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Research Paper

A multicenter prospective study of single nucleotide polymorphisms in the *PDCD1 (programmed cell death 1)* gene in patients with metastatic lung adenocarcinoma treated with pembrolizumab

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ABSTRACT

Introduction: Pembrolizumab, a monoclonal antibody targeting programmed death-1 (PD-1), improves outcomes in metastatic lung adenocarcinoma (MLA) expressing programmed death ligand-1 (PD-L1). Single nucleotide polymorphisms (SNPs) in the *PDCD1* gene may impair PD-1 signaling and reduce the efficacy of pembrolizumab. We evaluated the impact of rs11568821 C>T, rs10204525 C>T, rs7421861 A>G, rs2227981 G>A, and rs41386349 G>A on progression-free survival (PFS) in non-oncogene-addicted MLA patients treated with pembrolizumab-based regimens.

Materials and methods: Patients were prospectively enrolled: those expressing PD-L1 ⩾50% received pembrolizumab monotherapy, whereas those expressing PD-L1 <50% received pembrolizumab plus platinum-pemetrexed chemotherapy. Associations between SNPs and PFS (primary endpoint) were assessed using univariate and multivariable Cox models. Secondary endpoints included response rate and overall survival (OS).

Results: Among 141 evaluable patients, 71 received pembrolizumab and 70 received combination therapy. Median PFS was 20 months (95% confidence interval [CI], 13-27) overall, 25 months (95% CI, 16-34) with pembrolizumab, and 13 months (95% CI, 8-23) with combination therapy. Rs7421861 was significantly associated with PFS: 23 months (95% CI, 13-40) in A/A, 20 months (95% CI, 12-29) in A/G, and 6 months (95% CI, 3-11) in G/G patients. In multivariable analysis, G/G genotype showed the strongest effect (HR 2.85; 95% CI, 1.53-5.29; $p = 0.001$). Most G/G carriers (12/13) had stable or progressive disease, and OS was also inferior.

Discussion: The rs7421861G-allele is associated with poorer outcomes in pembrolizumab-treated MLA patients and warrants further validation.

1. Introduction

Immune checkpoint inhibitors (ICIs) targeting either programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1) have become standard treatments in the management of non-small cell lung cancer (NSCLC) [1]. The absence of driver somatic mutations, PD-L1 expression levels, and histology, are factors that influence the choice of first-line treatment strategy in metastatic disease. Specifically, patients with non-oncogene-addicted metastatic lung adenocarcinoma (MLA) with PD-L1 expression ⩾ 50% are typically offered monotherapy

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with the anti-PD-1 antibody pembrolizumab. In the same setting, but with PD-L1 expression <50%, the combination of a platinum-doublet chemotherapy and pembrolizumab is the choice [1].

These treatment strategies have significantly improved the overall management of MLA with the capability of inducing long-term responses and prolonged survival times. Unfortunately, a portion of these patients does not gain meaningful improvements in survival times because of primary or secondary resistance against anti-PD-1/PD-L1 [1]. In this context, accumulating evidence suggests that several biomarkers may play a role in predicting clinical outcomes and safety of anti-PD-1/PD-L1 therapy [2]. However, their predictive precision is still affected by intrinsic limitations of these markers, and likely, the combination of multiple biomarkers will provide more precise predictive information and will be used in the prediction process [2].

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variants in humans, and increasing research reports their important function in human diseases. SNPs causing a change in the amino acid sequence of a protein can impact the protein's structure, stability, and function, changing biological processes. They can also impact the regulation of gene expression at the transcriptional level, and several studies have established the relationship between polymorphisms in the *PDCD1* (PD-1) and *CD274* (PD-L1) genes and the risk of developing different types of cancer and other immune-related diseases. SNPs in the *PDCD1* gene can affect PD-1 and its role in immune checkpoint regulation, potentially impacting the effectiveness of cancer immunotherapies or ligand binding [3]. Several candidate SNPs in the *PDCD1* gene have been thoroughly investigated, and some of them have offered promising results in the context of the putative mechanism of primary and secondary resistance for anti-PD-1/PD-L1 therapy [3]. In clinical settings, several studies have addressed the predictive role of germline *PDCD1* SNPs in patients treated with ICIs [4,5]. However, many of these studies are limited by small sample size and because most do not correct for multiple testing, the results often remain statistically insignificant or clinically irrelevant.

In addition, these studies often enrolled a heterogeneous population of patients due to different clinical settings and/or series, including both anti-PD-1/PD-L1 therapies. Therefore, results may be specific for the investigated histology or drug(s) and may not apply to all ICI regimens [5].

According to this background, we conducted a prospective multicenter study to investigate the clinical impact of selected germline *PDCD1* SNPs in patients with non-oncogene-addicted MLA undergoing first-line anti-PD-1 pembrolizumab therapy.

2. Materials and methods

2.1. Patients and treatments

This prospective study was performed among five participating institutions in Central Italy between April 2020 and October 2024. Patients with MLA without driver mutations were considered eligible for study entry. Patients with central nervous system metastases were not excluded, provided that they underwent stereotactic radiotherapy of a single lesion documented by computed tomography and magnetic resonance imaging (MRI). According to current clinical practice, patients with PD-L1 expression ⩾50% received pembrolizumab, whereas patients with PD-L1 expression <50% received pembrolizumab and chemotherapy. All patients received 200 mg of pembrolizumab administered intravenously every 3 weeks. Chemotherapy consisted of cisplatin (75 mg per square meter of body-surface area) or carboplatin (area under the concentration–time curve, 5 mg per milliliter per minute) plus pemetrexed (500 mg per square meter), all administered intravenously every 3 weeks. All patients received premedication with folic acid, vitamin B_{12}, and glucocorticoids, administered according to local guidelines for the use of pemetrexed.

