PROJECT TITLE: ASSESSING THE COMBINED EFFECT OF MULTIPLE POLYMORPHISMS IN ORDER TO DEFINE THE GENETIC PREDISPOSITION TO MYELOPROLIFERATIVE NEOPLASMS

PROJECT CODE: PN-III-P1-1.1-PD-2016-1414

STAGE 2018: THE DEVELOPMENT AND IMPLEMENTATION OF REAL-TIME PCR TECHIQUES IN GENOTYPING A 16 SNPs PANEL IN PATIENTS WITH BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS AND CONTROLS.

The aim of this stage (2018) included the following activity:

GENOTYPING A 16 SNPs PANEL IN PATIENTS WITH BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS AND CONTROLS

BCR-ABL negative myeloproliferative neoplasms are hematological malignancies that originate from myeloid progenitors or precursors and consist of: Polycythemia vera (PV), essential thrombocythemia (TE) and primary myelofibrosis (PMF). All three are chronic diseases with a median overall surviaval of 6-7 years in case of primary myelofibrosis, surpassed by polycythemia vera and essential thrombocythemia (Tefferi and Vardiman, 2008).

Common complications of these diseases are: venous and arterial thrombosis (especially splenic thrombosis - potentially life threatening) in about 20-30% of patients at diagnosis, microcirculatory events (erythromelalgia, Raynaud or blue-toe syndrome, superficial thrombophlebitis, migraine, tinnitus) and bleedings (epistaxis, upper and lower digestive tract bleedings). After a variable amount of time, 10-15% of patients with polycythemia vera or essential thrombocythemia develop secondary

myelofibrosis. Approximately 5% of patients with myeloproliferative neoplasms progress into a blastic phase (acute myeloid leukemia), with a generally bad outcome and 80% of patients report a decrease in general health. All of the above make this group of hematological malignancies an important health issue, in spite of the relatively favorable prognostic (especially in patients with polycythemia vera and essential thrombocythemia).

BCR-ABL negative myeloproliferative neoplasms are characterized by some specific somatic mutations. The JAK2 (Janus kinase 2) V617F mutation, firstly described in 2005, by 4 distinct research groups, is present in almost all PV patients and in 50-60% out of the TE and PMF patients (Kralovics et al, 2005; James et al, 2005; Baxter et al, 2005; Levine et al, 2005). Mutations in the thrombopoietin receptor gene (myeloproliferative leukemia, MPL), described in 2006, are present in 5-10% of TE and PMF patients who are JAK2 V617F negative (Pikman et al, 2006; Pardanani et al, 2006). Lastly to mention, CALR (calreticuline) mutations characterize 60-80% of JAK2 V617F negative TE and PMF patients (Klampfl et al, 2013; Nangalia et al, 2013). While only a minority of PV patients present an exon 12 – JAK2 gene mutation, the remaining TE and PMF patients (10-15%), form a heterogeneous triple negative subgroup (negative for JAK2, MPL and CALR).

In the last years, reasearchers in this field have extended their focus also on the genetic predisposition of chronic myeloproliferative neoplasms (MPN). In 2008 a large epidemiological study showed that grade I relatives of MPN patients have a 5-10 times higher risk of developing these diseases then the rest of the population (Landgren et al, 2008). In 2009, three independent research groups have reported that the JAK2 46/1 haplotype is a major constitutuional driver of MPNs, especially for JAK2 V617F positive patients (Kilpivaara et al, 2009; Jones et al, 2009; Olcaydu et al, 2009). 2010 has been the year when my research team and I, have replicated these results on a 149 romanian patient group, by genotyping rs10974944 C>G SNPs, which mark the JAK2 46/1 haplotype through an own PCR-RFLP technique (Trifa et al, 2010a,b).

