Multidrug and toxin extrusion 1 and human organic cation transporter 1 polymorphisms in patients with castration-resistant prostate cancer receiving metformin (SAKK 08/09)

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BACKGROUND: This study was initiated to explore the impact of organic cation transporter 1 (OCT1) and multidrug and toxin extrusion transporter 1 (MATE1) genetic polymorphisms on toxicity, and clinical activity of metformin in patients with castration-resistant prostate cancer (CRPC).

METHODS: The SAKK 08/09 trial included 44 patients with CRPC to receive single-agent metformin 1000 mg two times a day until disease progression or unwanted toxicity. Drug pathway-associated gene polymorphisms of OCT1 (rs622342) and MATE1 (rs2289669) were assessed. The primary objective of this study was to define the relationship between mutations in OCT1, MATE1 and progression-free survival (PFS) at 12 weeks absolute PFS and PSA response in consenting patients of SAKK 08/09. The secondary objective of this study was to analyze the association between mutations in OCT1, MATE1, metformin-related toxicity, PSA response at 12 weeks and overall survival.

RESULTS: Thirty-six patients were evaluable for pharmacogenetic analysis. Homozygous carriers of the polymorphic OCT1 C-allele had no metformin-related toxicity as compared with 41.9% for any metformin-related toxicity in carriers of at least one wild-type A-allele ($P = 0.07$). Disease progression according to RECIST (Response Evaluation Criteria In Solid Tumors) was significantly more frequent in homozygous carriers of the polymorphic OCT1 C-allele (80%) as compared with carriers of at least one wild-type A-allele (28.6%) ($P = 0.002$). Disease progression according to RECIST was also more frequent in carriers of at least one polymorphic MATE1 A-allele (44%) as compared with homozygous carriers of the wild-type G-allele (12.5%) ($P = 0.07$). OCT1 and MATE1 were not associated with PFS.

CONCLUSIONS: The polymorphic OCT1 C-allele has been shown to be associated with less metformin-related toxicity and a higher risk of tumor progression in patients with CRPC receiving metformin as an anticancer treatment. Polymorphisms in metformin drug transporters are attractive molecular markers to serve as potential predictors of efficacy in future clinical studies.

INTRODUCTION

Androgen deprivation is the mainstay of systemic treatment of metastatic prostate cancer, but castration-resistant prostate cancer (CRPC) ultimately develops. Exploring pathways that could be cotargeted alongside the androgen receptor, eventually delaying the development of castration resistance is a current translational and clinical priority. Metformin, a widely used antidiabetic drug, has been shown to reduce the incidence of cancer in diabetic patients[1,2] and exert antitumor activity in preclinical prostate cancer models.[3–5] There is conflicting data on the impact of using metformin after the diagnosis of prostate cancer. In an observational study, cumulative duration of metformin treatment after prostate cancer diagnosis was associated with a significantly decreased risk of prostate cancer-specific and all-cause mortality in a dose-dependent manner.[6] However, in another recent retrospective analysis of 935 men with diabetes mellitus type 2, the use of metformin was not associated with an improvement of cancer-specific and all-cause mortality.[7] At the molecular level, most studies suggest that the primary target of metformin is the respiratory complex I,[8–10] but other targets have been proposed.[11] Metformin-induced reduction in oxidative phosphorylation causes energetic stress, which activates AMP-activated protein kinase (AMPK). The liver is a classic metformin target tissue that is exposed to high metformin levels following oral administration of the drug via the portal circulation. Hepatic energetic stress induced by metformin leads to an inhibition of gluconeogenesis, which lowers blood glucose levels if they are elevated at baseline, as is the case in type 2 diabetes. Reduction in glucose levels leads to a reduction in insulin levels, which may be relevant in prostate cancer, as high rates of insulin secretion are known to be associated with a significant increase in prostate cancer mortality.[12] The possibility that following oral dosing, metformin can accumulate in the prostate or other organs has been proposed,[13] but few studies have addressed this issue directly. In case of accumulation

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in prostate cancer cells, it would be expected to cause energetic stress and activate AMPK, resulting in a variety of downstream effects, including inhibition of the mammalian target of rapamycin pathway, that might have antineoplastic effects.