Enrolled patients underwent a pre-treatment blood sampling for SNP analysis. Re-staging procedures were scheduled every 12 weeks or earlier in the case of signs/symptoms of progression. Enrolled patients signed written informed consent, and the protocol was approved by the "Regione Marche" Ethical Committee.

2.2. PDCD1 SNPs selection and analysis

Five *PDCD1* SNPs were selected based on their functional effects and cumulative data on cancer risk susceptibility and putative interference on ICI mechanisms of action [3–6]. Rs2227981 G>A is a synonymous variant located in exon 5, widely studied for its associations with various diseases, including different types of cancer and autoimmune disorders. Despite being synonymous, it may influence *PDCD1* gene expression, at the mRNA or protein level [7,8]. Rs10204525 is located in the 3′-untranslated region of *PDCD1*, and the C>T change was found to affect both gene function and mRNA expression [9]. Rs11568821 is located in an enhancer-like region of intron 4; the C>T change may disrupt the binding site for RUNX1, which in turn may lead to aberrant PD-1 expression and deregulated lymphocyte activity [5,10,11]. Rs7421861 A>G is located in intron 1, where many regulatory components and splicing control elements, such as enhancers or silencers, are located [12,13]. Modifications of the splice site and/or mRNA secondary structure may induce aberrant splicing and alter the translational processes [12,14]. Rs41386349 is located in intron 4 in a putative enhancer-like region; the G>A change was found to impact the *PDCD1* transcriptional activity and alter the PD-1 expression [14,15].

For each patient, two tubes/K3-EDTA (6 ml each) of fresh peripheral blood were collected and stored at −20°C until use. QIAmp DNA Blood Midi Kit (Qiagen, Hilden, Germany) was used to extract the genomic DNA (gDNA) according to the manufacturer's instructions. The gDNA quantification was performed using NanoDrop™ (Thermo Fisher Scientific, Waltham, MA, USA). SNPs with a minor allele frequency (MAF) < 10% in the TSI (Toscani in Italy, Ensembl.org) population were excluded. The SNPs were genotyped by polymerase chain reaction (PCR) followed by high-resolution melting (PCR-HRM) analysis using the Rotor-Gene 6000 system (Corbett Research). The "Type-it HRM PCR Kit" (Qiagen, Hilden, Germany) was used to perform the analyses. To confirm accuracy, SNP genotypes were verified by pyrosequencing (PyroMark ID instrument, Biotage) in a randomly selected 15% of the samples. The amplification primers used in the PCR-HRM and pyrosequencing assays were designed by Primer3Plus (https://www.primer3plus.com/) (Supplementary Table S1). One primer of each pair was modified with a biotin group. The sequencing primers utilized in the pyrosequencing assay were designed using PSQ Assay Design Software V.1.0.6 (Biotage) (Supplementary Table S1).

2.3. Statistical analysis

The primary end-point of the study was the association analysis between *PDCD1* SNPs and progression-free survival (PFS). PFS was defined as the time from the date of the first cycle of therapy to disease progression or death, whichever happened first. Assuming the variant genotype frequency in a dominant model ⩾10%, an 80% power and a two-sided type I error of 5%, an association with PFS hazard ratio (HR) ⩾2 could be detected after 100 events (progressions) have occurred. Secondary end-points were considered association analyses between tumor response, overall survival (OS), and *PDCD1* genotypes. In the case of significant associations between *PDCD1* genotypes and PFS, an exploratory analysis was planned to characterize these effects in subgroups of patients treated with pembrolizumab alone and pembrolizumab plus chemotherapy.

Categorical variables in contingency tables were analyzed by the Chi-square test. The Kaplan-Meier method was used to estimate survival curves, and the log-rank test was used to compare survival times between groups. All reported *p*-values were two-sided, and confidence intervals (CIs) were at the 95% level. We used the Bonferroni correction

to account for multiple testing, and a two-tailed *p*-value < 0.01 (=0.05/5 SNPs) was considered statistically significant. A multivariable Cox proportional hazards model was then used to adjust for clinical and pathologic features, which were statistically significant in the univariate model. Analyses were performed using MedCalc Software (V.15 Ostend, Belgium).

3. Results

3.1. Study population

Between April 2020 and October 2024, 145 patients were enrolled. Four patients were not assessable: one patient did not receive pembrolizumab for medical reasons and three patients were staged with locally advanced, non-metastatic disease. The characteristics of the 141 fully assessable patients are listed in Table 1. There were 71 patients treated with pembrolizumab and 70 patients treated with pembrolizumab plus chemotherapy. First-line therapy achieved complete and partial responses in 91 patients (64.5%), whilst 50 patients (35.5%) showed stable disease or disease progression. At the time of analysis, 100 patients had disease progression (71%), and the remaining 41 (29%) were censored observations. In the whole group, median PFS was 20 months (95% CI, 13-27 months). The distribution of patient characteristics according to *PDCD1* genotypes is shown in Supplementary Table S2. No imbalance was observed, except for a marginally significant difference in the distribution of treatment groups among rs2227981 genotype carriers.

3.2. Progression-free survival analysis and PDCD1 SNPs

The genotype frequencies of *PDCD1* SNPs and the results of the univariate analysis for PFS are shown in Table 2. Among the five *PDCD1* polymorphisms, rs7421861 showed significant association with PFS. Notably, the strongest association with PFS was observed for the rs7421861 G/G genotype in the recessive model (Fig. 1). As shown in Table 3, clinical characteristics and *KRAS* status were investigated in univariate analysis. Eastern Cooperative Oncology Group (ECOG) performance status (PS) was significantly associated with PFS and it was included in the multivariate analysis (Table 3). The detrimental effect of rs7421861 on PFS was confirmed in both dominant and recessive models.

Table 1
Characteristics of the 141 patients and distribution according to treatment.

