4 Score (95% CI)	P value
HMR+	61 (56 - 75.5)	0.009	13.5 (11 - 14.5)	0.006
HMR-	49.8 (47.2 - 57.7)		9.7 (9.7 - 11)	

- Although cluster 6 did not correlate with HMR status, it did correlate with presence of the high-risk *U2AF1* mutation (mutated 19.3 vs. unmutated 6.3, *P*=0.0019), though not with other splicing factor mutations. Furthermore, levels of the cluster 6 cytokine tumour necrosis factor-alpha (TNF- α) were associated with HMR (HMR+ 61 pg/mL vs. HMR- 49 pg/mL, *P*=0.0088).
- No significant associations between cluster scores and driver mutations or clinical characteristics were identified.
- RAS-pathway mutations were associated with higher levels of the NF-kB-associated cytokines IL-8, IL-10, and IL-12P40 (**Figure 2**), though not with overall cluster 2 scores.

Figure 2. Levels of individual cytokines elevated in patients with RAS-activating mutations



mt=mutant; wt=wild type.

- For RAS-mt vs. RAS-wt, median cytokine levels comparisons were for IL-8 (38 pg/ml vs. 26 pg/ml, *P*=0.0093), IL-10 (13 pg/ml vs. 9.1 pg/ml, *P*=0.008) and IL12P40 (1 ng/ml vs 0.6 ng/ml, *P*=0.04) **(Figure 2).**
- There was no association between cytokine cluster scores and recent exposure to ruxolitinib.

CONCLUSIONS

- **We report associations between high-risk mutation profiles and cytokine signatures in patients with myelofibrosis.**
- **In this HMR+ and RAS mutant-enriched cohort of MF patients who were intolerant of or resistant to ruxolitinib, we identify a relationship between HMR mutations and an NF-κB directed pro-inflammatory cytokine signature.**
- **These results implicate the activation of a distinct biological signalling pathway operative in this molecularly-defined cohort**

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