The Utility of Genomics and Functional Imaging to Predict Sunitinib Pharmacokinetics and Pharmacodynamics: The Predict Su Study

Michael Michael (Michael.Michael@petermac.org)
Peter MacCallum Cancer Centre
Guy C. Toner
Peter MacCallum Cancer Centre
Vinod Ganju
Peninsula and Southeast Oncology
Emma Link
Peter MacCallum Cancer Centre
Mick Thompson
Peter MacCallum Cancer Centre
Anetta Matera
Peter MacCallum Cancer Centre
Rodney J Hicks
Peter MacCallum Cancer Centre
Ian Campbell
Peter MacCallum Cancer Centre
S Rowley
Peter MacCallum Cancer Centre
Christos S Karapetis
Flinders Medical Centre
Matthew Burge
Royal Brisbane and Women's Hospital
David W Pook
Monash Cancer Centre

Research Article

Keywords: Sunitinib, DMET, Hepatic functional imaging, Pharmacokinetics, Pharmacodynamics

Posted Date: June 26th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3069721/v1

Abstract

Purpose: Sunitinib has marked pharmacokinetic (PK) & pharmacodynamic (PD) interpatient variability. This study evaluated the utility of extensive excretory/metabolic/PD pharmacogenomics (PGx) with hepatic functional imaging (HNI) to explore associations with Sunitinib PK/PD (toxicity/response) and progression-free survival (PFS).

Methods: Eligible patients (pts) suitable for Sunitinb therapy. At baseline: (i) PGx: blood analyzed by the Affymetrix-DMET™-Plus-Array (1936 variants/225 genes) and Sanger sequencing (HNF1A, FLT3, VEGFR2, VEGFR3, RET, PDGFRα, TNFα). (ii) HNI: pts given IV 800MBq ^{99m}Tc-MIBI, imaging data analysed for hepatic extraction/excretion parameters (CL_{HNI}, $T_{1/2\text{-}HNI}$, 1hRET, HEF, $T_{d1/2}$). In cycles 1 and 2, bloods taken for sunitinib parent (SU), metabolite (SU12662), and Total SU (metabolite + parent) PK. Associations evaluated between (i) HNI parameters and (2) PGx, with Sunitinib PK, toxicity/response and PFS.

Results: N = 15 pts. The two most significant associations in either direction between PGx variants or HNI parameters (P <0.05) for: (i) PK included: (a) SU log$AUC_{0\text{-}14days}$ with HEF, ATP7B-(rs1801246), UGT8-(rs4148254), (b) SU log$AUC_{0\text{-}28days}$, with $T_{d1/2,}$ SLC15A1-(rs8187832), SLC10A2-(rs188096), (c) SU C_{ss}, with $T_{d1/2}$, SLC15A1-(rs8187832) (d) SU C_{trough} with TNFα-(rs1799724), ATP7B-(rs1801246), (e) Total SU log$AUC_{0\text{-}14days}$ with $T_{d1/2}$, TNFα-(rs1799724), (f) Total SU log$AUC_{0\text{-}28days}$ with $Td_{1/2}$ and SLCO3A1-(rs2283458), (g) Total SU C_{ss} and $Td_{1/2}$, UGT8-(rs4148254) and (h) Total SU C_{trough} with SLC16A1-(rs11585690). (ii) Toxicity (a) Diarrhea Gr1+ with HEF, VEFGR3-(rs307826), AKAP9-(rs7785971) (b) ≥Grade 3 AEs with CBR1-(rs998383) (iii) Overall response rate with SULT1E1-(rs1881668), GSTA2-(rs2180314) (Iv) PFS with CYP4Z1-(rs4926802) and CYP2A6-(rs28399442).

Conclusions: Exploratory associations were observed between Sunitinib PK/PD with hepatic functional imaging with extensive pharmacogenomics. Further validation is required

INTRODUCTION

Sunitinib is a selective multi-tyrosine kinase inhibitor,[1] approved for a variety of solid organ malignancies. It is metabolised by CYP3A4 to its active major metabolite (SU12662), upto 80% being eliminated via the biliary-fecal route by ABC-B1 (for parent and metabolite) and ABC-G2 (for metabolite only).[2] Their pharmacokinetics (PK) demonstrate marked interpatient variability ranging upto 80%.[3] The major Sunitinib toxicities have been well documented,[4] representing a major cause of drug discontinuation/interruptions.[5, 6] This has led to alternative regimens being used,[7], apart from the 50mg po daily/4 weeks, q6 weekly (4/2) schedule.[7]

Several PK studies have correlated SU (parent) or total (SU + metabolite) exposure with toxicity and efficacy [8–13]. Similarly, studies have assessed Sunitinb pharmacogenomics (PGx) with toxicity and efficacy,[14–17] usually with a targeted approach for the relevant drug handling and/or

pharmacodynamic (PD) genes.[14–18] Nevertheless, such biomarkers have not been validated for clinical use.

Novel approaches are required to individualise Sunitinib dosing in order to improve compliance, whilst maintaining response. These approaches need to be non-invasive, account for PK variability and be accessible. In regards to Sunitinib, in-vivo phenotypic probes for CYP3A4 activity have included the midazolam clearance test.[19] Similarly, hepatic functional nuclear imaging (HNI) probes such as technetium-99m-2-methoxy isobutyl isonitrile (^{99m}T-Sestimibi or –MIBI) have provided quantitative parameters of membrane transporter hepatic uptake and biliary excretion for Sunitinib.[20] Hence, combining robust HNI parameters with wide ranging PK/PD PGx analysis may have the potential to predict Sunitinib PK and toxicity/response, leading to individualised dosing.

The objectives of this study were therefore to: (i) Correlate ^{99m}T-MIBI HNI parameters with Sunitinib PK/toxicity. (ii) Correlate the combined PGx of excretory/metabolic/PD enzymes with Sunitinib PK, toxicity and response. (iii) Develop a non-invasive population model based on ^{99m}T-MIBI HNI and PGx, for optimal Sunitinib dosing. (iv) Exploratory: To assess the association of ^{99m}T-MIBI HNI parameters and PGx factors on progression-free survival (PFS) for patients with RCC and GIST.

PATIENTS AND METHODS

General Comments

Observational study in patients receiving Sunitinib for metastatic RCC or GIST. Patients underwent baseline ^{99m}T-MIBI HNI and blood sampling for pharmacogenomics. During cycles 1 and 2, blood was taken for SU, and SU12622 PK. Toxicities were documented in the first 2 cycles and patients were restaged at the end of cycle 2, (i.e., 12 weeks).

Patient Selection Criteria

Eligibility criteria: (i) Patients with untreated metastatic RCC or GIST (post imatinib therapy). (ii) Measurable/evaluable lesions according to RECIST version 1.1 criteria. (iii) Adequate organ function: (a) Hepatic: Se total bilirubin < 1.5xULN, ALT/AST and ALP ≤ 2.5xUNL in the absence, or ≤ 5xUNL in the presence of liver metastases, (b) Bone marrow: Platelets ≥ 100x10^9/L, Neutrophils ≥ 1.5x10^9/L (c) Renal: calculated creatinine clearance ≥ 45 ml/min. (iv) ECOG performance status 0–2. (v) Written, informed consent.

Exclusion criteria

(i) Medical co-morbidities that potentially compromise protocol compliance or ability to give consent. (ii) Conditions that compromise oral absorption. (iii) Female patients who were pregnant or breast-feeding. (iv) Unresolved toxicity > NCI-CTC Grade 2 from previous therapy. (iv) Co-administration of potent Cytochrome 3A4/5 inducers within 12 days, or potent inhibitors within 7 days of dosing.

Baseline Study Evaluations (within 2 weeks of trial entry)

1. **Clinical**: Bloods taken for haematology and serum biochemistry (Urea, Electrolytes, Creatinine, LFTs [Bili, AST/ALT, ALP, GGT, Albumin, Total protein], Lipase and Amylase), and Thyroid function tests. Radiological assessment of disease by CT or MRI, as appropriate.
2. **^{99m} Tc-MIBI (HNI)**: The methodology using intravenous ^{99m}Tc-MIBI, imaging, data acquisition and analysis was as described prior.[21] The analysis methodology and derived imaging parameters were as follows: (a) Non-compartmental analysis: (1) Terminal elimination half-life, ($T_{1/2\text{-HNI}}$), (2) Clearance (CL_{HNI}), (3) Percent (%) retention at 1 hour (1hRET). (b) Deconvolutional analysis (separating tracer activity within the intrahepatic vascular component from that taken up directly by hepatocytes): (1) hepatic extraction fraction (HEF) (i.e. fraction of drug presented to the liver that is excreted via the hepatobiliary route) and (2) deconvolution elimination half-life ($T_{d1/2}$).[21]
3. **Blood samples for Pharmacogenomic studies**: Two 7 mL aliquots of whole blood drawn, stored at −80°C, batched, and sent to the Cancer Genetics Laboratory (Prof IC, Peter MacCallum Cancer Centre). Genomic DNA was isolated and quantified as per prior report.[22] Samples were analysed in 2 ways:

1. **DMET-(Drug Metabolizing Enzymes and Transporters)-Plus-Array**. Blood was analyzed by DMET-Plus-Array (Afymetrix, CA, USA). The Array comprised of 1936 variants: 1931 single nucleotide polymorphisms (SNPs) and 5 copy number variants in 231 genes: coding for 47 phase I, 80 phase II enzymes, and membrane transporters.[23]
2. **Sanger sequencing**: Relevant PK/PD genes not covered by the DMET-Plus-Array were PCR amplified and then Sanger sequenced using the Sequenom MassARRAY iPLEX genotyping chemistry, enabling genotyping up to 36 variants in a single reaction.[22] The selected SNPs were those related to sunitinib PK/PD, common within the Western population and included: HNF1A, FLT3, VEGFR2, VEGFR3, RET, PDGFRα, TNFα. (**Supplementary Table 1**).

