Co-occurrence of myeloproliferative neoplasms and solid tumors is attributed to a synergism between cytoreductive therapy and the common *TERT* polymorphism rs2736100

Tunde Krahling,1,2 Katalin Balassa,1,2 Katalin Piroska Kiss,2 Andras Bors,2 Arpad Batai,3 Gabriella Halm,4 Miklos Egyed,4 Sandor Fekete,3 Peter Remenyi,3 Tamas Masszi,3,5 Attila Tordai,6 Hajnalka Andrikovics2

1Semmelweis University, Doctoral School, Budapest, Hungary
2Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, Budapest, Hungary
3Department of Haematology and Stem Cell Transplantation, St. Istvan and St. Laszlo Hospital, Budapest, Hungary,
4Department of Haematology, Kaposi Mor Hospital, Kaposvar, Hungary,
53rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
6Institute of Pathophysiology, Semmelweis University, Budapest, Hungary.

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**Corresponding author:** Hajnalka Andrikovics, Hungarian National Blood Transfusion Service, Laboratory of Molecular Diagnostics, Budapest, Karolina str. 19-21, 1113 Hungary
Tel.: (+36)-1-3724449; Fax: (+36)-1-3724448; Email: andrikovics.hajnalka@ovsz.hu

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Summary

**Background.** The germline telomerase reverse transcriptase (*TERT*) rs2736100_C variant was identified as a susceptibility factor for a variety of solid tumors and recently for myeloproliferative neoplasms (MPN). **Methods.** LightCycler melting curve analysis was applied to detect risk alleles of *TERT* rs2736100_C and Janus kinase 2 (*JAK2*) rs12343867_C tagging 46/1 haplotype in 584 BCR-ABL1 negative MPN, 308 acute and 86 chronic myeloid leukemia (AML and CML) patients and 400 healthy individuals. **Results.** *TERT* rs2736100_C showed an increased allele frequency in BCR-ABL1 negative MPN patients compared to controls (62.7±2.8% vs. 48.8±3.5%, p<0.0001) regardless of molecular background or disease type, but not in CML or AML. Combined *TERT* and *JAK2* hetero- or homozygosity conferred even higher risk for classic MPN. Common complications (thrombosis, myelofibrosis or leukemia) were not associated with the *TERT* variant, however adverse survival was noted in *TERT* variant carrier polycythemia vera patients. MPN patients with the *TERT* CC genotype had a higher probability (44.4%) to die from solid tumors compared to *TERT* AC/AA individuals (5.3%; p=0.004). *TERT* rs2736100_C carriers had increased risk of solid tumors independently from cytoreductive treatment [3.08 (1.03-9.26), p=0.045]. **Conclusions.** *TERT* rs2736100_C polymorphism predisposes to the development of BCR-ABL1 negative MPN with the co-occurrence of solid tumors especially with the usage of cytoreductive treatment. **Impact.** The high frequency of *TERT* variant in the classic MPN population highlights the importance of the avoidance of long-term cytoreductive treatment in MPN patients.
Introduction

Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders, which include four relatively common disease entities: the BCR-ABL1 positive chronic myeloid leukemia (CML), and the three classic, BCR-ABL1 negative disorders as polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The pathogenesis of the three, classic MPN entities involves the activation of the JAK2-STAT (Janus kinase 2 - signal transducer and activator of transcription) pathway by mutations affecting JAK2, calreticulin (CALR) or thrombopoietin receptor (MPL) genes in a mutually exclusive manner (1, 2). The most frequent somatic mutation in JAK2 gene is V617F, which occurs in 90-95% of PV and 50-60% of ET or PMF cases. The frequencies of CALR and MPL mutations vary between 25-35% and 5-10% in ET and PMF. All four types of MPNs evolve into acute myeloid leukemia (AML) in a considerable proportion of cases.

