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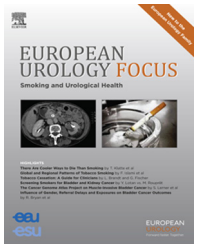

Platinum Priority – Kidney Cancer
Editorial by XXX on pp. x–y of this issue

Pharmacogenomic Markers of Targeted Therapy Toxicity in Patients with Metastatic Renal Cell Carcinoma

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Article info

Article history:
Accepted March 26, 2016

Associate Editor:
James Catto

Keywords:
Biomarker
Genomics
Polymorphisms
Renal cell carcinoma
Single-nucleotide polymorphism
Targeted therapy

Abstract

Background: Targeted therapy (TT) in metastatic renal cell carcinoma (mRCC) may be associated with a high rate of toxicity that undermines treatment efficacy and patient quality of life. Polymorphisms in genes involved in the pharmacokinetic pathways of TTs may predict toxicity.
Objective: To investigate whether selected single-nucleotide polymorphisms (SNPs) in three core genes involved in the metabolism and transport of sunitinib and the mTOR inhibitors everolimus and temsirolimus are associated with adverse events (AEs).
Design, setting, and participants: Germline DNA was extracted from blood or normal kidney tissue from mRCC patients of Caucasian ethnicity in two cohorts treated with either sunitinib (n = 159) or mTOR inhibitors (n = 62). Six SNPs in three candidate genes (*CYP3A4*: rs2242480, rs4646437, and rs2246709; *CYP3A5*: rs15524; and *ABCB1*: rs2032582 and rs1045642) were analyzed.
Outcome measurements and statistical analysis: Primary endpoints were grade ≥3 AEs for all patients; grade ≥3 hypertension in the sunitinib cohort, and any grade pneumonitis in the mTOR inhibitors cohort. A logistic regression model was used to assess the association between SNPs and AEs, with adjustment for relevant clinical factors.
Results and limitations: In total, 221 samples were successfully genotyped for the selected SNPs. In the sunitinib cohort, the *CYP3A4* rs464637 AG variant was associated with a lower risk of high-grade AEs (odds ratio 0.27, 95% confidence interval 0.08–0.88; p = 0.03), but no SNPs were associated with hypertension. In the mTOR inhibitor cohort, none of the selected SNPs was associated with analyzed toxicities.
Conclusions: We observed an association between *CYP3A4* polymorphisms and toxicity outcomes in mRCC patients treated with sunitinib, but not with everolimus or temsirolimus. Our findings are exploratory in nature, and further validation in independent and larger cohorts is needed.

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http://dx.doi.org/10.1016/j.euf.2016.03.017

Patient summary: We found that variants of *CYP3A4*, a gene involved in drug metabolism, are associated with sunitinib toxicity. This information may help in better selection of patients for targeted therapies in metastatic renal cell carcinoma.

1. Introduction

The introduction of targeted therapy (TT) in the management of metastatic renal cell carcinoma (mRCC) has led to improved outcomes at the expense of side effects associated with treatment [1]. Since mRCC remains an incurable disease, quality of life (QoL) is an important consideration for patients. During the last decade, two different types of TT agents have been used for the treatment of mRCC: vascular endothelial growth factor-TT (VEGF-TT), mainly TKIs (tyrosine kinase inhibitors); and mTOR inhibitors. These drugs are generally well tolerated, but major toxicities frequently arise. Some series show that up to 50% of patients can develop grade $\geq$3 toxicities, and a significant number experience adverse events (AEs) leading to treatment interruption, dose reduction, and drug discontinuation [2]. Therefore, individual variability in drug efficacy resulting in therapeutic failure is an important issue. Identification of genomic variants may aid in the development of strategies for patient selection that could lead to improved adherence to treatment and better QoL. Moreover, pharmacogenomics may reduce costs and improve optimal drug development [3].

The mechanism underlying TT toxicity is complex and not entirely understood [4]. While fatigue/asthenia, rash, and diarrhea are common to both sunitinib and mTOR inhibitors, other AEs are class-specific [1,2]. For example, sunitinib is associated with higher incidence of hypertension and hand-foot syndrome, while higher incidence of infections, pneumonitis, hypercholesterolemia, and hyperglycemia has been observed for mTOR inhibitors [2].

