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(54) Title: Method For Predicting Clinical Toxicity And Outcome

(57) Abstract: A method for determining the sensitivity of a patient with cancer to receptor tyrosine kinase inhibitor therapy is provided. The method comprising screening a nucleic acid sample to determine the identity of at least one single nucleotide polymorphism (SNP) genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof; and determining the sensitivity of a patient with renal cancer to receptor tyrosine kinase inhibitor therapy based on the identity of the SNP genotype. The sensitivity is selected from one or more of neutropenia, diarrhea, tumor response, early toxicity-necessitated treatment termination, overall survival and progression-free survival.



METHOD FOR PREDICTING CLINICAL TOXICITY AND OUTCOME**FIELD OF THE INVENTION**

[0001] The present invention relates stem cells. In particular the present invention relates to a method for culturing and maintaining a pluripotent stem cell in an undifferentiated state.

BACKGROUND OF THE INVENTION

[0002] Cancer therapy includes surgery, chemotherapy, targeted therapy, immunotherapy and radiation therapy. Targeted therapy is directed against cancer-specific molecules and signaling pathways within the tumor. Accordingly, drugs used in targeted therapy include drugs that inhibit angiogenesis and drugs that induce apoptosis. An example of a class of drugs used in targeted therapy is tyrosine kinase inhibitors (TKIs).

[0003] However, tumor response and individual survival vary among patients and therefore the therapeutic benefits of TKIs are not universal but individualized. In addition, despite its effectiveness in prolonging survival, life quality-compromising and potentially lethal toxicities are common. These include diarrhea, hand-foot syndrome, mucositis, hypertension, leucopenia, neutropenia and thrombocytopenia. Toxicities lead to dose interruption, dose reduction and prevent patients from benefitting fully from the drug. Toxicity presentation and severity, like survival, are also not universal but vary greatly among patients.

[0004] Therefore, there is a need to provide a method for determining the sensitivity of a patient with cancer to TKI therapy and a need to provide a method of identifying the most appropriate TKI therapy regimen for a patient. There is also a need for a kit to be used in such methods.

SUMMARY OF THE INVENTION

[0005] In a first aspect, there is provided a method for determining the sensitivity of a patient with cancer to receptor tyrosine kinase inhibitor therapy, comprising:

- a) isolating a nucleic acid sample from a biological sample obtained from the said patient;
- b) screening said nucleic acid sample to determine the identity of at least one single nucleotide polymorphism (SNP) genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof; and
- c) determining the sensitivity of a patient with renal cancer to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof,

wherein said sensitivity is selected from one or more of neutropenia, diarrhea, tumor response, early toxicity-necessitated treatment termination, overall survival and progression-free survival.

[0006] In a second aspect, there is provided a use of the method as described herein to identify the most appropriate receptor tyrosine kinase inhibitor therapy regimen for a patient with renal cancer.

[0007] In a third aspect, there is provided a kit for use according to the method as described herein, comprising components for the screening of said nucleic acid sample to determine the identity of at least one SNP genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof.

DEFINITIONS

[0008] The following words and terms used herein shall have the meaning indicated:

[0009] The term subject refers to living, multicellular vertebrate organisms, a category that includes both human and veterinary subjects for example, mammals, birds and primates.

[0010] The term nucleic acid refers to a deoxyribonucleotide or ribonucleotide polymer in either single or double stranded form, and unless otherwise limited, encompassing known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides.

[0011] The term polymorphism refers to a variant in a sequence of a gene, usually carried from one generation to another in a population. Polymorphisms can be those variations (nucleotide sequence differences) that, while having a different nucleotide sequence, produce functionally equivalent gene products, such as those variations generally found between individuals, different ethnic groups, geographic locations. The term polymorphism also encompasses variations that produce gene products with altered function, i.e., variants in the gene sequence that lead to gene products that are not functionally equivalent. This term also encompasses variations that produce no gene product, an inactive gene product, or increased or increased activity gene product.

[0012] Polymorphisms can be referred to, for instance, by the nucleotide position at which the variation exists, by the change in

amino acid sequence caused by the nucleotide variation, or by a change in some other characteristic of the nucleic acid molecule or protein that is linked to the variation (e.g., an alteration of a secondary structure such as a stem-loop, or an alteration of the binding affinity of the nucleic acid for associated molecules, such as polymerases, RNases, and so forth).