The rs2736100_C single nucleotide polymorphism (SNP) of the telomerase reverse transcriptase (TERT) gene was found to be a susceptibility factor for a variety of cancers (e.g. lung, bladder) (3) and recently for sporadic and familial MPN (4-6). The TERT gene encodes the catalytic subunit of telomerase, which is essential for the maintenance of telomere length. Short and dysfunctional telomeres limit normal stem cell proliferation and lead to genomic instability, predisposing both to hematologic malignancies and solid tumors (7, 8). In hematopoietic cancers, associations between telomere length, disease progression and prognosis have been noted (8, 9). We aimed to investigate the roles and the interactions of TERT rs2736100 and JAK2 rs12343867 (tagging 46/1 haplotype) polymorphisms in Hungarian BCR-ABL1-negative MPN, CML and AML patients concerning their frequencies and potential effects on clinical characteristics.
Materials and methods

Patients

Patient cohorts consisted of 584 individuals diagnosed with BCR-ABL1 negative, classic MPN [250 males / 334 females, median age at diagnosis: 60 years (range: 10-94)], 86 with BCR-ABL1 positive CML [44 males / 42 females, median age at diagnosis: 54 years (range: 21-85)] and 308 with AML [142 males / 166 females, median age at diagnosis: 51 years (range: 16-93)]. Patients were diagnosed and followed at the Department of Haematology and Stem Cell Transplantation, St. Istvan and St. Laszlo Hospital formerly National Medical Centre (Hungary). Laboratory and clinical data were collected retrospectively as described previously (10). In the classical MPN group, 209 patients suffered from PV, 281 from ET and 94 from PMF. Besides the occurrence of coagulation complications, myelofibrotic or leukemic transformation and the history on non-hematologic malignancies at diagnosis or during follow-up were recorded. The median follow-up was 5.8 years (range: 0-39 years). In the CML group, 77 patients were diagnosed in chronic, 6 in accelerated and 3 in blast phase. In the AML group, 195 patients suffered from de novo AML, 93 from myelodysplasia-related AML and 20 from therapy-related AML. 400 healthy Hungarian individuals were also studied as controls. Participants signed written informed consent, and the study was approved by the Hungarian National Ethics Committee in accordance with the Helsinki Declaration.

Molecular genetic methods

All analyses were performed using whole genomic DNA isolated from peripheral blood or bone marrow. The risk alleles of TERT rs2736100_C and JAK2 rs12343867_C were identified by melting curve analysis with the hybridization probe detection format on LightCycler 480II, (Roche Diagnostics). As JAK2 rs12343867_C tags 46/1 haplotype, its allele frequency is
presented as \textit{JAK2} 46/1 haplotype frequency (11). In case of \textit{TERT} rs2736100-C, amplification primers (named as LCF, LCR) and hybridization probes (named as ANC, SENS) designed using LightCycler Probe Design software (Roche Diagnostics) were the following: \textit{TERT-LCF}: 5’-GCT AAG CAT TAT TAA TAT TGT TTT CCG T-3’; \textit{TERT-LCR}: 5’-GCA ATA ACA AGA CAG AAG AAC C-3’; \textit{TERT-SENS} 5’-Cy5-GGC AAA GCT ACA GAA AC- Phosphate-3’-; \textit{TERT-ANC}: 5’-AAG GAG GAA AAG CAG GGC G-Fluorescein-3’.

Asymmetric polymerase chain reaction (PCR in a reaction volume of 20 ul) was performed with 3.3:1 (10 pmol : 3 pmol) forward (LCF) to reverse (LCR) primer ratio, with 5 pmol of labeled oligonucleotides (SENS and ANC each), 25 ng genomic DNA, 2x PCR Master Mix (Promega). Cycling conditions were as follows: 95°C for 3 min, followed by 60 cycles of denaturation at 95°C, annealing at 55°C, and extension at 72°C. After amplification a melting curve analysis was performed by cooling the samples to 35°C, then gradually heating them to 85°C. The decline of fluorescence was continuously monitored. Melting curves were converted to melting peaks with wild-type and variant alleles showing distinct melting points.