Metformin does not readily diffuse across cell membranes, but requires active transport. The organic cation transporter 1 (OCT1, encoded by SLC22A1) is responsible for active cellular uptake in hepatocytes and other cell types.[14] OCT2 (SLC22A2) is responsible for the basolateral transport of metformin into the proximal tubular cells in the kidney.[15] The OCTs are all uniporters that mediate facilitated diffusion of metformin in either direction.[16,17] At the apical side of the renal tubular cells, the H+/drug antiporter multidrug and toxin extrusion transporters 1 and 2 (MATE1 (SLC47A1) and MATE2 (SLC47A2)) facilitate the extrusion of metformin into the urine.[18,19] Both OCT1 and MATE1 have a major role in metformin pharmacokinetics,[14,20–24] pharmacodynamics and clinical activity/toxicity [22,24–31] of metformin. For example, genetic variation in OCT (SLC22A1 R61C, G401S, M420del, G465R) was associated with differences in metformin blood levels and glucose levels after an oral glucose tolerance test in healthy volunteers.[20,25] Both OCT1 (rs622342) and MATE1 (rs2289669) are frequent genetic polymorphisms, with allele frequencies of 37% and 44%, respectively.[27] Despite early evidence for clinical activity of metformin in cancer patients,[10,32] there is still no predictive molecular marker for the selection of patients for such a treatment. The present study was initiated to explore the impact of OCT1 and MATE1 genetic polymorphisms on toxicity and clinical activity of metformin in patients with CRPC.

MATERIALS AND METHODS

Patient population

The present study is the translational part of the prospective, multicenter single-arm phase 2 Swiss Group for Clinical Cancer Research (SAKK) trial 08/09, which included patients meeting the following major eligibility criteria: centrally confirmed, metastatic or locally advanced adenocarcinoma of the prostate, progressing on androgen deprivation therapy (ADT), castration-level testosterone $\leqslant 50$ ng dl^{-1}, no prior chemotherapy and no diagnosis of diabetes mellitus. Disease progression was defined as $\geqslant 25\%$ increase of PSA (absolute increase $\geqslant 2$ ng ml^{-1}) above nadir on ADT, measured on three successive occasions $\geqslant 1$ week apart. Patients had to be oligosymptomatic or asymptomatic, have a World Health Organization performance status of < 2, PSA < 114 ng ml^{-1}, PSA doubling time $\geqslant 155$ days and adequate organ function. Patients of the SAKK 08/09 study who consented to the pharmacogenomic analysis underwent blood sampling at baseline (before the start of study treatment). The trial was approved by the ethics committee, was registered (NCT01243385) and followed the current Guideline for Good Clinical Practice issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use[33] and the Declaration of Helsinki. Main results of the SAKK 08/09 trial have been published previously.[34]

Study treatment and assessments

Patients included into SAKK 08/09 received metformin continuously at 1000 mg two times a day in uninterrupted 4-weekly cycles. Metformin daily dose was increased in a stepwise manner, starting at 500 mg once daily and increased to the target dose of 1000 mg two times daily within 2 weeks from the start of treatment. Treatment was continued until progression, unacceptable toxicity or withdrawal of patient consent. ADT was continued in all nonsurgically castrated patients. Physical condition, safety and metformin-related toxicities were evaluated every 4 weeks during study treatment. Adverse events (AEs) were defined by the National Cancer Institute Common Terminology Criteria for Adverse Events v.4.0. The investigator assessed the potential relationship of each individual AE with metformin, considering possible interaction with continued ADT. Disease status was assessed every 12 weeks including computed tomography scans, bone scans and PSA in accordance with the Prostate Cancer Clinical Trials Working Group recommendations.[35] Metabolic parameters including body mass index, glycosylated hemoglobin, fasting glucose, insulin and C-peptide were assessed at baseline. The homeostatic model assessment index was determined (insulin (μU ml^{-1}) × glucose (mmol l^{-1})/22.5), with values $\geqslant 2$ indicating insulin resistance.[36]

