Platinum-based chemotherapy for pancreatic cancer: impact of mutations in the homologous recombination repair and Fanconi anemia genes

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Abstract

Background: Mutations in homologous recombination (HR) and Fanconi anemia (FA) genes may predispose to pancreatic cancer (PC) and enable the prediction of sensitivity to platinum-based chemotherapy. FOLFIRINOX is a standard treatment option for non-selected PC patients and could be effective due to undiagnosed DNA repair deficiency. Here, we aimed to determine the frequency of mutations in genes involved in the HR and FA pathways, evaluate their clinical implications, and determine the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) of PC patients treated with platinum.

Methods: We performed targeted DNA sequencing of 30 genes (*ABRAXAS1*, *ATM*, *ATR*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDKN2A*, *CHEK1*, *CHEK2*, *FANCC*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *MRE11A*, *NBN*, *PALB2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *RAD52*, *RAD54B*, *RBBP8*, *RINT1*, *SLX4*, and *XRCC2*) for 543 PC patients.

Results: In *BRCA/PALB2*-mutated patients with advanced PC (33 patients, 6.1%), the PFS and OS were higher for first-line platinum therapy than for non-platinum therapy [PFS: HR = 0.28, 95% confidence interval (CI) = 0.10–0.81, $p = 0.02$; OS: HR = 0.31, 95% CI = 0.08–1.16, $p = 0.08$]. Among 93 patients (17.1%) with mutations in other HR/FA genes, no statistically significant difference in PFS and OS was observed between first-line platinum therapy and non-platinum therapy (PFS: HR = 0.83, 95% CI = 0.43–1.62, $p = 0.59$; OS: HR = 0.58, 95% CI = 0.28–1.22, $p = 0.15$). For patients with early PC, no prognostic value was observed for *BRCA1/2*, *PALB2*, or other HR/FA genes mutations. Moreover, a personal history of breast, ovarian, pancreatic, or prostate cancer was identified as the only independent predictor of the risk of *BRCA/PALB2* mutations (HR = 5.83, 95% CI = 2.16–15.73, $p < 0.01$).

Conclusion: Mutations in the *BRCA1/2* and *PALB2* genes increase the sensitivity of PC to platinum agents. Thus, alterations in these genes in PC patients must be determined prior to anticancer therapy.

Keywords: *BRCA1/2*, homologous recombination deficiency, mutations, *PALB2*, pancreatic cancer, platinum-based chemotherapy

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Introduction

Pancreatic cancer (PC) is the seventh leading cause of cancer-related deaths worldwide, with a 5-year overall survival (OS) rate of less than 10%.[1,2]

The current standard of care for patients with metastatic pancreatic duct adenocarcinoma and a good performance status includes platinum-based chemotherapy regimen, FOLFIRINOX, which is used to treat non-selected patient populations.[3]

Germline and somatic genetic profiling of PC is a new trend in modern research and contributes to the optimization and individualization of currently available treatment options.[4–10] From the perspective of the clinical and therapeutic influence of mutations in PC, the *BRCA1* and *BRCA2* genes are the most studied.[4–10]

BRCA1/2 plays a central role in the homologous recombination (HR) pathway, which is necessary for error-free double-strand break repair. Significant intersections of the HR pathway and Fanconi anemia (FA) pathway, the latter of which performs the repair of DNA interstrand crosslinks (ICLs), have recently been deciphered. Key HR proteins, including the tumor suppressors, BRCA1 and BRCA2, also play important roles in ICL repair.[11]

Tumors with BRCA1/2 deficiency are defective in DNA repair by HR and are sensitive to interstrand DNA crosslinking agents, such as cisplatin and carboplatin, and poly(ADP-ribose) polymerase (PARP) inhibitors. Therefore, these agents are logical options for the treatment of BRCA1/2-deficient tumors and have been demonstrated to be clinically effective.[12]

Encouraging results have been reported regarding the use of platinum agents (with or without PARP inhibition) in PC patients with BRCA1/2-deficient tumors. As the germline and somatic alterations in these genes lead to disruption of the DNA damage repair pathway, alterations in other genes involved in the same functional process may also have a similar effect on sensitivity to therapy.[13–18] In the present study, we investigated the frequency and spectrum of mutations in 30 genes involved in the HR and FA pathways: *ABRAXAS1, ATM, ATR, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDKN2A, CHEK1, CHEK2, FANCC, FANCF, FANCG, FANCI, FANCL, FANCM, MRE11A, NBN, PALB2, PTEN, RAD50, RAD51C, RAD51D, RAD52, RAD54B, RBBP8, RINT1, SLX4,* and *XRCC2*. We also determined the effect of deleterious variants in these genes on the effectiveness of platinum drug treatment. This is the largest study of its kind in the Russian population of patients with PC.

Methods

Patients and samples
Patients with morphologically confirmed pancreatic adenocarcinoma who received treatment for PC from 2001 to 2019 at the N.N. Blokhin National Medical Research Center for Oncology were included in the study. The exclusion criteria were neuroendocrine or non-epithelial tumors of PC, metastases of other tumors to the pancreas, other periampullary cancers, and the absence of formalin-fixed and paraffin-embedded (FFPE) tissue or blood samples of sufficient quality. Medical records for family history, metastatic sites, treatments, and outcomes were collected.

This study included both retrospective and prospective cohorts. For the retrospective cohort of patients, the FFPE material (normal and/or tumor tissue) was collected; for the prospective cohort of patients, the peripheral blood sample and/or the FFPE material (normal and/or tumor tissue) were collected. An audit of the FFPE blocks was performed by a morphologist to select areas maximally enriched in tumor cells, as well as areas containing only normal tissue for subsequent DNA extraction.

This study was supported and approved by the Ministry of Health of the Russian Federation (Reg. No. ROSRID AAAA-A18-118112290058-0). Written informed consent was obtained from all participants included in the prospective part of the study. Ethical approval was obtained from the Ethical Committee of the N.N. Blokhin National Medical Research Center for Oncology.