MPN-patients were screened for \textit{JAK2} V617F mutation by real-time quantitative PCR (12). \textit{JAK2} V617F negative ET and PMF patients were tested for the presence of \textit{CALR} mutation by fragment analysis (1). Screening for \textit{MPL} mutations was performed by high resolution melting in \textit{JAK2} V617F and \textit{CALR} negative ET and PMF patients (13). The \textit{CALR} and \textit{MPL} positive cases were confirmed by Sanger sequencing. CML-patients were regularly monitored for \textit{BCR-ABL1} by real-time quantitative PCR (14). For AML-patients, standard karyotyping was performed as routine testing procedure.
Statistical analyses

Allele frequencies and 95% confidence intervals (AF±95%CI) for the risk alleles of TERT rs2736100_C and JAK2 rs12343867_C were presented. Dichotomous variables were compared using the Chi-square or Fischer’s exact tests, while continuous variables were conferred by using Mann-Whitney or Kruskal-Wallis tests. Odds ratios (OR) and 95% confidence intervals (±95%CI) for developing MPN or AML in different TERT and JAK2 genotype classes were calculated using logistic regression. Population attributable fraction (PAF) was defined as described (15, 16). Log-rank test was used to compare overall survival (OS) between subgroups. OS was censored at the time of hematopoietic transplantation (in 9 PMF and 3 secondary myelofibrotic cases). Statistical analyses were performed using SPSS 20.0 software package.
Results

1. TERT rs2736100_C as a BCR-ABL1 negative MPN predisposition factor

The distribution of acquired driver mutations in the tested BCR-ABL1 negative classic MPN cohort was the following. All PV patients were JAK2 V617F positive (n=209). In the ET cohort, 148 (53%) JAK2 V617F, 95 (34%) CALR and 9 (3%) MPL gene mutation positive and 29 (10%) triple negative cases; while in the PMF cohort 51 (54%) JAK2 V617F, 25 (27%) CALR and 7 (7%) MPL gene mutation positive and 11 (12%) triple negative cases were identified. In the classic MPN group, both TERT rs2736100 and JAK2 rs12343867 variants showed increased allele frequencies (AF±95%CI) among patients compared to controls (TERT rs2736100_C: 62.7±2.8% vs. 48.8±3.5%, p<0.0001; JAK2 rs12343867_C: 45.7±2.9% vs. 29.8±3.2%, p<0.0001). Beside the allelic model, the difference remained significant in all tested models (dominant, recessive, genotypic see Table 1).

In line with previous studies of classic MPN, carriership of the TERT variant (AC and CC genotypes combined) was associated similarly with both JAK2 V617F+ and CALR+ MPNs (p=0.5 for JAK2 V617F+ vs. CALR+ comparison). In contrast, the effect of the JAK2 rs12343867_C allele was more pronounced in the JAK2 V617F+ MPN (in JAK2 V617F+ MPN, JAK2 rs12343867_C AF: 49.8±3.5%; in CALR+ MPN AF: 35.8±6.2%, p=0<0.001 for JAK2 V617F+ vs. CALR+ comparison). Regarding the small subgroup of MPL positive MPN-patients, the AF of TERT rs2736100_C was also increased (62.5±17.1%, but not reaching the level of significance due to low case numbers). In contrast, the genotype distribution in the triple negative group was similar to those of controls (AF=51.3±11.2%). Dividing the triple negative cohort according to diagnosis (ET or PMF), a higher TERT variant AF (68.2±19.9%) was noted in the triple negative PMF, but no tendency for TERT AF increase was apparent in the triple negative ET group (AF=44.8±13.1%) compared to controls (statistical evaluation was hampered by low case numbers in the triple negative cohort).
In our BCR-ABL1 negative MPN cohort, the TERT variant showed an association with MPN both in hetero- and homozygous forms [OR: 2.2 (1.5-3.1) and 3.2 (2.2-4.7) respectively, both p<0.001]. TERT homozygosity increased MPN predisposition additively compared to TERT heterozygosity (p=0.009). Similarly to the TERT variant, hetero- or homozygosity for JAK2 polymorphism increased significantly the risk of MPN development [OR: 2.5 (1.9-3.4) and 3.4 (2.2-5.1) respectively, both p<0.001]. As the presence of a single TERT or JAK2 allele increased MPN susceptibility, we examined the combined effect of the number of risk alleles on the predisposition for MPN (Figure 1). A single TERT or JAK2 risk allele increased the risk of classic MPN by 2.3-fold (1.3-3.9) and each additional TERT or JAK2 risk allele further raised the risk separately. Harboring all four risk alleles (homozygous TERT and JAK2 individuals) conferred 9.6 times (4.4-21.1) higher risk for classic MPN development (Figure 1). In the classic MPN cohort, combined heterozygosity increased MPN risk sixfold, while combined homozygosity close to tenfold (Table 2). Interaction of the two variants was more outstanding in the JAK2 V617F+ subgroup compared to the CALR+ cohort where the effects of combined JAK2 hetero- and homozygosity did not differ significantly from each other. In the CALR+ subgroup, a single allele of JAK2 haplotype exerted a similar effect to that of JAK2 haplotype homozygosity (Table 2).

