Next generation sequencing (NGS) in the diagnosis, prognosis and features of the disease in Ph-negative myeloproliferative neoplasms

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Background

Over the past decade, high-throughput sequencing (NGS) has expanded our understanding of the genomic landscape of hematological malignancies. New biomarkers discovered during NGS are now widely used in clinical practice to improve diagnosis, stratify patients into prognostic groups, and select modern, including targeted, therapy in (Ph-negative MPNs).

Objective

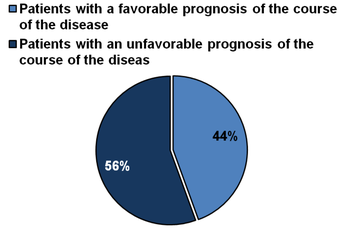
To assess possibilities of NGS in diagnosis and determination of prognostic features of disease course in Ph-MPNs patients.

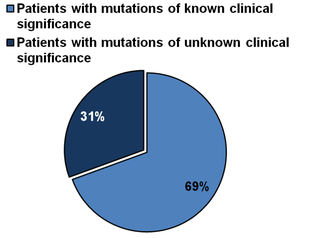
Materials & Methods

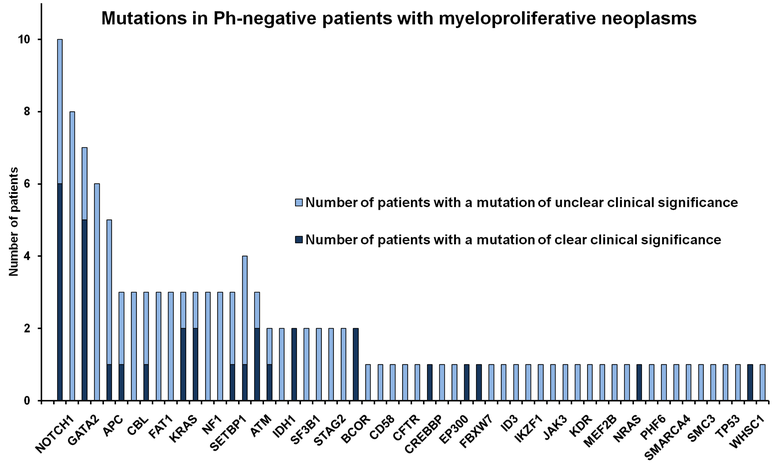
The pilot study included 36 patients (17 men and 19 women) from clinics in Moscow and St.Petersburg (Russia) aged 27 to 79 years (median Me=55 years). The diagnosis of myeloproliferative neoplasms (true polycythemia, essential thrombocythemia, primary myelofibrosis, myelodysplastic syndrome) not associated with the Philadelphia chromosome was previously established in all 36 patients. An NGS study was conducted to establish the clonal nature of the disease and to identify additional prognostically significant mutations. In all patients, sequencing was performed using a myeloid panel of 116 genes with an average reading depth of 200x or 1000x on a MiSeq instrument (Illumina). When analyzing the obtained data, a 3% allele frequency threshold (VAF) was used. The clinical significance of mutations was determined using the COSMIC and ClinVar databases.

Results

Genetic abnormalities were detected in all patients. On average, 10 mutations per one patient were revealed, including 4 somatic mutations in 92% (33/36) patients. In 70% (25/36), from 1 to 3 mutations with known clinical significance were found. In all patients without driver and somatic mutations (*ASXL1*, *EZH2* etc.) and chromosomal aberrations, somatic mutations (both clinically significant and unknown clinical significance) were detected, that served as clonality confirmation. All patients were divided into two groups - driver mutations, normal karyotype (16/36) (favorable prognosis) and triple-negative mutational status (20/36) with addition patients having mutations in epigenetic regulators genes (4/36) (unfavorable prognosis). In the first group NGS revealed additional mutations in 8/16 (50%) patients (*ASXL1, EZH2, IDH1*) and in 3/16 (19%) were noted mutations in *TET2* gene. In an unfavorable group, additional mutations were also found in genes of epigenetic regulation (*ASXL1, IDH1, SRSF2*) and in signal transduction genes (*RAS* genes, *PTPN11*) and *TET2*.







Summary

NGS data can confirm disease clonal nature, assess disease prognosis, select target therapy for patients with Ph-negative myeloproliferative neoplasms. To get new information it is possible to expand panel of sequenced genes, as well as study other genetic mechanisms underlying disease pathogenesis, e.g., changes gene expression level, microRNAs or transcription factors.

References

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The work was performed at the Research Center for Cellular and Molecular Pathology of Russian Research Institute of Hematology and Transfusiology of the Federal Medical and Biological Agency, St. Petersburg