Clinical determinants of TT toxicity, such as age, female gender, and low body-surface area, only partly explain the interindividual variability in drug toxicity [5]. Patients with similar clinical characteristics may exhibit wide variability in tolerability for the same drug according to their genetic background [6]. Single-nucleotide polymorphisms (SNPs) in the pharmacokinetic (PK) and pharmacodynamic (PD) pathways for TT agents have been postulated as a complementary explanation for this heterogeneous toxicity [3]. Not all TTs in the same class have the same toxicity profiles, and SNPs may contribute to shape these differences [7]. Sunitinib and mTOR inhibitors are significantly metabolized by cytochrome P450 proteins, predominantly CYP3A4, leading to variation in serum concentrations of the drugs [8,9]. Similarly, concentrations may differ according to polymorphisms in transporters such as ABCB1 [10]. Therefore, SNPs of genes involved in drug PK pathways affect the frequency and severity of drug toxicities in mRCC [11,12]. However, no individual SNP is currently used as a risk factor for TT toxicity in mRCC.

The aim of our study was to assess the association between six SNPs in three core genes implicated in the metabolic and transport pathways for sunitinib and mTOR inhibitors and the risk of grade $\geq$3 AEs and class-specific AEs such as hypertension in the sunitinib cohort and pneumonitis in the mTOR inhibitor cohort.

2. Patients and methods

2.1. Patients

The cohort comprised 221 mRCC patients who received at least one cycle (4 wk on treatment) of sunitinib or mTOR inhibitors as TT at the Dana-Farber/Harvard Cancer Center (DF/HCC) between January 2005 and December 2011 and for whom genotyping was successful. Patients were exclusively of Caucasian ethnicity to ensure no admixture due to ancestry [13]. All patients provided written informed consent. The institutional review board for DF/HCC approved the study. Clinical data were ascertained from medical records in a prospective database. High-grade and class-specific AEs (high-grade hypertension for sunitinib and all-grade pneumonitis for the mTOR inhibitors) were recorded during the treatment period and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

2.2. Blood sample collection, DNA extraction, and genotyping

Germline DNA was extracted from peripheral whole blood using a QIAamp DNA Blood mini kit (Qiagen, Valencia, CA, USA) or from formalin-fixed, paraffin-embedded blocks of normal kidney parenchyma (by an expert genitourinary pathologist) using a DNeasy 96 Blood & Tissue kit (Qiagen). Isolated DNA was genotyped for six polymorphisms in three candidate genes (Supplementary Table 1): *CYP3A4* (rs2242480, rs4646437, rs2246709), *CYP3A5* (rs15524), and *ABCB1* (rs2032582, rs1045642). The SNPs were selected from the European-American ancestry population of the HapMap database according to the following criteria: (1) involvement in the PK pathways for sunitinib and mTOR inhibitors; (2) assumed clinical relevance on the basis of previous reports [12]; (3) a minimal allele frequency of 5%; and (4) tagged across the gene (including both exons and introns) with a minimum correlation index (r2) of 80%.

Genotyping was performed using the iPlex Gold platform (Sequenom, San Diego, CA, USA) with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All SNP assays were combined into a 12-multiplex pool design, and all reactions were carried out in 384-well format. For quality control purposes, 5% of the duplicate samples were randomly selected and interspersed among plates. The concordance rate for duplicate genotyping was 100%. Analysis was restricted to SNPs passing quality filters; SNPs with a genotyping success rate <85% or with significant deviation from Hardy-Weinberg equilibrium (HWE) were excluded.

2.3. Statistical analysis

Patient characteristics were summarized as median with interquartile range (IQR) for continuous variables and as number and percentage for

Please cite this article in press as: de Velasco G, et al. Pharmacogenomic Markers of Targeted Therapy Toxicity in Patients with Metastatic Renal Cell Carcinoma. Eur Urol Focus (2016), http://dx.doi.org/10.1016/j.euf.2016.03.017