Similarly to previous reports (4, 5), in the Hungarian population the PAF for TERT rs2736100_C was larger (52.5%), than the PAF for JAK2 rs12343867_C (46.0%). Combined PAF for both common variants (74.4%) was estimated to explain the genetic background of the majority of our sporadic MPN.
2. **TERT rs2736100_C as susceptibility factor for coexisting solid tumors in classic MPN**

As a next step, we investigated the potential effect of *TERT* rs2736100_C on MPN signs and symptoms. In the whole classic MPN cohort, *TERT* rs2736100_C carriers displayed higher white blood cell count (9 vs. 11 G/L, \( p = 0.019 \)) compared to homozygous AA patients (dominant model). Similar observations were made in *JAK2* V617F+ (9 vs. 11 G/L, \( p = 0.001 \)) and in PV subgroups (10 vs. 11 G/L, \( p = 0.035 \)). The frequencies of different complications (splenomegaly, venous and arterial thrombosis, myelofibrotic or leukemic transformation) were not associated with the investigated *TERT* polymorphism either alone or in combination with the *JAK2* haplotype.

In spite of the relative lack of differences in the frequencies of life-threatening complications such as thrombosis, bleeding, myelofibrotic or leukemic transformation, we observed a tendency for adverse long-term overall survival according to *TERT* rs2736100 genotypes in the PV: the 15-year overall survival was 90.0±9.5% in patients with *TERT* rs2736100_AA, 62.7±8.5% with AC and 70.3±8.3% with CC genotype (\( p^{\text{global}} = 0.016, p^{\text{AAvsAC}} = 0.003, p^{\text{AAvsCC}} = 0.056 \), Figure 2A). In ET, the 15-year overall survival was 96.4±3.5% for AA, 78.9±6.7% for AC and 64.5±11.3% for CC genotype carriers (\( p^{\text{global}} = 0.229, p^{\text{AAvsAC}} = 0.6, p^{\text{AAvsCC}} = 0.2 \), Figure 2B). The survival was not different in PMF (Figure 2C). In the *BCR-ABL1*-negative MPN cohort, retrospective analyses comparing the cause of death in different *TERT* variant genotypes revealed that MPN patients with homozygous *TERT* rs2736100_C genotype had a higher probability (CC genotype: 44.4%; 12/27; 4 lung, 4 colorectal, 3 skin squamous cell carcinoma, 1 other type of tumor located in foramen jugulare) to die from solid tumors unrelated to the hematologic malignancy compared to wild type or heterozygous individuals (AA and AC genotypes: 5.3%; 2/38; \( n = 2 \) non-colorectal gastrointestinal cancers; \( p = 0.0004 \)).
Past medical history including the presence or absence of non-hematological malignancies was available in 356 BCR-ABL1-negative MPN cases. Solid tumors were present in 8.2% (4/49) of TERT wild type (rs2736100 AA genotype), in 16.2% (28/173) of heterozygous (AC genotype) and 23.1% (31/134) of homozygous (CC genotype; p=0.014 chi-squared test for trend). The most frequently developed solid tumors were the following: basal cell (n=10), skin squamous cell (n=9), colorectal (n=8), lung (n=7); prostate (n=7); and bladder carcinoma (n=5). As myelosuppressive treatment was suggested as the main cause of the well-documented increased frequency of solid tumors in MPN patients, we investigated the interaction of TERT germline variant with myelosuppressive treatment. Cytoreductive treatment increased the development of solid tumors in our cohort (no cytoreductive treatment: 6.0% (5/84); hydroxyurea treatment: 20.1% (48/239); busulphan or radiophosphorus treatment: 40.9% (9/22); p<0.001; 6% vs. 20.1% p=0.002). We performed multivariate analyses to test whether cytoreductive therapy and TERT genotypes were independent risk factors for non-hematological tumor formation. The analyses indicated that, TERT rs2736100_C genotype increased the risk of solid tumors independently from cytoreductive treatment [p=0.045; OR: 3.08 (1.03-9.26)]. TERT rs2736100_C genotype homozygosity reflected a greater risk [p=0.021; CC genotype: OR: 3.86 (1.23-12.08) vs. AC genotype: p=0.108; OR: 2.54 (0.82-7.94)].
3. **TERT rs2736100_C in CML and AML**