Germline genotyping of candidate mutations

Drug pathway-associated gene polymorphisms of OCT1 and MATE1 were assessed as has been described previously.[26–28] At baseline, 5 ml of blood was collected in EDTA tubes via phlebotomy. EDTA tubes were immediately frozen at − 20 °C until analysis. At the end of the study, all samples were collected and shipped to the Department of Pharmacy of the Erasmus MC (Rotterdam, The Netherlands) for central genotype analysis. The following germline mutations were analyzed in peripheral blood from all consenting patients: single-nucleotide polymorphisms (SNPs) rs622342 in the *SLC22A1* gene, coding for OCT1, and rs2289669 in the *SLC47A1* gene, coding for MATE1. All participants were genotyped using the tagging SNPs on the Illumina 550 k SNP array (Illumina, San Diego, CA, USA) for genotyping according to the manufacturer's instruction (Illumina). For this study, we selected the tagging SNPs in the genes for MATE1 (SLC47A1) and for OCT1 (SLC22A1), as described previously.[26–28] Hardy–Weinberg equilibrium was evaluated for each genotype. The average genotype call rate for all SNPs was $\geqslant 98\%$. SNPs with a missing call rate $\geqslant 5\%$ were excluded. The investigators performing genetic analyses (RHNS and MLB) were blinded to patient characteristics and clinical outcomes.

Statistical analysis

The primary objective of this study was to define the relationship between metformin drug pathway-associated polymorphic mutations in OCT1 (rs622342) and MATE1 (rs2289669), and progression-free survival (PFS) at 12 weeks in patients of the SAKK 08/09 trial receiving metformin. The secondary objective of this study was to define the relationship between polymorphic mutations in OCT1 (rs622342) and MATE1 (rs2289669) and metformin-associated AEs, PSA response at 12 weeks and overall survival. Statistical analysis was performed using genotype data as well as covariates such as patient age, gender, blood glucose concentrations and glycosylated hemoglobin. To analyze potential predictive markers, predefined covariates were submitted to multiple logistic regression analysis on the primary and secondary study end points. Based on the frequency polymorphic mutations in OCT1 (rs622342) and MATE1 (rs2289669), the full set of expected study patients ($n = 44$) was considered sufficient for the study purposes. No formal power analysis was performed, as the results of the analysis were considered exploratory or hypothesis generating. The association between OCT1 (rs622342), MATE1 (rs2289669) and time-variant clinical outcome including PFS and overall survival was explored by means of Kaplan–Meier analysis. Differences between subcategories were tested using log-rank statistics and the relation between multiple variables and time-variant clinical outcome by Cox-proportional hazard regression analysis. Multiple logistic modeling was applied for radiological (RECIST) and biochemical (PSA) tumor response adjusting for tumor grading and the time from the diagnosis of CRPC to the start of study treatment. Tumor grading was categorized into well-differentiated (Gleason < 7), moderately differentiated (Gleason 7) and poorly differentiated (Gleason 8–10). Checks for assumptions such as linearity and additivity, and checks for overfitting were performed for all relevant variables. All tests of significance were two-sided, with significance level 0.05. As no adjustment for multiple testing was applied, the results are considered exploratory and hypothesis generating. All statistical analyses were performed using Stata 11.0 software (Stata, College Station, TX, USA).

RESULTS

Patient, treatment and follow-up characteristics

A total of 44 patients were recruited into SAKK 08/09, and results have been published previously.[34] A single patient has not given informed consent for pharmacogenetic analysis, and seven patients had no blood available for genotyping, resulting in 36 evaluable patients for the pharmacogenetic subanalysis. Patient characteristics are outline in Table 1. No obvious differences in baseline characteristics and prognostic factors were found between all patients included into the SAKK 08/09 trial and those evaluable for pharmacogenetic testing. Clinical outcome was comparable between the overall study population and patients of the pharmacogenetic subgroup: disease stabilization at 12 weeks

Table 1. Patient demographics and clinical characteristics

Demographic or clinical characteristic	*No. of patients*	*%*
Age (years)		
Median	70.5	
Range	54.9–85.1	
⩾65	17	47.2
WHO PS		
0	26	72.2
1	10	27.8
Extent of disease		
Bone metastases	25	69.4
Lymph node metastases	16	44.4
Liver metastases	2	5.6
Other	8	22.2
Gleason score at diagnosis		
6	2	5.6
7	11	30.5
8–9	18	50.0
Unknown	5	13.9
Previous treatment		
LHRH	30	83.3
Orchiectomy	6	16.7
Tumor surgery		
Prostatectomy	15	41.7
TURP	8	22.2
No initial surgery	13	36.1
Radiotherapy/brachytherapy		
Yes	16	44.4
Baseline PSA (μg l^{-1})		
Median	28.7	
PSA doubling time (days)		
Median	88	

Abbreviations: LHRH, luteinizing hormone releasing hormone; PS, performance status; WHO, World Health Organization.

was 36.4% in all patients and 38.9% in patients in the pharmacogenetic subgroup; median PFS was 2.8 months (95% confidence interval: 2.7–3.2) in all patients and 2.8 months (95% confidence interval: 2.7–3.8) in patients in the pharmacogenetic subgroup.