Later, in 2014, the rs2736100 A>C TERT (telomerase) polymorphism, has been also described as a major driver of MPNs by a scientific research group from deCODE Iceland (Oddsson et al, 2014; Jager et al, 2014). To mention is that the same polymorphism has initially been described as having a major role in lung cancer predisposition and later also in other solid cancers, especially gliomas. It seems that

this particular polymorphism comes with an increased telomerase activity, respectively longer telomeres. In the general population, the presence of this polymorphism is associated with higher blood count of myeloid cells (erythrocytes, granulocytes, platelets). Both of these polymorphisms JAK2 46/1 haplotype and TERT rs2736100 A>C seem to have a major contribution in the development of BCR-ALB negative MPNs. The PAF (population attributable fraction) of these two polymorphisms has been evaluated at 40% and respectively at 50%.

A study from 2015 shows new polymorphisms in the context of genetic predisposition of BCR-ALB negative MPNs: JAK2 rs12339666 and MECOM rs2201862 for JAK2 V617F-negative patients, and HBS1L/MYB rs9376092 especially found in JAK2 V617F positive TE patients and CALR and MPL positive BCR-ALB negative MPNs (Tapper et al, 2015).

Later, in 2016, a GWAS study high lightens other new polymorphisms as risk factors in JAK2 V617F associated clonal hematopoiesis and MPNs (Hinds et al, 2016).

This being said, as project director, I would like to mention the results obtained so far by my team and I, on genetic predisposition in BCR-ALB negative MPNs. In 2010 we have published a study in Annals of Hematology, on 149 subjects, regarding the contribution of JAK2 46/1 haplotype in the development of JAK2 V617F positive MPNs, showing no correlations with JAK2 V617F negative MPNs (Trifa et al, 2010b). 2016 we have published in British Journal of Haematology the results of a study on 529 MPN-patients tested for JAK2 V617F and CALR (CALR hadn't been described in 2010). The study described the individual and combined contribution of the JAK2 46/1 haplotype and TERT rs2736100 A>C polymorphism in the development of MPNs (Trifa et al, 2016). We demonstrated that the JAK2 46/1 haplotype played a significant role in JAK2 V617F positive and CALR positive MPNs, with a much lesser extent in the latter. On the other hand, the TERT rs2736100 A>C polymorphism was associated in similar extent with all molecular and phonotypical types of NPMs. The two polymorphisms had a synergic effect on the development of the disease, with a peak in the case of both homozygote genotypes.

Finally in 2017, out study in American Journal of Haematology showed the individual and combined effect of five polymorphisms (JAK2 46/1, TERT rs2736100, MECOM rs2201862, HBS1L-MYB rs9376092 and THRB-RARB rs4858647) on 939

MPN patients. The MECOM rs2201862 polymorphism was associated with all MPN phenotypes and both molecular subtypes (JAK2 V617F and CALR positive), with a greater effect on the CALR positive patients. The HBS1L-MYB rs9376092 polymorphism was positively associated only with ET (on behalf of JAK2 V617F and not CALR). Lastly the THRB-RARB rs4858647 polymorphism had only a weak correlation with PMF. To sum up the study, JAK2 46/1, TERT rs2736100 and MECOM rs2201862 polymorphisms have the greatest contribution in the development of MPNs. The study had also proposed a statistic model for the prediction of these diseases by evaluating genotypes for these three polymorphisms.

Results of 2018 stage: THE DEVELOPMENT AND IMPLEMENTATION OF REAL-TIME PCR TECHIQUES IN GENOTYPING A 16 SNPs PANEL IN PATIENTS WITH BCR-ABL NEGATIVE MYLOPROLIFERITIVE NEOPLSMS AND CONTROLS.

In this stage we have elaborated the genotyping protocols by real-time PCR for the 16 SNPs panel in order to genotype the study subjects and controls.

Genotyping these polymorphisms is based on the TaqMan chemistry, using specific hydrolysis probes for each SNP. Each SNP assay contains a mix of two probes, one for each specific allele and another for the extern primers used in the amplification of the SNPs containing DNA fragments, basically forming multiplex real-time PCR type reactions.

Each of the two probes is marked with a distinct fluorochrome. The fluorochromes are masked by a quencher and can only be freed if the probe hybridizes with the targeted DNA. The wild-type allele is marked with the FAM fluorochrome, while the variant allele is marked with the VIC fluorochrome in all SNPs of the Open Array panel. The genotyping mix uses ROX as a passive reference stain.