DNA isolation
DNA was extracted from peripheral blood using a DNeasy Blood & Tissue Kit (Qiagen, Germany). All FFPE samples were subjected to histological control to verify and select only normal or tumor cells for extraction. DNA from FFPE tissues was isolated using a blackPREP FFPE DNA Kit (Analytik Jena, Germany) according to the manufacturer's instructions. The quantity of nucleic acids was controlled using a Qubit 4.0 Fluorometer (Invitrogen, Life Technologies Corporation, USA) using a Qubit dsDNA HS Assay Kit (Invitrogen, Life Technologies Corporation), according to the manufacturer's protocol.

Gene panel
To assess genes known or suspected to play a role in PC development or treatment, we designed a customized panel for targeted DNA sequencing using the NimbleDesign Software (Roche, Switzerland). The analysis of germline and

somatic variants was restricted to 30 genes (exons ± 20 bp in bordering introns) linked to inherited cancer risk, including those related to the HR and FA pathways: *ABRAXAS1, ATM, ATR, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDKN2A, CHEK1, CHEK2, FANCC, FANCF, FANCG, FANCI, FANCL, FANCM, MRE11A, NBN, PALB2, PTEN, RAD50, RAD51C, RAD51D, RAD52, RAD54B, RBBP8, RINT1, SLX4,* and *XRCC2.*

Next generation sequencing (NGS)

DNA (100 ng per sample for DNA from peripheral blood or 500 ng per sample for DNA from FFPE tissues) was sheared to 200 bp using a Covaris S220 System (Thermo Fisher Scientific, USA) and subjected to library preparation with KAPA HyperPlus Kit (Roche, Switzerland), according to the manufacturer's instructions. Libraries were validated prior to sequencing using quantitative polymerase chain reaction (PCR). Sequencing was performed on a NextSeq 500 System (Illumina, USA) and MiniSeq System (Illumina) in paired-end mode. Read length was 151 bp. The obtained coverage was at least $500\times$ for tumor tissues and at least $200\times$ for blood and normal tissues.

Variant calling and characterization

First, Illumina reads were trimmed and filtered, and the remaining adapters were removed using Trimmomatic 0.38. Then the reads were mapped to the human genome (GRCh37.75) using BWA 0.7.17.[19] The derived Binary Alignment Map (BAM) files were sorted, grouped, and reordered using Picard-tools 2.21.3 (http://broadinstitute. github.io/picard/) and Samtools 1.10. Thereafter, the FixMateInformation tool (Picard-tools) was run to fix paired and read information, and the PCR and optical duplicates were marked with MarkDuplicatesWithMateCigar (Picard-tools). Base quality score recalibration was not performed because of the small panel size (30 genes, 139 kb).

Variant calling was performed using HaplotypeCaller (GATK 4.0.8.1),[20] freeBayes 1.3.2,[21] and VarScan 2.4.3,[22] in two ways: joint calling simultaneously for all samples and separately for each sample. The search was limited to the regions defined in the manifest file provided by Roche (with 50 bp padding). The derived HaplotypeCaller Variant Call Format (VCF) files were split into indels and single nucleotide variations, and then hard-filtered using VariantFiltration tool from the GATK package (excluding variants with low mapping quality, low confidence, strand bias, positional bias, etc.). For freeBayes and VarScan, we tightened the default start-up parameters (minimal alternate allele coverage = 5, mapping and base calling quality = 20). In most cases, we relied on the results derived with HaplotypeCaller and freeBayes because of the high false-positive rate for VarScan. In addition, we marked substitutions in error-prone motifs (e.g. GGGTG > GGGGG, CCCG > CCCC). The derived variant list was annotated using Annovar (June 2020 version).

Mutect2 (GATK 4.0.8.1) was used to identify somatic mutations. When the matched normal tissue (or blood) samples were available, Mutect2 was run in paired mode. Otherwise, we launched Mutect2 in 'tumor-only' mode. We supplied Mutect2 with gnomAD 2.1.1[23] population frequency data. Next, we tried to eliminate FFPE artifacts using the LearnReadOrientationModel, GetPileupSummaries, and CalculateContamination tools according to the GATK best practice workflow for somatic short variant discovery. Finally, VCFs generated with Mutect2 were transferred to the FilterMutectCalls tool (GATK) and then annotated using Annovar. In addition, we manually examined paired tumor-normal samples to include variants missed by Mutect2.

Pathogenicity scoring

Germline variants. A phased pipeline was constructed to evaluate the pathogenicity of each rare variant that passed quality control. Variants were classified as 'Pathogenic' (P), 'Likely Pathogenic' (LP), 'Variant of Uncertain Significance' (VUS), 'Likely Benign' (LB), or 'Benign' (B). The interpretation of variants was based on a recent publication[24] and the recommendations of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP).[25]

The pipeline included several successive stages:

> Stage 1: For further analysis, we included only non-synonymous variants (except synonymous variants in canonical splice sites) with a maximal population frequency <1%.
> Stage 2: Variants matching a known variant annotated in ClinVar with a review status of at

least two stars[26] were categorized as the designated pathogenicity category given by ClinVar (P, LP, VUS, LB, or B).

Stage 3: Variants not annotated in ClinVar (Stage 1) were evaluated using InterVar (default settings; http://wintervar.wglab.org/)[27] and categorized by their designated pathogenicity category (P, LP, VUS, LB, or B).

Stage 4: Variants of uncertain significance, as determined by either ClinVar or InterVar and non-annotated variants, which were high-impact variants (frameshift indels, stop gain/loss, or canonical splice sites), were categorized as LP. Frameshift indels and stop gain were manually reviewed considering the 50 bp rule[28,29] for evaluating pathogenicity and categorized as LP or VUS.

Step 5: The VUS category was further evaluated by an *in silico* prediction algorithm to categorize VUS variants as 'damaging' (VUS_D) or 'not damaging' (VUS_ND). Several programs were used: SIFT, Polyphen2 HDIV, Polyphen2 HVAR, LRT, MutationTaster, MutationAssessor, FATHMM, PROVEAN, VEST3, MetaSVM, MetaLR, M-CAP, REVEL, MutPred, CADD, and DANN. The variant was categorized as 'VUS_D' if 2/3 of all algorithms and 2/3 of ensemble algorithms (VEST3, MetaSVM, MetaLR, M-CAP, REVEL, CADD, DANN) predicted that it is deleterious; otherwise, the variant was categorized as 'VUS_ND'.