Sunitinib Therapy.

Sunitinib was administered at 50mg p.o. daily, for 28 days, q 6-weekly, (4/2 schedule). The management of the toxicities was as per the standard practice. To standardize dosing, there were protocol recommended dose interruptions/reductions for significant toxicities.

Patient Evaluations During Sunitinib Treatment and Followup

During cycles 1 and 2, patients were reviewed on days 1, 14 and 28 for the following: (i) Physical examination and ECOG PS assessment on day 1, (ii) Treatment compliance, (iii) Bloods taken for haematology and biochemistry. (iv) Adverse event assessment, as per the Common Toxicity Criteria Adverse Events version 4.0 CTCAE v4.0. Thyroid function tests repeated on day 1 of cycle 2.

On Cycle 2 day 42, an end of study visit was completed. Evaluations were as above, and patients were restaged: response evaluation was as per RECIST version 1.1.

Beyond cycle 2 (12 weeks), management was as per standard practice and with no study-mandated data collection.

Data on PFS was collected retrospectively from the medical record.

Sunitinib Pharmacology Studies

Serial 3 mL blood samples were taken, preceding the Sunitinib dose, from Cycles 1 and 2, on days 1, 14 and 28. Samples were collected into tubes containing K2EDTA anticoagulant and gently inverted 15 times. They were centrifuged immediately at 1000g/10 minutes/4°C to provide plasma for analysis. The plasma was taken off, protected from light in amber cryovials and stored at -70°C.

The analysis was carried out by Inotiv (West Lafayette, IN, 47906, USA). Sunitinib parent (SU) and metabolite (SU12662) were extracted from potassium EDTA human plasma by liquid/liquid extraction at alkaline pH with ethyl acetate. Before the extraction, a deuterated internal standard of SU (SU11248) was added. The organic layer was collected, transferred to a new plate, and evaporated to dryness. The residue was then reconstituted with an ammonium formate/acetonitrile mixture and injected into an LC/MS/MS system using a C18 column with an ammonium formate/acetonitrile mobile phase.

PK parameters were calculated for Cycles 1 and 2 by non-compartmental analysis using Win-Non-Lin Professional 5.2 (Pharsight Corporation). The parameters were derived for the following moieties: (a) Sunitinib parent (SU), (b) metabolite (SU12662) and (c) Total SU (parent + metabolite). The parameters for each moiety included (a) $AUC_{(0-14days)}$ and $AUC_{(0-28days)}$, (b) Concentration at steady state (C_{ss}) (as per $AUC_{(0-28days)}/[\tau \times 28]$, where dosing interval τ = 24hrs. Where $AUC_{(0-28)}$ was unable to be derived, the following was used $AUC_{(0-14days)}/[\tau \times 14]$), (c) C_{trough}, on days 14.

Statistical Methods

Quality control and general methods:

All statistical analyses were performed in SAS and R using standard validated statistical procedures. Statistical tests were performed using a two-sided significance level of 5%. Two sided, exact, 95% confidence intervals (CIs) were calculated for rates. No adjustments were made for multiple testing.

Descriptive statistics were used to describe patient characteristics measured at baseline and during the study. For continuous variables, as number of patients, median, minimum and maximum, and for qualitative variables as counts and percentages. AUC measures were transformed to a log scale: analysed as log(AUC). Transformations for other variables were assessed based on their observed distributions.

The correlation of ^{99m}Tc-MIBI HNI with the Sunitinib PK and toxicity:

Associations were assessed to examine the strength/direction of the linear relationship between HNI with PK and toxicity parameters. Linear regression models to predict PK parameters from HNI parameters were

fitted to estimate the relationship between pairs of parameters. Logistic regression was used to assess whether HNI parameters could predict presence/absence of toxicity. Due to the absence of linear regression associations, the Pearson correlation coefficient or the nonparametric Spearman rank correlation were not performed.

The correlation of PGxs with Sunitinib PK, toxicity and response:

The observed SNP genotypes were assessed for deviations from Hardy-Weinberg equilibrium. Exact logistic regression was used to assess the association between SNP genotypes with toxicity and response in 2 degree of freedom tests. Linear and logistic regressions was used to assess the relationship between the number of minor alleles at each SNP with PK, toxicity and response parameters, as appropriate, in 1 degree of freedom tests.

The development and validation of a population-dosing model based on imaging and PGx factors:

The genotypic and imaging parameters that showed some evidence of an association with toxicity/response (as above) were tested for inclusion as covariates in logistic regression models for the prediction of toxicity and response. Patient baseline demographics and other measured variables were also considered. The improvement in model fit, as assessed by chi-squared statistics determined whether a variable was included in the analysis.

Assess the association of HNI and PGx factors on PFS:

It was intended that for each malignancy, PFS was to be estimated using the Kaplan-Meier method. However, as there were only 15 patients in total, the results were combined. Estimates and 95% CIs for the percentage of event-free patients at 1 year were specifically reported. Patients without events were censored at the time of last tumor assessment. A close-out date, the earliest of dates of last contact among patients still alive and being followed up, was applied. The assessment of individual prognostic factors for time to event estimates were made using the Mantel-Cox logrank test and Cox proportional hazards regression. The latter was used for multiple variant analysis to identify independent prognostic factors.

Sample size:

A sample size of 60 patients was required for a power of > 95% to detect a significant positive correlation between a PK and HNI variable at the $P < 0.05$ level of significance, provided the true correlation coefficient is ≥ 0.7 (95% CI 0.54–0.81). This would have allowed detection with 88% power of a common SNP (with minor allele frequency [MAF] of 0.25) associated with 30% higher AUC levels of drug (assuming standard deviation in log(AUC) of 0.4). To account for patient drop out, an additional 10 patients, hence a total of 70 patients, were intended to be accrued.

Overall, 13 patients had been recruited in nearly 4 years due to slow recruitment and changes in the approved therapies for RCC: hence the sample size was revised to 18, to ensure recruitment was completed within 4 years. With 18 patients, assuming 3 drop out, 15 patients would provide power of 35% to detect a significant positive PK-HNI variable coefficient ≥ 0.7, (assuming 0.4 is not of interest) and 34% power to detect a SNP-AUC association (i.e. 30% higher AUC). Therefore, these data would enable assessment of associations of this magnitude and still be of clinical interest.

RESULTS

Patients

Overall, 15 patients were recruited across 5 centres, patient demographics are summarised in Table 1: 10 patients had metastatic RCC and 5 GIST.

Table 1
Patient demographics

Baseline characteristics	N	Mean (SD) or %	Median (Range)
Diagnostic Category			
Metastatic renal cell carcinoma	10	67%	
Metastatic gastrointestinal stromal tumour	5	33%	
Months from diagnosis to registration	15	27.1 (43.6)	7.8 (0.3 -156.6)
Age at registration (years)	15	62 (10)	65 (48–78)
Sex: M:F	11:4	73%:27%	
N target lesions			
2	7	47%	
3	5	33%	
≥ 3	8	53%	
WCC (x10^9/L)	15	7.6 (3.6)	6.9 (4.2–19.3)
Neutrophils (x10^9/L)	15	5.45 [(3.27)	4.70 (3.02–16.2)
Neutrophil to WCC ratio	15	0.70 (0.09)	0.72 (0.52–0.84)
Bilirubin (µmol/L)	15	12 [7]	10 (6–35)
ALP (U/L)	15	90 (33)	83 (43–186)
ALT (U/L)	15	24 (18)	18 (4–69)
AST (U/L)	14	25 (11)	25 (11–46)
GGT (U/L)	15	49 (43)	26 (15–158)
Total Protein (g/L)	13	70 (7)	70 (56–82)
Albumin (g/L)	15	39 (4)	39 (30–48)
Lipase (U/L)	13	38 (23)	35 (11–97)

1. SD = Standard deviation.

2. Sunitinib ceased early by AE, N = 1; Treatment cycle delayed by 1 week.

N = 1.

