Association of \(ABCB1\) and \(FLT3\) Polymorphisms with Toxicities and Survival in Asian Patients Receiving Sunitinib for Renal Cell Carcinoma

Ying-Hsia Chu\(^1\), Huihua Li\(^2\), Hui Shan Tan\(^3\), Valerie Koh\(^4\), Johnathan Lai\(^1\), Wai Min Phyo\(^1\), Yukti Choudhury\(^1\), Ravindran Kanesvaran\(^3\), Noan Minh Chau\(^3\), Chee Keong Toh\(^3\), Quan Sing Ng\(^4\), Puay Hoon Tan\(^4\), Balram Chowbay\(^5,6,7\), Min-Han Tan\(^1,3\)*

\(^1\) Institute of Bioengineering and Nanotechnology, Singapore, Republic of Singapore, \(^2\) Health Services Research, Singapore General Hospital, Singapore, Republic of Singapore, \(^3\) Division of Medical Oncology, National Cancer Centre Singapore, Singapore, Republic of Singapore, \(^4\) Department of Pathology, Singapore General Hospital, Singapore, Republic of Singapore, \(^5\) Laboratory of Clinical Pharmacology, Division Medical Sciences, National Cancer Centre Singapore, Singapore, Republic of Singapore, \(^6\) Clinical Pharmacology Core, SingHealth, Singapore, Republic of Singapore, \(^7\) Clinical Sciences, Duke-NUS Graduate Medical School, Singapore, Republic of Singapore

☯ These authors contributed equally to this work.

* mhtan@ibn.a-star.edu.sg

Abstract

Sunitinib is a tyrosine kinase inhibitor used as first-line treatment for metastatic renal cell carcinoma (mRCC). Asian ethnicity has been previously associated with lower clearance and greater toxicities for sunitinib treatment, relative to Caucasian ethnicity. Research focusing on identifying corresponding biomarkers of efficacy and toxicity has been hitherto conducted in Caucasian populations, and few of the reported associations have been externally validated. Our work thus aims to investigate candidate biomarkers in Asian patients receiving sunitinib, comparing the observed genotype effects with those previously reported in Caucasian populations. Using data from 97 Asian mRCC patients treated with sunitinib, we correlated 7 polymorphisms in \(FLT3\), \(ABCB1\), \(VEGFR2\), \(ABCG2\) and \(BIM\) with patient toxicities, response, and survival. We observed a stronger association of \(FLT3\) \(738T\) genotype with leucopenia in our Asian dataset than that previously reported in Caucasian mRCC patients (odds ratio [OR] = 8.0; \(P = 0.03\)). We observed significant associations of \(FLT3\) \(738T\) (OR= 2.7), \(ABCB1\) \(1236T\) (OR= 0.3), \(ABCB1\) \(3435T\) (OR= 0.1), \(ABCB1\) \(2677T\) (OR= 0.4), \(ABCG2\) \(421A\) (OR= 0.3) alleles and \(ABCB1\) \(3435\), \(1236\), \(2677\) TTT haplotype (OR= 0.1) on neutropenia. Primary resistance (OR= 0.1, \(P = 0.004\)) and inferior survival (progression-free: hazard ratio [HR]= 5.5, \(P = 0.001\); overall: HR= 5.0, \(P = 0.005\)) were associated with the \(ABCB1\) \(3435\), \(1236\), \(2677\) TTT haplotype. In conclusion, \(ABCB1\) and \(FLT3\) polymorphisms may be helpful in predicting sunitinib toxicities, response and survival benefit in Asian mRCC patients. We have also validated the association between \(FLT3\) \(738T\) and sunitinib-induced leucopenia previously reported in Caucasian populations, but have not validated other reported genetic associations.
Introduction

Sunitinib is a tyrosine kinase inhibitor that targets vascular endothelial growth factor receptors (VEGFR1, VEGFR2 and VEGFR3), platelet-derived growth factors (PDGFRα and PDGFβ), Fms-like tyrosine kinase 3 (FLT3) and the RET protein [1–4]. It is used as a standard treatment of metastatic renal cell carcinoma (mRCC) in the first-line setting. Although sunitinib has demonstrated benefits in comparison with interferon therapy [4], clinical outcomes including best radiological response, survival and toxicities are heterogeneous, with 25% of patients achieving complete or partial response and 57% exhibiting severe adverse effects in the recent COMPARZ trial [5]. Sunitinib-associated toxicities include diarrhea, hand-foot syndrome, mucositis, hypertension, leucopenia, neutropenia and thrombocytopenia, as well as abnormalities in hepatic, renal, pancreatic and left ventricular function [4]. In the landmark phase 3 trial, toxicities led to dose interruption in 38% and dose reduction in 32% of patients [4]. Asian patients have been noted to experience higher toxicities from sunitinib therapy. For instance, the incidences of grade 3 to 4 thrombocytopenia (37.7%), neutropenia (29.5%) and anemia (21.9%) reported in Korean patients [6] were more than double of the incidences reported in Western patients [4, 7, 8]. This might be related to a previous observation that Asian ethnicity is associated with decreased sunitinib clearance as compared to Caucasians [9].

Since the FDA approval of sunitinib in 2006, genetic biomarkers have received intense research attention as a promising measure to personalize sunitinib use by profiling individual toxicity and response predisposition. To this end, several prior studies (S1 Table) have correlated survival outcome and toxicity incidences with several single nucleotide polymorphisms (SNPs) in the genes that encode sunitinib targets (such as VEGFR1 [10], VEGFR2 [11, 12], PDGFRα [11], FLT3 [11] and FLT4 [13, 14]), other proteins involved in proangiogenic pathways (VEGFA [14–17], FGFR2 [13] and eNOS [17]), hepatic xenobiotic-metabolizing enzymes (CYP3A5 [18] and CYP1A1 [11, 19]), hepatic enzyme modulators (NR1/3 [13] and NR1/2 [13]), as well as transcellular multidrug efflux pumps such as ABCG2 [11, 20] and ABCB1 [8, 11, 21]. However, few of these reported associations have been replicated, except for an association between VEGFR3 3971 GG genotype and better progression-free survival in two independent studies [13, 18]. The majority of these previous studies were conducted in North America and Europe. In comparison, little work has been done to study Asian populations, an interesting demographic to study given the high incidences of grade 3 to 4 toxicities present in Asian populations [6].