Stage 6: Variant calls for all P/LP/VUS(VUS_D and VUS_ND) were further manually reviewed by visual verification using the Integrative Genomic Viewer (IGV) to exclude sequencing and analysis artifacts.[30]

Stage 7: All P/LP/VUS_D variants were manually reviewed for the final confirmation of P, LP, or VUS_D status. The review included an analysis of the literature for confirmation of pathogenicity, gene-specific database review, and evaluation of the impact of mutations on gene function.

Somatic variants. The pipeline was created to evaluate the pathogenicity of each rare somatic variant that passed quality control. Variants were classified as P/LP/VUS/LB/B. The classification of variants was based on standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists (AMP-ASCO-CAP) guidelines.[31]

The pipeline included several successive stages:

Stage 1: For further analysis, we filtered out somatic variants with a population frequency threshold >0.5%, according to gnomAD 2.1.1.[23]

Stage 2: Variants matching a known variant annotated in ClinVar with a review status of at least two stars[26] were categorized as the designated pathogenicity category given by ClinVar. We did not use COSMIC (Catalogue of Somatic Mutations in Cancer) pathogenicity prediction data as it is only based on one predictive algorithm, FATHMM.

Stage 3: Variants not annotated in ClinVar (Stage 1) were evaluated using VIC (Variant Interpretation for Cancer),[32] which classifies variants according to the AMP-ASCO-CAP guidelines, and were categorized by their designated pathogenicity category (P, LP, VUS, LB, or B).

Stage 4: Variants of uncertain significance, as determined by either ClinVar or VIC and non-annotated variants, which were high-impact variants (frameshift indels, stop gain/loss, or canonical splice sites), were categorized as LP. Frameshift indels and stop gain were manually reviewed considering the 50 bp rule[28,29] for evaluating pathogenicity and categorized as LP or VUS.

Stages 5 to 7 are the same as those outlined in the 'Germline variants' section.

Assessments

We evaluated the frequency and structure of P/LP/VUS_D mutations in HR and FA genes in patients with PC, their clinical and morphological characteristics, as well as the objective response rate (ORR), progression-free survival (PFS), and OS based on platinum use. PFS was defined as the time from the start of chemotherapy to objective disease progression, while OS was defined as the time from the start of chemotherapy to the patient's death (or last contact for censored observations).

Statistical analysis

The chi-square and Fisher criteria were used for comparative analysis of nominal and serial
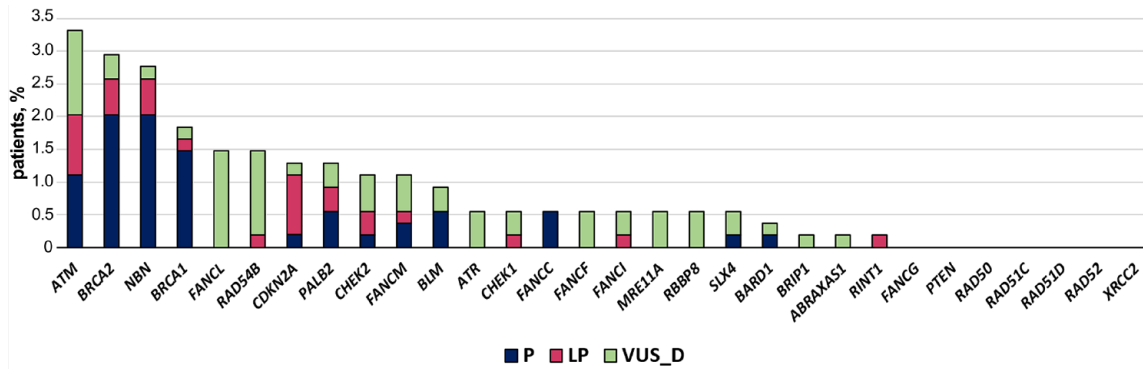
Figure 1. Frequency of pathogenic (P), likely pathogenic (LP), and variants of uncertain significance which evaluated by *in silico* prediction algorithms as 'damaging' (VUS_D; see 'Pathogenicity scoring' section) in pancreatic cancer patients ($n = 543$).

variables. The Yates-corrected chi-square statistic was used in the analyses of 2×2 contingency tables. The Mann–Whitney criterion (comparing two groups) or Kruskal–Wallis criterion (comparing more than two groups) was used for comparative analysis of quantitative variables with abnormally distributed samples. The *t*-criterion was used for normally distributed samples using the Shapiro–Wilk criterion. A log-range test was used for the comparative analysis of survival. The Kaplan–Maier method was used to calculate survival.

Cox regression analysis was used to assess the impact of traits on prognosis. The analysis involved two-stage selection of traits. First, a series of univariate regression analyses was performed. Thereafter, a multivariate regression analysis with forced inclusion of variables was performed for traits that showed a statistically significant impact on prognosis. If the trait had a statistically significant effect on prognosis based on multivariate regression analysis, we treated this as an independent prognostic factor for the selected group of patients.

To assess the effect of potential predictors on the risk of carrying a germinal mutation, logistic regression was carried out with mutations in *BRCA1/2*, *PALB2*, or other HR/FA genes as dependent variables. Regression results were analyzed using a regression coefficient exp(*B*), 95% confidence interval (CI), and *p* values.

Statistical analysis of the obtained results was performed using Microsoft Excel 2007 software (Microsoft, USA) and IBM SPSS Statistics v.20.0 (IBM, USA).

Results

Peripheral blood samples or FFPE tissues were obtained from 543 PC patients who were treated at the N.N. Blokhin National Medical Research Center for Oncology, Ministry of Health of the Russian Federation, between 2001 and 2019. The non-tumor material only (peripheral blood or FFPE normal tissues) was evaluated in 427 patients, tumor-only material was investigated in 80 patients, and non-tumor and tumor materials were investigated in 36 patients.

Genetic alterations in PC patients

We assessed the frequency of P, LP, and VUS_D variants in 30 genes involved in the HR and FA pathways: *ABRAXAS1*, *ATM*, *ATR*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDKN2A*, *CHEK1*, *CHEK2*, *FANCC*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *MRE11A*, *NBN*, *PALB2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *RAD52*, *RAD54B*, *RBBP8*, *RINT1*, *SLX4*, and *XRCC2*. Overall, 69 of 543 (12.7%) patients with PC had P/LP variants, and 54 of 543 (9.9%) patients had VUS_D variants (Figure 1). A list of the identified mutations is presented in Supplementary Table 1. Mutations were not detected in *FANCF*, *FANCG*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *RAD52*, and *XRCC2*.