	Number of patients (%)			
Variable	**Total**	**P + C**	**P**	***p*-value**
Age				
>70 years	66 (46.8)	31 (44.3)	35 (49.3)	0.6
≤70 years	75 (53.2)	39 (55.7)	36 (50.7)	
Gender				
Male	97 (68.8)	48 (68.6)	49 (69)	1.0
Female	44 (31.2)	22 (31.4)	22 (31)	
***KRAS* status**				
Wild-type	98 (69.5)	54 (77.1)	44 (62)	0.07
Mutated	43 (30.5)	16 (22.9)	27 (38)	
Number of metastatic sites				
1–2	102 (72.3)	49 (70)	53 (74.6)	0.6
≥ 3	39 (27.7)	21 (30)	18 (25.4)	
Cerebral metastasis				
Positive	28 (19.9)	15 (21.4)	13 (18.3)	0.7
Negative	113 (80.1)	55 (78.6)	58 (81.7)	
ECOG-PS				
0	97 (68.8)	46 (65.7)	51 (71.8)	0.5
1 and 2	44 (31.2)	24 (34.3)	20 (28.2)	

Abbreviations: ECOG-PS, Eastern Cooperative Group Performance Status; P + C, pembrolizumab + chemotherapy; P, pembrolizumab.

3.3. Secondary end-points

In the whole population of 141 patients, there were 90 (64%) patients with complete or partial response (responders) and 51 (36%) patients with stable disease or disease progression (non-responders). Table 4 summarizes the distribution of responders and non-responders across the genotypes evaluated. Finally, a significant association between rs7421861 genotypes and tumor response was observed; notably, 12 out of 13 carriers of the rs7421861 G/G genotype were non-responders.

Regarding OS, none of the studied variants except rs7421861 showed a significant impact on this endpoint (data not shown). The rs7421861 G-allele showed a significant association with unfavorable OS, and this effect remained statistically significant across codominant, dominant, and recessive models (Fig. 2), suggesting a robust, genotype-dependent impact on prognosis.

3.4. Exploratory PDCD1 SNPs analysis in treatment groups

The clinical impact of the rs7421861 G-allele on PFS was analyzed in the 71 patients treated with pembrolizumab and the 70 patients who received pembrolizumab plus chemotherapy. As shown in Fig. 3, statistically significant differences in PFS were observed according to rs7421861 genotypes, with G-allele carriers showing unfavorable outcomes. In both treatment groups, patients' clinical characteristics and *KRAS* status were not significantly associated with PFS in univariate analysis (data not shown).

4. Discussion

Over the last decades, PD-1 and PD-L1 have been identified as immune checkpoint proteins that play a critical role in the maintenance of immune tolerance. For this reason, functional genetic variants in immune response-related genes that regulate T lymphocyte function and alter immune status have been investigated for the pathogenesis of various cancers [6]. More recently, there has been renewed interest in *PDCD1* and *CD274* SNPs because of the introduction of anti-PD-1 and anti-PD-L1 checkpoints inhibitors for cancer therapy in clinical practice [4,5]. These compounds are now widely employed in the systemic therapy of lung cancer patients, and the potential predictive role of genetic variants in their targets has aroused great interest for translational research.

In the last years, the clinical impact of *CD274* and *PDCD1* polymorphisms in patients with advanced NSCLC treated with anti-PD-1 and anti-PD-L1 checkpoint inhibitors has been investigated in some retrospective studies, but mainly including Asian patients [16–21]. Notably, in these studies, analyses of *PDCD1* polymorphisms are underrepresented [20,21]. Patients with different NSCLC histologic subtypes were included and no subgroup analyses were performed for adenocarcinoma or squamous cell carcinoma patients. In some of these studies, *CD274* rs2282055 and *PDCD1* rs2227981 variants showed a potential correlation with PFS during treatment with nivolumab. However, these results are at risk of bias due to the limited sample sizes and the heterogeneity of the study populations.

In our study, a homogeneous population of MLA was prospectively accrued and the detrimental effect on PFS seemed to be predominant in carriers of the rs7421861 G/G genotype. The consistency across models (co-dominant, dominant, and recessive) supports the robustness of the association, although the recessive model appears the most biologically

Table 2
PDCD1 polymorphisms analysis.

PDCD1 variant	Number of pts	median PFS	Univariate Log-Rank months (range)	*p*-value HR (95%CI)	Bonferroni (m = 5)	FDR (m = 15)
rs41386349*						
G/G	74	19 (14–28)	reference			
G/A	58	13 (8.5–27)	0.89 (0.5–1.27)	0.08	0.40	0.12
A/A	8	NR	0.26 (0.12–0.57)			
Dominant model						
G/G	74	19 (14–28)	reference			
G/A + A/A	66	20 (14–33)	1.02 (0.68–1.54)	0.8	>1	1.00
Recessive model						
G/G + G/A	132	16 (12–25)	reference			
A/A	8	NR	0.25 (0.11–0.53)	**0.03**	0.15	0.09
rs11568821**						
C/C	104	19 (13–28)	reference			
C/T	26	12 (6.5–25)	1.37 (0.79–2.37)	0.07	0.35	0.11
T/T	9	33 (25.5–33)	0.33 (0.14–0.71)			
Dominant model						
C/C	104	19 (13–28)	reference			
C/T + T/T	35	23 (8.5–31)	0.94 (0.58–1.52)	0.7	>1	1.00
Recessive model						
C/C + C/T	130	16 (12–26)	reference			
T/T	9	33 (23–33)	0.29 (0.13–0.65)	0.06	0.30	0.10
rs10204525						
C/C	106	16 (11–25)	reference			
C/T	32	31 (12–51)	0.71 (0.44–1.11)	0.18	>1	0.27
T/T	3	NR	0.37 (0.11–1.03)			
Dominant model						
C/C	106	16 (11–25)	reference			
C/T + T/T	35	31(13–51)	0.66 (0.42–1.03)	0.09	0.45	0.14
Recessive model						
C/C + C/T	138	19 (13–28)	reference			
T/T	3	NR	0.34 (0.11–1.12)	0.2	>1	0.30
rs7421861						
A/A	76	23 (13–40)	reference			
A/G	52	20 (12–29)	1.33 (0.87–2.04)	**0.001**	**0.005**	**0.004**
G/G	13	6 (3–11)	2.94 (1.17–7.38)			
Dominant model						
A/A	76	23 (13–40)	reference			
A/G + G/G	65	14 (7–25)	1.55 (1.02–2.30)	**0.02**	0.10	**0.04**
Recessive model						
A/A + A/G	128	22 (14–29)	reference			
G/G	13	6 (3–11)	2.62 (1.06–6.46)	**0.0008**	**0.004**	**0.004**
rs2227981						
A/A	43	27 (11–40)	reference			
A/G	58	16 (12–29)	1.41 (0.87–2.24)	0.2	>1	0.30
G/G	40	20 (7.5–26)	1.49 (0.88–2.51)			
Dominant model						
A/A	43	27 (11–40)	reference			
A/G + G/G	98	20 (12–26)	1.43 (0.94–2.22)	0.1	0.50	0.15
Recessive model						
A/A + A/G	101	19 (13–33)	reference			
G/G	40	20 (7.5–26)	1.22 (0.77–1.93)	0.3	>1	0.45

Abbreviations/Notes: HR, Hazard Ratio; pts, patients; CI, Confidence interval; NR not reached; Bold = values in bold in the table are statistically;sig post-adjust = significant after adjustment (correction); Bonferroni $p < 0.01$ = with Bonferroni correction, significant if $p < 0.01$ (0.05/5 tests). *one missing genotype; **two missing genotypes.

plausible given the magnitude of effect observed in G/G carriers. This finding may be indicative of an allele-dosage effect, which is also reflected in the results of the survival analysis (Fig. 1). Notably, Dimitrakopoulou F. *et al.* [21] reported data on the analysis of *PDCD1* gene polymorphisms in 90 patients with NSCLC treated with anti-PD-1 immunotherapy, and found that rs2227981 and rs7421861 were associated with longer time to disease progression. Both these variants have been included in the present investigation and according to our results, the rs7421861 showed a similar clinical impact in MLA patients treated with anti-PD-1 pembrolizumab.

It is plausible that alterations in PD-1 expression and/or protein structure are among the mechanisms that underpin the clinical impact of rs7421861 genotypes on immunotherapy with PD-1 ICIs [22,23].

Rs7421861 is located in intron 1, where numerous known splicing and regulatory elements are located. In silico analyses reported by Eldafashi *et al.* [22] showed that the rs7421861 G-allele was significantly associated with PD-1 downregulation in immune cells (blood or spleen); conversely, the rs7421861 A-allele was associated with high PD-1 expression.

Intriguingly, the rs7421861 G-allele was found to be associated with high serum levels of the soluble form of PD-1 (sPD-1) [24–26]. The sPD-1 is structurally similar to membrane-bound PD-1 and is released by