Baseline characteristics	N	Mean (SD) or %	Median (Range)
Diagnostic Category			
Amylase (U/L)	11	59 (28)	56 (29–111)
TSH (mU/L)	15	1.20 (0.97)	1.00 (0.11–3.50)
T4 (µg/100mL)	12	14.4 (3.2)	15.8 (8.3–19.1)
T3 (µg/100mL)	9	4.5 [0.5]	4.6 (3.6–5.1)
Sunitinib Exposure: Cycle 1 N = 15, Cycle 2 N = 11			
Cycle 1 completed as per protocol (50mg daily, 4 weeks, 2 weeks off)		10 (67%)	
Missed doses		4 (27%)	
Sunitinib stopped prematurely due to AEs		1 (7%)	
Cycle 2 completed as per protocol (50mg daily, 4 weeks, 2 weeks off)		4 (36%)	
Missed doses		1 (9%)	
Dose modifications		3 (27%)	
Dose modifications and missed dose		1 (9%)	
Other protocol deviations [2]		2 (18%)	
1. SD = Standard deviation.			
2. Sunitinib ceased early by AE, N = 1; Treatment cycle delayed by 1 week.			
N = 1.			

Sunitinb Exposure and Toxicity.

Of the 15 patients enrolled, (Table 1), 10 (67%) completed the planned Cycle 1 treatment, i.e., 50mg daily (4/2 schedule), 4 patients had missed doses, and 1 patient stopped therapy prematurely by treatment-related AEs. Eleven patients commenced cycle 2: only 4 completing the planned cycle, the remainder had dose modifications and/or missed doses.

The treatment-related toxicity was as expected (**Supplementary Table 2**). One patient each had grade 3 thrombocytopenia and hypertension in Cycle 1, respectively. The overall Grade 3 + adverse event rate was 13% (95% confidence interval 2%-40%). No grade 4 toxicities were observed.

^{99m}Tc-MIBI Hepatic Nuclear Functional Imaging (HNI)

Baseline HNI parameters were available from all 15 patients (Table 2). The interpatient variability for each parameter as expected was wide: ranging from 17.8% for 1hRET to 46.6% for HEF.

Table 2
Baseline ^{99m}Tc MIBI Hepatic nuclear functional imaging (HNI) parameters.

Baseline characteristics	N	Mean (SD)	Median (Range)	CV (%)
Clearance (CL_{HNI})[1] (%/min)	15	1.4 (0.4)	1.3 (0.8–2.3)	27.4
Terminal Half-life ($T_{1/2-HNI}$) (min)	15	54.8 (14.2)	52.2 (30.5–84.1)	26.0
% Retention at 60 min (1hrRET) (%)	15	69.5 (12.4)	67.0 (52.9–94.7)	17.8
Hepatic Extraction Fraction (HEF) (%)	15	38.8 (18.1)	32.2 (17.0-93.8)	46.6
Deconvolutional half-life ($T_{d1/2}$) (min)	15	3.52 (1.48)	3.34 (0.43–6.94)	42.1

1. CL = Clearance, SD = Standard deviation, HNI = hepatic nuclear functional imaging. CV = Coefficient of Variation = 100%x(SD/Mean)

Pharmacogenomics

(i) Sanger Sequencing

Overall, 7 genes were sequenced for 17 variants (**Supplementary Table 1**). The wild type alleles ranged from 13% for VEGFR2 (rs2071559) to 93% for PDGFRα (rs35597368). Homozygote variant alleles were found in upto 2 patients subject to the gene.

(ii) DMET-Plus-Array

Of the 1936 variants evaluated: (i) 13 markers had deviations from Hardy Weinberg equilibrium (exact P-values < 0.05), hence these markers were not analysed further (ii) 1251 markers had a MAF (minor allele frequency) = 0, thus not further analysed. (iii) 667 markers had a MAF > 0.00, without deviations from the Hardy Weinberg Equilibrium, therefore carried forwards to analysis. Of these, 605 markers had a MAF ≥ 0.05.

SU (parent), Su12662 (metabolite) and Total SU (parent + metabolite) Pharmacokinetics

The PK parameters for SU, Su12662 and Total SU are summarised in Table 3. Due to patient drop-offs and missed doses, a smaller cohort had values for $AUC_{(0-28days)}$ relative to $AUC_{(0-14day)}$. The relationship

between each moiety's $AUC_{(0-28days)}$ relative to $AUC_{(0-14days)}$ was nonlinear, with the caveats of small sample size. The ratio of the SU versus metabolite AUCs was approximately 3:1, as observed prior.[19, 24, 25]

Table 3
Sunitinib parent (SU), metabolite (SU12662) and Total SU (parent + metabolite) pharmacokinetics in Cycles 1 and 2.

Parameter	N	Mean (SD)[3]	Median (Range)	CV (%)
SU cycle 1				
$AUC_{0-14days}$ (hr.ng/mL)[1]	14	11280.85 (3447.08)	10752.56 (7146.38 -19155.45)	30.56
$AUC_{0-28days}$ (hr.ng/mL)	11	34667.49 (9843.05)	34690.47 (19511.82-57824.39)	28.39
Log $AUC_{0-14days}$	14	9.29 (0.29)	9.28 (8.87–9.86)	3.12
Log $AUC_{0-28days}$	11	10.42 (0.28)	10.45 (9.88–10.97)	2.69
C_{SS} (ng/mL)[2]	13	49.67 (15.93)	51.62 (21.27–86.05)	30.82
C_{Trough} (ng/mL)	12	62.54 (17.51)	60.05 (40.60–104.00)	28.00
SU cycle 2				
$AUC_{0-14days}$ (hr.ng/mL)	2	11038.10 (5458.21)	11038.10 (7178.56 -14897.63)	49.45
$AUC_{0-28days}$ (hr.ng/mL)	2	34179.02 (13551.40)	34179.02 (24596.73-43761.30)	39.65
Log$AUC_{0-14days}$	2	9.24 (0.52)	9.24 (8.88–9.61)	5.63
Log$AUC_{0-28days}$	2	10.40 (0.41)	10.40 (10.11–10.69)	3.94
C_{ss} (ng/mL)	2	50.86 (20.17)]	50.86 (36.60-65.12)	39.66
C_{Trough} (ng/mL)	9	63.98 (18.86)	68.50 (32.60–91.10)	29,48
Su12662 cycle 1				
$AUC_{0-14days}$ (hr.ng/mL)	14	3354.49 (1229.28)	3451.81 (1497.01–5613.30)	36.65
$AUC_{0-28days}$ (hr.ng/mL)	12	9634.02 (2982.29)	9904.13 (4047.72 -13858.58)	30.96
Log $AUC_{0-14days}$	14	8.05 (0.39)	8.15 (7.31–8.63)	4.84
1. Natural log transformation				
2. C_{ss} = $AUC_{days0-28}$/(Tau x 28). Tau = 24hrs. Assume steady state reached by 28 days of administration. Where $AUC_{days0-28}$ was unable to be calculated,				
$AUC_{days0-14}$ was utilised.				
3. SD = Standard deviation, CV = Coefficient of variability = 100%x[SD]/[Mean]				

Parameter	N	Mean (SD)[3]	Median (Range)	CV (%)
SU cycle 1				
		9.12 (0.36)	9.19 (8.31–9.54)	
$LogAUC_{0-28days}$	12			3.95
C_{ss} (ng/mL)	14	13.87 (4.57)	14.09 (6.02–20.62)	32.95
C_{Trough} (ng/mL)	12	18.75 (5.32)	19.40 (10.10–27.90)	28.37
Su12662 cycle 2				
$AUC_{0-14days}$ (hr.ng/mL)	2	3367.74 (1467.39)	3367.74 (2330.14–4405.34)	43.57
$AUC_{0-28days}$ (hr.ng/mL)	2	8147.14 (3341.29)	8147.14 (5784.49 -10509.79)	41.01
Log $AUC_{0-14days}$	2	8.07 (0.450)	8.07 (7.75–8.39)	5.58
$LogAUC_{0-28days}$	2	8.96 (0.42)	8.96 (8.66–9.26)	4.69
C_{ss} (ng/mL)	2	12.12 (4.97)	12.12 (8.61–15.64)	41.01
C_{Trough} (ng/mL)	9	18.88 (6.26)	18.60 (10.00-30.10)	33.16
Total SU cycle 1				

1. Natural log transformation

2. $C_{ss} = AUC_{days0-28}/(Tau \times 28)$. Tau = 24hrs. Assume steady state reached by 28 days of administration. Where $AUC_{days0-28}$ was unable to be calculated,

$AUC_{days0-14}$ was utilised.