[0013] The term Single Nucleotide Polymorphism (SNP) refers to a single base (nucleotide) difference in a specific location in the DNA sequence among individuals in a population. A subset of SNPs give rise to changes in the encoded amino acid sequence; these are referred to as coding SNPs, or cSNPs.

[0014] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0015] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter

from the genus, regardless of whether or not the excised material is specifically recited herein.

[0016] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0018] **Fig. 1** shows (A) Patients grouped according to the ABCB1 3435C/T, 1236C/T, 2677G/T haplotype; median progression-free survival for homozygous carriers of the TTT haplotype was 2.4 months and for other cases was 8.4 months ($P = 0.001$). (B) Patients grouped according to the ABCB1 3435C/T, 1236C/T, 2677G/TA haplotype; median overall survival for homozygous carriers of the TTT haplotype was 4.6 months and for other cases was 19.6 months ($P = 0.005$).

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0019] In a first aspect there is provided a method for determining the sensitivity of a patient with cancer to receptor tyrosine kinase inhibitor therapy. The method may comprise: isolating a nucleic acid sample from a biological sample obtained from the said patient; screening said nucleic acid sample to determine the identity of at least one single nucleotide polymorphism (SNP) genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof; and determining the sensitivity of a patient with renal cancer to receptor tyrosine kinase inhibitor therapy based on the

identity of at least one SNP genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof, wherein said sensitivity is selected from one or more of neutropenia, diarrhea, tumor response, early toxicity-necessitated treatment termination, overall survival and progression-free survival.

[0020] In one embodiment, the cancer may be selected from the group consisting of renal cancer, neuroendocrine cancer, breast cancer, prostate cancer, cervical cancer, ovarian cancer, gastric cancer, colorectal cancer, pancreatic cancer, liver cancer, brain cancer, lung cancer, hematological malignancies, melanoma and sarcomas.

[0021] In another embodiment, the renal cancer is metastatic renal cell carcinoma (mRCC). In some embodiments the patient may suffer from mRCC. Alternatively, the patient suffers from mRCC and undergoes receptor tyrosine kinase inhibitor treatment.

[0022] In some embodiments, the biological sample is obtained from the group consisting of frozen tissue, tissue biopsies, circulating cells, bodily fluids and cheek swab.

[0023] The biological sample may be non-neoplastic or neoplastic.

[0024] In one embodiment the bodily fluids may be selected from the group consisting of blood, saliva, ascites, effusions and urine.

[0025] The receptor tyrosine kinase inhibitor may be a multikinase inhibitor for receptor tyrosine kinase including but not limited to sunitinib, pazopanib, axitinib, sorafenib, regorafenib, tivozanitinib or combinations thereof.

[0026] In some embodiments the patient is a mammal or a human. The human may be an ethnic Asian, or an ethnic Caucasian, or an ethnic African.

[0027] In another embodiment, for determining the risk of diarrhea during receptor tyrosine kinase inhibitor treatment, the method may further comprise: (a) screening for at least one SNP genotype selected from the group consisting of rs1128503 and rs1045642, and (b) determining the risk of diarrhea based on the screening result

obtained in step (a). In some embodiments, in step (b), with respect to rs1128503, the risk of diarrhea during receptor tyrosine kinase inhibitor treatment may be higher for a CC genotype than a CT or TT genotype. In some embodiments, in step (b), with respect to rs1045642, the risk of diarrhea during receptor tyrosine kinase inhibitor treatment may be higher for a CC genotype than a CT or TT genotype.

[0028] In some embodiments, for determining the risk of neutropenia during receptor tyrosine kinase inhibitor treatment, the method may further comprise: (a) screening for at least one SNP genotype selected from the group consisting of rs1933437, rs2032582, rs1045642, rs1128503 and rs2231142, and (b) determining the risk of neutropenia based on the screening result obtained in step (a). In step (b), with respect to rs1933437, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment may be higher for a TT genotype than a CC or CT genotype. In step (b), with respect to rs2032582, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment may be higher for a AA or AG or GG genotype than a AT or GT or TT genotype. In step (b), with respect to rs1045642, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment may be higher for a CC or CT genotype than a TT genotype. In step (b), with respect to rs1128503, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment may be higher for a CC or CT genotype than a TT genotype. In step (b), with respect to rs2231142, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment may be higher for a CC or AC genotype than a AA genotype. In step (b), with respect to the three SNPs including rs1045642, rs2032582 and rs1128503, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment may be higher for allele combinations other than T allele homozygosity at all of the three SNP sites than for a TTT/TTT genotype.