ATM gene. The largest number of P, LP, and VUS_D variants was found in the *ATM* gene in 18/543 (3.3%) patients. The P variant c.G5932T (p.E1978X) was the most common and was found in three patients. The LP variant, c.T8565G (p.S2855R), was detected in two patients. Other variants were not found to be repeated.

BRCA2 gene. The P, LP, and VUS_D variants in *BRCA2* were found in 16/543 (2.9%) patients. The c.T5286G (p.Y1762X) variant was the most frequent and was found in four patients. Another recurring variant was c.A7879T (p.I2627F), which was found in three patients. All other variants were identified once.

NBN gene. Fifteen of the 543 (2.8%) patients carried P, LP, and VUS_D variants in the *NBN* gene. The c.657_661del (p.K219fs) variant was detected in 11 patients, while the c.C643T (p.R215W) variant was found in 3 patients. In one case, the VUS_D variant c.C191G (p.P64R) was found.

BRCA1 gene. The P, LP, and VUS_D variants in the *BRCA1* gene were found in 10/543 (1.8%) patients, eight of which harbored the P variant, c.5266dupC (p.Q1756fs). The c.G4036A (p.E1346K) variant and exon13_exon22del were found in one case each.

FANCL gene. Eight patients (1.5%) harbored VUS_D variants in the *FANCL* gene. No P or LP variants were found. The non-frameshift deletion, c.712_714del (p.238del), was the most common variant and was detected in six cases. The c.G637A (p.D213N) and c.C800A (p.P267H) variants were found in one case each.

RAD54B gene. Eight patients (1.5%) harbored the LP or VUS_D variants in the *FANCL* gene. The c.A1889T (p.D630V) classified as VUS_D was found in five cases. The c.G1721A (p.G574E) and c.G2342A (p.R781K) variants were found in two and one cases, respectively.

PALB2 gene. The P, LP, and VUS_D variants in *PALB2* were found in 7/543 (1.3%) patients. All of these were unique.

CDKN2A gene. The P, LP, and VUS_D variants in *CDKN2A* were found in 7/543 (1.3%) patients. All of these variants were unique.

CHEK2 gene. Six patients (1.1%) harbored P, LP, or VUS_D variants in the *CHEK2* gene. The c.C433T (p.R145W) and c.C972G (p.C324W) variants were found in two cases each. The remaining variants were identified once.

FANCM gene. Six patients (1.1%) harbored the P, LP, or VUS_D variants in the *FANCM* gene. All of these were unique.

Other genes. For each gene, mutations were found in less than 1.0%: *BLM* (5 patients, 0.9%), *ATR* (3 patients, 0.6%), *CHEK1* (0.6%), *FANCC* (0.6%), *FANCI* (0.6%), *MRE11A* (0.6%), *RBBP8* (0.6%), *SLX4* (0.6%), *BARD1* (0.4%), *BRIP1* (0.2%), *ABRAXAS1* (0.2%), and *RINT1* (0.2%). Mutations were not detected in *FANCF*, *FANCG*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *RAD52*, and *XRCC2*.

Efficacy of platinum therapy

Patients' characteristics. We analyzed 277 patients with metastatic or locally advanced PC with known HR and FA genes statuses. These patients were assigned to three groups: mutations in *BRCA1/2* or *PALB2* genes, mutations in other HR/FA genes, and wild-type HR/FA genes (see Table 1). All three groups did not have any statistically significant differences in the main prognostic indicators, such as sex, age, T stage, N stage, diameter of primary tumor, localization in the head/body or body/tail of the pancreas, liver metastases, canceromatosis, ascites, baseline carbohydrate antigen (CA) 19-9, and ECOG (Eastern Cooperative Oncology Group) status. Females were more common in the *BRCA/PALB2*-mutated group than in the wild-type HR/FA genes group (71.4% *versus* 51.7%, respectively). No patients in the *BRCA/PALB2*-mutated group had ECOG 2 status or more, while 14 patients (7.0%) in the wild-type HR/FA genes group had an ECOG 2 status or higher.

ORR. We compared the ORR dependence of first-line platinum use between the three groups of patients. In the *BRCA/PALB2*-mutated group, 12 patients were treated with first-line platinum (57.1%). In the groups with mutations in other HR/FA genes and wild-type HR/FA genes, 25 (45.5%) and 90 (44.8%) patients were, respectively, treated with first-line platinum chemotherapy (Table 2). Most patients in all the groups received FOLFIRINOX, but four patients in the wild-type HR/FA genes group, two patients in *BRCA/PALB2*-mutated group, and two patients in the other HR/FA genes mutated group received gemcitabine plus cisplatin or oxaliplatin (GemPt).

In the *BRCA/PALB2*-mutated group, no statistically significant difference in ORR was observed, although ORR was numerically higher for platinum-based therapy than non-platinum therapy (58.3% *versus* 25.0%, $p = 0.20$). All objective responses occurred with the use of the FOLFIRINOX regimen, and no responses were

Table 1. Characteristics of patients with locally advanced or metastatic PC depending on the mutational status of the HR/FA genes.

Characteristic	*BRCA/PALB2*-mutated group ($n = 21$)	Other HR/FA genes mutated group ($n = 55$)	Wild-type HR/FA genes group ($n = 201$)	*p* value
Female, *n* (%)	15 (71.4)	27 (49.1)	104 (51.7)	0.19
Age in years, median (minimum–maximum)	60 (34–76)	62 (36–81)	61 (27–90)	0.44
ECOG status $\geqslant 2$, *n* (%)	0	8 (14.5)	14 (7.0)	0.07
T stage, *n* (%)				
$T_1 1 – T_2$	1 (4.8)	9 (16.4)	21 (10.4)	0.29
$T_3 – T_4$	20 (95.2)	46 (83.6)	180 (89.6)	
Primary tumor in millimeters, median (minimum–maximum)	47.5 (0–115)	39 (0–105)	40 (0–100)	0.62
Primary tumor location in the head of the pancreas, *n* (%)	7 (33.3)	22 (40.0)	79 (39.3)	0.86
Metastases in regional lymph nodes, *n* (%)	10 (47.6)	27 (49.1)	91 (45.3)	0.87
Distant metastases, *n* (%)	11 (52.4)	32 (58.2)	105 (52.8)	0.77
Liver metastases, *n* (%)	6 (28.6)	16 (29.1)	86 (42.8)	0.11
Peritoneal metastases, *n* (%)	1 (4.8)	3 (5.5)	12 (6.0)	0.97
Ascites, *n* (%)	0	3 (5.5)	12 (6.0)	0.52
CA 19-9 level in IU/ml, median (minimum–maximum)	452 (47–8907)	1302 (3–77,283)	332 (1–215,868)	0.47
Neutrophil-to-lymphocyte ratio > 5, *n* (%)	2 (9.5)	2 (3.7)	8 (4.0)	0.48