We identified three candidate polymorphisms, 1236C/T, 3435C/T and 2677G/T of ABCB1 for their demonstrated effect on the functionality of the multi-specificity transporter encoded [22, 23]. Sunitinib is a substrate of ABCB1 and another efflux transporter encoded by ABCG2; brain accumulation of sunitinib has been observed to increase significantly in ABCB1 knockout and ABCB1/ABCG2 double knockout mice, despite bioavailability after oral dosing remaining similar to that of wild type mice [24]. Recently, ABCB1 1236C/T and 2677G/T were found to be associated with the clearance of sunitinib in a study involving 114 cancer patients in the Netherlands [25]. Currently, the three ABCB1 polymorphisms have been associated with hand-foot syndrome and survival in sunitinib receivers in exploratory studies [11, 13, 19] in Europe. Given these findings and known interethnic allele frequency variations (for instance, the ABCB1 1236 T allele was found in 71.9% and 41% of a Chinese [26] and German population [27] respectively), we were interested in investigating the correlation of ABCB1 polymorphisms with sunitinib treatment outcomes in Asian patients.

Recently, a 2,903-base-pair deletion polymorphism in intron 2 of the BIM gene was found to be associated with unfavorable outcomes upon treatment with multiple tyrosine kinase inhibitors (TKIs). For example, inferior imatinib response in chronic myelogenous leukemia...
and shorter progression-free survival in EGFR—mutated non—small-cell lung cancer treated with gefitinib or erlotinib was observed in an Asian population [28]. The likely underlying mechanism is alternate splicing leading to loss of the pro-apoptotic BCL2-homology domain 3 (BH3) [28]. The involvement of BIM in sunitinib activity has been suggested by several prior animal and in vitro studies—Naik et al. demonstrated that destruction of tumor vasculature by VEGF-blocking antibodies was BIM-dependent [29] and Yang et al. noted that there was upregulation of BIM along with other proapoptotic genes in human medulloblastoma cell lines treated with sunitinib [30]. The investigation of the association of BIM deletion with outcomes in sunitinib-receiving patients is therefore a subject of interest to us.

This study aimed to evaluate genetic polymorphisms to investigate their association with sunitinib toxicities and survival benefits in Asian renal cancer patients. We have selected candidate polymorphisms based on previously reported effects in Caucasian patients in order to compare the genotype effects seen in each ethnicity. We anticipated that the increased prevalence of high-grade toxicities in Asians would yield increased statistical power in determining relevant genetic markers.

Materials and Methods

Patients and treatment

A total of 97 mRCC patients who received sunitinib between 2006 and 2014 at the National Cancer Centre Singapore (NCCS) were included in this retrospective study. The study was approved by the Institutional Review Board (Singapore Health Services) and written informed consent was obtained from each patient. Sample size estimation is detailed in S5 Table. The majority of patients (79/97) received sunitinib at a starting dose of 37.5 mg daily over 4 consecutive weeks followed by a 2 week break. This attenuation and deviation from the drug label-recommended dosage of 50mg daily was established as routine at NCCS after severe to life-threatening toxicities were frequently noted when sunitinib was initiated at 50mg daily. Efficacy outcomes as determined through a national retrospective analysis have been comparable [31]. 12 patients in this study received a starting dose of 50mg daily. 6 patients received a starting dose of 25mg daily due to advanced age or an aversion to the expected toxicities.

Follow-up and data collection

Sunitinib toxicities and best radiological response were evaluated based on CTCAE version 3.0 [32] and RECIST criteria version 1.1 [33]. Laboratory assessments of serum creatinine, total bilirubin, albumin, aspartate transaminase (AST), alanine transaminase (ALT), hemoglobin, leucocytes and platelets and clinical examinations for hand-foot syndrome and diarrhea were conducted at baseline (before starting sunitinib) and at two time points in each cycle: after 4 weeks of daily sunitinib and after 2 weeks of sunitinib-free rest (before starting the next cycle). Patient characteristics including age, gender, self-reported ethnicity, body weight and height and Eastern Cooperative Oncology Group (ECOG) performance status were also collected. Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic score [34] was calculated for each patient with the available data. All collected data was de-identified by a third party before being used in statistical analysis. The follow-up period ended at the end of April, 2014.

Toxicity definitions

The toxicities assessed include leucopenia, neutropenia, thrombocytopenia, hepatotoxicity, diarrhea and hand-foot syndrome. Blood cell counts from the electronic medical system and physician notes from the first sunitinib cycle were assessed for leucopenia (<3000/μL),
neutropenia (<2000/μL), thrombocytopenia (<15000/μL), hand-foot syndrome (documented physical examination findings) and diarrhea (documented patient complaints). Hepatotoxicity was defined as elevation of AST (>33IU/L) or ALT (>36IU/L) above a normal baseline (AST≤33IU/L and ALT≤36IU/L) during the first two cycles.

**Survival endpoint definition**

Progression-free survival (PFS) was defined as the time from the date of sunitinib initiation to the date of sunitinib termination when sunitinib was terminated due to radiological or clinical evidence of progressive disease (PD), severe toxicities or death, with termination due to PD and death due to PD as events. Dose reduction did not count as an endpoint for PFS. Overall survival (OS) was defined as the time from the date of sunitinib initiation to the date of death or to the date of the last follow-up for censored cases.

**Genotyping**

We genotyped 6 SNPs in 4 genes, including FLT3 738 T/C, VEGFR2 1191C/T, ABCG2 421C/A, ABCB1 3435C/T, ABCB1 1236T/C, ABCB1 2677G/TA, as well as an intron 2 deletion polymorphism of BIM [28]. The SNPs were selected based on minor allele frequency higher than 0.1 in Han Chinese, previously reported associations with sunitinib toxicities (S1 Table) and presumed function in sunitinib pharmacokinetics or pharmacodynamics. Primers for genotyping the SNPs and the BIM deletion are provided in S6 Table [15, 35, 36].

Germline DNA was obtained from the buffy coat or from formalin-fixed tissue of benign kidney obtained from nephrectomy. The labeling on blood tubes and tissue slides were de-identified by a third party before they were used for DNA extraction. Genotyping was done by PCR amplification of the flanking region of each SNP followed by direct sequencing.