3. SD = Standard deviation, CV = Coefficient of variability = 100%x[SD]/[Mean]

Parameter	N	Mean (SD)[3]	Median (Range)	CV (%)
SU cycle 1				
Log $AUC_{0-14days}$	14	9.55 (0.29)	9.57 (9.13–10.10)	3.03
$LogAUC_{0-28days}$	11	10.66 (0.27)	10.72 (10.07–11.09)	2.5
C_{ss} (ng/mL)	13	63.21 (18.28)	67.15 (28.03–97.68)	28.92
C_{Trough} (ng/mL)	12	81.29 (20.68)	80.40 (53.50 -131.90)	25.44
Total SU cycle 2				
Log $AUC_{0-14days}$	2	9.51 (0.50)	9.51 (9.16–9.87)	5.26
$LogAUC_{0-28days}$	2	10.61 (0.41)	10.61 (10.32–10.90)	3.86
C_{ss} (ng/mL)	2	62.99 (25.14)	62.99 (45.21–80.76)	39.91
C_{Trough} (ng/mL)	9	82.86 (22.67)	91.20 (45.00 -111.50)	27.36
1. Natural log transformation				
2. C_{ss} = $AUC_{days0-28}$/(Tau x 28). Tau = 24hrs. Assume steady state reached by 28 days of administration. Where $AUC_{days0-28}$ was unable to be calculated,				
$AUC_{days0-14}$ was utilised.				
3. SD = Standard deviation, CV = Coefficient of variability = 100%x[SD]/[Mean]				

The PK parameters (non-log transformed) demonstrated marked interpatient variability (as expressed by CV%), ranging from 28–49%, as per prior reports.[3, 20, 26, 25]

Overall Response

The responses are summarised in **Supplementary Table 3**. In the RCC cohort (N = 10, 2 not reported): 3 had partial response, 3 stable disease, with a disease control rate of 63% (95% CI 24%-91%). In the GIST cohort, 2 patients with stable disease: disease control rate of 40% (5%-85%).

The Associations Between ^{99m}Tc-MIBI HNI Parameters, PGx with Sunitinib PK (Cycle 1).

The associations, strongest 2 in either direction ($P < 0.05$), between Sunitinib (SU, Su12262 and Total SU) PK in cycle 1 with HNI parameters and PGx, are summarised in Table 4. **Supplementary Table 4** summarises the complete Sanger and DMET variant (per allele analysis) associations ($P < 0.05$).

Table 4

Associations (P < 0.05) of baseline variables, HNI, and pharmacogenomics (the two strongest gene variants [Sanger and DMET] in either direction) with cycle 1 Sunitinib parent (SU), metabolite (Su12662) and Total SU (parent + metabolite) PK. Note: The complete PGx association set is summarised in Supplementary Table 4.

Pharmacogenomics	**Parameter**	**N**	**Estimate (Std Err)**[1]	**P Value**
SU log$AUC_{0-14days}$[2]				
HNI	HEF	14	-0.01 (0.00)	0.05
Pharmacogenomics	**Enzymes, (dbSNP accession rs nos.)**			
Sanger	TNFa-857C > T (rs1799724)[3]	14	. .	0.04
	TNFa-857C > T (rs1799724)[4]	14	0.34 (0.11)	0.009
DMET[5]	ATP7B_c.2973G > A(T991T) (rs1801246)	14	0.61 (0.26)	0.04
	SLC16A1_c.*145T > G (rs11585690)	13	0.58 (0.25)	0.04
	CYP2D6_-1584C > G (rs1080985)	14	-0.32 (0.14)	0.04
	UGT8_c.677C > T(P226L) (rs4148254)	14	-0.44 (0.19)	0.04
SU log$AUC_{0-28days}$				
Patient factors	ALT	11	-0.01 (0.01)	0.05
HNI	$T_{d1/2}$	11	-0.11 (0.04)	0.02
Pharmacogenomics	**Enzymes, (dbSNP accession rs nos.)**			
Sanger	TNFa − 238G > A (rs361525) [3]	11	.	0.004
DMET[5]	SLC15A1_c.1527C > T(N50) (rs8187832)	11	0.60 (0.24)[3]	0.03

Pharmacogenomics	Parameter	N	Estimate (Std Err)[1]	P Value
SU log$AUC_{0-14days}$[2]				
	SLC15A1_c.1352C > A(T45) (rs8187838)	11	0.60 (0.24)	0.03
	SLC10A2_c.511G > T(A171) (rs188096)	11	-0.59 (0.24)	0.04
	CYP2B6_12740G > C(P72P) (rs2279341)	11	-0.59 (0.24)	0.04
SU C_{ss}				
HNI	$T_{d1/2}$ (min)	13	-6.93 (1.91)	0.04
Pharmacogenomics	**Enzymes, (dbSNP accession rs nos.)**			
Sanger	VEFGR3 (FLT4) 1501A > G (rs307826)[3]	13		0.007
	TNFa − 308G > A (rs1800629)[3]	13		0.01
DMET[5]	SLC15A1_c.1527C > T(N50) (rs8187832)	13	39.41 (12.60)	0.01
	SLC15A1_c.1352C > A(T45) (rs8187838)	13	39.41 (12.60)	0.01
SU C_{trough}				
Pharmacogenomics	**Enzymes, (dbSNP accession rs nos.)**			
Sanger	TNFa − 857C > T (rs1799724)[3]	12		0.02
	TNFa − 857C > T (rs1799724)[4]	12	17.9 (7.03)	0.03

Pharmacogenomics	Parameter	N	Estimate (Std Err)[1]	P Value	
SU $logAUC_{0-14days}$[2]					
DMET[5]	SLC25A27_c.298 + 766C > G (rs9369629)	11	43.41 (11.78)	0.005	
	ATP7B_c.2973G > A(T991T) (rs1801246)	12	45.23 (12.78)	0.005	
Su12662 $logAUC_{0-14days}$					
Patient factors		WCCs	14	-0.06 (0.03)	0.04
		Neutrophils	14	-0.07 (0.03)	0.02
HNI		HEF	14	-0.01 (0.00)	0.05
Pharmacogenomics		**Enzymes, (dbSNP accession rs nos.)**			
Sanger		VEFGR3 (1501A > G) (rs307826)[4]	14	-0.34 (0.14)	0.04
DMET[5]		ABCC3_c.3890G > A(R1297) (rs11568591)	14	0.52 (0.22)	0.04
		PPARD_c.-101-1463A > C (rs1003973)	14	0.52 (0.16)	0.01
		SLC10A2_c.*315G > T (rs279941)	14	-0.80 (0.36)	0.05
		SLC10A2_c.511G > T(A171 (rs188096)	14	-0.80 (0.36)	0.05
Su12662 $logAUC_{0-28days}$					
Patient factors		WCCs	11	-0.06 (0.02)	0.01
		Neutrophils	11	-0.07 (0.02)	0.009

Pharmacogenomics	Parameter	N	Estimate (Std Err)[1]	P Value	
SU $logAUC_{0-14days}$[2]					
Pharmacogenomics		Enzymes, (dbSNP accession rs nos.)			
Sanger		HNF1A (79A > C) (rs1169288)[3]	11	. .	0.04
		RET (2251G > A) (rs1799939) [3]	11	. .	0.03
DMET[5]		SULT1A2_c.704A > C(N235) rs1059491	9	0.39 (0.049)	< .0001
		AOX1_T > C(rs7563682) (rs7563682)	12	0.33 (0.14)	0.04
		SLC10A2_c.511G > T(A171) (rs188096)	12	-0.89 (0.28)	0.01
		CYP2B6_12740G > C(P72P) (rs2279341)	12	-0.89 (0.28)	0.01
Su12662 C_{ss}					
HNI		$T_{d1/2}$	13	-1.40 (0.53)	0.02
Pharmacogenomics		Enzymes (dbSNP accession rs nos.)			
DMET[5]		UGT8_c.677C > T(P226L) (rs4148254)	14	-8.72 (2.62)	0.006
Su12662 C_{trough}					
Pharmacogenomics		Enzymes (dbSNP accession rs nos.)			
Sanger		VEFGR3 (FLT4) 1501A > G (rs307826)[4]	12	-4.68 (2.01)	0.04
DMET[5]		SLC28A3_c.267G > A(T89T) (rs7867504)	10	7.23 (1.62)	0.0021

Pharmacogenomics	Parameter	N	Estimate (Std Err)[1]	P Value	
SU $logAUC_{0-14days}$[2]					
		CYP7A1_-1744G > A (rs12542233)	12	5.95 (1.67)	0.0052
		SLC22A2_c.390G > T(T130) (rs624249)	12	-6.19 (1.68)	0.0042
		UGT2B4_c.*225T > C (rs1966151)	12	-6.52 (1.40)	0.0009
Total SU $logAUC_{0-14days}$					
HNI		$T_{d1/2}$	14	-0.08 (0.04)	0.04
		HEF	14	-0.01 (0.00)	0.03
Pharmacogenomics		**Enzymes (dbSNP accession rs nos.)**			
Sanger		TNFa − 857C > T (rs1799724)[4]	14	0.33 (0.11)	0.01
DMET[5]		CYP4F2_11602C > T (rs2074900)	14	0.26 (0.08)	0.01
Total SU $logAUC_{0-28days}$					
Patient factors		ALT	11	-0.1	0.02
HNI		$Td_{1/2}$	11	-0.10 (0.02)	0.002
Pharmacogenomics		**Enzymes (dbSNP accession rs nos.)**			
Sanger		HNF1A 79A > C (rs1169288)[3]	11		0.04
		TNFa − 238G > A rs361525[3]	11		0.04
DMET[5]		MAOA_c.1410C > T (rs1137070)	3	-0.03 (0.00)	0.004
		SLCO3A1_c.1513-1102G> (rs2283458)	11	-0.39 (0.11)	0.005