CA, carbohydrate antigen; ECOG, Eastern Cooperative Oncology Group; FA, Fanconi anemia; HR, homologous recombination; PC, pancreatic cancer.

recorded on the GemPt regimen. There were no cases of disease progression on platinum-based chemotherapy in the *BRCA/PALB2*-mutated group, and the remaining 41.7% of the patients had stable disease.

The ORR for the group with mutations in other HR/FA genes was similar irrespective of platinum use (24.0% *versus* 29.4%, *p* = 0.73). There were 9 (36.0%) cases of stable disease and 10 (40.0%) cases of disease progression on platinum-based chemotherapy in this group.

Notably, the ORR for the wild-type HR/FA genes group was higher for platinum-based therapy than for non-platinum therapy (24.4% *versus* 10.5%, *p* = 0.06). There were 14 cases (15.6%) of disease progression on platinum-based therapy.

When patients who received first-line platinum-based therapy were compared, mutations in *BRCA1/2* or *PALB2* were identified to be associated with higher ORR than mutations in other HR/FA genes or wild-type HR/FA genes (58.3%, 24.0%, and 24.4%, respectively, *p* = 0.04).

PFS and OS. We analyzed PFS and OS for platinum-based regimens among the three groups of patients. The median follow-up time was 12.7 months (range, 1–101 months). Median PFS in the *BRCA/PALB2*-mutated group for platinum use ($n = 12$) compared with non-platinum use ($n = 8$) was 12.7 *versus* 4.4 months (HR = 0.28, 95% CI = 0.10–0.81, *p* = 0.02), respectively (Figure 2(a)). Median PFS in the group with mutations in other HR/FA genes for platinum use ($n = 26$) compared with non-platinum use

Table 2. ORR due to the use of platinum in the first-line chemotherapy depending on the mutational status of the HR/FA genes.

Group	First-line chemotherapy	Objective responses, n (%)	p value
BRCA/PALB2-mutated group (n = 20)	With platinum (n = 12): FOLFIRINOX (n = 10) GemPt (n = 2)	7 (58.3): 7 (70.0) 0	0.20
	Without platinum (n = 8)	2 (25.0)	
Other HR/FA genes mutated group (n = 42)	With platinum (n = 25): FOLFIRINOX (n = 23) GemPt (n = 2)	6 (24.0): 5 (21.7) 1 (50.0)	0.73
	Without platinum (n = 17)	5 (29.4)	
Wild-type HR/FA genes group (n = 147)	With platinum (n = 90): FOLFIRINOX (n = 86) GemPt (n = 4)	22 (24.4): 19 (22.1) 3 (75.0)	0.06
	Without platinum (n = 57)	6 (10.5)	

FA, Fanconi anemia; GemPt, gemcitabine plus cisplatin or oxaliplatin; HR, homologous recombination; ORR, objective response rate.

($n = 20$) was 8.0 *versus* 9.3 months (HR = 0.83, 95% CI = 0.43–1.62, $p = 0.59$), respectively (Figure 2(b)). Median PFS in the wild-type HR/FA genes group for platinum use ($n = 94$) compared with non-platinum use ($n = 67$) was 9.2 *versus* 7.6 months (HR = 0.80, 95% CI = 0.55–1.17, $p = 0.25$), respectively (Figure 2(c)).

The median OS in the *BRCA/PALB2*-mutated group was higher for platinum-based regimens than non-platinum-based regimens (22.9 *versus* 9.0 months, HR = 0.31, 95% CI = 0.08–1.16, $p = 0.08$); however, this trend did not reach statistical significance (Figure 3(a)). For the group with mutations in other HR/FA genes, median OS for platinum use compared with non-platinum use was 16.8 *versus* 11.2 months (HR = 0.58, 95% CI = 0.28–1.22, $p = 0.15$), respectively (Figure 3(b)). Median OS in the wild-type HR/FA genes group for platinum use compared with non-platinum use was 20.5 *versus* 12.1 months (HR = 0.52, 95% CI = 0.33–0.80, $p < 0.01$), respectively (Figure 3(c)).

### Prognostic role of HR/FA genes mutations in patients with localized PC

Patients' characteristics. We analyzed 266 patients with resectable PC who had known HR/FA genes status (Table 3) and assigned them to three groups: mutations in *BRCA1/2* or *PALB2* genes, other HR/FA genes mutations, and wild-type HR/FA genes. None of the three groups had statistically significant prognostic differences in age, resectability, regional lymph node metastasis, diameter of primary tumor, localization in the head/body or body/tail of the pancreas, patients after chemo- or radiotherapy, and non-radical resections. T1–T2 PC was more common in the *BRCA/PALB2*-mutated group ($p = 0.04$) than in the other groups. Females were also more common in the *BRCA/PALB2*-mutated group than in the group with mutations in other HR/FA genes and the wild-type HR/FA genes group (83.3%, 37.5%, and 57.1%, respectively; $p = 0.01$).

Distant outcomes. The median follow-up was 19.8 months (range, 1–166.7 months). Median follow-up times for *BRCA/PALB2*-mutated, other HR/FA genes mutated, and wild-type HR/FA genes groups were 39.6, 17.6, and 19.7 months ($p = 0.53$), respectively.