**Statistical analysis**

Genotype associations with toxicity events or best radiological response were first analyzed using univariate logistic regression. Genotypes generating $P<0.20$ were further analyzed using multivariate logistic regression including patient age, gender, baseline ECOG status and starting dose as covariates. PFS and OS were estimated using the Kaplan-Meier method [37]. Univariate associations of genotypes and patient characteristics with PFS and OS were analyzed using either a two-tailed log rank test [38] or a Cox proportional hazard test depending on the property of the variable. Genotypes generating $P<0.20$ were further analyzed using a multivariate Cox regression model by including patient characteristics which had univariate $P$ values of less than 0.05 as covariates and PFS or OS as the depending variable. Only patients for whom sunitinib was the first line treatment for mRCC were included in PFS and OS analyses. In all analyses, missing data were kept missing except for baseline ECOG status, which was replaced with the median value. With an exploratory purpose, multiple testing correction was not done.

**Results**

**Patient characteristics and genotype frequencies**

The demographic and baseline clinical characteristics of the 97 patients included in this study are listed in Table 1. The polymorphism frequencies of the 6 SNPs and the BIM deletion are listed in Table 2. Hardy-Weinberg equilibrium held for all 6 SNPs and the BIM deletion ($P>0.05$) [39]. After verifying pairwise linkage disequilibrium for ABCB1 3435C/T, ABCB1 1236T/C and ABCB1 2677G/TA using a Chi-square test ($P<0.05$ in each pair) and phasing with PLINK [40], haplotype TTT was found to be the most common haplotype. It was found
in 51 patients, among whom 8 were homozygous carriers. A complete list of haplotypes and their frequencies is provided in S2 Table.

**Correlation of genotypes to toxicities**

Univariate and multivariate logistic regression analyses for associations between genetic markers and clinical outcomes are listed in Table 3 (non-significant results are provided in S3 Table). It is noteworthy that the FLT3 738 TT genotype was associated with an 8.0-fold increase in the risk of leucopenia ($P = 0.03$) and a 2.7-fold increase in the risk of neutropenia ($P = 0.04$). The $ABCB1$ 1236 $T$ allele, $ABCB1$ 3435 $T$ allele, $ABCB1$ 2677 $T$ allele, $ABCB1$ 3435, 1236, 2677
TTT haplotype and the ABCG2 421 A allele were correlated with a 3-fold (P = 0.03), 10-fold (P = 0.01), 3-fold (P = 0.04), 10-fold (P = 0.03) and 3-fold (P = 0.03) decrease in the risk of neutropenia respectively. The ABCB1 1236 T and ABCB1 3435 T alleles were correlated with a 25-fold (P = 0.0005) and 3-fold (P = 0.02) decrease in the risk of diarrhea respectively. No genotypes were correlated with thrombocytopenia, hepatotoxicity or hand-foot syndrome. The VEGFR2 1191C/T genotype and BIM deletion were not associated with the toxicity endpoints.

Correlation of genotypes with best radiological response and patient survival

Primary sunitinib resistance, defined as the condition in which progressive disease is the best radiological response observed, was more common in carriers of the ABCB1 3435 TT genotype (P = 0.02), ABCB1 2677 TT genotype (P = 0.01) and the ABCB1 3435, 1236, 2677 TTT haplotype (P = 0.004) (Table 4). Median PFS of the 81 patients who received sunitinib as the first-line therapy was 8.1 months and median OS was 19.5 months. As shown in Table 5 (non-significant results are provided in S4 Table), after including starting dose as a covariate based on univariate P < 0.05, the ABCB1 3435, 1236, 2677 TTT haplotype was correlated with inferior PFS (P = 0.001) and OS (P = 0.005) (survival curves are provided in Fig 1).

Discussion

We observed that the FLT3 738 TT genotype predisposed to sunitinib-related leucopenia, an association which had previously been previously reported by van Erp et al. in Caucasian patients [11]. The effect size we observed i.e. an 8.0-fold increase in risk was greater than the 2.4-fold increase previously reported [11]. This may be related to interethnic differences in allele frequencies of other potentially leucopenia-predisposing genotypes such as the CYPIA1 2455A/G (the G allele is present in 3% of Caucasians and 26% of Chinese based on NCBI data) noted by van Erp et al. [11] but not included in this study. Houk et al. also correlated Asian ethnicity with a 13% decrease in sunitinib clearance and 15% increase in peak serum sunitinib concentration and area under curve compared to a control group that was composed of >85% Caucasians [9]. The increased drug exposure, for which interethnic differences in
Table 3. Factors associated with toxicities of sunitinib.