MATE1 and OCT1 genotypes and metformin-related toxicity

Distribution for OCT1 (SLC22A1) AA (wild type), AC (heterozygous mutant) and CC (homozygous mutant) genotypes was 11 (30.6%), 20 (55.6%) and 5 (13.8%) patients, respectively. Distribution for MATE1 (SLC47A1) GG (wild type), GA (heterozyous mutant) and AA (homozygous mutant) genotypes was 9 (25.0%), 23 (63.9%) and 4 (11.1%) patients, respectively. There was no evidence for deviation from Hardy–Weinberg equilibrium for the studied gene polymorphisms ($P > 0.8$). Specific categories of metformin-related toxicity (i.e. diarrhea, bloating, anorexia, nausea and fatigue) were not significantly associated with OCT1 or MATE1 genotypes, with the exception of bloating that was less frequent in carriers of at least one MATE1 mutant A-allele (Table 2). Homozygous carriers of the polymorphic OCT1 C-allele had no metformin-related toxicity as compared with 41.9% for any metformin-related toxicity in carriers of at least one wild-type A-allele ($P = 0.07$). Metformin-related toxicity was not different in homozygous carriers of the MATE1 polymorphic A-allele as compared with carriers of at least one wild-type G-allele (50% versus 34.4%, $P = 0.54$), but carriers of at least one MATE1 A-allele also had a nonsignificantly lower risk for metformin-associated toxicity compared towith carriers of the MATE1 wild-type GG-genotype (70% versus 44%, $P = 0.16$).

MATE1 and OCT1 genotypes and clinical activity of metformin

Disease progression according to RECIST was significantly more frequent in homozygous carriers of the polymorphic OCT1 C-allele (80%) as compared with carriers of at least one wild-type A-allele (28.6%) ($P = 0.002$). Similarly, disease progression according to RECIST was more frequent in carriers of at least one polymorphic MATE1 A-allele (44%) as compared with homozygous carriers of the wild-type G-allele (12.5%) ($P = 0.07$). When using logistic regression analysis adjusting for tumor grading, the type of ADT used (LHRH agonist, orchiectomy) and the time from being diagnosed with CRPC, there was a trend for the association between the polymorphic OCT1 C-allele and disease progression (odds ratio (OR) = 7.9, $P = 0.09$). Increase of PSA during trial treatment was substantially higher in homozygous carriers of the polymorphic OCT1 C-allele (+67 ng ml^{-1}) as compared with carriers of at least one OCT1 wild-type A-allele (+30 ng ml^{-1}), but this was not statistically significant ($P = 0.08$). Similarly, increase of PSA during treatment with metformin was higher in carriers of at least one polymorphic MATE1 A-allele (+ 79 ng ml^{-1}) as compared with homozygous carriers of the wild-type MATE1 G-allele (+53 ng ml^{-1}), but this was not statistically significant ($P = 0.26$). Waterfall plots of the association between PSA change during metformin treatment, OCT1 and MATE1 genotypes are outline in Figure 1a and b, respectively. Of the 36 patients evaluable for pharmacogenetic analysis, 21 (58.3%) were progressive after 12 weeks of treatment with metformin. Carriers of the polymorphic OCT1 C-allele had a higher risk of progressive disease at 12 weeks using logistic regression, but this was not statistically significant (OR = 3.3 for the OCT1 CC-genotype, $P = 0.31$). OCT1 and MATE1 genotypes were not significantly associated with PFS in patients receiving metformin, as illustrated in Figure 2. Similarly, OCT1 and MATE1 genotypes were not significant covariates in the multiple Cox regression model on PFS, including patient age ('elderly' as defined above) and tumor grading ('low to moderately' and 'poorly' differentiated).