The median disease-free survival (DFS) for *BRCA/PALB2*-mutated, other HR/FA genes mutated, and wild-type HR/FA genes groups were 25.2, 11.0, and 15.0 months ($p = 0.43$), respectively. Between *BRCA/PALB2*-mutated and wild-type HR/FA genes groups, no statistically significant difference was observed
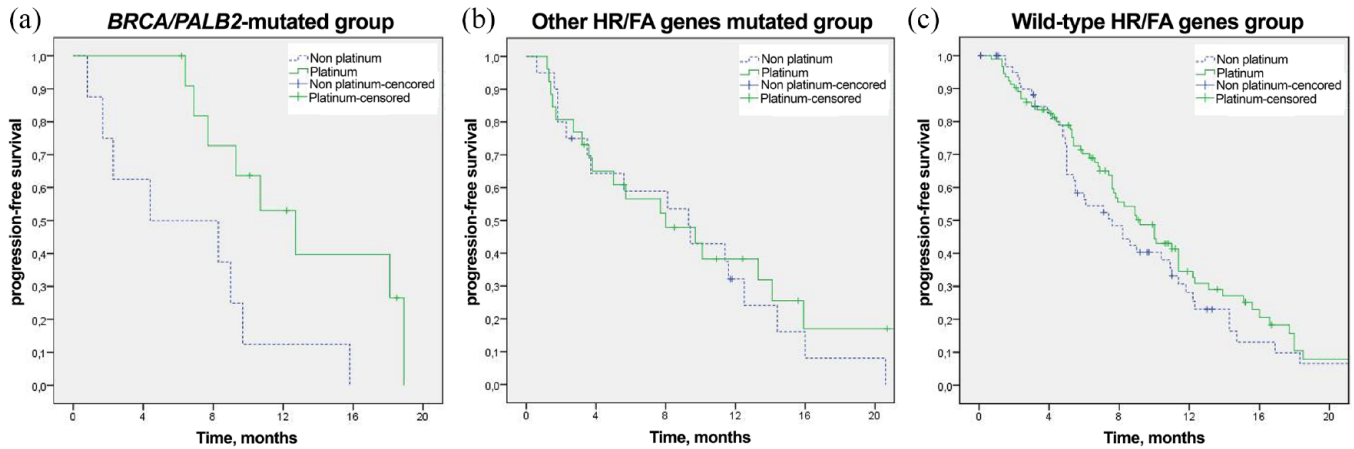
Figure 2. Progression-free survival for patients administered first-line platinum and non-platinum chemotherapy: (a) patients with *BRCA/PALB2* mutations (*n* = 20), (b) patients with mutations in other HR/FA genes (*n* = 46), and (c) patients with wild-type HR/FA genes (*n* = 161).
FA, Fanconi anemia; HR, homologous recombination.
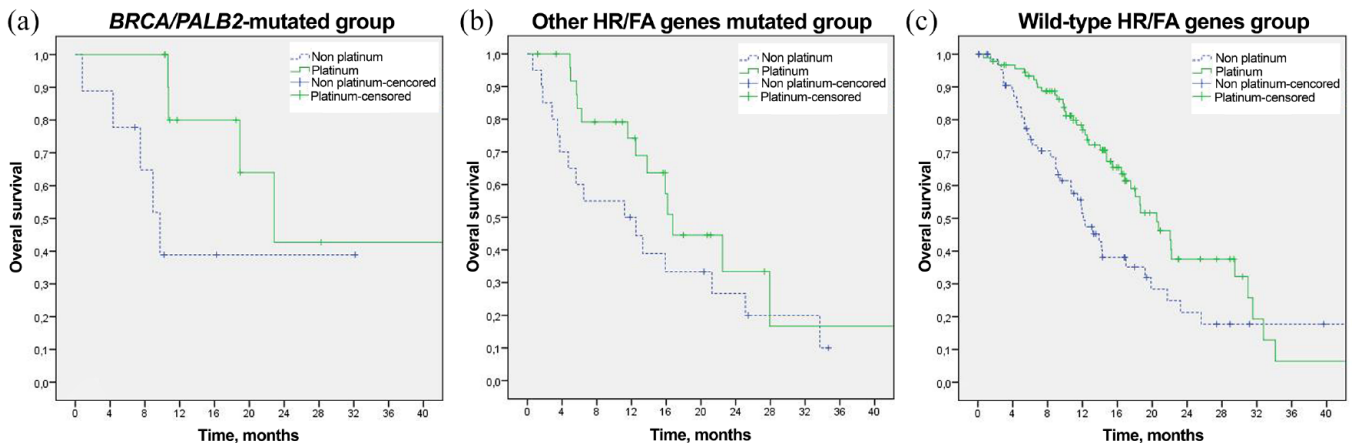
Figure 3. Overall survival of patients administered first-line platinum and non-platinum chemotherapy: (a) patients with *BRCA/PALB2* mutations (*n* = 20), (b) patients with mutations in other HR/FA genes (*n* = 46), and (c) patients with wild-type HR/FA genes (*n* = 161).
FA, Fanconi anemia; HR, homologous recombination.

(HR = 0.93, 95% CI = 0.45–1.89, p = 0.83). The group with mutations in other HR/FA genes also showed no statistically significant difference from the wild-type HR/FA genes group (HR = 1.31, 95% CI = 0.86–2.01, p = 0.21) (Figure 4(a)).

The median OS for *BRCA/PALB2*-mutated, other HR/FA genes mutated, and wild-type HR/FA genes groups were 26.5, 18.2, and 22.1 months (p = 0.51), respectively (Figure 4(b)). The *BRCA/PALB2*-mutated group had no statistically significant difference compared with the wild-type HR/FA genes group (HR = 0.73, 95% CI = 0.32–1.66, p = 0.45). The other HR/FA genes mutated group had no statistically significant difference compared with the wild-type HR/FA genes group (HR = 1.22, 95% CI = 0.76–1.96, p = 0.41).

Prospects for selecting patients with PC to determine potential mutations in the BRCA1/2 and PALB2 genes. We analyzed the factors that could help to identify patients with PC that would most likely benefit from *BRCA/PALB2* mutation testing.

We performed a series of logistic regressions in which mutations in the *BRCA1/2* and *PALB2* genes were used as dependent variables (see Table

Table 3. Characteristics of patients with local PC depending on the mutational status of the HR/FA genes.

Characteristic	BRCA/PALB2-mutated group (n = 12)	Other HR/FA genes mutated group (n = 35)	Wild-type HR/FA genes group (n = 219)	p value
Female, n (%)	10 (83.3)	13 (37.1)	125 (57.1)	0.01
Age in years, median (minimum–maximum)	62 (54–70)	63 (39–73)	61 (30–80)	0.95
T stage, n (%)				
T_1–T_2	5 (41.7)	5 (14.3)	31 (14.2)	0.04
T_3	7 (58.3)	30 (85.7)	188 (85.8)	
Primary tumor in millimeters, median (minimum–maximum)	25 (15–45)	39 (23–60)	39 (5–200)	0.18
Primary tumor location in the head of the pancreas, n (%)	10 (83.3)	27 (77.1)	170 (77.6)	0.89
Metastases in regional lymph nodes, n (%)	5 (41.7)	19 (54.3)	93 (42.5)	0.42
Radiologic resectability, n (%)				
Resectable	9 (75.0)	21 (60.0)	142 (64.8)	0.72
Borderline resectable	3 (25)	14 (40.0)	77 (35.2)	

FA, Fanconi anemia; HR, homologous recombination; PC, pancreatic cancer.
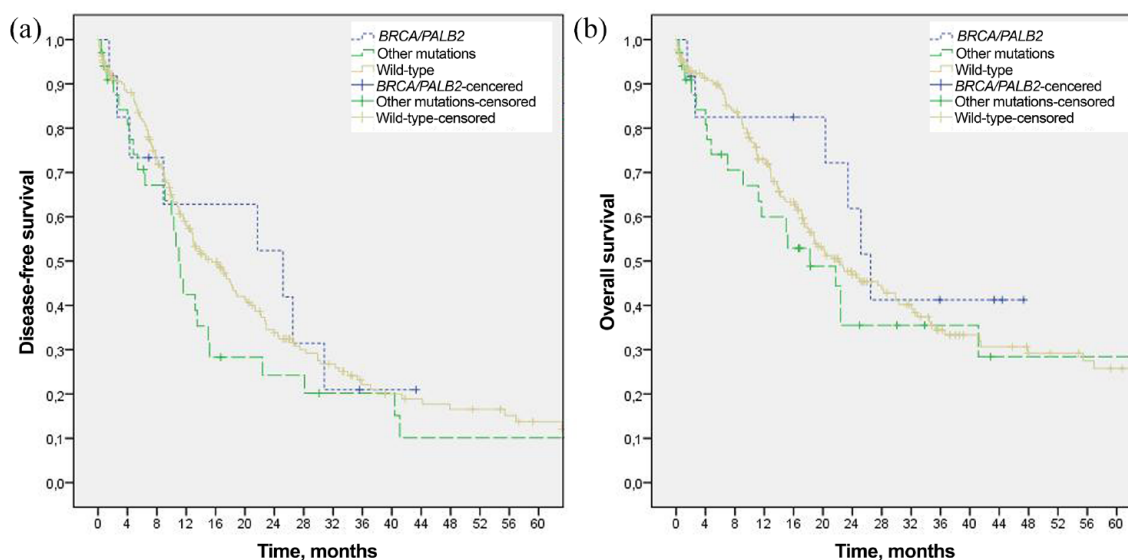
Figure 4. Disease-free survival (a) and overall survival (b) in patients with local PC who had mutations in the *BRCA1/2* or *PALB2* genes (n = 12), mutations in other HR/FA genes (n = 35), and wild-type HR/FA genes (n = 219).
FA, Fanconi anemia; HR, homologous recombination; PC, pancreatic cancer.

Table 4. Logistic regression results that aim to identify factors for the increased risk of *BRCA1/2* or *PALB2* mutations.

Characteristic	Risk of *BRCA/PALB2* mutations			
	Univariate analyses		Multivariate analysis	
	Odds ratio (95% confidence interval)	*p* value	Odds ratio (95% confidence interval)	*p* value
Age >55 years	0.95 (0.92–0.99)	<0.01	1.45 (0.66–3.14)	0.35
Female	1.67 (0.82–3.42)	0.16	–	–
Presence of relative with ovarian, breast, or prostate cancer	1.56 (0.79–3.11)	0.20	–	–
Number of relative with ovarian, breast, or prostate cancer			–	–
0	–	0.30		
1	2.03 (0.83–4.94)	0.12		
2 or more	1.36 (0.62–2.96)	0.44		
Presence of the first degree relative with ovarian, breast, or prostate cancer	1.79 (0.90–3.54)	0.10	–	–
Personal history of ovarian, breast, or prostate cancer	5.26 (1.99–13.96)	<0.01	5.83 (2.16–15.73)	<0.01

4). The tested variables were sex; age; family history of breast; ovarian, pancreatic, or prostate cancer; and a personal history of multiple primary cancers. We discovered that younger age combined with a personal history of breast, ovarian, pancreatic, or prostate cancer was associated with a higher risk of harboring *BRCA/PALB2* mutations. Family history of cancer was not significantly correlated with *BRCA/PALB2* mutational status. Multivariate regression analysis demonstrated that only personal history of multiple primary cancers can serve as an independent predictor of the risk of *BRCA/PALB2* mutations (HR = 5.83, 95% CI = 2.16–15.73, *p* < 0.01). At the same time, patients with PC and no personal history of prior cancer had BRCA/PALB2 mutations in 4.7% of cases.

Discussion

We performed NGS for 543 patients with PC and identified HR/FA genes mutations in 123 cases.

Thirty-three patients (6.1%) had mutations in *BRCA1/2* and *PALB2* genes (*BRCA1*, 10 patients; *BRCA2*, 16 patients; *PALB2*, 7 patients). The remaining 90 patients had mutations in other HR or FA genes. Nine patients had concurrent mutations in the two genes (Supplementary Table 1). None of the patients simultaneously harbored mutations in *BRCA1*, *BRCA2*, or *PALB2*. The most common mutation in *BRCA2* was c.T5286G, and these data are consistent with the OVATAR study results in patients with ovarian cancer.[33] This nucleotide replacement has not been described for other cohorts and is likely to be specific to the Russian population.

All three groups of patients demonstrated different clinical courses of the disease. Patients with *BRCA1/2* and *PALB2* mutations tended to benefit more from platinum-based chemotherapy than patients without these mutations. In our study, no patients with *BRCA/PALB2* mutations showed disease progression as the best response to

platinum-based chemotherapy. In contrast, 40% of patients without *BRCA/PALB2* mutations showed disease progression on platinum-based chemotherapy. The relative rarity of other HR or FA genes mutations did not allow for a separate evaluation of each gene. Therefore, we assigned patients with mutations in these genes to a single group. However, these patients did not benefit from platinum-based therapy.

The most commonly used platinum-based regimen was FOLFIRINOX. Among patients with *BRCA/PALB2* mutations, all objective responses were revealed on FOLFIRINOX therapy. Two patients who received platinum plus gemcitabine had stable disease. Therefore, we could not evaluate the correlation between platinum regimen and its efficacy in patients with mutations due to the low number of patients treated with platinum–gemcitabine combination.

We found that the *BRCA/PALB2*-mutated group had higher PFS when platinum-based chemotherapy was used compared with non-platinum chemotherapy. In contrast, there were no differences in PFS when platinum and non-platinum regimens were compared in other HR/FA genes mutated and wild-type HR/FA genes groups.

Our data are consistent with the published studies with respect to higher platinum efficacy in patients with *BRCA/PALB2* mutations. For example, in a retrospective study by Wattenberg *et al.*,[34] the use of chemotherapy in 26 patients with PC and mutations in *BRCA1/2* or *PALB2* genes had 58% response rate, including 60% responses to the FOLFIRINOX regimen. In contrast, in the respective control group without mutations in these genes, the response rate was only 21%.

There are few data estimating the predictive value of HR/FA genes mutations, besides *BRCA1/2*, in PC. At the same time, talazoparib, a PARP-inhibitor, demonstrated efficacy in patients with *BRCA1/2* and *PALB2* mutations, but not in the patients with mutations in other HR/FA genes.[35] Similarly, the PARP-inhibitor olaparib improved survival in the patients with *BRCA1/2* mutations, but not in the patients with other HR/FA genes mutations.[36] These findings doubt the clinical utility of identifying HR/FA genes mutations other than *BRCA1/2* in routine clinical practice for PC. Our data are partially consistent with the recently published analyses that demonstrated inconsistent results of predictive value of other HR/FA genes

mutations as biomarkers of platinum response.[37] In contrast, in our study, *ATM* mutations were rather common (18/543 – 3.3%). Moreover, ATM inhibitors are currently in clinical trials.[38] Thus, these mutations may serve as emerging biomarkers.

In our study, *BRCA1/2*, *PALB2*, and other HR/FA genes mutations did not affect the prognosis of patients with resected PC. The predictive value of HR/FA genes mutations in patients with local PC could not be assessed because only a few patients received platinum in the neoadjuvant or adjuvant setting. However, *BRCA1/2* and *PALB2* can be considered as potential biomarkers for the choice of chemotherapy regimen in neoadjuvant and adjuvant settings. The 'Know Your Tumor' project revealed a trend toward higher OS in patients with resected *BRCA1/2*-associated PC if they had received platinum-based chemotherapy compared with non-platinum chemotherapy.[39]

The main limitation of our study is the determination of mutations predominantly in normal tissue without verifying all germline mutations for loss of heterozygosity in the tumor. Loss of the second allele of the mutated gene is important for tumor cells. A recent study by the Memorial Sloan Kettering Cancer Center demonstrated that 33% of tumor cells remained monoallelic with germline *BRCA1/2* mutations coincident with the wild-type allele in tumor cells.[40] This could be a possible explanation for the decreased efficacy of platinum agents in PC compared with ovarian cancer.[41] Specifically, in the POLO trial, 17% of patients with germline mutations in *BRCA1/2* genes progressed on platinum regimens.[42] However, in our study, all patients with mutations in *BRCA1/2* and *PALB2* had objective responses or stabilization as the best treatment response to first-line platinum therapy, and no patients progressed.

Germline mutations in PC are relatively rare. Furthermore, their testing is expensive and not covered by medical insurance in many countries. We analyzed the clinical factors that could help identify patients most likely to benefit from such testing. Unfortunately, age, sex, and family history failed to predict a higher risk of harboring these alterations. Nevertheless, we demonstrated that a personal history of breast, ovarian, pancreatic, or prostate cancer was associated with a six-fold increase in the risk of these mutations. At the same time, patients with PC and no personal history of prior cancer had *BRCA/PALB2* mutations in 4.7% of cases.

Our results confirm recently published data that personal history of prior cancer is a surrogate marker of higher risk of germline *BRCA1/2* mutations.[37] Nevertheless, the clinically meaningful rate of *BRCA1/2* or *PALB2* mutations in patients with PC without a personal history of prior cancer points at the importance of genetic testing beyond this marker. The National Comprehensive Cancer Network guidelines also recommend genetic testing for all the patients with PC.[43]

Some data suggest that non-tested PC patients with a family history of PC, breast, ovarian, or prostate cancer benefit from platinum-based chemotherapy compared with patients without a family history of cancer.[44] However, other studies did not confirm the role of family history as a surrogate marker of platinum efficacy.[37] Although the role of family history as a predictor of higher risk of mutations in HR/FA genes cannot be denied, our study failed to confirm this issue. We assume that the results of our study are related to the insufficient knowledge of family history by patients in our population.

Conclusion

We demonstrated that mutations in *BRCA1/2* and *PALB2* genes account for up to 6.1% of PC cases. These mutations were found to increase tumor sensitivity to platinum agents. Herein, we highlight the need to determine *BRCA1/2* and *PALB2* alterations in all patients with PC, as stated in most current clinical recommendations.[45] Further research is needed to estimate the role of other HR/FA mutations and the loss of heterozygosity in PC.

Author contributions

Marina Emelyanova: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

Elena Pudova: Investigation; Methodology; Writing – review & editing.

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George Krasnov: Formal analysis; Software; Writing – original draft.

Anna Popova: Investigation; Writing – review & editing.

Ivan Abramov: Investigation; Methodology; Writing – review & editing.

Vladimir Mikhailovich: Investigation; Writing – review & editing.

Maxim Filipenko: Investigation; Writing – review & editing.

Sofia Menshikova: Formal analysis; Writing – review & editing.

Sergey Tjulandin: Conceptualization; Funding acquisition; Project administration; Writing – review & editing.

Ilya Pokataev: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Project administration; Resources; Supervision; Visualization; Writing – original draft; Writing – review & editing.

Supplemental material

Supplemental material for this article is available online.

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