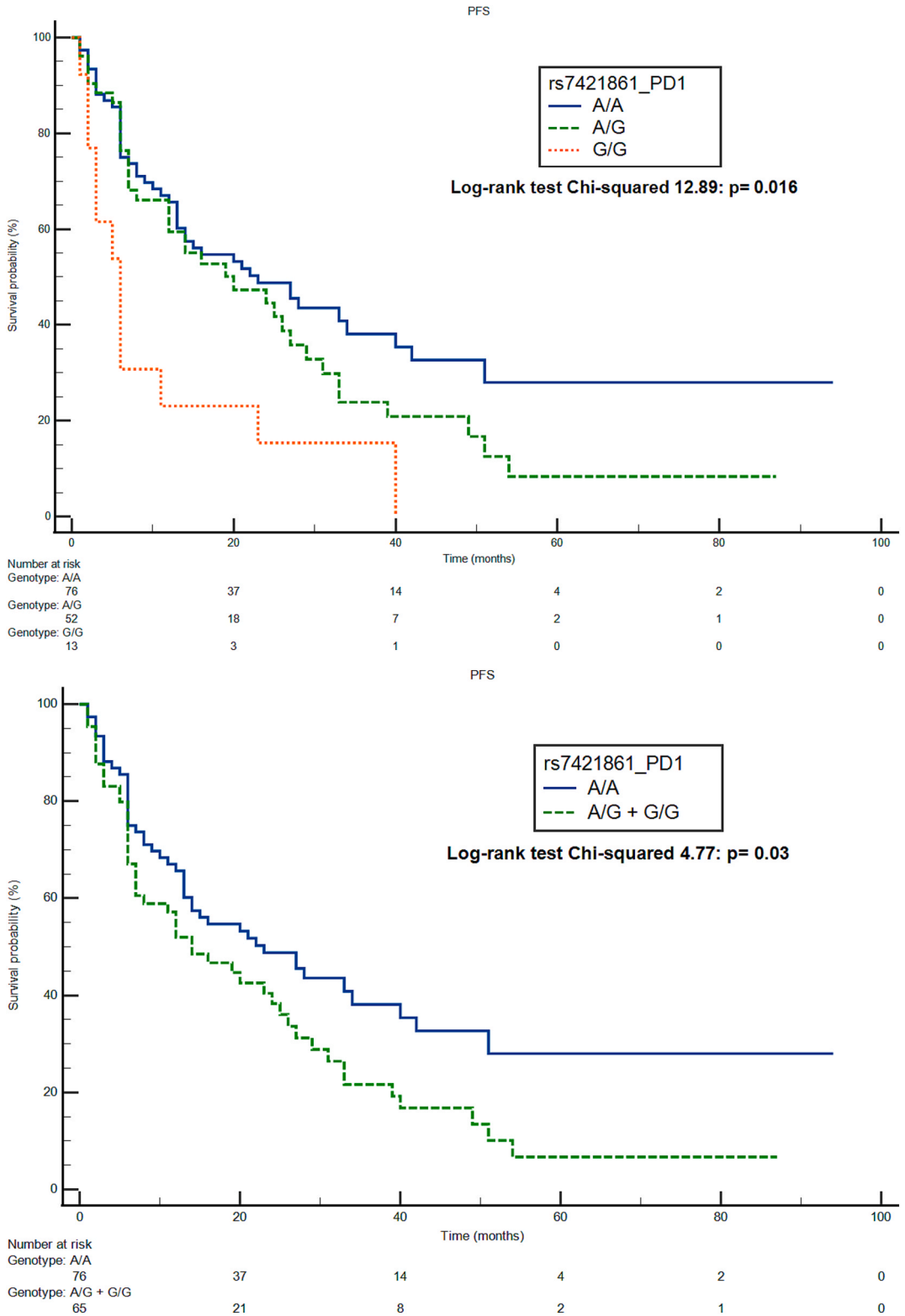


Fig. 1. Log-rank and Kaplan-Meier PFS curves according to rs7421861 genotypes. A) Three groups in the codominant model. B) Two groups in the dominant model. C) Two groups in the recessive model.

proteolytic cleavage or as a splice variant that is no longer membrane-bound. Notably, as reported by Hassel JC *et al.* [27] the concentration of sPD-1 inversely correlated with lymphocyte counts and the frequency of PD-1 positive T cells. In NSCLC patients, high sPD-1 levels were found to be associated with unfavorable PFS [27–29]. Therefore, it can be speculated that anti-PD-1 antibodies, such as nivolumab or pembrolizumab, may have affinity towards sPD-1, thus forming a sPD-1/anti-PD-1 complex. In this status, sPD-1 may act as a "decoy", potentially impairing antibody activity [30].

Rs7421861 is located within intron 1 of the *PDCD1* gene, a region

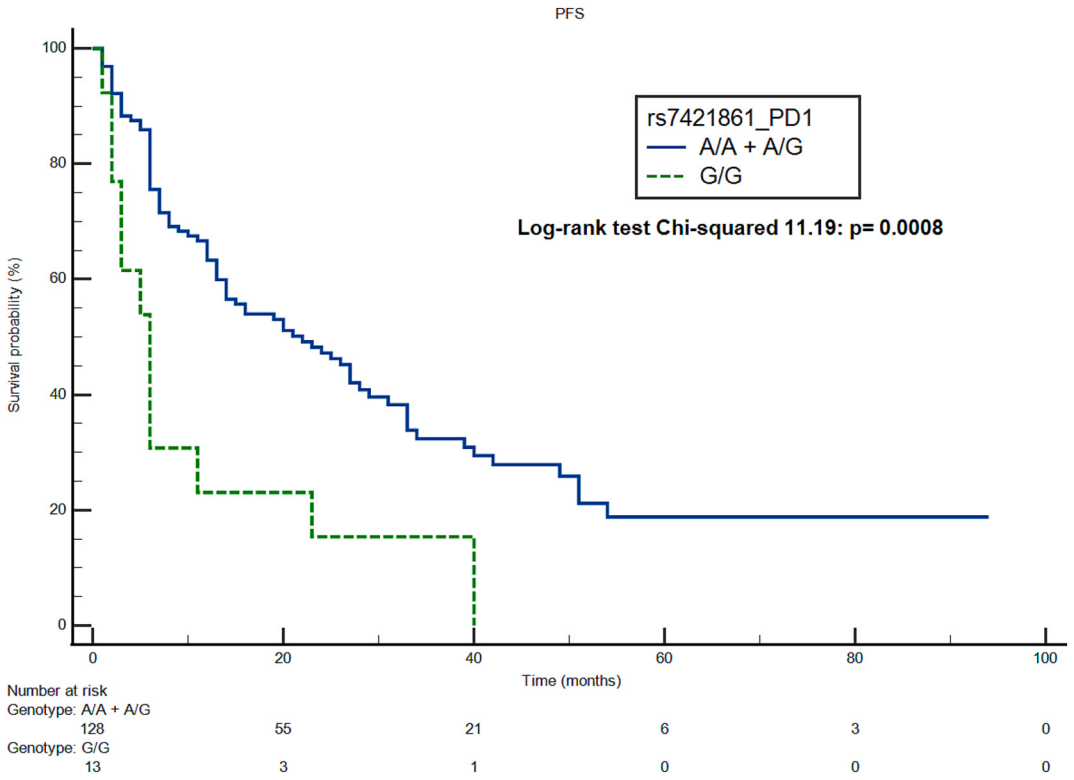


Fig. 1. (*continued*).

Table 3
Univariate and multivariate analyses for progression-free survival.

Variable	HR (95% CI)	*p*-value
Univariate analysis		
Age		
>70 years vs. ≤ 70 years	1.21 (0.85–1.92)	0.2
Gender		
Male vs. Female	0.89 (0.58–1.39)	0.6
Cerebral metastasis		
Positive vs. Negative	1.54 (0.96–2.47)	0.09
First-line therapy		
Chemotherapy plus P vs. P	1.41 (0.94–2.12)	0.07
ECOG-PS		
1–2 vs. 0	1.70 (1.07–2.70)	**0.009**
Number of metastatic sites		
1–2 vs. ≥ 3	0.98 (0.62–1.54)	0.9
***KRAS* status**		
KRAS mutated vs. *KRAS* wild type	1.13 (0.72–1.78)	0.5
Multivariate analyses		
Dominant model		
PDCD1 rs7421861		
GG + GA vs. A/A	1.50 (1.01–2.25)	**0.04**
ECOG-PS		
1–2 vs. 0	1.73 (1.14–2.61)	**0.01**
Recessive model		
PDCD1 rs7421861		
GG vs GA + A/A	2.85 (1.53–5.29)	**0.001**
ECOG-PS		
1–2 vs. 0	1.84 (1.21–2.78)	**0.004**

Abbreviations: HR, Hazard Ratio; CI, confidence interval; P, pembrolizumab; ECOG-PS, Eastern Cooperative Group Performance Status.

Table 4
Analysis of *PDCD1* polymorphisms according to treatment response rate.

***PDCD1* variant**	**Number of pts**	**CR + PR (90 pts) Number of pts (%)**	**SD + DP (51 pts) Number of pts (%)**	***p*-value**
rs41386349*				
G/G	74	48 (54)	26 (51)	0.07
G/A	58	35 (39)	23 (45)	
A/A	8	6 (7)	2 (4)	
rs11568821**				
C/C	104	68 (76.5)	36 (72)	0.3
C/T	26	14 (15.5)	12 (24)	
T/T	9	7 (8)	2 (4)	
rs10204525				
C/C	106	66 (73.3)	40 (78.5)	0.7
C/T	32	22 (24.5)	10 (19.5)	
T/T	3	2 (2.2)	1 (2)	
rs7421861				
A/A	76	53 (59)	23 (45)	**0.0005**
A/G	52	36 (40)	16 (31.5)	
G/G	13	1 (1)	12 (23.5)	
rs2227981				
A/A	43	25 (27.8)	18 (35.5)	0.9
A/G	58	38 (42.2)	20 (39)	
G/G	40	27 (30)	13 (25.5)	

Abbreviations: Pts, patients; CR, complete response; PR, partial response; SD, stable disease; DP, disease progression. *one missing genotype; **two missing genotypes.

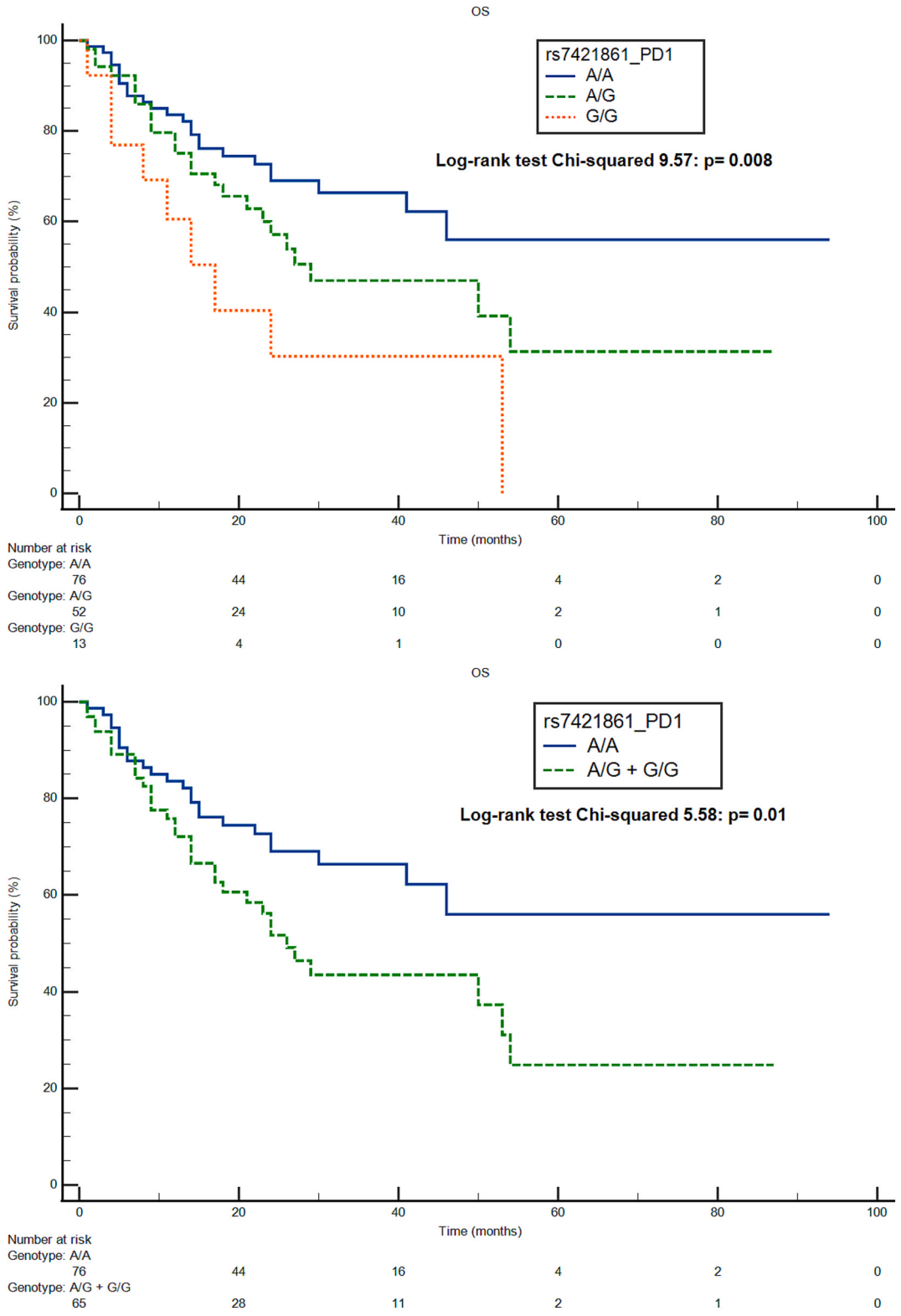


Fig. 2. Log-rank and Kaplan-Meier OS curves according to rs7421861 genotypes. A) Three groups in the codominant model. B) Two groups in the dominant model. C) Two groups in the recessive model.

with multiple regulatory and potential splicing control elements that may influence *PDCD1* mRNA expression. Using JASPAR [31], a database of curated non-redundant transcription factor binding profiles, integrated into UCSC Genome Browser (hg38 assembly, https://genome.ucsc.edu/), we found that rs7421861 is located in a predicted binding site for the transcription factors ZNF343, MYOD1, ASCL1 and TCF4. Among these, TCF4 and ASCL1 are noteworthy. TCF4 is a master regulator of multiple transcriptional programs and may directly promote immune cell evasion and resistance to immune checkpoint inhibitor therapy [32]. ASCL1 is involved in several signaling pathways and

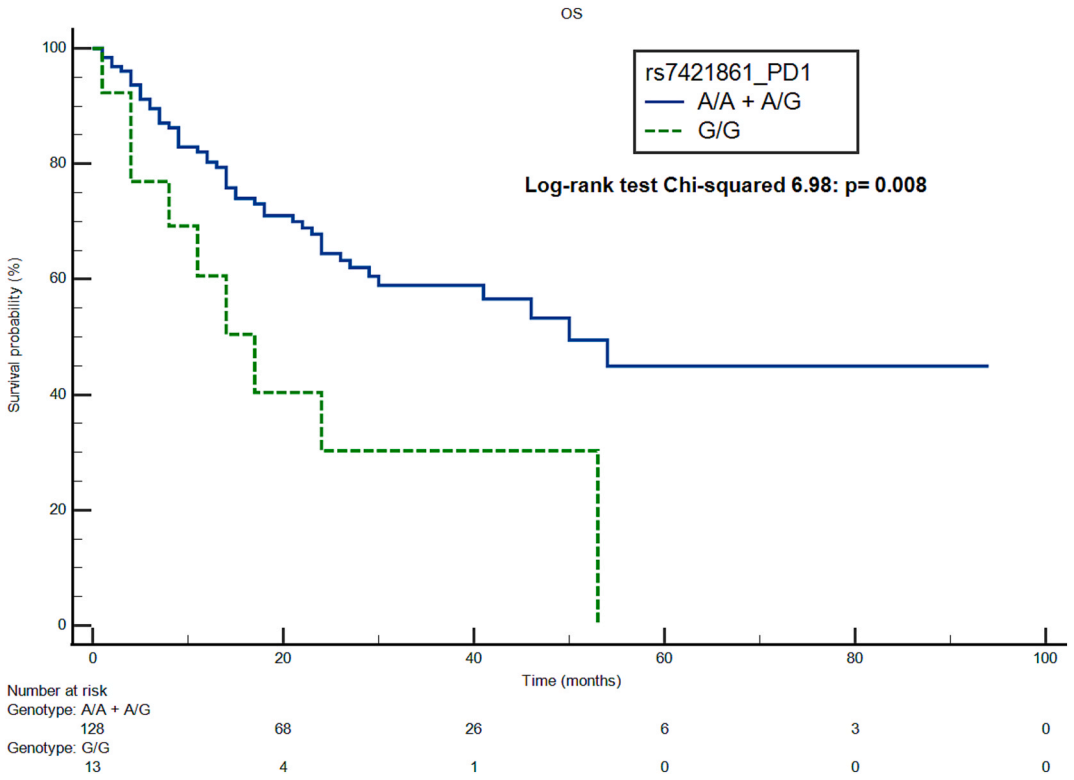


Fig. 2. (*continued*).

its overexpression seems to be implicated in the development of small cell lung carcinoma (SCLC) [33].

Recently, it has been reported that the long non-coding RNA (lncRNA) AC131097.3 is co-expressed with PD-1. LncRNA AC131097.3 is physically adjacent to *PDCD1*. Both genes exhibit coordinated epigenetic regulation in HNSCC (Head and Neck Squamous Cell Carcinoma) tissues [34]. The co-expression pattern and contextual methylation associations suggest that lncRNA AC131097.3 plays a potential role in HNSCC immune responses, particularly in relation to PD-1 signaling and immune checkpoint regulation [34]. Multiple sources also indicate that AC131097.3 may be implicated in immune escape mechanisms alongside PD-1 [35]. A suggested hypothesis is that rs7421861 in *PDCD1* intron 1 may influence local chromatin architecture through splicing disruption, potentially affecting the co-regulated adjacent lncRNA AC131097.3. This hypothesis remains inferential and should be experimentally validated.

To the best of our knowledge, this is the first prospective multicenter study that addressed the role of *PDCD1* SNPs in a homogeneous population of patients with MLA undergoing first-line therapy with the anti-PD-1 pembrolizumab. Notably, the effect on PFS of the rs7421861 G-allele was retained in the exploratory analysis, when patients treated with pembrolizumab alone or pembrolizumab plus chemotherapy were analyzed separately. Intriguingly, the detrimental effect of the rs7421861 G-allele seemed attenuated in the combination group. It could be speculated that its impact may be mitigated in the presence of lower PD-L1 expression levels and/or in the presence of chemotherapy.

We must acknowledge potential limitations of the study. Somatic *KRAS* mutations were included in the present analysis, but additional somatic mutations, such as inactivating mutations in *STK11*, *TP53*, *KEAP1*, *JAK1/2* and *B2M*, or activating mutations in *MAPK*, *PI3K/Akt/mTOR*, and *Wnt/β-catenin* pathways, could have a predictive and prognostic role [30,36]. We must also consider that the panel of polymorphisms we studied may be incomplete and that other germline *PDCD1/CD274* variants could have additional predictive and prognostic value. Finally, it is to be considered that we focused on MLA patients only, therefore, confirmatory studies should evaluate whether *PDCD1* genotypes may display a clinical impact also in patients with squamous histology. This choice was based on the distinct genomic features of the two major histologic subtypes of NSCLC and on the variability in therapeutic strategies and survival outcomes [37]. Therefore, in this prospective investigation we aimed at maintaining a high level of homogeneity in the study population.

In conclusion, our findings indicate that a host genetic feature may play a role in conditioning the effectiveness of an immune checkpoint inhibitor. Further studies are warranted to confirm the clinical impact of rs7421861 and to clarify its potential application as a host-related biomarker in clinical practice.

CRediT authorship contribution statement

Rita Chiari: Writing – original draft, Supervision, Investigation, Data curation, Conceptualization. **Marco Russano:** Writing – review & editing, Investigation, Data curation. **Giacomo Leva:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Rita Emili:** Writing – review & editing, Investigation, Data curation. **Matteo Fiorenti:** Writing – review & editing, Investigation, Data curation. **Bruno Vincenzi:** Writing – review & editing, Investigation, Data curation. **Vincenzo Catalano:** Writing – review & editing, Investigation, Data curation. **Mariagrazia De Lisa:** Writing – review & editing, Investigation, Data curation. **Alfredo Santinelli:** Writing – review & editing, Methodology, Data curation. **Anna Elisa Gulini:** Writing – review & editing, Methodology, Data curation. **Giulia Marcantognini:** Writing – review & editing, Investigation, Data curation. **Renato Bisonni:** Writing – review & editing, Investigation, Data curation. **Francesco Graziano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Annamaria Ruzzo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation,

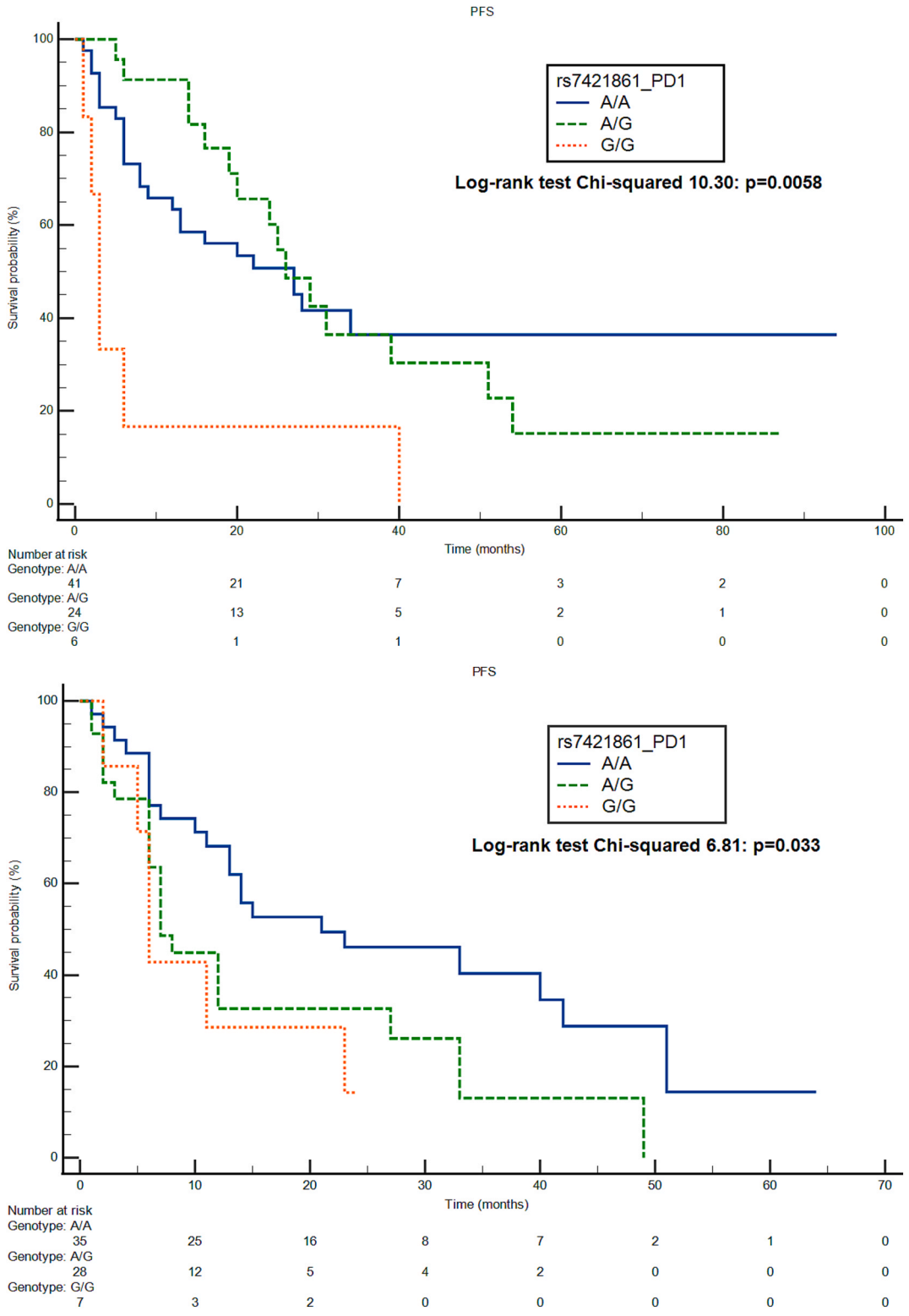


Fig. 3. Log-rank and Kaplan-Meier PFS curves according to rs7421861 genotypes. A) Patients receiving pembrolizumab monotherapy. B) Patients receiving pembrolizumab plus chemotherapy.

Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2026.109383.

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