Genetic polymorphism in *ATIC* is associated with effectiveness and toxicity of pemetrexed in non-small-cell lung cancer

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ABSTRACT

Patients with advanced non-small-cell lung cancer who are treated with pemetrexed display a wide variation in clinical response and toxicity. In this prospective, multicentre cohort study, we investigated the association with treatment effectiveness and toxicity of 10 polymorphisms in nine candidate genes, covering the folate pathway (*MTHFR*), cell transport (*SLC19A1/ABCC2/ABCC4*), intracellular metabolism (*FPGS/GGH*) and target enzymes (*TYMS/DHFR/ATIC*) of pemetrexed. Adjusted for sex, ECOG performance score and disease stage, the association between *ATIC* (rs12995526) and overall survival (HR 1.59, 95% CI 1.06 to 2.39) was significant. Regarding toxicity, this *ATIC* polymorphism was significantly associated with severe laboratory (p=0.014) and clinical (p=0.016) chemotherapy-related adverse events, severe neutropenia (p=0.007) and all-grade diarrhoea (p=0.034) in multivariable analyses.

BACKGROUND

Pemetrexed is widely used in the treatment of advanced non-small-cell lung cancer (NSCLC) as first-line treatment in combination with a platinum agent, and recently also immunotherapy, second-line therapy and maintenance treatment.[1] Pemetrexed shows a substantial variation in clinical effectiveness and toxicity, which cannot be predicted for individual patients. Importantly, toxicity is related to the pharmacokinetic (PK) parameters of pemetrexed, which have a wide interpatient variability.[2]

Here, we aimed to investigate whether polymorphisms of genes associated with the pharmacodynamics (figure 1), which cover the folate pathway (*MTHFR*), cell transport (*SLC19A1/ABCC2/ABCC4*), intracellular metabolism (*FPGS/GGH*) and target molecules (*TYMS/DHFR/ATIC*) of pemetrexed, are associated with clinical effectiveness and toxicity of pemetrexed in a large cohort of patients exposed to this drug. Additionally, we explored the relationship of these pharmacogenetic single nucleotide polymorphisms (SNPs) with the PK of pemetrexed.

MATERIALS AND METHODS

Pharmacogenetic data were available from the 'PEmetrexed and biomaRkerS: an observatiONAL study' (PERSONAL), a prospective multicentre cohort study in the Netherlands. Adult patients with locally advanced or metastatic (stage IIIB/IV) non-squamous NSCLC receiving per standard of care (online supplemental methods) platinum-combined pemetrexed therapy as first-line treatment, followed by maintenance pemetrexed if indicated, or pemetrexed monotherapy as second-line treatment, were recruited from October 2012 until November 2014. The Institutional Review Board of the Erasmus University Medical Center approved this study and all patients provided written informed consent.

Adverse events (AEs) were registered weekly during the entire treatment period and graded according to the National Cancer Institute - Common Terminology Criteria for Adverse Events (NCI-CTCAE) V.4.03 (grade ≥3 was marked as severe toxicity).[3] Clinical effectiveness endpoints were overall survival (OS), progression-free survival (PFS) and best tumour response according to the Response Evaluation Criteria In Solid Tumors (RECIST) V.1.1.

DNA isolation and genotyping are described in the online supplemental methods.

Within the *TYMS* gene, two polymorphisms were selected and combined as one genotype, resulting in a high-expression (3RG/3RG), intermediate expression (3RG/3RC, 2R/3RG) and low expression genotype (2R/2R, 2R/3RC, 3RC/3RC).[4]

For details on SNP selection and statistical analyses, see online supplemental methods. We used our recently developed population-PK model as a base model for the current pharmacokinetic/pharmacodynamic analyses,[5] in which SNPs were included as covariables on pemetrexed clearance. Cox regression analysis was applied in treatment-naïve patients to test the association between polymorphisms and OS/PFS. Adjustment for sex, Eastern Cooperative Oncology Group (ECOG) performance score and disease stage was performed. Polymorphisms were tested against toxicity endpoints using cause-specific Cox regression analyses. If the patient died before completion of four cycles of chemotherapy, censoring for death was performed to take this into account as a competing risk.[6] In univariable analyses, correction for multiple testing was applied using the false discovery rate (FDR) Benjamini-Hochberg procedure (significance level $p<0.1$). A two-sided $p<0.05$ was regarded as significant in multivariable analyses.