[0029] In some embodiments, for determining the risk of early toxicity-necessitated treatment termination during receptor

tyrosine kinase inhibitor treatment, the method may further comprise: (a) screening for the SNP genotype of rs1128503, and (b) determining the risk of early toxicity-necessitated treatment termination based on the genotyping result obtained in step (a).

[0030] In step (b), with respect to rs1128503, the risk of early toxicity-necessitated treatment termination during receptor tyrosine kinase inhibitor treatment may be higher for a CC or CT genotype than a TT genotype.

[0031] In some embodiments, for determining the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment, the method may further comprise: (a) screening for at least one SNP genotype selected from the group consisting of rs1045642, rs2032582 and rs1128503, and (b) determining the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment based on the genotyping result obtained in step (a).

[0032] In step (b), with respect to rs1045642, the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment may be higher for a TT genotype than a CC or CT genotype.

[0033] In step (b), with respect to rs2032582, the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment may be higher for a TT genotype than a AA or AG or GG or AT or GT genotype.

[0034] In step (b), with respect to the three SNPs including rs1045642, rs2032582 and rs1128503, the risk of primary tumour resistance to receptor tyrosine kinase inhibitor therapy may be higher for a TTT/TTT genotype than for allele combinations other than T allele homozygosity at all of the three SNP sites.

[0035] In some embodiments, for determining overall survival, during receptor tyrosine kinase inhibitor treatment, the method may further comprise: (a) screening for at least one SNP genotype selected from the group consisting of rs1045642, rs2032582 and rs1128503, and (b) determining overall survival, based on the genotyping result obtained in step (a).

[0036] In step (b), with respect to the three SNPs including rs1045642, rs2032582 and rs1128503, overall survival during receptor tyrosine kinase inhibitor treatment, may be lower for a TTT/TTT genotype than for allele combinations other than T allele homozygosity at all of the three SNPs.

[0037] In some embodiments, for determining progression-free survival, during receptor tyrosine kinase inhibitor treatment, the method may further comprise: (a) screening for at least one SNP genotype selected from the group consisting of rs1045642, rs2032582 and rs1128503, and (b) determining progression-free survival, based on the genotyping result obtained in step (a).

[0038] In step (b), with respect to the three SNP genotypes rs1045642, rs2032582 and rs1128503, progression-free survival during receptor tyrosine kinase inhibitor treatment may be lower for a TTT/TTT genotype than for allele combinations other than T allele homozygosity at all of the three SNPs.

[0039] In another aspect, there is provided the use of the method as described herein to identify the most appropriate receptor tyrosine kinase inhibitor therapy regimen for a patient with renal cancer.

[0040] In another aspect, there is provided a kit for use in accordance with the method as described herein, comprising components for the screening of said nucleic acid sample to determine the identity of at least one SNP genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof.

EXPERIMENTAL SECTION

[0041] **Example 1**

[0042] ***Evaluation of genetic markers for determining toxicity, response and survival in patients with cancer***

[0043] **Materials and Methods**

[0044] Patients and treatment

[0045] A total of 97 mRCC patients receiving sunitinib between 2006 and 2014 at National Cancer Centre Singapore (NCCS) were included in this retrospective study, which was approved by the Institutional Review Board (Singapore Health Services). 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 from the drug label-recommended dosage of 50mg daily was established as routine at NCCS after severe to life-threatening toxicities were frequently noted in early use of sunitinib when initiated at 50mg daily. Twelve patients in this study received a starting dose of 50mg daily. Six patients started with 25mg daily due to advanced age or patients' aversion to expected toxicities.

[0046] Follow-up and data collection

[0047] Sunitinib toxicities and tumor response were evaluated by independent physicians in every treatment cycle based on Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 and Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1. Laboratory assessments of serum creatinine, total bilirubin, albumin, aspartate transaminase, alanine transaminase, hemoglobin, leucocytes and platelets, and clinical examinations for mucositis, hand-foot syndrome, fatigue 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 were also collected including age, gender, ethnicity, body weight and height, and Eastern Cooperative Oncology Group (ECOG) performance status. Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic score was calculated for each patient with available data. All the collected data were de-identified by a third party before being used in statistical analysis. The follow-up period ended at the end of April, 2014.

[0048] Toxicity definitions

[0049] The toxicities analyzed included leucopenia, neutropenia, thrombocytopenia, diarrhea, hand-foot syndrome and early toxicity-necessitated treatment termination. Blood cell counts from the electronic medical system and the physician notes from the first sunitinib cycle were assessed for leucopenia ($<3000/\mu\text{L}$), neutropenia ($<2000/\mu\text{L}$), thrombocytopenia ($<150000/\mu\text{L}$), hand-foot syndrome (documented physical examination finding) and diarrhea (documented patient complaints). Early toxicity-necessitated treatment termination was defined as interruption of the first or second treatment cycle consented by the patient and the physician based on recognition of intolerable or life-threatening toxicities without evidence of progressive disease and failure to resume treatment as far as the patient was followed. For patients experiencing any toxicity grade ≥ 2 , cycle interruptions were frequently required; in such cases, a resumed treatment with 25% dose reduction would be applied for safety and life quality considerations.

[0050] Survival endpoint definition

[0051] Progression free survival was defined as the time from the date of sunitinib initiation to the date of sunitinib termination if sunitinib was terminated due to radiological or clinical evidence of progressive disease, toxicities or death. Overall survival 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.

[0052] Genotyping

[0053] 6 SNPs were genotyped, as listed in Table 1, in 4 genes: sunitinib targets *FLT3* and *VEGFR2*, and multi-drug effluxers *ABCG2* and *ABCB1*. These polymorphisms were selected based on minor allele frequency higher than 0.1 in Han Chinese, previously reported associations with sunitinib toxicities (Table 2), and presumed function in sunitinib pharmacokinetics or pharmacodynamics. Primers

for genotyping the SNPs and the BIM deletion are provided in Table 3.

[0054] Table 1. Polymorphisms genotyped and allele frequencies

Polymorphism	rs number	Variation	n ^a	Genotype distribution			VAF ^d
				wt/ wt	wt/ var	var/ var	
VEGFR2 1191 C/T	rs2305948	V297I	94	64	30	0	0.160 (T)
FLT3 738 T/C	rs1933437	M227T	95	47	43	5	0.279 (C)
ABCB1 1236 T/C	rs1128503	G412G	93	35	49	9	0.360 (C)
ABCB1 2677 G/TA	rs2032582	A893S/T	96	25	44 ^b	27 ^c	0.375 (T); 0.135 (A)
ABCB1 3435 C/T	rs1045642	I1145I	96	34	50	12	0.385 (T)
ABCG2 421 C/A	rs2231142	Q141K	95	50	38	7	0.274 (A)
^a Patients successfully genotyped.							
^b include 34 GT and 10 AG individuals.							
^c include 2 AA, 12 AT and 13 TT individuals.							
^d Variant allele frequencies.							

[0055] Table 2. Previous analyses on the 6 SNPs selected for this study.

SNP	Significantly associated outcomes	Cohort size	Publication
VEGFR2 1191 C/T	Any toxicity grade>2 increased with T allele.	(n= 183)	van Erp et al., 2009
FLT3 738 T/C	Leucopenia increased with T allele.	(n= 188)	van Erp et al., 2009
ABCB1 1236 T/C	Poorer PFS and OS with the TT	(n= 88)	Beuselinck et al., 2013

	genotype. Less dose reductions with the <i>TT</i> genotype.	(n= 96)	Beuselinck et al., 2014
<i>ABCB1</i> 2677G/TA	Less dose reductions with <i>TT</i> or <i>TA</i> genotypes.	(n= 96)	Beuselinck et al., 2014
<i>ABCB1</i> haplotype ^a	HFS increased with <i>TTT</i> haplotype. PFS increased with <i>TCG</i> haplotype.	(n= 219) (n= 136)	van Erp et al., 2009 van der Veldt et al., 2011
<i>ABCG2</i> 421 C/A	More thrombocytopenia , neutropenia, and HFS with the <i>AA</i> genotype.	(n= 65)	Kim et al., 2013
Abbreviations: OS, overall survival; PFS, progression-free survival; HFS, hand-foot syndrome.			
^a <i>ABCB1</i> haplotype composed of 3435 C/T, 1236 T/C and 2677 G/TA.			

