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ELEVENTH ANNUAL MEETING

THE ROLE OF VARIANTS OF UNCERTAIN SIGNIFICANCEFROM NGS DATA FOR PATIENTS WITH PH-NEGATIVE MYELOPROLIFERATIVENEOPLASMS

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INTRODUCTION

In the last decade the next generation sequencing (NGS) has played an increasing role in the diagnosis of Ph-negative patients with myeloproliferative neoplasms (Ph-MPN). However, some mutations are characterized according to American College of Medical Genetics and Association for Molecular Pathology recommendations, as variants of uncertain significance. The role of such mutations in the pathogenesis and prognosis of the disease is not clear

AIM

To study the role of variants of uncertain significance from NGS data of patients with Ph-negative myeloproliferative neoplasms.

METHOD

Study included 36 patients from clinics in Moscow and Saint-Petersburg (15 men and 21 women) with diagnosis PV, ET, PMF. Cohort median age- 52 (IQR 27–85). Panel of 118 genes with an average reading depth of 200x or 1000x on MiSeq (Illumina) was used. NGS panel had detection limit of 3% variant allele frequency. The clinical significance of mutations was determined using COSMIC, ClinVar, Varsome.

RESULTS

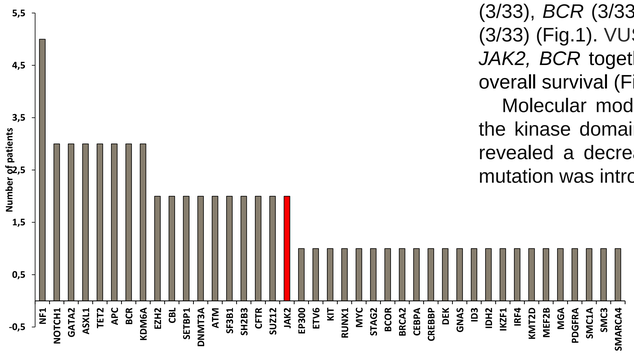


Fig.1 Mutations with unknown clinical significance

Variants of uncertain significance (VUS) were found in 92% (33/36) patients, Me=2.The most frequent mutations were in genes of epigenetic regulation- *ASXL1* (3/33), *TET2* (3/33), *KDM6A* (3/33); signal transduction - *NF1* (5/33), *NOTCH1* (3/33), *BCR* (3/33); transcription factor *GATA2* (3/33) and tumor suppressor *APC* (3/33) (Fig.1). VUS of genes involved into signaling - *NF1, K(N)RAS, NOTCH1(2), JAK2, BCR* together with driver mutations significantly (P≤0,001) influenced on overall survival (Fig 2).

Molecular modeling demonstrated that identified VUS in *JAK2* L977V affects the kinase domain structures (Fig 3.) Docking performed for the kinase domain revealed a decrease in the affinity of the ruxolitinib molecule when the L977V mutation was introduced (Fig 4).

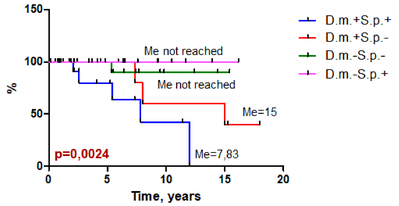


Fig.2 Overall survival at Ph-MPN depending on pathogenic mutations and VUS in the signaling pathway genes

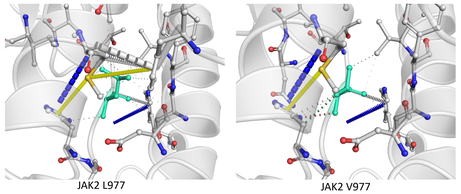
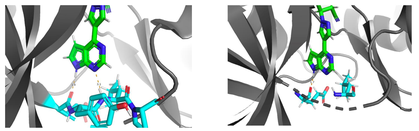


Fig.3 Effect of the L977V mutation on the stability of the kinase domain



Binding of ruxolitinib to the kinase domain of JAK2 wt E930 (glutamic acid) and L932 (leucine)

Binding of ruxolitinib to the kinase domain of JAK2 L977V E930 (glutamic acid)

Fig.4 Effect of L977V on binding of ruxolitinib to the kinase domain of JAK2

CONCLUSIONS

The influence of mutations in genes associated with signaling pathways on disease progression of the Ph-MPN patients has been shown, however, this requires further study. The effect of the identified mutation on the structure of *JAK2* domain and on binding to ruxolitinib indicates the need for precise classification of VUS in oncohematology.

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