Pharmacogenomics	Parameter	N	Estimate (Std Err)[1]	P Value	
SU log$AUC_{0-14days}$[2]					
Total SU C_{ss}					
HNI		$Td_{1/2}$	13	-5.85 (2.02)	0.01
Pharmacogenomics		**Enzymes (dbSNP accession rs nos.)**			
Sanger		VEFGR3 (FLT4) 1501A > G rs307826[3]	13		0.01
		TNFa − 238G > A (rs361525)[3]	13		0.03
DMET[5]		UGT8_c.677C > T(P226L) (rs4148254)	13	-37.43 (9.39)	0.0021
		ABCC3_c.4509A > G(E1503) (rs1051640)	13	-26.38 (7.43)	0.0046
		ABCC5_c.*1243G > A (rs562)	11	15.54 (4.2)	0.0074
Total SU C_{trough}					
Pharmacogenomics		**Enzymes (dbSNP accession rs nos.)**			
Sanger		TNFa − 857C > T (rs1799724)[4]	12	21.77 (7.69)	0.02
DMET[5]		SLC16A1_c.*145T > G (rs11585690)	11	52.89 (12.70)	0.0024
		SLC22A14_4739C > T (rs6792261)	11	52.89 (12.70)	0.0024

1. Estimate: If > 0, as HNI parameter, patient demographic increases or minor allele presence then PK variable increases.

If ≤ 0 then as HNI parameter, patient demographic increases or minor allele presence the PK variable decreases.

2. Natural log transformation

Pharmacogenomics	Parameter	N	Estimate (Std Err)[1]	P Value
SU $logAUC_{0-14days}$[2]				
3. For patients with one copy of the mutant allele compared to those with no copies of the mutant allele (heterozygote versus wild type, respectively).				
4. Per mutant allele				
5. Per allele analysis				

Correlations ($P < 0.05$) identified in terms of HNI included: (i) an increase in HEF with a decrease in the $logAUC_{(0-14days)}$, for SU, Su12262 and Total SU, respectively and (ii) an increase in $T_{d1/2}$ with a decrease in (a) SU $logAUC_{(0-28days)}$, and C_{ss}, (b) Su12662 $C_{ss,}$ and (c) Total SU $logAUC_{0-14days}$, $logAUC_{0-28days}$ and $C_{ss,}$ respectively

In terms of PGx from the Sanger sequencing, several variants correlated with PK for example: (i) TNFα (rs1799724) with SU $logAUC_{(0-14days)}$, and SU C_{trough}, (ii) VEGFR3 (FLT4, rs307826) with Su12262 $logAUC_{(0-14days)}$, (iii) TNFα (rs361525) with SU $logAUC_{(0-28days)}$ and Su12262 $logAUC_{(0-28\ days)}$, (iv) TNFα -857C > T (rs1799724) with Total SU C_{trough}, respectively.(Table 4)

In terms of the DMET Array genes, several variants correlated in a positive manner with drug PK, (Table 4) including: (i) ATP7B (rs1801246) with SU $logAUC_{(0-14days)}$, (ii) SLC15A1 (rs8187832) and SU $logAUC_{(0-28days)}$, (iii) ATP7B (rs1801246) with SU C_{trough} (iv) SLC15A1 (rs8187832) with SU C_{ss} and (v) SLC16A1 (rs11585690) with Total SU C_{trough}, respectively.

Negative correlations between DMET Array gene variants and drug PK, included (i) (ii) UGT8 (rs4148254) with Total SU C_{ss} and Su12662 C_{ss} (iii) UGT2B4 (rs1966151) with Su12662 C_{trough}, respectively.

Associations with TreatmentRelated Toxicity

As all patients experienced an AE of grade 1 or higher toxicity (**Supplementary Table 2**), we were unable to identify predictors for this category. The predictors for therapy-induced diarrhea G1+ (4 patients Grade 1/2 [27%] and no G3/G4) are summarized in Table 5, (**Supplementary Table 5** details all the relevant PGx variants). Elevated serum ALT and AST and HNI HEF were associated with increased diarrhea (Odds ratio [OR] > 1.0 resp, $P < 0.05$). In terms of gene variants, correlations with increased toxicity, included VEGFR3 (rs307826), SLC7A8 (rs2236135), ABCC3 (rs1051640), (OR > 10, $P < 0.05$, respectively). In terms of the decreased risk of Grade 1 + diarrhea, correlates included CDA (rs1048977) and CYP51A1 (rs7797834), (OR < 1.0, $P < 0.05$), as well as increased drug exposure.

Table 5

Associations (P < 0.05) of baseline variables, HNI, pharmacokinetics and pharmacogenomics (the five strongest gene variants [Sanger and DMET] in either direction) with G1 + treatment-related diarrhea. Note: The complete PGx association set is summarised in Supplementary Table 5.

Parameter		N	Odds Ratio (95% CI)[2]	P Value
Patient demographics	ALT	15	1.09 (1.00-1.27)	0.02
	AST	14	1.13 (0.99–1.35)	0.05
HNI parameters	HEF	15	1.08 (1.00- .22)	0.03
Su Cycle 1 PK	C_{ss}	13	0.84 (0.63–0.98)	0.007
	C_{trough}	12	0.77 (0.45–0.98)	0.05
Su12662 Cycle 1 PK	Log $AUC_{0-14days}$[1]	14	0.03 (0.00-1.08)	0.05
	Log $AUC_{0-28days}$	12	0.03 (0.00-1.87)	0.05
	C_{ss}	14	0.72 (0.46–0.99)	0.03
Total SU Cycle 1 PK	Log $AUC_{0-28days}$	11	0.00 (0.00-1.55)	0.04
	C_{ss}	13	0.84 (0.47–0.98)	0.007
	C_{trough}	12	0.81 (0.46–0.98)	0.04
Pharmacogenomics	**Enzymes, (dbSNP accession rs nos.)**	**N**	**Odds Ratio (95% CI)**	**P Value**
Sanger	VEFGR3 (FLT4), 1501A > G, (rs307826)[3]	15	21.53 (2.87-I)	0.004
DMET[4]	SLC7A8_c.*665T > C (rs2236135)	15	19.965 (0.871->999.999)	0.033
	ABCC3_c.4509A > G(E1503 (rs1051640)	15	14.622 (2.039->999.999)	0.011

1. Natural log transformation

2. OR: Odds Ratio. If > 1.0, as the parameter increases or minor allele presence, greater risk of toxicity. If ≤ 1.0 then as parameter increases or minor allele presence then lesser risk of toxicity.

3. For patients with one copy of the mutant allele compared to those with no copies of the mutant allele (heterozygote versus wild type, respectively).

4. DMET Plus Array chip: per allele analysis

Parameter		N	Odds Ratio (95% CI)[2]	P Value
	CDA_c.435C > T(T145T) (rs1048977)	15	0.072 (< 0.001–0.947)	0.0425
	AKAP9_c.11546 + 58T > A (rs7785971)	15	0.065 (< 0.001–0.988)	0.03
	CYP51A1_c.1359T > C(H45 (rs7797834)	15	0.065 (< 0.001–0.988)	0.03
1. Natural log transformation				
2. OR: Odds Ratio. If > 1.0, as the parameter increases or minor allele presence, greater risk of toxicity. If ≤ 1.0 then as parameter increases or minor allele presence then lesser risk of toxicity.				
3. For patients with one copy of the mutant allele compared to those with no copies of the mutant allele (heterozygote versus wild type, respectively).				
4. DMET Plus Array chip: per allele analysis				

In terms of grade 3 + toxicities (**Supplementary Table 5**), CBR1 (rs998383), ARSA (rs743616), and RALBP1 (rs12680) all demonstrated strong correlations with increased toxicity (OR > 10, P < 0.05). No correlations with HNI and PK were observed.

Correlations with Overall Response and Progression Free Survival

As per above, the RCC and GIST populations were amalgamated to identify hypothesis generating correlations for response and progression-free survival (**Supplementary Tables 6 and 7**, respectively). In terms of the objective response, several DMET genes correlates were identified. The strongest positive correlations included CHST5 (rs2641806) and GSTA2 (rs2180314), (OR 9.7, P < 0.05). The strongest negative correlations were SULT1E1 (rs1881668) and SLC13A1 (rs2204295), (OR 0.057 and 0.123, respectively, P < 0.05). In terms of Disease Control Rate (DCR) several DMET correlates were also identified.