We tested 86 CML and 308 AML patients to investigate possible association between the *TERT* variant and diseases. The allele frequency of *TERT* rs2736100_C did not show significant difference neither in the CML nor in the AML group compared to controls (CML AF: 45.9±7.6%, p=0.56; AML AF: 52.1±4.0%, p=0.22; Table 1). Genotype distribution was examined in the group of AML patients with and without normal karyotype, but no statistically significance differences were found in the different AML subgroups (Table 1). In AML with complex karyotype changes (≥ 3 chromosomes affected), *TERT* rs2736100_C homozygosity showed a tendency to occur more frequently [40% (16/40) in AML with complex karyotype vs. 25% (63/251) in AML with no complex karyotype or 25% (101/400) in controls, both p=0.06].
Discussion

Alterations in the maintenance of telomere length are associated with increased risk of different cancers. Several germline polymorphisms of the TERT gene influence telomerase activity and consequentially telomere length. Although the exact mechanism how the intronic TERT gene rs2736100_C variant exerts its functional effect is unknown, several lines of evidence suggest that TERT rs2736100_C associates with longer telomere length (17, 18).

In recent years, genome wide association studies (GWAS) revealed that the TERT locus (located at 5p15.33) is one of the genomic regions (besides 8q24 and 9p21), which are associated with multiple solid tumors (19). Associations with cancers affecting skin (20), lung (21-23), colon (24), prostate (25), breast (26), testicle (27), bladder (28), brain (29) were reported. Predisposition to hematological malignancies such as acute lymphoid leukemia (18), and chronic lymphoid leukemia (30, 31), and recently MPN (4-6) were also linked to the TERT locus. Similarly to previous reports, in the present study, we confirmed the significant association between classic MPN and TERT rs2736100_C besides the JAK2 46/1 haplotype (tagged by rs12343867_C). Our data further support the previously reported increased allele frequencies of TERT rs2736100_C in each diagnostic subgroup of classic MPN (PV, ET, PMF) and in each MPN subgroups with different driver mutations (JAK2 V617F+, CALR+) (4, 5). In our cohort similarly to the Italian cohort (4), the TERT rs2736100_C allele increased MPN susceptibility both in JAK2 V617F+ or CALR+ MPN to a similar extent, while the effect of the JAK2 rs12343867_C allele was more pronounced in the JAK2 V617F+ group. Our results are in line with earlier observations that TERT and JAK2 risk alleles have independent and additive impact on MPN predisposition suggesting different underlying pathomechanisms in the background of MPN susceptibility owing to TERT and JAK2 variants. We also investigated CML and AML cases to further explore the potential role of TERT variant in the
development of other myeloid neoplasms, and we observed no association of \textit{TERT} rs2736100\textsubscript{C} allele with CML or AML susceptibility, similarly to the findings by Oddsson et al. (5). On the other hand, AML has a heterogeneous genetic, etiologic background, and we cannot exclude associations of \textit{TERT} variant allele with distinct AML subtypes (e.g. AML with complex karyotype).

In Icelandic and Japanese populations (5, 32), \textit{TERT} rs2736100\textsubscript{C} was associated with increase in peripheral blood cell counts (erythrocytes, thrombocytes and granulocytes-monocytes) in healthy individuals, therefore we hypothesized that \textit{TERT} rs2736100\textsubscript{C} variant might influence blood cell counts in MPN. However, no remarkable effect of \textit{TERT} variant was observed on hematological parameters, (except the elevated WBC in the entire classic MPN, PV and \textit{JAK2 V617F}\textsuperscript{+} cohorts), indicating that type and the burden of acquired driver and additional mutations had greater impact on hematological parameters than the germline \textit{TERT} variant.

Concerning major hematological complications (such as venous thrombosis, myelofibrotic and acute leukemic transformations) no association with the \textit{TERT} variant was found in our classic MPN cohort. In contrast, we observed that \textit{TERT} rs2736100\textsubscript{C} variant altered long term overall survival in carriers in PV. In the background, more frequent occurrence of non-hematological malignancies was revealed to be responsible for this adverse survival in our classic MPN cohort. \textit{BCR-ABL1}-chromosome negative and positive MPN patients were previously described to present with an increased risk of developing a subsequent non-hematological cancer (33). According to the general assumption, these subsequent non-hematological tumors were simply attributed to the application of myelosuppressive regimens. In line with the general assumption, an increased incidence of solid tumors was noted in patients treated with hydroxyurea, and to a greater extent in busulphan or radio-isotope phosphorus-32 (\textsuperscript{32}P) in our classic MPN cohort. Skin tumors were the most common
secondary tumors in our MPN cohort. The cutaneous side-effects of long-term hydroxyurea
treatment are well-known including skin tumors. Furthermore colorectal, lung, prostate and
bladder cancers were the most frequently observed co-occurring solid tumors in our classic
MPN cohort, all of which were recently associated with the presence of the TERT
rs2736100_C variant. Multivariate analyses showed that cytoreductive therapy and TERT
genotypes were independent risk factors for non-hematological tumor formation in BCR-
ABL1-negative classic MPN.

In a recent large epidemiological study, an increased number of solid tumors were reported
in MPN compared to the general population even 3-year prior to the MPN diagnosis (34).
This observation highlights a general predisposition to solid tumors in MPN without
cytoreductive treatment suggesting common, shared pathogenic background. Chronic
inflammation had been hypothesized to be a shared candidate trigger and driver both for MPN
and solid tumor development (34, 35). Our observations suggest that the common TERT
germline variant that predisposes both to MPN and solid tumors could also explain the
frequent co-occurrence. The high frequency of TERT rs2736100_C hetero and homozygous
carriers in the general (70%) and in the classic MPN population (85%) highlights the
importance of the well-known therapeutic suggestion to avoid long-term cytoreductive
treatment in young MPN patients.

The pathogenic role of TERT in the pathogenesis of MPN is further supported by recent
studies that investigated the potential value of a TERT inhibitor (imetelstat, a lipid-conjugated
oligonucleotide targeting the RNA template of TERT) in conventional therapy-
refractory/intolerant ET (36) and in primary myelofibrosis (37). Although myelosuppression
(thrombocytopenia) was reported as an “off-target” side effect of antisense oligonucleotide
treatment (independent of the antisense sequence), the observed hematologic, morphologic
and molecular remissions were promising for future MPN treatment (38).
In summary, we confirmed that, *TERT* rs2736100_C polymorphism predisposes to the development of *BCR-ABL1*-negative MPN regardless of the molecular background (JAK2 V617F+ and CALR+) or disease type (PV, ET, PMF) in an independent large cohort. Furthermore, we propose that this genetic predisposing factor contributes to the increased risk of non-hematological malignancies in MPN and the usage of cytoreductive therapies further add to this risk.
References


Table 1. Genotyping of TERT rs2736100 polymorphism in control and patient groups.