|                          | Group         | Prevalence<sup>b</sup> | Univariate           | Multivariate<sup>a</sup> |
|--------------------------|---------------|-------------------------|-----------------------|---------------------------|
|                          |               | OR (95% CI)             | <i>P</i>              | OR (95% CI)               | <i>P</i>  |
| **Leucopenia (n = 85)**  |               |                         |                       |                           |         |
| Age                      | Male vs. 11/85| 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
| Gender                   | Female 5/20   | 3.3 (0.9, 12.4)         | 0.08                  |                           |         |
| Baseline ECOG            | 0 1/25        | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | 1 9/44        | 6.2 (1.1, 117.6)        | 0.09                  |                           |         |
|                          | 2 1/12        | 2.2 (0.1, 58.7)         | 0.59                  |                           |         |
|                          | 3 0/4         | NR                      | 0.99                  |                           |         |
| Starting dose (mg)       | ≤37.5 0/4     | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | 37.5 10/70    | NR                      | 0.99                  |                           |         |
|                          | 50 1/11       | NR                      | 0.99                  |                           |         |
| FLT3 738 T/C             | CC+CT 2/42    | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | TT 8/41       | 4.9 (1.1, 33.6)         | 0.06                  | 8.0 (1.3, 51.0)           | 0.03    |
| BIM i2del<sup>d</sup>    | Wild type 4/29| 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | Deletion 1/10 | 0.7 (0.1, 5.5)          | 0.76                  | NR                        | 0.39    |
| **Neutropenia (n = 88)** |               |                         |                       |                           |         |
| Age                      | Male 40/88    | 1.0 (1.0, 1.1)          | 0.24                  |                           |         |
| Gender                   | Female 13/20  | 2.8 (1.0, 8.4)          | 0.05                  |                           |         |
| Baseline ECOG            | 0 13/25       | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | 1 22/46       | 0.9 (0.3, 2.3)          | 0.74                  |                           |         |
|                          | 2 5/12        | 0.7 (0.2, 2.6)          | 0.56                  |                           |         |
|                          | 3 0/5         | NR                      | 0.99                  |                           |         |
| Starting dose (mg)       | ≤37.5 1/5     | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | 37.5 32/72    | 3.2 (0.5, 64.3)         | 0.31                  |                           |         |
|                          | 50 7/11       | 7.0 (0.7, 165.7)        | 0.13                  |                           |         |
| FLT3 738 T/C             | CC+CT 15/45   | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | TT 23/41      | 2.6 (1.1, 6.2)          | 0.04                  | 2.7 (1.1, 7.2)            | 0.04    |
| ABCG2 421 C/A            | CC+AC 23/42   | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | AA 16/44      | 0.5 (0.2, 1.1)          | 0.09                  | 0.3 (0.1, 0.9)            | 0.03    |
| ABCB1 1236 T/C           | CC+CT 27/52   | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | TT 11/32      | 0.5 (0.2, 1.2)          | 0.12                  | 0.3 (0.1, 0.9)            | 0.03    |
| ABCB1 2677G/TA           | Other 20/35   | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | TT+AT+GT 20/53| 0.5 (0.2, 1.1)         | 0.08                  | 0.4 (0.1, 0.9)            | 0.04    |
| ABCB1 3435 C/T           | CC+CT 38/75   | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | TT 1/12       | 0.1 (0.0, 0.5)          | 0.02                  | 0.1 (0.0, 0.4)            | 0.01    |
| ABCB1 haplotype<sup>c</sup> | Other 38/79 | 1.0 (0.9, 1.0)          | 0.44                  |                           |         |
|                          | TTT/TTT 1/8   | 0.2 (0.0, 0.5)          | 0.09                  | 0.1 (0.0, 0.5)            | 0.03    |
| BIM i2del<sup>d</sup>    | Wild type 13/27| 1.0 (0.9, 1.0)         | 0.44                  |                           |         |
|                          | Deletion 6/11 | 1.3 (0.3, 5.5)          | 0.72                  | NR                        | 0.93    |
| **Diarrhea (n = 95)**    |               |                         |                       |                           |         |
| Age                      | Male 20/95    | 1.0 (1.0, 1.0)          | 0.62                  |                           |         |
| Gender                   | Female 5/21   | 1.2 (0.4, 3.7)          | 0.73                  |                           |         |

(Continued)
polymorphism frequencies could potentially play a role, may have an influence on the effect sizes of genotype-toxicity associations.

The \textit{ABCB1} 2677T allele was associated with reduced neutropenia risk and inferior radiological response and the \textit{ABCB1} 1236 T allele was associated with reduced risk of neutropenia and diarrhea. A trend was observed for the association of the \textit{ABCB1} 1236 T allele with inferior PFS \((P = 0.09)\) and OS \((P = 0.07)\), which was previously reported in Caucasians [13]. This association appears to be in accordance with the findings of Diekstra et al., whose study correlated \textit{ABCB1} 1236 TT and \textit{ABCB1} 2677 TT to increased clearance of sunitinib and its active metabolite in 114 cancer patients using univariate analyses that did not include demographic covariates [25]. It is also congruent with Beuselinck et al.’s findings that mRCC patients who received sunitinib as first-line therapy and carried \textit{ABCB1} 1236 TT or \textit{ABCB1} 2677 TT/TA require fewer dose reductions due to toxicities compared to carriers of other genotypes [21]. One plausible hypothesis is that increased clearance leads to decreased drug exposure, reduced toxicity and inferior response. However, Diekstra et al. noted that the effect size of a single genetic polymorphism on clearance is much smaller than that of inter-individual variability and is thus inadequate to directly guide dosing [25]. Therefore, the discovery of a panel of genetic markers that collectively offers adequate predictive power and the addition of non-genetic (eg. demographic) markers into the model remain to be investigated.

Our observation that the \textit{ABCG2} 421 AA genotype was associated with reduced risk of neutropenia (which we defined as \(<2000/\mu L\) being equivalent to grade 1 and above as described in CTCAE version 3.0 [32]) appears to be inconsistent with the observation of Kim et al. [20] that grade 3 or grade 4 neutropenia is significantly more common in carriers of this genotype. In comparison with the Korean cohort \((n = 65)\) studied by Kim et al. [20], among whom 61.5%

| Group                  | Prevalenceb | OR (95% CI) | P       | OR (95% CI) | P       |
|------------------------|-------------|-------------|---------|-------------|---------|
| Baseline ECOG          |             |             |         |             |         |
| 0                      | 6/27        | 1           |         |             |         |
| 1                      | 9/50        | 0.8 (0.2, 2.6) | 0.66   |             |         |
| 2                      | 4/12        | 1.8 (0.4, 7.9) | 0.47   |             |         |
| 3                      | 1/6         | 0.7 (0.0, 5.6) | 0.76   |             |         |
| Starting dose (mg)     |             |             |         |             |         |
| \(<37.5\)             | 2/5         | 1           |         |             |         |
| 37.5                   | 15/78       | 0.4 (0.1, 2.9) | 0.28   |             |         |
| 50                     | 3/12        | 0.5 (0.1, 5.2) | 0.54   |             |         |
| \textit{ABCB1} 3435 T/C |             |             |         |             |         |
| CC                     | 11/34       | 1           |         |             |         |
| TT+CT \textit{ABCB1} 1236 T/C |     | 9/60     | 0.4 (0.1, 1.0) | 0.05 | 0.3 (0.1, 0.8) | 0.02 |
| CC                     | 7/9         | 1           |         |             |         |
| TT+CT \textit{BIM} i2deI |         | 13/82     | 0.1 (0.0, 0.3) | 0.0006 | 0.04 (0.0, 0.2) | 0.0005 |
| Wild type              | 7/33        | 1           |         |             |         |
| Deletion               | 5/12        | 2.7 (0.6, 11.2) | 0.18 | 3.1 (0.6, 16.5) | 0.17 |

Abbreviations: OR, ratio of the odds that the event occurs; CI, confidence interval; NR, not reached; PR, partial response; SD, stable disease.

\(a\) Including age, gender, starting dose and baseline ECOG status as covariates.