Correlates with reduced risk of progression included (**Supplementary Table 7**): PDGFRα (rs1800810) (HR < 0.04, P < 0.05), and CYP4Z1 (rs4926802) (HR = 0, P < 0.05). Increased risk was correlated with HNF1A (rs1169288) (HR = 3.65) and CYP2A6 (rs28399442) (P < 0.05, respectively).

Multivariate Models for the Prediction of Sunitinib Cycle 1 PK and PD

In terms of PK, a multi-parameter model was able to predict: (i) SU $AUC_{(0-14days)}$ with HEF and TNFα (rs1799724), being independent predictors (model P = 0.02), (ii) Su12262 $AUC_{(0-14days)}$ with $T_{d1/2}$ and VEFGR3 (rs307826-per allele) (model P = 0.009), and (iii) Su12262 $AUC_{(0-28days)}$ with $T_{d1/2}$ and RET

(rs1799939- per allele) (Model P = 0.003). Due to the limited events, multivariate models were not generated for toxicity or PFS.

DISCUSSION

The study reported here, represents the first attempt to correlate the combination of metabolic end-organ phenotyping (^{99m}Tc-MIBI scans) and extensive metabolic/excretory/PD PGx with Sunitinib PK, toxicity and efficacy. The patient cohort was of limited size due to recruitment and regulatory environment issues. Nevertheless, the treatment compliance, toxicity profile,[5, 27] and PK parameters,[10, 19, 20] were as expected.

The pretherapy derived ^{99m}Tc-MIBI parameters (Table 2) in this study were similar to those in our initial trial, with analogous wide interpatient variability.[21] In another cohort, the ^{99m}Tc-MIBI scan elimination coefficient (kH) showed a 12-fold interpatient variation: not correlated with patient hepatic biochemistries, or hepatic metastasis.[28] The MIBI kH has been correlated with vinorelbine clearance (P = 0.01),[29] and the MIBI 1hrRET with SN38 AUC in irinotecan-treated patients.[21]

The DMET-Plus-Array system has examined pharmacogenomic-PK/PD correlates for several anticancer agents: frequently generating novel genomic associations, often in the absence of direct mechanistic linkages.[22, 30, 31]. For example, in paclitaxel-treated patients, a multivariate analysis revealed that SNPs in VKORC1, SLC22A14, and DCK were associated with paclitaxel toxicities, quite distinct to other reports.[32] In docetaxel-treated patients, 28 SNPs were associated with its AUC (P < 0.05), but only the CYP39A1 (rs7761731) variant with grade 4 neutropenia (P = 0.049).[30]

The Sunitinib PK correlates observed from the study reported here included (Table 4): (i) an increase in HEF with a decrease in the log$AUC_{(0-14days)}$, for SU, Su12262 and Total SU, respectively (iii) an increase in $T_{d1/2}$ with a decrease in (a) SU log$AUC_{(0-28days),}$ and C_{ss}, (b) Su12662 $C_{ss,}$ and (c) Total SU log$AUC_{0-14days}$, log$AUC_{0-28days}$ and $C_{ss,}$ respectively. Only one other study has evaluated such phenotypic probes in Sunitinib-treated patients: whereby patients underwent both the hepatic ^{99m}Tc-MIBI scan and the midazolam CL test.[20] No significant correlation was observed between MIBI kH with SU, metabolite or total C_{trough} levels.[20] Similarly, in the study reported here, no correlation was found between the HNI parameters and C_{trough} values.

Several gene variants in this study correlated with Sunitinib PK. For the PD genes, in particular variants of TNFα and VEGF3 HNF1A (P < 0.05), (Table 4 **and Supplementary Table 4**). Positive correlations for the DMET genes included variants in the transporters (i.e., SLC15A1, SLC22A2, SLC16A1) and phase II enzymes (SULT1A2). Whilst negative correlations were observed for variants in UGT-8 and – 2B4 as well as ABCC3. The prior reported Sunitinb PGx studies have been characterised by variable sized cohorts and inconsistent findings. In one multicentre study (N = 114 RCC patients), of the gene variants investigated, only CYP3A4*22 was an independent negative factor for SU CL.[16] Another study analyzed the impact of 14 common SNPs in the CYP3A4/5, NR1-I2 and -I3, ABCB1 and –G2 genes on Sunitinib drug exposure:

ABCG2 (421C > A) (P = 0.014) was the only parameter accounting for total drug exposure.[33] The impact of the common ABCB1 SNPs (1236C > T, 2677G > T/A and 3435C > T) had also been inconsistent.[34]

With regards to Sunitinib toxicity, in the study reported here there was a negative correlation between Grade 1 + diarrhea with drug exposure ($P \leq 0.05$, $OR \leq 1.0$), (Table 5): most likely reflecting the small cohort size and low frequency of the toxicities. In contrast, several reports had observed positive correlations between PK and toxicity.[8, 10, 35]. From the report here, other predictors of increased therapy-induced diarrhea G1 + included elevated serum ALT and AST and MIBI HEF (OR > 1.08 resp, P < 0.05). Several PK/PD SNPs also demonstrated correlations with increased Grade 1 + diarrhea and Grade 3 + toxicities (OR > 10, P < 0.05). (Table 5, **Supplementary Table 5**),

There have been multiple studies correlating PGx with Sunitinib toxicity, focusing mainly on the ABC membrane transporters, CYP-450 subfamilies and a few PD genes.[14, 15, 36, 37] They have been mostly in RCC, varying markedly in terms of sample size, ethnicity, and variants assessed. One meta-analysis evaluated ABCG2 and -B1 SNPs with sunitinib toxicity and efficacy in 1081 RCC patients,[36]: ABCG2 (rs2231142) was associated with increased thrombocytopenia and hand-foot syndrome in Asians (P = 0.006), whilst ABCB1 (rs1128503) with a reduced risk of hypertension.[36] Another study (N = 219), evaluated 31 SNPs in 12 candidate genes, finding an increased risk of: (i) leucopenia with CYP1A1 (2455 G > A), FLT3 (738T > C) or CAG in the NR1I3 (5719C/T, 7738A/C, 7837T/G) haplotype, (ii) Grade 2 + toxicity with VEGFR2 (1191 T > C) or a copy of TT in the ABCG2 (-15622C/T, 1143C/T) haplotype.[14] As noted above, these correlations have not been consistent.[15, 18, 33]

In the study reported here, the RCC and GIST populations were merged to evaluate hypothesis generating correlates with response (ORR and DCR) and PFS, (**Supplementary Tables 6 and 7**). In terms of response these included membrane transporters (SLC13A1, SCLO5A1, ABCB4, ABCG1), phase II enzymes (SULT1E1) and PD genes (HNF1A79A > C), (P < 0.05). Several SNP correlates were also identified for the risk of disease progression (P < 0.05).

Several studies had reported that increased PK exposure parameters,[33, 35] had been associated with overall survival,[33] time to progression,[35] and PFS.[8, 10] Similarly several reports correlated PK/PD PGx with Sunitinib efficacy: albeit of variable size and genotypes assessed.[17, 18, 36, 38, 39] A meta-analysis in RCC patients observed that the common variants in ABCB1 (rs1128503 and rs2032582) were both correlated with worse PFS (P = 0.011 and = 0.003, respectively), but not OS.[36] Significant correlations had also been found for (i) PFS with variants in FGFR2 and NR1/2,[40] NR1I3,[17] and VEGFR3,[15, 41] (ii) OS with NR1/3, VEGFR3,[40, 41] VEGFR2, VEGFR4,[38] and VEGFA,[41]. Nevertheless, as noted above, these correlations had not been confirmed by others.[39]

There are several limitations to the study reported here. The data here was generated from a small cohort (N = 15) treated with the Sunitinib 4/2 schedule. The limited sample size and the low proportion of patients with significant toxicity reduced the ability to define the impact of low frequency gene variants. Hence, together with the extensive PGx evaluated, the data analysis can potentially lead to the generation of type I errors. These associations hence require further validation in larger cohorts allowing for the

increased incidence of different genotypes. It is therefore appropriate to consider the identified significant HNI and PGx covariates, as exploratory instead of conclusive.

One alternative approach is the use of therapeutic drug monitoring (TDM) to individualize dosing. There is sufficient evidence confirming the concentration-efficacy and concentration-toxicity relationships in the indications of GIST and RCC.[42] TDM approaches have included sampling for C_{trough},[43] or after the T_{max} with extrapolation to trough levels.[44] Other approaches have included toxicity-adjusted dosing in combination with TDM.[45]

In conclusion, the study reported here represents the first attempt to correlate the combination of end organ phenotyping (^{99m}Tc-MIBI scans) and extensive metabolic/excretory/PD pharmacogenomics (DMET Array and Sanger sequencing) with Sunitinib parent/metabolite PK, toxicity, and efficacy. Exploratory correlations were observed that require further validation.