The hybridization process leads to the hydrolysis of the probe, the fluorochrome thus being freed and excited. The emitted light signal will be quantified after each amplification cycle. The quantity of the emitted fluorescence is proportional with the number of targeted DNA sequences matching each specific probe.

The fluorescent signal is converted into a graphic signal in shape of amplification curves (one for each allele). In this sense, homozygotes have one amplification curve, specific to the wild-type or variant allele, whereas homozygotes have both amplification curves. In the end the results appear as an amplification plot, grouped in three clusters for each SNP: wild-type homozygotes, heterozygotes, variant homozygotes. The genotyping soft has an auto-call option which generates automatically the genotype of each sample, if all steps have been followed optimally.

Due to Open Array panels, patented by Thermo Fisher, genotyping more then one SNP at once is possible. These kind of panels are custom made and can contain probes for 16, 32, 64 and more probes. For this project we have ordered an Open Array panel for 16 SNPs containing the probes for the following SNPs:

- JAK2 rs10974944
- TERT rs2736100
- MECOM rs2201862
- MECOM rs3851379
- HBS/MYB1L1 rs9376092
- THRB/RARB rs4858647
- SH2B3 rs3184504
- TET2 rs1548483
- CHEK2 rs555607708
- CHEK2 rs17879961
- ATM rs1800056
- GFIB rs621940

Bioinformatic quality control of the genomic sequences for these particular SNPS has been favorable except for one. The initial panel should have contained the PINT rs58270997 SNP, which didn't pass the quality control. We will try evaluating this SNP in the next step individually, through a Taqman assay, by common real-time PCR technique. In the mean time this SNP has been replaced by CHEK2 rs17879961, which has been previously described as a moderate risc factor for many human neoplasms. Besides the mentioned 12 SNPs, the pannel contains another 4 probes for internal control (selected by the pannel producer).

The ordered panel came in shape of 10 array plates. Each one intended for 144 samples. The Open Array panels use specific reactieves and supplies:

- QuantStudio™ 12K Flex System OpenArray® Accessories Kit (12 lids and plugs, 12 immersion fluid syringes, and 2 carriers)
- Open Array Genotyping Master Mix
- OpenArray® 384-Well Sample Plates
- OpenArray® AccuFill™ System Tips

All reactions were run on a real-time PCR system dedicated to Open-Array panels: QuantStudio 12K Flex 12K.

After amplification condition optimization, we have genotyped 1000 samples of MPN patients and 400 controls.

All included study subjects had a positive molecular marker, JAK2 V617F or CALR, thus providing evidence of the malignant nature of the affection. DNA samples have been collected at the time of diagnosis, the same time as samples have been collected for molecular evidence. The controles are individuals from general population with no evidence of any hematological malignancies.

In conclusion:

- we have fulfilled 100% the aim of the activity of the 2018 stage: **GENOTYPING A 16 SNPs PANEL IN PATIENTS WITH BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS AND CONTROLS**

Also, we have accomplished all 100% deliverables proposed in the work plan for the 2018 stage, as following:

1. Protocols for genotyping the 16 SNPs panels for patients with BCR-ABL negative myeloproliferative neoplasms and controls
2. Genotype distribution and allele frequencies for the 16 analyzed SNPs in patients with BCR-ABL negative myeloproliferative neoplasms and controls.

Following, the obtained results will be analyzed in order to build a mathematical model for the genetic predisposition of BCR-ABL negative myeloproliferative neoplasms, taking into account the genotypes and alleles for the genotyped SNPs through Open Array panel by real-time PCR. All of this will be written down and submitted in order to be published in a famous hematology paper. The above mentioned compose the 2019 stage.

STAGE 2019: THE DEVELOPMENT AND IMPLEMENTATION OF REAL-TIME PCR TECHIQUES IN GENOTYPING A 5 SNPs PANEL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND CONTROLS.