\(b\) Number of cases affected by toxicity/ total number of cases in the group.

\(c\) \textit{ABCB1} 3435C/T, 1236C/T, 2677G/TA haplotype.

\(d\) A 2,903-bp deletion polymorphism in intron 2 of \textit{BIM} previously associated with resistance to tyrosine kinase inhibitors [28]. As we were unable to genotype formalin-fixed tissues with the current method, only 45 patients were typed.

doi:10.1371/journal.pone.0134102.t003
were first-line sunitinib receivers, 83.5% of our mostly Chinese cohort of patients were first-line sunitinib receivers. Furthermore, 81.4% of our patients started treatment with a reduced dose (37.5mg daily) from the standard course (50mg daily). Although further studies are required for clarification, these differences may possibly explain the discordant observations.

The limitations of this study include the retrospective nature of our data collection and the attenuated dosing regimens adopted in Singapore to reduce toxicity. Indeed, we observed lower toxicity incidences as compared to that of the recent COMPARZ trial [5]. For example, 13%, 49%, 46% and 21% of our cohort developed leucopenia, thrombocytopenia, neutropenia and diarrhea respectively. However, the survival outcome we observed (median PFS: 8.1 months; median OS: 19.5 months) is similar to that observed previously by van der Veldt et al. [19] (median PFS: 10.0 months; median OS: 16.3 months), whose study of a cohort of 136

### Table 4. Factors associated with the clinical benefit of sunitinib (best response being PR or SD) (n = 90).

| Group                      | Prevalence^b | Univariate |          | Multivariate^a |
|----------------------------|--------------|------------|----------|----------------|
|                            | OR (95% CI)  | P          | OR (95% CI) | P              |
| Age                        |              |            |          |                |
| Male                       | 46/71        | 1          |          |                |
| Female                     | 13/19        | 1.2 (0.4, 3.7) | 0.77    |                |
| Baseline ECOG              |              |            |          |                |
| 0                          | 20/25        | 1          |          |                |
| 1                          | 31/49        | 0.4 (0.1, 1.3) | 0.15    |                |
| 2                          | 5/11         | 0.2 (0.0, 0.9) | 0.05    |                |
| 3                          | 3/5          | 0.4 (0.1, 3.4) | 0.35    |                |
| Starting dose (mg)         |              |            |          |                |
| ≤37.5                      | 1/4          | 1          |          |                |
| 37.5                       | 49/75        | 5.7 (0.7, 117.5) | 0.14    |                |
| 50                         | 9/11         | 13.5 (1.1, 378.2) | 0.06    |                |
| VEGFR2 1191 C/T            |              |            |          |                |
| CC                         | 42/60        | 1          |          |                |
| CT                         | 14/27        | 0.5 (0.2, 1.2) | 0.11    | 0.5 (0.2, 1.3) | 0.16 |
| FLT3 738 T/C               |              |            |          |                |
| CC+CT                      | 29/46        | 1          |          |                |
| TT                         | 29/43        | 1.2 (0.5, 2.9) | 0.66    | 1.1 (0.4, 2.8) | 0.84 |
| ABCG2 421 C/A              |              |            |          |                |
| CC+AC                      | 25/39        | 1          |          |                |
| AA                         | 32/49        | 1.1 (0.4, 2.5) | 0.91    | 0.8 (0.3, 2.1) | 0.62 |
| ABCB1 1236 T/C             |              |            |          |                |
| CC+CT                      | 37/54        | 1          |          |                |
| TT                         | 19/33        | 0.6 (0.3, 1.5) | 0.30    | 0.5 (0.2, 1.3) | 0.13 |
| ABCB1 2677 G/TA            |              |            |          |                |
| Other                      | 55/79        | 1          |          |                |
| TT                         | 4/11         | 0.3 (0.1, 0.9) | 0.04    | 0.1 (0.0, 0.6) | 0.01 |
| ABCB1 3435 C/T             |              |            |          |                |
| CC+CT                      | 54/78        | 1          |          |                |
| TT                         | 5/12         | 0.3 (0.1, 1.1) | 0.07    | 0.2 (0.0, 0.7) | 0.02 |
| ABCB1 haplotype^c          |              |            |          |                |
| Other                      | 57/82        | 1          |          |                |
| TTT/TTT                    | 2/8          | 0.2 (0.0, 0.7) | 0.02    | 0.1 (0.0, 0.3) | 0.004 |
| BIM i2del^d                |              |            |          |                |
| Wild type                  | 25/33        | 1          |          |                |
| Deletion                   | 8/10         | 1.3 (0.3, 9.6) | 0.78    | 1.0 (0.2, 8.4) | 0.97 |

Abbreviations: OR, ratio of the odds that the event occurs; CI, confidence interval; NR, not reached; PR, partial response; SD, stable disease.

^a Including age, gender, starting dose and baseline ECOG status as covariates.

^b Number of cases with PR or SD as the best response observed / total number of cases in the group.

^c ABCB1 3435C/T, 1236C/T, 2677G/TA haplotype.

^d A 2,903-bp deletion polymorphism in intron 2 of BIM previously associated with resistance to tyrosine kinase inhibitors [28]. As we were unable to genotype formalin-fixed tissues with the current method, only 45 patients were typed.

Abbreviations: OR, ratio of the odds that the event occurs; CI, confidence interval; NR, not reached; PR, partial response; SD, stable disease.
Table 5. Survival analyses in mRCC patients receiving sunitinib as first-line treatment (n = 81).