Declarations

Author contributions

Wrote Manuscript: M Michael, GC Toner, V Ganju, CS Karapetis, M Burge, E Link, RJ Hicks, A Matera, M Thompson, I Campbell, S Rowley, DW Pook,

Performed Research: M Michael, GC Toner, V Ganju, CS Karapetis, M Burge, E Link, RJ Hicks, A Matera, M Thompson, I Campbell, S Rowley, DW Pook,

Analysed Data, and Contributed Analytical Tools: M Michael, E Link, M Thompson, RJ Hicks, IG Campbell, S Rowley.

Conflicts of interest: All contributing authors have no competing financial

interests in relation to the work described.

Ethics approval: The study was approved by the Peter MacCallum Cancer Centre Ethics Committee on the 3rd Sept 2010, followed shortly after the Institutional Ethics Committees at all other study sites.

This study was carried out in compliance with the protocol and with adherence to Good Clinical Practice, as described in the following documents:

ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996. Directive 91/507/EEC,

Rules Governing Medicinal Products in the European Community.

Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Patients), Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000, Washington 2002; Appendix 11

Funding Information

Funded by the Australian National Health and Medical Research Council Grant 628564.

Research Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable reques

References

1. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, Murray LJ, Carver J, Chan E, Moss KG, Haznedar JO, Sukbuntherng J, Blake RA, Sun L, Tang C, Miller T, Shirazian S, McMahon G, Cherrington JM (2003) 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. Clin Cancer Res 9 (1):327–337
2. Hu S, Chen Z, Franke R, Orwick S, Zhao M, Rudek MA, Sparreboom A, Baker SD (2009) Interaction of the multikinase inhibitors sorafenib and sunitinib with solute carriers and ATP-binding cassette transporters. Clin Cancer Res 15 (19):6062–6069. doi:1078-0432.CCR-09-0048 [pii] 10.1158/1078 –0432.CCR-09-0048
3. Lankheet NA, Kloth JS, Gadellaa-van Hooijdonk CG, Cirkel GA, Mathijssen RH, Lolkema MP, Schellens JH, Voest EE, Sleijfer S, de Jonge MJ, Haanen JB, Beijnen JH, Huitema AD, Steeghs N (2014) Pharmacokinetically guided sunitinib dosing: a feasibility study in patients with advanced solid tumours. Br J Cancer 110 (10):2441–2449. doi:10.1038/bjc.2014.194
4. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, Li JZ, Bello CL, Theuer CP, George DJ, Rini BI (2006) Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol 24 (1):16–24
5. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG (2006) Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 368 (9544):1329–1338. doi:10.1016/S0140-6736(06)69446-4
6. Gore ME, Szczylik C, Porta C, Bracarda S, Bjarnason GA, Oudard S, Hariharan S, Lee SH, Haanen J, Castellano D, Vrdoljak E, Schoffski P, Mainwaring P, Nieto A, Yuan J, Bukowski R (2009) Safety and efficacy of sunitinib for metastatic renal-cell carcinoma: an expanded-access trial. Lancet Oncol 10 (8):757–763. doi:10.1016/S1470-2045(09)70162-7
7. Kang HJ, Lee S (2020) Tolerability of Alternative Dosing Schedules for Sunitinib: A Systematic Review and Meta-Analysis. Yonsei Med J 61 (10):837–843. doi:10.3349/ymj.2020.61.10.837

8. Noda S, Otsuji T, Baba M, Yoshida T, Kageyama S, Okamoto K, Okada Y, Kawauchi A, Onishi H, Hira D, Morita SY, Terada T (2015) Assessment of Sunitinib-Induced Toxicities and Clinical Outcomes Based on Therapeutic Drug Monitoring of Sunitinib for Patients With Renal Cell Carcinoma. Clin Genitourin Cancer 13 (4):350–358. doi:10.1016/j.clgc.2015.01.007
9. Ravaud A, Bello CL (2011) Exposure-response relationships in patients with metastatic renal cell carcinoma receiving sunitinib: maintaining optimum efficacy in clinical practice. Anticancer Drugs 22 (5):377–383. doi:10.1097/CAD.0b013e3283442039
10. Numakura K, Fujiyama N, Takahashi M, Igarashi R, Tsuruta H, Maeno A, Huang M, Saito M, Narita S, Inoue T, Satoh S, Tsuchiya N, Niioka T, Miura M, Habuchi T (2018) Clinical implications of pharmacokinetics of sunitinib malate and N-desethyl-sunitinib plasma concentrations for treatment outcome in metastatic renal cell carcinoma patients. Oncotarget 9 (38):25277–25284. doi:10.18632/oncotarget.25423
11. Teo YL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A (2015) Association of drug exposure with toxicity and clinical response in metastatic renal cell carcinoma patients receiving an attenuated dosing regimen of sunitinib. Target Oncol 10 (3):429–437. doi:10.1007/s11523-014-0349-2
12. Houk B, Bello C, Michaelson M, Bukowski R, Redman B, Hudes G, Wilding G, Motzer R (2007) Exposure-response of sunitinib in metastatic renal cell carcinoma (mRCC): A population pharmacokinetic/pharmacodynamic (PKPD) approach. J Clin Oncol 25, No. 18S (June 20 Supplement):5027 (Abstr)
13. Houk BE, Bello CL, Poland B, Rosen LS, Demetri GD, Motzer RJ (2009) Relationship between exposure to sunitinib and efficacy and tolerability endpoints in patients with cancer: results of a pharmacokinetic/pharmacodynamic meta-analysis. Cancer Chemother Pharmacol. doi:10.1007/s00280-009-1170-y
14. van Erp NP, Eechoute K, van der Veldt AA, Haanen JB, Reyners AK, Mathijssen RH, Boven E, van der Straaten T, Baak-Pablo RF, Wessels JA, Guchelaar HJ, Gelderblom H (2009) Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity. J Clin Oncol 27 (26):4406–4412. doi:JCO.2008.21.7679 [pii] 10.1200/JCO.2008.21.7679
15. Garcia-Donas J, Esteban E, Leandro-Garcia LJ, Castellano DE, Gonzalez del Alba A, Climent MA, Arranz JA, Gallardo E, Puente J, Bellmunt J, Mellado B, Martinez E, Moreno F, Font A, Robledo M, Rodriguez-Antona C (2011) 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 12 (12):1143–1150. doi:10.1016/S1470-2045(11)70266-2
16. Diekstra MH, Klumpen HJ, Lolkema MP, Yu H, Kloth JS, Gelderblom H, van Schaik RH, Gurney H, Swen JJ, Huitema AD, Steeghs N, Mathijssen RH (2014) Association analysis of genetic polymorphisms in genes related to sunitinib pharmacokinetics, specifically clearance of sunitinib and SU12662. Clin Pharmacol Ther 96 (1):81–89. doi:10.1038/clpt.2014.47

17. van der Veldt AA, Eechoute K, Gelderblom H, Gietema J, Guchelaar HJ, van Erp NP, van den Eertwegh AJ, Haanen JB, Mathijssen RH, Wessels JA (2011) Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. Clin Cancer Res 17 (3):620–629. doi:10.1158/1078-0432.CCR-10-1828
18. Diekstra MH, Swen JJ, Boven E, Castellano D, Gelderblom H, Mathijssen RH, Rodriguez-Antona C, Garcia-Donas J, Rini BI, Guchelaar HJ (2015) CYP3A5 and ABCB1 polymorphisms as predictors for sunitinib outcome in metastatic renal cell carcinoma. Eur Urol 68 (4):621–629. doi:10.1016/j.eururo.2015.04.018
19. de Wit D, Gelderblom H, Sparreboom A, den Hartigh J, den Hollander M, Konig-Quartel JM, Hessing T, Guchelaar HJ, van Erp NP (2014) Midazolam as a phenotyping probe to predict sunitinib exposure in patients with cancer. Cancer Chemother Pharmacol 73 (1):87–96. doi:10.1007/s00280-013-2322-7
20. Kloth JS, Klumpen HJ, Yu H, Eechoute K, Samer CF, Kam BL, Huitema AD, Daali Y, Zwinderman AH, Balakrishnar B, Bennink RJ, Wong M, Schellens JH, Mathijssen RH, Gurney H (2014) Predictive value of CYP3A and ABCB1 phenotyping probes for the pharmacokinetics of sunitinib: the ClearSun study. Clin Pharmacokinet 53 (3):261–269. doi:10.1007/s40262-013-0111-4
21. Michael M, Thompson M, Hicks RJ, Mitchell PL, Ellis A, Milner AD, Di Iulio J, Scott AM, Gurtler V, Hoskins JM, Clarke SJ, Tebbut NC, Foo K, Jefford M, Zalcberg JR (2006) Relationship of hepatic functional imaging to irinotecan pharmacokinetics and genetic parameters of drug elimination. J Clin Oncol 24 (26):4228–4235. doi:10.1200/JCO.2005.04.8496
22. Michael M, Liauw W, McLachlan SA, Link E, Matera A, Thompson M, Jefford M, Hicks RJ, Cullinane C, Hatzimihalis A, Campbell IG, Rowley S, Beale PJ, Karapetis CS, Price T, Burge ME (2021) Pharmacogenomics and functional imaging to predict irinotecan pharmacokinetics and pharmacodynamics: the predict IR study. Cancer Chemother Pharmacol 88 (1):39–52. doi:10.1007/s00280-021-04264-8
23. Deeken J (2009) The Affymetrix DMET platform and pharmacogenetics in drug development. Curr Opin Mol Ther 11 (3):260–268
24. Reck M, Frickhofen N, Cedres S, Gatzemeier U, Heigener D, Fuhr HG, Thall A, Lanzalone S, Stephenson P, Ruiz-Garcia A, Chao R, Felip E (2010) Sunitinib in combination with gemcitabine plus cisplatin for advanced non-small cell lung cancer: a phase I dose-escalation study. Lung Cancer 70 (2):180–187. doi:10.1016/j.lungcan.2010.01.016
25. Michaelson MD, Zhu AX, Ryan DP, McDermott DF, Shapiro GI, Tye L, Chen I, Stephenson P, Patyna S, Ruiz-Garcia A, Schwarzberg AB (2013) Sunitinib in combination with gemcitabine for advanced solid tumours: a phase I dose-finding study. Br J Cancer 108 (7):1393–1401. doi:10.1038/bjc.2013.96
26. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, Raymond E (2006) Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 24 (1):25–35. doi:10.1200/JCO.2005.02.2194