The aim of this stage (2019) included the following activity:

GENOTYPING A 5 SNPs PANEL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND CONTROLS

Chronic myeloid leukemia (CML) in a classic myeloproliferative neoplasm characterized by the proliferation that originates from myeloid progenitors or precursors. CML was is the first cancer for which a genetic alteration has been described. The Philadelphia chromosome in patients with CML has been described for the first by Peter Nowell and David Hungerford in 1960. The two scientists observed that patients with CML had a shorter chromosome; initially they believed it was the chromosome 21 not 22 and they thought it was a deletion involved not a translocation. This is no wonder given the karyotyping techniques back then. Besides this, although a translocation is involved the amount of transferred genetic material is not equivalent: chromosome 9 gets a substantial amount whereas chromosome 22 receives only a small fragment, leaving it considerable shorter than normal. Only after another 13 years had the true nature of the Philadelphia chromosome been uncovered. In 1973 Rowley reported 9 patients suffering from CML, that had an addition to the chromosome 9 along side the deletion on chromosome 22. This is how the scientist proves that a recurrent translocation is in fact involved, between chromosomes 9 and 22 (Rowley, 1973). Due to more modern karyotyping techniques, scientists could find out the exact breaking points of the two chromosomes, describing it as follows: t(9;22)(q34.1;q11.2).

Another 10 years had passed, so that the involvement of this translocation was understood; we are talking about a fusion of 2 genes: *ABL* on chromosome 9 and *BCR* on chromosome 22, the first time such an event was described in a human cancer. This BCR-ABL fusion brings a part of the ABL gene under the control of the promoter gene BCR, following an activation of the ABL tyrosine kinase which leads to a continuous signaling in the signaling pathways of myeloid precursors. The majority of patients are diagnosed during the chronic phase. Back in the days the

disease would inevitably progress to an accelerated, blastic phase with high mortality rate and an overall survival of 2-3 years. Treatment was very limited. The appearance of Imatinib, the first tyrosine kinase inhibitor (TKI) marked a breakthrough in CML treatment and medicine. Imatinib competes with ATP while merging with the BCR-ABL hybrid protein, inhibiting it. This causes cellular death of the cells which carry the BCR-ABL mutation. TKIs are nowadays standard treatment in CML, being able even to cure the disease, notion named as TFR (treatment free remission). Further on, next generation TKIs appeared, like dasatinib, nilotinib or bosutinib changing the course of a once lethal disease.

A higher incidence of CML has been observed in relatives of patients, although the genetic basis of this phenomenon has not yet been as clearly understood as in case of other myelopriferative neoplasms like polycythemia vera (PV), essential thrombocythemia (TE) and primary myelofibrosis.

In the case of these last mentioned diseases three independent research groups have reported that the JAK2 46/1 haplotype is a major constitutional driver of MPNs, especially for JAK2 V617F positive patients (Kilpivaara et al, 2009; Jones et al, 2009; Olcaydu et al, 2009). 2010 has been the year when my research team and I, have replicated these results on a 149 Romanian patient group, by genotyping rs10974944 C>G SNPs, which mark the JAK2 46/1 haplotype through an own PCR-RFLP technique (Trifa et al, 2010a,b). This SNP was part of the Open Array panel applied in this project for testing 1000 patients and 400 controls.

Later, in 2014, the rs2736100 A>C TERT (telomerase) polymorphism, has been also described as a major driver of MPNs by a scientific research group from deCODE Iceland (Oddsson et al, 2014; Jager et al, 2014). To mention is that the same polymorphism has initially been described as having a major role in lung cancer predisposition and later also in other solid cancers, especially gliomas. It seems that this particular polymorphism comes with an increased telomerase activity, respectively longer telomeres. In the general population, the presence of this polymorphism is associated with higher blood count of myeloid cells (erythrocytes, granulocytes, platelets). This polymorphism was also included in the Open Array panel applied in this project for testing 1000 patients and 400 controls.

Other studies from 2015 and 2016 show new polymorphisms in the context of genetic predisposition of BCR-ALB negative MPNs, in the following genes:

MECOM, HBS1L/MYB, SH2B3, TET, ATM, CHEK2 (Tapper et al, 2015; Hinds et al, 2016).

Regarding CML, a study published in 2015 analyzed 16.561 polymorphisms in 1916 genes on 437 patients and 1.144 controls. The study identified 5 SNPs (rs14178, rs6651394, rs6668196, rs3777744, rs3768641) in the following genes: PSMB10, TNFRSF10D, PSMB2, PPARD, CYP26B1.

Results of 2019 stage: THE DEVELOPMENT AND IMPLEMENTATION OF REAL-TIME PCR TECHIQUES IN GENOTYPING A 5 SNPs PANEL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND CONTROLS.

This stage contained many deliverables, as followed:

1. The contribution of the 16 analyzed SNPs in the development of BCR-ALB negative MPNs – a mathematic model regarding genetic predisposition in these diseases.

The contribution of the 16 SNPs genotyped in the last stage,

- JAK2 rs10974944
- TERT rs2736100
- MECOM rs2201862
- MECOM rs3851379
- HBS/MYB1L1 rs9376092
- THRB/RARB rs4858647
- SH2B3 rs3184504
- TET2 rs1548483
- CHEK2 rs555607708
- CHEK2 rs17879961
- ATM rs1800056
- GFIB rs621940

was statistically analyzed.

The JAK2 rs10974944 polymorphism, marker of the JAK2 46/1 haplotype, is the most important figure in genetic predisposition in PV, TE and PMF. Its weight lies in

the risk score of genetic predisposition for developing these entities and its contribution is maximal in the development of JAK2 V617F positive diseases.

According to the results obtained in this study, the TERT rs2736100 polymorphism comes in second regarding genetic predisposition in PV, TE and PMF. In contrast to the JAK2 rs10974944 polymorphism, the TERT rs2736100 has an equal contribution in each of the three diseases, independent of their molecular marker, JAK2 V617F or CALR.

The two polymorphisms would explain over 70% of the attributable fraction of genetic variation in the development of chronic myeloproliferations. The MECOM rs2201862 polymorphism was associated with all MPN phenotypes and both molecular subtypes (JAK2 V617F and CALR positive), with a greater effect on the CALR positive patients. This polymorphism came in third in the mathematic model for prediction of the development of chronic myeloproliferations. SH2B3 rs3184504 and TET2 rs1548483 polymorphisms have been associated with MPNs, regardless of their phenotypic or molecular background, occupying the following positions after JAK2 rs10974944, TERT rs2736100 and MECOM rs2201862, in the mathematic model of MPN prediction. The ATM rs1800056 polymorphism is more frequent in patients with MPNs, regardless of their phenotypic or molecular background, with a low frequency in the population.

ATM rs1800056 polymorphism was seen more frequently in MPN patients, regardless of the phenotype or molecular subtype. However, it has a low frequency in the population.

The HBS1L-MYB rs9376092 polymorphism was positively associated only with ET (on behalf of JAK2 V617F and not CALR). This way we could only include this polymorphism in the predictive mathematic model for ET, where it came in 4th, after JAK2 rs10974944, TERT rs2736100 and MECOM rs2201862.

The THRB-RARB rs4858647 polymorphism had only a weak correlation with PMF.

CHEK2 polymorphisms had a low frequency in the population analyzed in this study.

2. Oral presentations and posters at national and international conferences.

I was an invited speaker at the MPN&MPNr-EuroNet Fourteenth Meeting, held in Belgrade from the 8th to the 10th of May of this year. My presentation included my research activity regarding genetic predisposition of BCR-ABL negative myeloproliferative neoplasms and partial results obtained during this project.

I have participated at The 12th International Congress on Myeloproliferative Neoplasms, held in New York, 24-25th of October, at the European Hematology Conference in Amsterdam in June 2019 and at the Focus on MPN&MDS meeting in Belgrade in September 2019.