| Factor                  | Median (months) | Univariate HR (95% CI) | P | MultivariateHR (95% CI) | P |
|-------------------------|-----------------|-------------------------|---|------------------------|---|
| **Progression-free survival** |                 |                         |   |                        |   |
| Age                     |                 |                         |   |                        |   |
| Female                  | 20              | 10.0                    |   | 1.0 (0.6, 2.1)         | 0.69 |
| Male                    | 61              | 8.1                     |   | 1.1 (0.6, 2.1)         | 0.08 |
| Baseline ECOG           |                 |                         |   |                        |   |
| 0                       | 21              | 16.1                    |   | 1.0 (0.6, 2.3)         | 0.08 |
| 1                       | 43              | 6.9                     |   | 2.2 (1.1, 4.4)         | 0.08 |
| 2                       | 11              | 3.3                     |   | 2.8 (1.1, 7.0)         | 0.08 |
| 3                       | 6               | 12.9                    |   | 2.8 (0.8, 10.3)        | 0.08 |
| Starting dose (mg)      |                 |                         |   |                        |   |
| ≤37.5                   | 5               | 1.8                     |   | 1.0 (0.6, 2.3)         | 0.08 |
| 37.5                    | 71              | 8.3                     |   | 0.2 (0.1, 0.8)         | 0.08 |
| 50                      | 5               | 17.3                    |   | 0.1 (0.0, 0.6)         | 0.08 |
| MSKCC                   |                 |                         |   |                        |   |
| Good                    | 7               | 12.6                    |   | 1.0 (0.6, 2.3)         | 0.08 |
| Intermediate            | 33              | 10.0                    |   | 1.3 (0.5, 3.6)         | 0.08 |
| Poor                    | 23              | 5.5                     |   | 2.3 (0.8, 6.4)         | 0.08 |
| ABCB1 1236 T/C          |                 |                         |   |                        |   |
| CC+CT                   | 51              | 11.7                    |   | 1.0 (0.6, 2.3)         | 0.08 |
| TT                      | 28              | 3.6                     |   | 1.7 (0.9, 3.0)         | 0.08 |
| Other                   | 71              | 8.4                     |   | 1.0 (0.6, 2.3)         | 0.08 |
| ABCB1 2677 G/TA         |                 |                         |   |                        |   |
| TT                      | 10              | 2.7                     |   | 2.3 (0.9, 6.0)         | 0.08 |
| Other                   | 71              | 8.4                     |   | 1.0 (0.6, 2.3)         | 0.08 |
| ABCB1 3435 C/T          |                 |                         |   |                        |   |
| CC+CT                   | 70              | 8.4                     |   | 1.0 (0.6, 2.3)         | 0.08 |
| TT                      | 10              | 2.7                     |   | 1.7 (0.8, 3.9)         | 0.08 |
| Haplotype b             |                 |                         |   |                        |   |
| Other                   | 74              | 8.4                     |   | 1.0 (0.6, 2.3)         | 0.08 |
| TTT/TTT                 | 6               | 2.4                     |   | 4.9 (1.8, 13.6)        | 0.08 |
| BIM i2del c             |                 |                         |   |                        |   |
| Wild type               | 30              | 12.3                    |   | 1.0 (0.6, 2.3)         | 0.08 |
| Deletion                | 10              | 7.9                     |   | 1.6 (0.7, 3.9)         | 0.08 |
| **Overall survival**    |                 |                         |   |                        |   |
| Age                     |                 |                         |   |                        |   |
| Female                  | 20              | 19.9                    |   | 1.0 (0.6, 2.3)         | 0.87 |
| Male                    | 61              | 16.3                    |   | 1.1 (0.6, 2.0)         | 0.06 |
| Baseline ECOG           |                 |                         |   |                        |   |
| 0                       | 21              | 32.9                    |   | 1.0 (0.6, 2.3)         | 0.06 |
| 1                       | 43              | 19.6                    |   | 1.9 (0.9, 3.9)         | 0.06 |
| 2                       | 11              | 5.7                     |   | 3.0 (1.3, 7.1)         | 0.06 |
| 3                       | 6               | 15.7                    |   | 2.7 (0.9, 8.0)         | 0.06 |
| Starting dose (mg)      |                 |                         |   |                        |   |
| ≤37.5                   | 5               | 4.6                     |   | 1.0 (0.6, 2.3)         | 0.06 |
| 37.5                    | 71              | 19.5                    |   | 0.2 (0.1, 0.4)         | 0.06 |
| 50                      | 5               | 47.4                    |   | 0.1 (0.0, 0.3)         | 0.06 |
| MSKCC                   |                 |                         |   |                        |   |
| Good                    | 7               | 41.4                    |   | 1.0 (0.6, 2.3)         | 0.10 |
| Intermediate            | 33              | 19.6                    |   | 2.2 (0.8, 6.5)         | 0.10 |
| Poor                    | 23              | 14.4                    |   | 3.1 (1.0, 9.1)         | 0.10 |
| ABCB1 1236 T/C          |                 |                         |   |                        |   |
| CC+CT                   | 51              | 20.0                    |   | 1.0 (0.6, 2.3)         | 0.07 |
| TT                      | 28              | 10.4                    |   | 1.7 (0.9, 2.9)         | 0.07 |
| Other                   | 71              | 19.6                    |   | 1.0 (0.6, 2.3)         | 0.07 |
| ABCB1 2677 G/TA         |                 |                         |   |                        |   |
| TT                      | 10              | 5.9                     |   | 2.9 (1.3, 6.7)         | 0.07 |
| Other                   | 71              | 19.6                    |   | 1.0 (0.6, 2.3)         | 0.07 |
| ABCB1 3435 C/T          |                 |                         |   |                        |   |
| CC+CT                   | 70              | 19.5                    |   | 1.0 (0.6, 2.3)         | 0.07 |
| TT                      | 10              | 7.2                     |   | 1.6 (0.7, 3.6)         | 0.07 |
| Haplotype b             |                 |                         |   |                        |   |
| Other                   | 74              | 19.6                    |   | 1.0 (0.6, 2.3)         | 0.07 |
| TTT/TTT                 | 6               | 4.6                     |   | 3.9 (1.3, 11.7)        | 0.07 |
mRCC patients employed the standard 50mg daily dose and calculated PFS and OS from the day of sunitinib initiation. Furthermore, we included starting dose in the multivariate analyses for each genotype correlation with toxicities, response and survival to avoid confounding effect produced by uneven dosing in the genotype models.

**Conclusion**

Based on our findings, ABCB1 and FLT3 polymorphisms may be helpful in predicting sunitinib toxicities, response and survival benefit in Asian mRCC patients. We have validated the predisposition to leucopenia associated with FLT3 polymorphism as has been previously reported in Caucasian populations.

**Supporting Information**

S1 Table. Previously reported SNPs with effect on outcomes of sunitinib treatment. (DOC)
S2 Table. ABCB1 haplotype frequencies estimated with and without assuming associations. (DOC)

S3 Table. Factors with non-significant association with toxicities of sunitinib. (DOC)

S4 Table. Genotypes with non-significant associations with survival in mRCC patients receiving sunitinib as first-line treatment. (DOC)

S5 Table. Sample size estimation for the validation of previous associations. (DOC)

S6 Table. Primers for Genotyping. (DOC)

Author Contributions
Conceived and designed the experiments: YHC MHT. Performed the experiments: YHC VK JL WMP YC PHT. Analyzed the data: YHC HL MHT. Contributed reagents/materials/analysis tools: HST RK NMC CKT QSN PHT BC. Wrote the paper: YHC HL MHT.

References
1. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, et al. In Vivo Antitumor Activity of SU11248, a Novel Tyrosine Kinase Inhibitor Targeting Vascular Endothelial Growth Factor and Platelet-derived Growth Factor Receptors: Determination of a Pharmacokinetic/Pharmacodynamic Relationship. Clinical Cancer Research. 2003; 9(1):327–37. PMID: 12538485

2. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006; 368(9544):1329–38. Epub 2006/10/19. doi: 10.1016/s0140-6736(06)69446-4 PMID: 17046465.

3. Faivre S, Delbald C, Vera K, Robert C, Lozahic S, Lassau N, et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol. 2006; 24(1):25–35. Epub 2005/11/30. doi: 10.1200/jco.2005.02.2194 PMID: 16314617.

4. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med. 2007; 356(2):115–24. Epub 2007/01/12. doi: 10.1056/NEJMoa065044 PMID: 17215529.

5. Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, et al. Pazopanib versus Sunitinib in Metastatic Renal-Cell Carcinoma. New England Journal of Medicine. 2013; 369(8):722–31. doi: 10.1056/NEJMoa1303989 PMID: 23964934.

6. Kim HS, Hong MH, Kim K, Shin SJ, Ahn JB, Jeung HC, et al. Sunitinib for Asian Patients with Advanced Renal Cell Carcinoma: A Comparable Efficacy with Different Toxicity Profiles. Oncology. 2011; 80(5–6):395–405. doi: 10.1159/000330361 PMID: 21829041.

7. Motzer RJ, Rini BI, Bukowski RM, Curti BD, George DJ, Hudes GR, et al. Sunitinib in patients with metastatic renal cell carcinoma. JAMA: the journal of the American Medical Association. 2006; 295(21):2516–24. Epub 2006/06/08. doi: 10.1001/jama.295.21.2516 PMID: 16757724.

8. van der Veldt AAM, Boven E, Helgason HH, van Wouwe M, Berkhof J, de Gast G, et al. Predictive factors for severe toxicity of sunitinib in unselected patients with advanced renal cell cancer. British journal of cancer. 2008; 99(2):259–65. doi: 10.1038/sj.bjc.6604456 PMID: 18594533.

9. Houk BE, Bello CL, Kang D, Armita Me M. A population pharmacokinetic meta-analysis of sunitinib malate (SU11248) and its primary metabolite (SU12662) in healthy volunteers and oncology patients. Clin Cancer Res. 2009; 15(7):2497–506. Epub 2009/03/05. doi: 10.1158/1078-0432.ccr-08-1893 PMID: 19258444.

10. Beuselinck B, Karadamoun A, Lambrechts D, Claes B, Wolter P, Couchy G, et al. VEGFR1 single nucleotide polymorphisms associated with outcome in patients with metastatic renal cell carcinoma treated with sunitinib—a multicentric retrospective analysis. Acta oncologica (Stockholm, Sweden). 2014; 53(1):103–12. Epub 2013/02/21. doi: 10.3109/0284186X.2013.770600 PMID: 23421954.
11. van Erp NP, Eechoute K, van der Veldt AA, Haanen JB, Reyners AK, Mathijssen RH, et al. Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity. J Clin Oncol. 2009; 27 (26):4406–12. Epub 2009/08/12. doi: 10.1200/jco.2008.21.7679 PMID: 19667267.

12. Maeng CH, Yi JH, Lee J, Hong JY, Choi MK, Jung HA, et al. Effects of single nucleotide polymorphisms on treatment outcomes and toxicity in patients treated with sunitinib. Anticancer research. 2013; 33 (10):4619–26. Epub 2013/10/15. PMID: 24123039.

13. Beuselinck B, Karadimou A, Lambrechts D, Claes B, Wolter P, Couchy G, et al. Single-nucleotide polymorphisms associated with outcome in metastatic renal cell carcinoma treated with sunitinib. British journal of cancer. 2013; 108(4):887–900. Epub 2013/03/07. doi: 10.1038/bjc.2012.548 PMID: 23462807; PubMed Central PMCID: PMCPMC3590652.

14. Scartozzi M, Bianconi M, Faloppi L, Loretelli C, Bittoni A, Del Prete M, et al. VEGF and VEGFR polymorphisms affect clinical outcome in advanced renal cell carcinoma patients receiving first-line sunitinib. British journal of cancer. 2013; 108(5):1126–32. Epub 2013/03/21. doi: 10.1038/bjc.2012.501 PMID: 23511629; PubMed Central PMCID: PMCPMC3619056.

15. Kim JJ, Vaziri SA, Rini BI, Elson P, Garcia JA, Wirka R, et al. Association of VEGF and VEGFR2 single nucleotide polymorphisms with hypertension and clinical outcome in metastatic clear cell renal cell carcinoma patients treated with sunitinib. Cancer. 2012; 118(7):1946–54. Epub 2011/09/02. doi: 10.1002/cncr.26491 PMID: 21882181.

16. Ruikrok P, Bylina E, Klimeczak A, Switaj T, Falkowski S, Kroc J, et al. The outcome and predictive factors of sunitinib therapy in advanced gastrointestinal stromal tumors (GIST) after imatinib failure—one institution study. BMC Cancer. 2012; 12:107. Epub 2012/02/24. doi: 10.1186/1471-2407-12-107 PMID: 22439647; PubMed Central PMCID: PMC3361487.

17. Eechoute K, van der Veldt AA, Oosting S, Kappers MH, Wessels JA, Gelderblom H, et al. Polymorphisms in endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) predict sunitinib-induced hypertension. Clinical pharmacology and therapeutics. 2012; 92(4):503–10. Epub 2012/09/06. doi: 10.1002/cpt.12.136 PMID: 22948895.

18. Garcia-Donas J, Esteban E, Leandro-Garcia LJ, Castellano DE, del Alba AG, Climent MA, et al. Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicentre, observational, prospective study. Lancet Oncol. 2011; 12(12):1143–50. Epub 2011/10/22. doi: 10.1016/s1470-2045(11)70266-2 PMID: 22015057.

19. van der Veldt AA, Eechoute K, Gelderblom H, Gietema J, Guchelaar HJ, van Erp NP, et al. Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. Clin Cancer Res. 2011; 17(3):620–9. Epub 2010/11/26. doi: 10.1158/1078-0432.ccr-10-1828 PMID: 21097692.

20. Kim HR, Park HS, Kwon WS, Lee JH, Tanigawara Y, Lim SM, et al. Pharmacogenetic determinants associated with sunitinib-induced toxicity and ethnic difference in Korean metastatic renal cell carcinoma patients. Cancer chemotherapy and pharmacology. 2013; 72(4):825–35. Epub 2013/09/10. doi: 10.1007/s00280-013-2258-y PMID: 24013576.

21. Beuselinck B, Lambrechts D, Van Brussel T, Wolter P, Cardinaels N, Joniau S, et al. Efflux pump ABCB1 single nucleotide polymorphisms and dose reductions in patients with metastatic renal cell carcinoma treated with sunitinib. Acta oncoplogica (Stockholm, Sweden). 2014;1–10. Epub 2014/05/31. doi: 10.1007/s12038-014-1922-7 PMID: 24874929.

22. Salama NN, Yang Z, Bui T, Ho RJ. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. J Pharm Sci. 2006; 95(10):2293–308. Epub 2006/08/03. doi: 10.1002/jps.20717 PMID: 16883550.

23. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science (New York, NY). 2007; 315 (5811):525–8. Epub 2006/12/23. doi: 10.1126/science.1133608 PMID: 17185560.

24. Tang SC, Lagas JS, Lankheet NA, Poller B, Hillebrand MJ, Rosing H, et al. Brain accumulation of sunitinib is restricted by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) and can be enhanced by oral elacridar and sunitinib coadministration. International journal of cancer International du cancer. 2012; 130(1):223–33. Epub 2011/02/26. doi: 10.1002/ijc.26000 PMID: 21351087.

25. Diekstra MH, Klumpen HJ, Loikema MP, Yu H, Kloth JS, Gelderblom H, et al. Association Analysis of Genetic Polymorphisms in Genes Related to Sunitinib Pharmacokinetics, Specifically Clearance of Sunitinib and SU12662. Clinical pharmacology and therapeutics. 2014. Epub 2014/02/26. doi: 10.1038/clpt.2014.47 PMID: 24566734.

26. Singh O, Chan JY, Lin K, Heng CCT, Chowbay B. SLC22A1-ABCB1 Haplotype Profiles Predict Imatinib Pharmacokinetics in Asian Patients with Chronic Myeloid Leukemia. PLoS ONE. 2012; 7(12):e51771. doi: 10.1371/journal.pone.0051771 PMID: 23272163.
27. Cascorbi I, Gerloff T, Johna A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clinical pharmacology and therapeutics. 2001; 69(3):169–74. Epub 2001/03/10. doi:10.1067/mcp.2001.114164 PMID: 11240981.

28. Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nature medicine. 2012; 18(4):521–8. Epub 2012/03/20. doi:10.1038/nm.22426421.

29. Naik E, O’Reilly LA, Asselin-Labat ML, Merino D, Lin A, Cook M, et al. Destruction of tumor vasculature and abated tumor growth upon VEGF blockade is driven by proapoptotic protein Bim in endothelial cells. The Journal of experimental medicine. 2011; 210(7):1351–8. Epub 2011/06/08. doi:10.1084/jem.20100951 PMID: 21646395; PubMed Central PMCID: PMCPMC3135358.

30. Yang F, Jove V, Xin H, Hedvat M, Van Meter TE, Yu H. Sunitinib induces apoptosis and growth arrest of medulloblastoma tumor cells by inhibiting STAT3 and AKT signaling pathways. Molecular cancer research. MCR. 2010; 8(1):35–45. Epub 2010/01/08. doi:10.1158/1541-7786.mcr-09-0220 PMID: 20053726; PubMed Central PMCID: PMCPMC2808420.

31. Tan HS, Li H, Hong YW, Toh CK, Wong A, Lopes G, et al. Efficacy and Safety of an Attenuated-Dose Sunitinib Regimen in Metastatic Renal Cell Carcinoma: Results From a Prospective Registry in Singapore. Clinical genitourinary cancer. 2014. Epub 2014/12/30. doi:10.1016/j.clgc.2014.11.004 PMID: 25541325.

32. Common Terminology Criteria for Adverse Events Version 3.0: National Cancer Institute; [updated August 9, 2006]. Available from: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

33. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European journal of cancer (Oxford, England: 1990). 2009; 45(2):228–47. Epub 2008/12/23. PMID: 19097774.

34. Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumdar M. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol. 2002; 20(1):289–96. Epub 2002/01/05. PMID: 11773181.

35. Onizuka M, Kunii N, Toyosaki M, Machida S, Ohgiya D, Ogawa Y, et al. Cytochrome P450 genetic polymorphisms influence the serum concentration of calcineurin inhibitors in allogeneic hematopoietic SCT recipients. Bone marrow transplantation. 2011; 46(8):1113–7. Epub 2010/11/26. doi:10.1038/bmt.2010.273 PMID: 21102498.

36. Jeannesson E, Albertini L, Siest G, Gomes AM, Ribeiro V, Aslanidis C, et al. Determination of ABCB1 polymorphisms and haplotypes frequencies in a French population. Fundamental & clinical pharmacology. 2007; 21(4):411–8. Epub 2007/07/20. doi:10.1111/j.1472-8206.2007.00507.x PMID: 17635180.

37. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Journal of the American statistical association. 1958; 53(282):457–81.

38. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer chemotherapy reports Part 1. 1966; 50(3):163–70. Epub 1966/03/01. PMID: 5910392.

39. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. American journal of epidemiology. 2009; 169(4):505–14. Epub 2009/01/08. doi:10.1093/aje/kwn339 PMID: 19126586; PubMed Central PMCID: PMCPMC2640163.

40. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–75. Epub 2007/08/19. doi:10.1086/519795 PMID: 17701901; PubMed Central PMCID: PMC1950838.