27. Motzer RJ, Hoosen S, Bello CL, Christensen JG (2006) Sunitinib malate for the treatment of solid tumours: a review of current clinical data. Expert Opin Investig Drugs 15 (5):553–561. doi:10.1517/13543784.15.5.553
28. Wong M, Evans S, Rivory LP, Hoskins JM, Mann GJ, Farlow D, Clarke CL, Balleine RL, Gurney H (2005) Hepatic technetium Tc 99m-labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer. Clin Pharmacol Ther 77 (1):33–42. doi:10.1016/j.clpt.2004.09.002
29. Wong M, Balleine RL, Blair EY, McLachlan AJ, Ackland SP, Garg MB, Evans S, Farlow D, Collins M, Rivory LP, Hoskins JM, Mann GJ, Clarke CL, Gurney H (2006) Predictors of vinorelbine pharmacokinetics and pharmacodynamics in patients with cancer. J Clin Oncol 24 (16):2448–2455. doi:10.1200/JCO.2005.02.1295
30. Uchiyama T, Kanno H, Ishitani K, Fujii H, Ohta H, Matsui H, Kamatani N, Saito K (2012) An SNP in CYP39A1 is associated with severe neutropenia induced by docetaxel. Cancer Chemother Pharmacol 69 (6):1617–1624. doi:10.1007/s00280-012-1872-4
31. Ravegnini G, Urbini M, Simeon V, Genovese C, Astolfi A, Nannini M, Gatto L, Saponara M, Ianni M, Indio V, Brandi G, Trino S, Hrelia P, Biasco G, Angelini S, Pantaleo MA (2019) An exploratory study by DMET array identifies a germline signature associated with imatinib response in gastrointestinal stromal tumor. Pharmacogenomics J 19 (4):390–400. doi:10.1038/s41397-018-0050-4
32. Sissung TM, English BC, Venzon D, Figg WD, Deeken JF (2010) Clinical pharmacology and pharmacogenetics in a genomics era: the DMET platform. Pharmacogenomics 11 (1):89–103. doi:10.2217/pgs.09.154
33. Narjoz C, Cessot A, Thomas-Schoemann A, Golmard JL, Huillard O, Boudou-Rouquette P, Behouche A, Taieb F, Durand JP, Dauphin A, Coriat R, Vidal M, Tod M, Alexandre J, Loriot MA, Goldwasser F, Blanchet B (2015) Role of the lean body mass and of pharmacogenetic variants on the pharmacokinetics and pharmacodynamics of sunitinib in cancer patients. Invest New Drugs 33 (1):257–268. doi:10.1007/s10637-014-0178-2
34. Zhang Y, Mai H, Guo G, Bi G, Hao G, Li Y, Wang X, Cheng L, Wang J, Dong R, Liu Z, Chen L, Qu H (2018) Association analysis of SNPs present in plasma with adverse events and population pharmacokinetics in Chinese sunitinib treated patients with renal cell carcinoma. Oncotarget 9 (18):14109–14123. doi:10.18632/oncotarget.23881
35. Houk BE, Bello CL, Poland B, Rosen LS, Demetri GD, Motzer RJ (2010) Relationship between exposure to sunitinib and efficacy and tolerability endpoints in patients with cancer: results of a pharmacokinetic/pharmacodynamic meta-analysis. Cancer Chemother Pharmacol 66 (2):357–371. doi:10.1007/s00280-009-1170-y
36. Sun F, Chen Z, Yao P, Weng B, Liu Z, Cheng L (2021) Meta-Analysis of ABCG2 and ABCB1 Polymorphisms With Sunitinib-Induced Toxicity and Efficacy in Renal Cell Carcinoma. Front Pharmacol 12:641075. doi:10.3389/fphar.2021.641075

37. Ravegnini G, Nannini M, Zenesini C, Simeon V, Sammarini G, Urbini M, Gatto L, Saponara M, Biasco G, Pantaleo MA, Venturoli N, Hrelia P, Angelini S (2017) An exploratory association of polymorphisms in angiogenesis-related genes with susceptibility, clinical response and toxicity in gastrointestinal stromal tumors receiving sunitinib after imatinib failure. Angiogenesis 20 (1):139–148. doi:10.1007/s10456-016-9534-5
38. Liu R, Wang X, Li W, Shou T, Zhou L, Li Y, Bai M, Pei Q (2017) Influence of VEGFR single nucleotide polymorphisms on the efficacy of sunitinib therapy against renal cell carcinoma. Oncol Lett 13 (1):201–205. doi:10.3892/ol.2016.5396
39. Liu X, Swen JJ, Boven E, Castellano D, Gelderblom H, Mathijssen RH, Rodriguez-Antona C, Garcia-Donas J, Rini BI, Guchelaar HJ (2017) Meta-analysis on the association of VEGFR1 genetic variants with sunitinib outcome in metastatic renal cell carcinoma patients. Oncotarget 8 (1):1204–1212. doi:10.18632/oncotarget.13597
40. Beuselinck B, Karadimou A, Lambrechts D, Claes B, Wolter P, Couchy G, Berkers J, Paridaens R, Schoffski P, Mejean A, Verkarre V, Lerut E, de la Taille A, Tourani JM, Bigot P, Linassier C, Negrier S, Berger J, Patard JJ, Zucman-Rossi J, Oudard S (2013) Single-nucleotide polymorphisms associated with outcome in metastatic renal cell carcinoma treated with sunitinib. Br J Cancer 108 (4):887–900. doi:10.1038/bjc.2012.548
41. Scartozzi M, Bianconi M, Faloppi L, Loretelli C, Bittoni A, Del Prete M, Giampieri R, Maccaroni E, Nicoletti S, Burattini L, Minardi D, Muzzonigro G, Montironi R, Cascinu S (2013) VEGF and VEGFR polymorphisms affect clinical outcome in advanced renal cell carcinoma patients receiving first-line sunitinib. Br J Cancer 108 (5):1126–1132. doi:10.1038/bjc.2012.501
42. Demlova R, Turjap M, Pes O, Kostolanska K, Jurica J (2020) Therapeutic Drug Monitoring of Sunitinib in Gastrointestinal Stromal Tumors and Metastatic Renal Cell Carcinoma in Adults-A Review. Ther Drug Monit 42 (1):20–32. doi:10.1097/FTD.0000000000000663
43. Crumbaker M, Guminski A, Gurney H, Sabanathan D, Wong S, Pavlakis N (2018) Real-world experience of the feasibility and tolerability of the 2/1 dosing schedule with sunitinib in the treatment of patients with advanced renal cell carcinoma in Australia. Asia Pac J Clin Oncol 14 (2):e45-e49. doi:10.1111/ajco.12686
44. van Eerden RAG, Oomen-de Hoop E, Noordam A, Mathijssen RHJ, Koolen SLW (2021) Feasibility of Extrapolating Randomly Taken Plasma Samples to Trough Levels for Therapeutic Drug Monitoring Purposes of Small Molecule Kinase Inhibitors. Pharmaceuticals (Basel) 14 (2). doi:10.3390/ph14020119
45. Sabanathan D, Zhang A, Fox P, Coulter S, Gebski V, Balakrishnar B, Chan M, Liddle C, Gurney H (2017) Dose individualization of sunitinib in metastatic renal cell cancer: toxicity-adjusted dose or therapeutic drug monitoring. Cancer Chemother Pharmacol 80 (2):385–393. doi:10.1007/s00280-017-3362-1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx