Co-occurrence of Myeloproliferative Neoplasms and Solid Tumors Is Attributed to a Synergism Between Cytoreductive Therapy and the Common TERT Polymorphism rs2736100

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Abstract

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 with 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 polychytemia vera patients. MPN patients with the TERT CC genotype had a higher probability (44.4%) to die from solid tumors compared with 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. Cancer Epidemiol Biomarkers Prev; 25(1); 1–7. ©2015 AACR.

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, essential thrombocythemia, and primary myelofibrosis. The pathogenesis of the three, classic MPN entities involves the activation of the JAK2-STAT pathway by mutations affecting Janus kinase 2 (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% to 95% of polycythemia vera and 50% to 60% of essential thrombocythemia or primary myelofibrosis cases. The frequencies of CALR and MPL mutations vary between 25% and 35% and 5% and 10% in essential thrombocythemia and primary myelofibrosis. 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; ref. 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 polycythemia vera, 281 from essential thrombocythemia, and 94 from primary myelofibrosis. Besides the occurrence of coagulation complications, myelofibrotic or leukemic transformation and the history on nonhematologic 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.

Four-hundred 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 JAK2 46/1 haplotype frequency (11). In case of 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: TERT-LCF: 5'-GCA TAT TAA TAT TGT TTT CCG T-3'; TERT-LCR: 5'-GCA ATA ACA CAG CAG AAC CAC C-3'; TERT-SENS: 5'-Cys5-GGC AAA CCT ACA GAA AC- Phosphate-3'; TERT-ANC: 5'-AAG GAG GAA AAG CAG GGG C-Fluorescein-3'. Asymmetric PCR (reaction volume of 20 μl) was performed with 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, 2× PCR Master Mix (Promega). Cycling conditions were as follows: 95°C for 3 minutes, 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 JAK2 V617F mutation by real-time quantitative PCR (12). JAK2 V617F negative essential thrombocythemia and primary myelofibrosis patients were tested for the presence of CALR mutation by fragment analysis (1). Screening for MPL mutations was performed by high resolution melting in JAK2 V617F and CALR-negative essential thrombocythemia and primary myelofibrosis patients (13). The CALR- and MPL-positive cases were confirmed by Sanger sequencing. CML patients were regularly monitored for 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 χ2 or Fisher exact tests, while continuous variables were conferred by using Mann–Whitney or Kruskal–Wallis tests. Odds ratios (OR) and 95% CIs (±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 primary myelofibrosis and 3 secondary myelofibrotic cases). Statistical analyses were performed using SPSS 20.0 software package.

Results

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 polycythemia vera patients were JAK2 V617F positive (n = 209). In the essential thrombocythemia cohort, 148 (53%) JAK2 V617F, 95 (34%) CALR, and 9 (3%) MPL gene mutation positive and 29 (10%) triple-negative cases; whereas, in the primary myelofibrosis 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 with 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, carriage 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 = 0.001 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 (essential thrombocythemia or primary myelofibrosis), a higher TERT variant AF (68.2 ± 19.9%) was noted in the triple-negative primary myelofibrosis, but no tendency for TERT AF increase was apparent in the triple-negative essential thrombocythemia group (AF = 44.8 ± 13.1%) compared with 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
Cohorts 

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<tr>
<th>n</th>
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<th>CC</th>
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<th>OR (AA)</th>
<th>OR (AC)</th>
<th>OR (CC)</th>
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<td>111</td>
<td>188</td>
<td>101</td>
<td>48.8 ± 3.5</td>
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<td>1.70 (1.36–2.10)</td>
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<td>38</td>
<td>159</td>
<td>104</td>
<td>61.7 ± 4.1</td>
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<td>44</td>
<td>66.5 ± 6.9</td>
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<td>1.85 (1.31–2.63)</td>
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Abbreviations: CALR, calreticulin gene mutation positive; JAK2 V617F, Janus kinase 2 gene V617F mutation positive; MPN, myeloproliferative neoplasm; neg., negative; NK-AML, AML with normal karyotype; TERT, telomerase reverse transcriptase gene.

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 with homozygous AA patients (dominant model). Similar observations were made in JAK2 V617F+ (9 vs. 11 G/L, \( P = 0.001 \)) and in polycythemia vera subgroups (10 vs. 11 G/L, \( P = 0.035 \)). The frequencies of different complications (splanchnal, 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 OS according to TERT rs2736100 genotypes in the polycythemia vera: the 15-year OS 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 (\( P_{\text{global}} = 0.016 \), \( P_{\text{AAAC}} = 0.003 \), \( P_{\text{AAAC}} = 0.056 \), Fig. 2A). In essential thrombocythemia, 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 (\( P_{\text{global}} = 0.229 \), \( P_{\text{AAAC}} = 0.6 \), \( P_{\text{AAAC}} = 0.2 \), Fig. 2B). The survival was not different in primary myelofibrosis (Fig. 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 with wild-type or heterozygous individuals (AA and AC genotypes: 5.3%; 2/38; \( n = 2 \) noncolorectal gastrointestinal cancers; \( P = 0.0004 \)).

Past medical history including the presence or absence of nonhematological 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; \( P = 0.014 \chi^2 \) 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]; busulfan or radiochromosome 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 nonhematologic 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)].

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 with 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 significant 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 consequently 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), and brain (29) were reported. Predisposition to hematologic 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 (polychromatia vera, essential thrombocytopenia, primary myelofibrosis) and in each MPN subgroups with different driver mutations (JAK2 V617F+, CALR+, refs. 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 TERT rs2736100_C allele with CML or AML susceptibility, similarly to the findings by Oddsson and colleagues (5). On the other hand, AML has a heterogeneous genetic, etiologic background, and we cannot exclude associations of TERT variant allele with distinct AML subtypes (e.g., AML with complex karyotype).

In Icelandic and Japanese populations (5, 32), TERT rs2736100_C was associated with increase in peripheral blood cell counts (erythrocytes, thrombocytes, and granulocytes-monocytes) in healthy individuals; therefore, we hypothesized that TERT rs2736100_C variant might influence blood cell counts in MPN. However, no remarkable effect of TERT variant was observed on hematologic parameters, (except the elevated WBC in the entire classic MPN, polychromatia vera and JAK2 V617F+ cohorts), indicating that type and the burden of acquired driver and additional mutations

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<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
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<td>CC</td>
<td>3.98 (1.55–10.22)</td>
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**Table 2. Interaction of TERT rs2736100 and JAK2 rs12343867 polymorphisms in different classic MPN patient subgroups differing in driver mutations (JAK2 V617F or CALR)**

Abbreviations: CALR+, calreticulin gene mutation positive; 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; TERT, telomerase reverse transcriptase gene.
had greater impact on hematologic parameters than the germ-line TERT variant.

Concerning major hematologic complications (such as venous thrombosis, myelofibrotic and acute leukemic transformations) no association with the TERT variant was found in our classic MPN cohort. In contrast, we observed that TERT rs2736100_C variant altered long-term overall survival in carriers in polycythemia vera. In the background, more frequent occurrence of nonhematological malignancies was revealed to be responsible for this adverse survival in our classic MPN cohort. BCR-ABL1 chromosome–negative and positive MPN patients were previously described to present with an increased risk of developing a subsequent nonhematologic cancer (33). According to the general assumption, these subsequent nonhematologic 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 (32P) 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 cyto-reductive therapy and TERT genotypes were independent risk factors for nonhematologic tumor formation in BCR-ABL1–negative classic MPN.

In a recent large epidemiologic study, an increased number of solid tumors were reported in MPN compared with the general population even 3 years 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 essential thrombocythemia (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

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<th>Time from diagnosis (years)</th>
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**Figure 2.**
OS according to TERT rs2736100_C/A genotype in the following MPN diagnostic subgroups: polycythemia vera (PV, A), essential thrombocytopenia (ET, B), and primary myelofibrosis (PM, C). Global $P$ value is indicated by bold face characters on each panel, while $P$ values for pairwise comparisons are marked with italic.
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 (polycythemia vera, essential thrombocythemia, primary myelofibrosis) in an independent large cohort. Furthermore, we propose that this genetic predisposing factor contributes to the increased risk of nonhematologic malignancies in MPN and the usage of cytoreductive therapies further add to this risk.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: T. Krahling, H. Andrikovics
Development of methodology: T. Krahling, K. Balassa, H. Andrikovics
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Batai, G. Halm, S. Fekete, T. Masszi, A. Tordai, H. Andrikovics

References

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Krahling, H. Andrikovics
Writing, review, and/or revision of the manuscript: T. Krahling, K. Balassa, A. Batai, G. Halm, S. Fekete, T. Masszi, A. Tordai, H. Andrikovics

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