DISCUSSION

Prior research has explored biological factors that may influence antineoplastic activity of biguanides, and has revealed that mutations of genes involved in sensing energetic stress, mutations in genes encoding components of respiratory complex I or hormonal factors associated with obesity may be important.[37–39] We believe this is the first study to examine polymorphisms of genes influencing metformin pharmacokinetics in the context of a clinical trial investigating antineoplastic activity of the drug. We found that certain polymorphic variants of OCT1 and MATE1 were associated with substantially increased risk of experiencing early biochemical disease progression in patients with advanced CRPC receiving metformin. However, this did not translate into a different PFS according to the RECIST criteria. Interestingly, the polymorphic OCT1 C-allele was associated with a substantial reduction in metformin-related toxicity, whereas this was not found for the polymorphic MATE1 A-allele. Previously, Christensen *et al.*[22] have described the impact of dysfunctional alleles in OCT1 on steady-state metformin plasma concentrations in 159 diabetic patients of the South Danish Diabetes Study. The authors found a 'gene-dose' effect, with decreasing steady-state metformin plasma concentrations as the number of dysfunctional OCT1 alleles increased, and this pharmacokinetic effect also translated into a pharmacodynamic effect of metformin on the absolute decrease

Table 2. OCT1 and MATE1 genotypes and metformin-assossiated toxicity

	OCT1 rs622342 A>C				*MATE1 rs2289669 G>A*			
Toxicity	*WT*	*HET*	*HOM*	P-*value*	*WT*	*HET*	*HOM*	P-*value*
Diarrhea				0.29				0.22
Grade 0	10 (91%)	15 (75%)	5 (100%)		6 (67%)	21 (91%)	3 (75%)	
Grade I–III	1 (9%)	5 (25%)	0		3 (33%)	2 (9%)	1 (25%)	
Bloating				0.75				0.05
Grade 0	10 (91%)	19 (95%)	5 (100%)		7 (78%)	23 (100%)	4 (100%)	
Grade I–IV	1 (9%)	1 (5%)	0		2 (22%)	0.000	0.000	
Anorexia				0.38				0.73
Grade 0	8 (73%)	17 (85%)	5 (100%)		7 (78%)	20 (87%)	3 (75%)	
Grade I–III	3 (27%)	3 (15%)	0		2 (22%)	3 (13%)	1 (25%)	
Nausea				0.56				0.73
Grade 0	9 (82%)	16 (80%)	5 (100%)		7 (78%)	20 (87%)	3 (75%)	
Grade I–III	2 (18%)	4 (20%)	0		2 (22%)	3 (13%)	1 (25%)	
Fatigue				0.07				0.36
Grade 0	6 (55%)	17 (85%)	5 (100%)		8 (89%)	19 (83%)	2 (50%)	
Grade I –III	5 (45%)	3 (15%)	0		1 (11%)	4 (17%)	2 (50%)	

Abbreviations: HET, heterozygous mutant; HOM, homozygous mutant; MATE1, multidrug and toxin extrusion transporter 1; OCT1, organic cation transporter 1; *P*-value, Wilcoxon's rank-sum test; WT, wild type.