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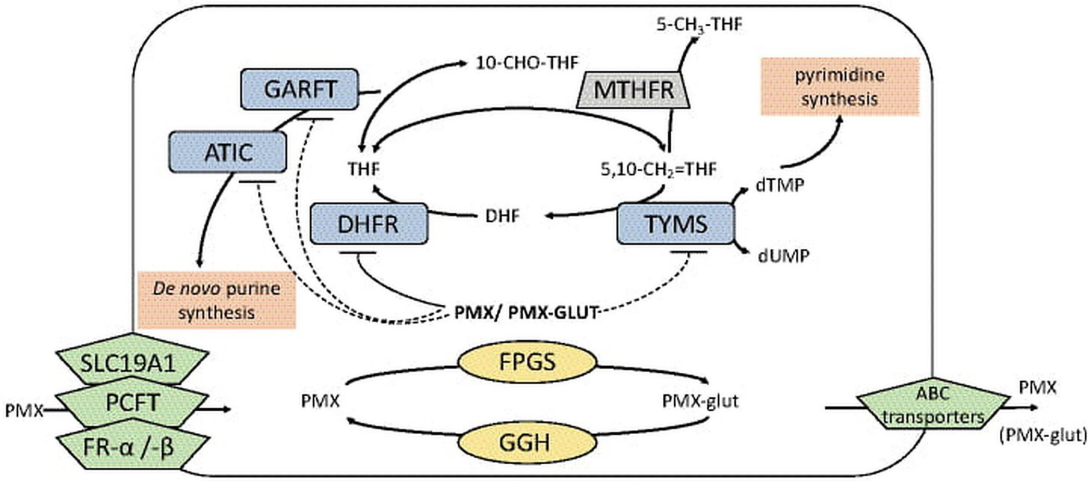

Figure 1 Important proteins involved in the working mechanism of pemetrexed. Green boxes: enzymes involved in the cell transport of pemetrexed. The most important import transporter reduced folate carrier (RFC) is encoded by SLC19A1. Pemetrexed and its polyglutamates are excreted from the cell via ABC transporters, but polyglutamates to a lesser extent. Yellow boxes: FPGS is responsible for the polyglutamylation of pemetrexed and GGH for the deglutamylation. Blue boxes: TYMS, DHFR, GARFT and ATIC are the target enzymes of pemetrexed. The dashed lines represent the increased inhibitory ability of the pemetrexed polyglutamates compared with pemetrexed. Grey box: MTHFR has a major impact on the regulation of the folic acid pathway due to conversion of 5,10-methylenetetrahydrofolate to 5-methyl-THF, which is the methyl donor for methylation of dUMP to dTMP for de novo dTMP synthesis. PMX, pemetrexed; PMX-glut, pemetrexed polyglutamates; dTMP, deoxythymidylate; dUMP, deoxyuridine monophosphate.

RESULTS

One hundred sixty-three patients were recruited, of whom 161 (99%) provided blood samples for pharmacogenetic analysis (online supplemental table 1). Half of the patients were male with a mean age of 63.3±9. Most patients had metastatic NSCLC (87%) and received first-line platinum-combined chemotherapy (91%). Treatment-naïve patients (n=147) had a median OS of 7.7 months and PFS of 4.7 months. Forty-four patients (30%) continued with pemetrexed maintenance after induction treatment. The results of the pharmacogenetic analyses are demonstrated in table 1.

None of the polymorphisms were associated with tumour response (online supplemental table 2). In the univariable analyses, only the *ATIC* polymorphism (rs12995526) was significantly correlated with OS after FDR correction (table 2). Adjusted for sex, disease stage and ECOG performance score, the association between *ATIC* and OS remained (HR 1.59, 95% CI 1.06 to 2.39). Patients with a homozygous variant genotype (CC) had a significantly shorter OS compared with patients with CT/TT genotypes (6.2 months, 95% CI 3.4 to 9.0 vs 9.0 months, 95% CI 5.6 to 12.3, p=0.012), but this association was not found for PFS (online supplemental figure 1).

Detailed information about frequencies of treatment-related AEs and univariable analyses between SNP polymorphisms and toxicity are provided in online supplemental tables 3 and 4. In multivariable analyses (table 2), the homozygous variant genotype of *ATIC* was significantly associated with a 1.9-fold higher risk of severe laboratory and clinical AEs, a 2.0-fold higher risk of developing diarrhoea and a 2.3-fold higher risk of severe neutropenia. Univariably, the CC genotype of *ATIC* was also associated with experiencing severe fatigue and severe anaemia, and having at least one mutant *SLC19A1* allele was associated with an almost sevenfold lower risk of experiencing severe anorexia. These associations could not be tested multivariably due to a too low number of events.

Treatment-naïve patients with the CC genotype of *ATIC* had more dose reductions (OR 4.16, 95% CI 1.59 to 10.93, p=0.004), which was not significantly associated with OS. They continued less often with maintenance treatment than patients with the CT/TT genotypes (20% vs 33%, p=0.09). Receiving maintenance therapy was associated with improved OS (HR 0.59, 95% CI 0.40 to 0.87, p=0.01). Patients who experienced severe clinical toxicities during induction treatment received less often maintenance treatment than patients without these toxicities (19% vs 39%, p=0.01).

No significant associations were observed between the selected SNPs and pemetrexed clearance in the pharmacokinetic/pharmacodynamic analyses (online supplemental table 5).

DISCUSSION

We have found new associations between a genetic polymorphism in a gene encoding for pemetrexed target enzyme ATIC and overall survival, as well as pemetrexed-induced (severe) toxicity. None of the investigated polymorphisms could explain a part of the interpatient variability in pemetrexed PK.

Patients with homozygous variant *ATIC* alleles had a 1.6-fold higher risk of death and they experienced approximately two times more treatment-related toxicities than patients with CT/TT genotypes. Patients with this genotype also had an approximately four times higher risk of receiving dose reductions due to toxicity and they received less maintenance treatments. According to our data, a lower OS in patients with homozygous variant alleles of *ATIC* may be explained by more severe treatment-related toxicity leading to dose reductions and less maintenance treatment. However, a decreased OS is not mediated by increased tumour growth due to increased purine synthesis or decreased activations of AMP-activated protein kinase, as *ATIC* genotype was not associated with tumour response and/or PFS. The effect of the intronic polymorphism rs12995526 on *ATIC* functionality has not been clarified yet. Recently, Zhang *et al* did find an association between the CC genotype of the same *ATIC* polymorphism and worse tumour response, but no survival analyses were performed and therefore we cannot easily compare these findings with our results.[7] However, the specific ethnic Han Chinese population together with the high number

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Table 1 Investigated single nucleotide polymorphisms in the total cohort (n=161)

Gene	rsID	Variant	Assay ID		WT (%)	HET (%)	HVAR (%)	MAF	HWE
SLC19A1	rs1051298	c.*746C>T	C__26854602_10		58 (36.0)	81 (50.3)	22 (13.7)	39%	0.45
GGH	rs3780126	c.109+1307C>T	C__26361922_20		65 (40.4)	74 (46.0)	22 (13.7)	37%	0.90
FPGS	rs1544105	g.2572C>T	C___8342611_10		53 (32.9)	79 (49.1)	29 (18.0)	43%	0.95
ABCC2	rs717620	c.-24C>T	C___2814642_10		100 (62.1)	56 (34.8)	5 (3.1)	20%	0.39
ABCC4	rs7317112	c.75-23516T>C	C__29165801_20		73 (45.3)	80 (49.7)	8 (5.0)	30%	0.02†
ATIC	rs12995526	c.815-102T>C	Assay-by-Design		39 (24.2)	79 (49.1)	43 (26.7)	49%	0.82
DHFR	rs1650697	c.-473T>C	C__27863089_10		90 (55.9)	57 (35.4)	14 (8.7)	26%	0.26
MTHFR	rs1801133	c.665C>T	C___1202883_20		72 (44.7)	77 (47.8)	12 (7.5)	31%	0.16
*TYMS**	rs45445694	5′UTR TSER*2/TSER*3	*See Reference*[4]	Genotypes				25%	0.34
	rs183205964	c.-86G>C		2R/2R	37 (23.0)				
				2R/3RC	42 (26.1)				
				2R/3RG	28 (17.4)				
				3RC/3RC	13 (8.1)				
				3RC/3RG	24 (14.9)				
				3RG/3RG	13 (8.1)				
				Undetermined	4 (2.5)				

**TYMS* was categorised into a high expression genotype (3RG/3RG), an intermediate expression genotype (3RG/3RC, 2R/3RG) and a low expression genotype (2R/2R, 2R/3RC, 3RC/3RC) depending on the 5′-UTR variable number tandem repeat (VNTR) polymorphism and the C/G polymorphism within the third VNTR.
†Since *ABCC4* 2168T>C was not in Hardy-Weinberg equilibrium, this SNP was excluded from all further analyses.
HET, heterozygous; HVAR, homozygous variant; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; rsID, reference SNP ID number; TSER, thymidylate synthase enhancer region; WT, wild type.

of never smokers (66%), in contrast to our population (never smokers 3%), probably results in genetically different tumours, which might alter tumour behaviour and response to treatment.

Although this could not be confirmed multivariably, the CT+TT genotype of *SLC19A1* was univariably associated with a sevenfold lower risk of severe anorexia. Adjei *et al* showed an association between CT+TT genotype and a shorter PFS/OS.[8] Although the influence of the SNP, located in the 3′-UTR region, on gene functionality and expression is unknown, one could speculate that the CT+TT genotype may lead to a decreased influx of pemetrexed into the cell and thereby lower toxicity and less effectiveness.

To our knowledge, this is the largest NSCLC patient population treated with first-line pemetrexed in which pharmacogenetic analyses have been performed. However, a limitation of our study is still the relatively small sample size, which may have led to missed (weaker) associations between SNP genotypes and treatment outcomes due to a lack of statistical power.

A recent shift in the treatment paradigm of advanced NSCLC has led to the common use of platinum and pemetrexed plus pembrolizumab induction regimen followed by pemetrexed plus pembrolizumab maintenance. Although the survival has improved with the combination treatment of chemo/immunotherapy,[9] the

Table 2 Association between polymorphisms and PFS and OS in treatment-naïve patients and toxicity in all patients

					Univariable			Multivariable*	
Endpoint	**SNP**	**rsID**	**Model**	**Genotype**	**HR (95% CI)**	**P value**	**Adjusted P value†**	**HR (95% CI)**	**P value**
Clinical effectiveness (n=147)									
OS	*ATIC*	rs12995526	Recessive	CC vs CT+TT	1.65 (1.11 to 2.45)	0.010	0.080	1.59 (1.06 to 2.39)	0.025
	TYMS	rs45445694 rs183205964	High vs intermediate +low	3G/3G vs 3G/3C, 2R/3G+2R/2R, 2R/3C, 3C/3C	1.79 (0.97 to 3.28)	0.062	0.244		
PFS	*FPGS*	rs1544105	Dominant	TT +CT vs CC	0.65 (0.46 to 0.93)	0.012	0.096	0.72 (0.50 to 1.05)	0.084
Toxicity (n=161)									
Clinical									
Any event, grade 3/4	*ATIC*	rs12995526	Recessive	CC vs CT+TT	1.80 (1.10 to 2.96)	0.012	0.096	1.86 (1.12 to 3.07)	0.016
Diarrhoea, all grade	*ATIC*	rs12995526	Recessive	CC vs CT+TT	2.01 (1.07 to 3.78)	0.012	0.096	1.99 (1.05 to 3.77)	0.034
Fatigue, severe	*ATIC*	rs12995526	Recessive	CC vs CT+TT	3.33 (1.47 to 7.56)	0.004	0.032‡		
Anorexia, Severe	*SLC19A1*	rs1051298	Dominant	TT+CT vs CC	0.15 (0.03 to 0.72)	0.008	0.064‡		
Laboratory									
Any event, grade 3/4	*ATIC*	rs12995526	Recessive	CC vs CT+TT	1.89 (1.15 to 3.10)	0.011	0.088	1.87 (1.14 to 3.09)	0.014
Neutropenia, grade 3/4	*ATIC*	rs12995526	Recessive	CC vs CT+TT	2.24 (1.25 to 4.03)	0.007	0.056	2.25 (1.25 to 4.06)	0.007
Anaemia, severe	*ATIC*	rs12995526	Recessive	CC vs CT+TT	4.38 (1.67 to 11.52)	0.003	0.024‡		

*For clinical effectiveness endpoints adjusted for sex, disease stage and ECOG performance score. For toxicity endpoints adjusted for sex and age.
†Significant p<0.1 after false discovery rate correction.
‡Only tested univariably due to the number of events.
OS, overall survival; PFS, progression-free survival; rsID, reference SNP ID number.

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combination also leads to more severe toxicity and withdrawal of induction treatment.[9 10] To date, germline genetic aberrations in genes involved in the PD-1 pathway have no clinical utility in predicting PD-1 inhibitor-associated toxicities.[11] But now, polymorphism analysis of *ATIC* (rs12995526) could provide valuable information on which patients are more vulnerable to severe pemetrexed-related toxicities. Our suggestion that decreased survival in patients with the CC genotype of *ATIC* may be a result of increased toxicity is alarming, but warrants further validation.

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Contributors SV, RM, SK, JA and BHS were involved in study design. SV, CvdL, NvW and RC were involved in recruiting patients and collecting data. Genetic analyses were performed by RvS and NvD and pharmacogenetic analyses by SV and SK. Statistical analyses were performed by SV and BHS. All authors were involved in data interpretation and manuscript writing.

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Competing interests JA reports personal fees and non-financial support from Eli Lilly, outside the submitted work. In addition, JA has a patent allogenic tumor cell lysate licensed to Amphera pending, and a patent for combination immunotherapy in cancer and biomarker for immunotherapy pending; BHS reports grants from ZonMw during the conduct of the study.

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