3. Elaborating a manuscript in order to be published in a famous hematology paper.

We have elaborated a manuscript based on the contribution of SH2B3 rs3184504 and TET2 rs1548483 polymorphisms in the development of MPN.

4. Genotyping protocols for the 5 SNPs panels for patients with CML and controls.

We elaborated individual protocols for genotyping each of the following SNPs: rs14178, rs6651394, rs6668196, rs3777744, rs3768641, in the next mentioned genes: PSMB10, TNFRSF10D, PSMB2, PPARD, CYP26B1. We used real-time PCR and Taqman probes for the genotyping of each SNP.

Genotyping these polymorphisms is based on the TaqMan chemistry, using specific hydrolysis probes for each SNP. Each SNP assay contains a mix of two probes, one for each specific allele and another for the extern primers used in the amplification of the SNPs containing DNA fragments, basically forming multiplex real-time PCR type reactions.

Each of the two probes is marked with a distinct fluorochrome. The fluorochromes are masked by a quencher and can only be freed if the probe hybridizes with the targeted DNA. The wild-type allele is marked with the FAM fluorochrome, while the variant allele is marked with the VIC fluorochrome in all SNPs of the Open Array panel. The genotyping mix uses ROX as a passive reference stain.

The hybridization process leads to the hydrolysis of the probe, the fluorochrome thus being freed and excited. The emitted light signal will be quantified after each amplification cycle. The quantity of the emitted fluorescence is

proportional with the number of targeted DNA sequences matching each specific probe.

The fluorescent signal is converted into a graphic signal in shape of amplification curves (one for each allele). In this sense, homozygotes have one amplification curve, specific to the wild-type or variant allele, whereas homozygotes have both amplification curves. In the end the results appear as an amplification plot, grouped in three clusters for each SNP: wild-type homozygotes, heterozygotes, variant homozygotes. The genotyping soft has an auto-call option which generates automatically the genotype of each sample, if all steps have been followed optimally.

5. Genotype distribution and allele frequencies for the 5 analyzed SNPs in patients with CML and controls

We have genotyped the mentioned 5 SNPs in 250 CML patients and 200 controls.

In conclusion:

- we have fulfilled 100% the aim of the activity of the 2019 stage: **GENOTYPING A 5 SNPs PANEL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND CONTROLS**

Also, we have accomplished all 100% deliverables proposed in the work plan for the 2018 stage, as following:

1. The contribution of the 16 analyzed SNPs in the development of BCR-ALB negative MPNs – a mathematic model regarding genetic predisposition in these diseases.
2. Oral presentations and posters at national and international conferences.
3. Elaborating a manuscript in order to be published in a famous hematology paper.
4. Protocols for genotyping the 5 SNPs panels for patients with CML and controls
5. Genotype distribution and allele frequencies for the 5 analyzed SNPs in patients with CML and controls

Following, the results obtained after genotyping the specific 5 SNPs in patients with CML, will be analyzed in order to build a mathematical model for the genetic predisposition of CML.

STAGE 2020: BUILDING MATHEMATICAL MODELS FOR THE GENETIC PREDISPOSITION TO CHRONIC MYELOID LEUKEMIA

The aim of this stage (2020) contained one activity:

BUILDING MATHEMATICAL MODELS FOR THE GENETIC PREDISPOSITION TO CHRONIC MYELOID LEUKEMIA AND DISSEMINATION OF FINAL RESULTS

This stage comprised several deliverables, as follows:

1. The effective contribution of the 5 SNPs to the occurrence of chronic myeloid leukemia - a mathematical model regarding the genetic predisposition to this disease.

Statistical analysis shows a modest contribution of PSMB10 rs14178 and TNFRSF10D rs6651394 polymorphisms to the occurrence of CML. However, the other three polymorphisms - PSMB2 rs6668196, PPARD rs3777744, and CYP26B1 rs3768641, show no statistically significant correlation. Thus, PSMB10 rs14178 and TNFRSF10D rs6651394 polymorphisms explain a minor part of the inter-individual variability regarding the predisposition to CML.

2. An original article published in a prestigious journal of hematology

In the previous stage I drafted a manuscript focused on SH2B3 rs3184504 and TET2 rs1548483 polymorphisms. Because this manuscript would have contained a large amount of data, I decided in the end to split the data and publish two articles. Thus, in stage 2020, I published the results on the correlation between SH2B3 rs3184504 and PV, ET, PMF and CML, in the form of "Original article". The article was published in Romanian Journal of Laboratory Medicine, which is an ISI journal with impact factor. The article can be found here:
http://www.rrml.ro/articole/articol.php?year=aop&vol=aop&poz=4

The other article will focus on TET2 rs1548483 polymorphism and especially on the interaction between this polymorphism and others analyzed in previous stages of the project. This manuscript is under production and will be submitted in June to an ISI journal with impact factor, such as Genes.

3. Oral presentations or posters at national and international conferences

I was supposed to present results from the project at the conference Diagnosis and therapy of sporadic and hereditary myeloproliferative diseases, which should have been held between 22nd-24th of April, 2020, in Ljubljana, Slovenia. Given the Covid-19 pandemics, the conference has been postponed to the end of the year.

The conclusions of the study:

The main important conclusions of the study are the following:

1. There is an important genetic background for the occurrence of non-BCR-ABL myeloproliferative neoplasms (polycythemia vera, essential thrombocythemia, primary myelofibrosis), as I demonstrated on a large cohort comprising around 1.700 patients.

2. JAK2 rs10974944 polymorphism, which tags the JAK2 46/1 haplotype, is the most important determinant in the genetic predisposition to polycythemia vera, essential thrombocythemia and primary myelofibrosis. It has the most important contribution in the genetic risk score for the prediction of these diseases. Its contribution is the greatest in the case of JAK2 V617F-positive diseases.

3. TERT rs2736100 polymorphism holds the second position in the genetic predisposition to polycythemia vera, essential thrombocythemia and primary myelofibrosis. Unlike JAK2 rs10974944, TERT rs2736100 exerts a similar influence on all three diseases, regardless of the molecular marker displayed - JAK2 V617F or CALR.

4. MECOM rs2201862 was correlated with all myeloproliferative neoplasms phenotypes, namely JAK2 V617F and CALR-positive. This effect was stronger in the case of CALR mutations. MECOM rs2201862 holds the third position in the genetic predisposition score to myeloproliferative neoplasms.

5. SH2B3 rs3184504 and TET2 rs1548483 were correlated with myeloproliferative neoplasms, regardless of phenotypes or molecular subtypes. They hold the forth and fifth position in the genetic predisposition score to myeloproliferative neoplasms, after JAK2 rs10974944, TERT rs2736100 and MECOM rs2201862.

6. HBS1L-MYB rs9376092 was correlated only in case of essential thrombocythemia, while in case of polycythemia vera the effect was reversed. HBS1L-MYB rs9376092 was correlated with JAK2 V617F mutation only. Thus, I could include this polymorphisms in the prediction score for essential thrombocythemia only, where it holds the fourth position, after JAK2 rs10974944, TERT rs2736100 and MECOM rs2201862.

7. ATM rs1800056 is encountered more frequently in patients with myeloproliferative neoplasms, regardless of phenotypes or molecular subtypes. However, it has a reduced frequency in the population.

8. CHEK2 polymorphisms were almost absent in this study. Thus, their relevance for the myeloproliferative neoplasms is reduced in the Romanian population.

9. In the case of THRB-RARB rs4858647, there was a weak correlation with primary myelofibrosis only.

10. PSMB10 rs14178 and TNFRSF10D rs6651394 polymorphisms have a modest contribution to the occurrence of chronic myeloid leukemia.

I believe the results we obtained in this project contribute to the international efforts in understanding the genetic factors involved in myeloproliferative neoplasms. A part of the results have been already published, and the remainder will be published in another journal. Thus, the project will have generated one supplementary article in addition to the one assumed at the beginning of the project.

In conclusion, I consider this project was a succes, generating significant results in the field of genetic predisposition to myeloproliferative neoplasms.

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