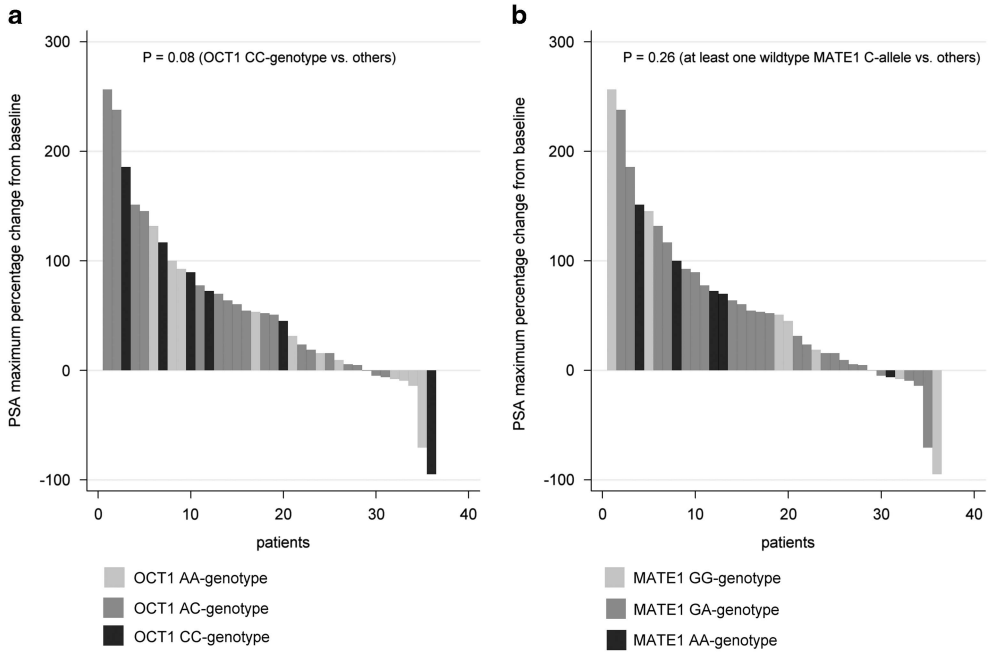

Figure 1. Biochemical responses to metformin as assessed by the change in PSA plasma concentrations in patients with Castration-resistant prostate cancer receiving single-agent metformin. OCT1 (**a**) and MATE1 (**b**) are separately illustrated. OCT1, organic cation transporter 1; MATE1, human multidrug and toxin extrusion transporter.

in glycated hemoglobin after several months of treatment.[22] Similarly, Tarasova *et al.*[29] assessed the relationship between several germline mutations of OCT1, OCT2 and metformin-related gastrointestinal adverse events. The OCT1 polymorphic G-allele (rs628031) was associated with less toxicity from metformin, in accordance with previous results from Christensen *et al.*,[22] showing dysfunctional OCT1 mutations to result in lower metformin plasma concentrations. The group of Shu *et al.*[25] assessed the impact of dysfunctional OCT1 mutations on the activity of metformin in mice and humans. Both in the animal model and in the diabetic patients, dysfunctional OCT1 mutations resulted in a reduced uptake of metformin and a significantly lower effect of metformin on the glucose tolerance test.[25] The present study results are in accordance with these previous observations, in this case showing that dysfunctional OCT1 mutations not only result in less metformin-related toxicity but also in less clinical activity of metformin, approximated by the increase of PSA in patients with CRPC.

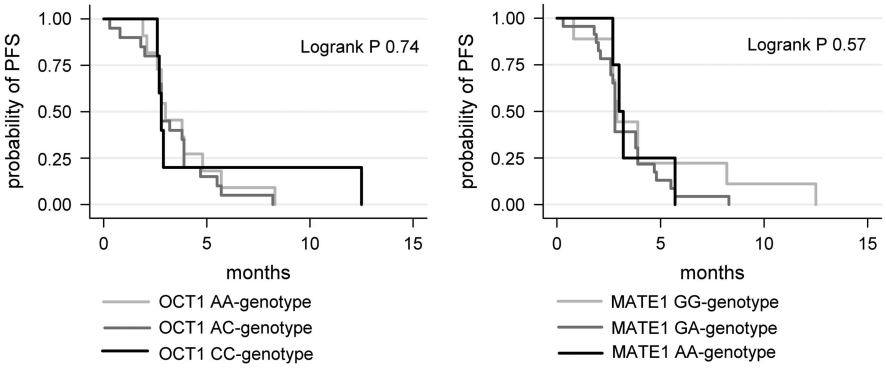

Figure 2. Kaplan–Meier estimates of progression-free survival (PFS) for patients with castration-resistant prostate cancer (CRPC) to the investigational agent metformin. OCT1, organic cation transporter 1; MATE1, human multidrug and toxin extrusion transporter.

Specific genetic variations in MATE1 were previously associated with the glucose-lowering effect of metformin in 116 incidental metformin users, that is, with a reduced decrease in glycosylated hemoglobin levels.[26] We were not able to detect any significant effect of the polymorphic MATE1 A-allele on metformin-related toxicity, but there was a statistical trend for the polymorphic MATE1 A-allele to be associated with an increased risk of PSA progression, potentially because of lower steady-state plasma concentrations of MATE1.

The present study is clearly limited by the small number of patients, by the fact that we did not have metformin plasma or tissue concentrations in the study patients. Also, we only studied a limited subset of genes that influence metformin pharmacokinetics. However, our findings are sufficient to justify further research into the hypothesis that polymorphisms influencing metformin pharmacokinetics influence antineoplastic activity of the drug, and respective molecular analyses have been implemented into a prospective randomized trial adding metformin to enzalutamide in patients with CRPC. To our knowledge, there has only been a single study that examined metformin levels in relationship to antineoplastic activity.[40] That study, which examined short-term metformin exposure in relation to changes in Ki67 labeling of breast cancer, was also limited by small sample size and did not explore polymorphims, but did detect a relationship between changes in Ki67 and serum drug level. As many clinical trials of metformin are ongoing, both for prostate cancer and other malignancies, it will be important to analyze polymorphisms as well as drug levels in relation to any clinical benefit that may be observed.

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