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>n</th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
<th>'C' AF ± 95% CI (%)</th>
<th>P allelic</th>
<th>OR (95% CI)</th>
<th>P (AA)</th>
<th>OR (AC) (95% CI)</th>
<th>OR (CC) (95% CI)</th>
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<tr>
<td>Controls</td>
<td>400</td>
<td>111</td>
<td>188</td>
<td>101</td>
<td>48.8 ± 3.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>MPN (total)</td>
<td>584</td>
<td>77</td>
<td>282</td>
<td>225</td>
<td>62.7 ± 2.8</td>
<td>&lt;0.0001</td>
<td>1.77 (1.47 - 2.12)</td>
<td>1</td>
<td>2.17 (1.54-3.06)</td>
<td>3.23 (2.22-4.69)</td>
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<tr>
<td>PV</td>
<td>209</td>
<td>26</td>
<td>106</td>
<td>77</td>
<td>62.2 ± 4.7</td>
<td>&lt;0.0001</td>
<td>1.73 (1.36 - 2.20)</td>
<td>1</td>
<td>2.41 (1.48-3.93)</td>
<td>3.26 (1.94-5.47)</td>
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<td>ET</td>
<td>281</td>
<td>38</td>
<td>139</td>
<td>104</td>
<td>61.7 ± 4.1</td>
<td>&lt;0.0001</td>
<td>1.70 (1.36 - 2.11)</td>
<td>1</td>
<td>2.16 (1.41-3.32)</td>
<td>3.01 (1.90-4.76)</td>
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<td>PMF</td>
<td>94</td>
<td>13</td>
<td>37</td>
<td>44</td>
<td>66.5 ± 6.9</td>
<td>&lt;0.0001</td>
<td>2.09 (1.50 - 2.91)</td>
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<td>1.31 (0.86-3.30)</td>
<td>3.72 (1.89-7.31)</td>
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<td>JAK2 V617F+</td>
<td>408</td>
<td>49</td>
<td>198</td>
<td>161</td>
<td>63.7 ± 3.4</td>
<td>&lt;0.0001</td>
<td>1.85 (1.51 - 2.25)</td>
<td>1</td>
<td>2.39 (1.41-3.93)</td>
<td>3.61 (2.38-5.49)</td>
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<td>CALR+</td>
<td>120</td>
<td>17</td>
<td>55</td>
<td>48</td>
<td>62.9 ± 6.2</td>
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<td>1.78 (1.33 - 2.40)</td>
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<td>1.91 (1.06-3.45)</td>
<td>&lt;0.001 3.10 (1.68-5.74)</td>
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<td>MPL+</td>
<td>16</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>62.5 ± 17.1</td>
<td>0.1501</td>
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<td>5.90 (0.75-64.74)</td>
<td>0.123 5.50 (0.63-47.83)</td>
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<td>10</td>
<td>19</td>
<td>11</td>
<td>51.3 ± 11.2</td>
<td>0.7254</td>
<td>1.11 (0.70 - 1.75)</td>
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<td>1.02 (0.59-1.75)</td>
<td>0.680 1.21 (0.49-2.97)</td>
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<td>86</td>
<td>25</td>
<td>43</td>
<td>18</td>
<td>45.9 ± 7.6</td>
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<td>0.89 (0.64 - 1.24)</td>
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<td>1.02 (0.59-1.75)</td>
<td>0.489 0.79 (0.41-1.54)</td>
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<td>AML (total)</td>
<td>308</td>
<td>71</td>
<td>153</td>
<td>84</td>
<td>52.1 ± 4.0</td>
<td>0.2178</td>
<td>1.14 (0.93 - 1.41)</td>
<td>1</td>
<td>1.27 (0.88-1.84)</td>
<td>0.215 1.30 (0.86-1.97)</td>
</tr>
<tr>
<td>NK-AML</td>
<td>122</td>
<td>28</td>
<td>64</td>
<td>30</td>
<td>50.8 ± 6.4</td>
<td>0.6088</td>
<td>1.09 (0.82 - 1.45)</td>
<td>1</td>
<td>1.35 (0.82-2.23)</td>
<td>0.582 1.18 (0.66-2.11)</td>
</tr>
<tr>
<td>Non NK-AML</td>
<td>169</td>
<td>40</td>
<td>80</td>
<td>49</td>
<td>52.7 ± 5.4</td>
<td>0.2431</td>
<td>1.17 (0.91 - 1.51)</td>
<td>1</td>
<td>1.18 (0.76-1.85)</td>
<td>0.241 1.35 (0.82-2.21)</td>
</tr>
</tbody>
</table>
Table 2. Interaction of *TERT* rs2736100 and *JAK2* rs12343867 polymorphisms in different classic MPN patient subgroups differing in driver mutations (*JAK2* V617F or *CALR*).

<table>
<thead>
<tr>
<th></th>
<th>Entire classic MPN</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td><strong>MPN vs. control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>2.24</td>
<td><strong>0.006</strong></td>
<td>3.62</td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>5.96</td>
<td><strong>&lt;0.001</strong></td>
<td>8.25</td>
</tr>
<tr>
<td><strong>CC</strong></td>
<td>6.52</td>
<td><strong>&lt;0.001</strong></td>
<td>9.60</td>
</tr>
<tr>
<td><strong>JAK2 V617F+ MPN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>2.49</td>
<td><strong>0.012</strong></td>
<td>3.63</td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>7.76</td>
<td><strong>&lt;0.001</strong></td>
<td>11.73</td>
</tr>
<tr>
<td><strong>CC</strong></td>
<td>9.91</td>
<td><strong>&lt;0.001</strong></td>
<td>14.08</td>
</tr>
<tr>
<td><strong>CALR+ MPN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>2.27</td>
<td>0.095</td>
<td>4.36</td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>3.98</td>
<td><strong>0.004</strong></td>
<td>5.47</td>
</tr>
<tr>
<td><strong>CC</strong></td>
<td>2.54</td>
<td>0.156</td>
<td>4.69</td>
</tr>
</tbody>
</table>
Abbreviations for Tables 1 and 2.

95%CI: 95% confidence interval; AML: acute myeloid leukemia; CALR+: calreticulin gene mutation positive; CML: chronic myeloid leukemia; ET: essential thrombocythemia; JAK2 V617F: Janus kinase 2 gene Val617Phe mutation positive; MPL+: thrombopoietin receptor gene mutation positive; MPN: myeloproliferative neoplasm; neg.: negative; NK-AML: AML with normal karyotype; OR: Odds ratio; PMF: primary myelofibrosis; PV: polycythemia vera; TERT: telomerase reverse transcriptase gene.
Figure 1. Increasing MPN susceptibility according to the number of \( TERT \) rs2736100 and \( JAK2 \) rs12343867 risk alleles. Heterozygous genotype counts as single, while homozygous genotype as double risk alleles.

Figure 2. Overall survival according to \( TERT \) rs2736100 \_C\_A genotype in the following MPN diagnostic subgroups: polycythemia vera (Panel A), essential thrombocythemia (Panel B) and primary myelofibrosis (Panel C). Global \( p \) value is indicated by bold face characters on each panel, while \( p \) values for pairwise comparisons are marked with italic.
Figure 1

OR (95% CI)

Number of TERT/JAK2 risk alleles
Figure 2A

Probability of survival vs. Time from diagnosis (years)

- AA (n=25)
- CC (n=71)
- AC (n=97)

- AA vs. CC p=0.056
- AC vs. CC p=0.133
- AA vs. AC p=0.003

p=0.016
Figure 2B

AA (n=37)

AC (n=122)

CC (n=98)

Time from diagnosis (years)

Probability of survival

AC vs. CC
p=0.1

AA vs. AC
p=0.6

AA vs. CC
p=0.2

p=0.229
Figure 2C

Probability of survival vs. Time from diagnosis (years) for AA (n=13), AC (n=33), and CC (n=41). The p-value is 0.812.
Co-occurrence of myeloproliferative neoplasms and solid tumors is attributed to a synergism between cytoreductive therapy and the common TERT polymorphism rs2736100

Tunde Krahling, Katalin Balassa, Katalin P Kiss, et al.

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