[0056] Table 3. Primers for genotyping

Gene	Polymorphism	rs Number	Forward Primer	Reverse Primer
<i>VEGFR2</i>	1191 C/T	rs2305948	5' TCTTGGTCATC AGCCCACTG3' (S EQ ID NO. 1)	5' AAACCCAGTCTGG GAGTGAG3' (SEQ ID NO. 2)
<i>FLT3</i>	738 T/C	rs1933437	5' GCAGCTGTAAA GAAGAAAGTCCAG 3' (SEQ ID NO. 3)	5' TGCATTCCCTGCC CAGTT3' (SEQ ID NO. 4)
<i>ABCB1</i>	1236 T/C	rs1128503	5' TGTCTGTGAAT TGCCTTGAA3' (SEQ ID NO. 5)	5' GGCATAGAGCCT CTGCATC3' (SEQ ID NO. 6)
<i>ABCB1</i>	2677 G/TA	rs2032582	5' GTACCCATCAT TGCAATAGCA3' (SEQ ID NO. 7)	5' TTTAGTTTGACTC ACCTTCCCAG3' (SEQ ID NO. 8)
<i>ABCB1</i>	3435 C/T	rs1045642	5' GAGCCCATCCT GTTTGACTGC3' (SEQ ID NO. 9)	5' CATTAGGCAGTGA CTCGATGAAGGC3' (SEQ ID NO. 10)

ABCG2	421 C/A	rs2231142	5' GGATGATGTTG TGATGGGCACTC3' ' (SEQ ID NO. 11)	5' CCAGACCTAACTC TTGAATGACCCTG3' (SEQ ID NO. 12)
BIM	BIM i2del ^a	-	(1) 5' CTTAGCGT AATG TCGTCAGGG3' (SEQ ID NO. 13) (2) 5' AGGCTTCA GTGAGGTAAATCA C3' (SEQ ID NO. 14)	5' GCTCCTCTGTGAG GCCAGCCTG3' (SEQ ID NO. 15)
a a 2,903-bp deletion polymorphism in intron 2 of <i>BIM</i> associated with resistance to tyrosine kinase inhibitors.				

[0057] Germline DNA was obtained from peripheral blood, formalin-fixed tissue of benign kidney from nephrectomy and immortalized lymphocytes. The labeling on blood tubes and tissue slides were de-identified by a third party before being used for DNA extraction or cell immortalization. Genotyping was done by PCR amplification of the flanking region of each SNP followed by direct sequencing.

[0058] Statistical analysis

[0059] Genotype associations with toxicity events or responsive tumor 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. Progression-free survival and overall survival were estimated by means of Kaplan-Meier method, while the associations of genotypes and patient characteristics with PFS and OS were analyzed using two-tailed log rank test. The effects of genotypes on the progression-free survival and overall survival were then evaluated using multivariate Cox regression model by including genotypes and patient characteristics with $P < 0.05$ in univariate analysis. Only those patients for whom sunitinib was the first line treatment for mRCC were included in progression-free survival and overall

survival analyses. In all analyses, missing data were kept missing except for baseline ECOG status, which was replaced with the median value. As this was designed as a validation study of previously observed correlations rather than an exploratory evaluation, multiple testing correction was not done.

[0060] **Results**

[0061] Patient characteristics and genotype frequencies

[0062] The demographic and baseline clinical characteristics of the 97 patients included in this study are listed in Table 4. The polymorphism frequencies of the 6 SNPs are listed in Table 1. Hardy-Weinberg equilibrium held for all the 6 SNPs ($P > 0.05$). After verifying pairwise linkage disequilibrium for *ABCB1* 3435C/T, *ABCB1* 1236C/T and *ABCB1* 2677G/TA by Chi-square test ($P < 0.05$ in each pair) and phasing with PLINK, haplotype TTT was the most common haplotype and was found in 51 patients, among whom 8 were homozygous carriers. A complete list of haplotypes and their frequencies is provided in Table 5.

[0063] Table 4. Patient demographics and baseline characteristics (n = 97)

Characteristic	No.	%
Median age when initiating sunitinib, years	58	
Range	18 - 79	
Gender		
Male	75	77.3
Female	22	22.7
Ethnicity		
Chinese	86	88.7
Malay	7	7.2
Indian	4	4.1
Baseline ECOG performance status		
0	27	27.8
1	51	52.6
2	13	13.4

3	6	6.2
Median body surface area, m²		
Range	1.18 -	1.92
Line of therapy		
First line	81	83.5
Second or third line	16	16.5
Starting sunitinib dose, mg daily		
<37.5	6	6.2
37.5	79	81.4
50	12	12.4
Baseline chemistry and hematology (n < 97 because of missing data)		
Median aspartate transaminase, U/L (n = 93)		
Range	11 -	70
Median alanine transaminase, U/L (n = 93)		
Range	8 -	135
Median creatinine, µM (n = 96)		
Range	33 -	649
Median hemoglobin, g/dL (n = 96)		
Range	5.9 -	15.8
Median leukocyte, K/µL (n = 96)		
Range	1.4 -	24.1
Median thrombocyte, K/µL (n = 96)		
Range	117 -	799

[0064] Table 5. ABCB1 haplotype frequencies estimated with and without assuming associations (n = 92)

3435 C/T	1236 T/C	2677 G/TA	Without association	With association
C	C	A	0.028	0.096
C	C	G	0.114	0.222
C	C	T	0.083	0.000
C	T	A	0.049	0.029
C	T	G	0.199	0.214
C	T	T	0.146	0.059
T	C	A	0.017	0.000
T	C	G	0.070	0.037
T	C	T	0.051	0.008
T	T	A	0.030	0.000

<i>T</i>	<i>T</i>	<i>G</i>	0.122	0.032
<i>T</i>	<i>T</i>	<i>T</i>	0.089	0.303

[0065] Correlation of genotypes to toxicities

[0066] Univariate and multivariate logistic regression analyses for associations between genetic markers and clinical outcomes are listed in Table 6. Of note, the *FLT3* 738 *TT* genotype was associated with an 8.0-fold increase of the risk of leucopenia ($P=0.03$) and a 2.7-fold increase of the risk of neutropenia ($P=0.04$). The *ABCB1* 1236 *T* allele was correlated to a 3-fold decrease of the risk of neutropenia ($P=0.03$), a 25-fold decrease of the risk of diarrhea ($P=0.0005$) and a 10-fold decrease of the risk of early toxicity-necessitated treatment termination ($P=0.04$). The *ABCB1* 3435 *T* allele was associated with a 10-fold decrease of the risk of neutropenia ($P=0.01$) and a 3-fold decrease of the risk of diarrhea ($P=0.02$). The *ABCB1* 2677 *T* allele was correlated to a 3-fold decrease of the risk of neutropenia ($P=0.04$). The *ABCB1* 3435, 1236, 2677 *TTT* haplotype was correlated to a 10-fold decrease of the risk of neutropenia ($P=0.03$). The *ABCG2* 421 *A* allele was associated with a 3-fold decrease of the risk of neutropenia ($P=0.03$). No genotypes were correlated with thrombocytopenia or hand-foot syndrome. The *VEGFR2* 1191*C/T* genotype was not associated with the toxicity endpoints.

[0067] Table 6. Factors associated with toxicities of sunitinib. Only those with multivariate $P \leq 0.05$ are listed.

Group		Prevalence ^b	Univariate		Multivariate ^a	
			OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Leucopenia (n=85)						
Age		11/85	1.0 (0.9, 1.0)	0.44		
Gender	Male vs.	6/65	1			
	Female	5/20	3.3 (0.9, 12.4)	0.08		
Baseline ECOG						
	0	1/25	1			
	1	9/44	6.2 (1.1, 117.6)	0.09		
	2	1/12		0.59		

	3	0/4	2.2 (0.1, 58.7)	0.99			
			NR				
Starting dose (mg)	<37.5	0/4	1				
	37.5	10/70	NR	0.99			
	50	1/11	NR	0.99			
FLT3 738 T/C	CC+CT	2/42	1			1	
	TT	8/41	4.9 (1.1, 33.6)	0.06		8.0 (1.3, 51.0)	0.03
Neutropenia (n=88)							
Age		40/88	1.0 (1.0, 1.1)	0.24			
Gender	Male	27/68	1				
	Female	13/20	2.8 (1.0, 8.4)	0.05			
Baseline ECOG	0	13/25	1				
	1	22/46	0.9 (0.3, 2.3)	0.74			
	2	5/12		0.56			
	3	0/5	0.7 (0.2, 2.6)	0.99			
			NR				
Starting dose (mg)	<37.5	1/5	1				
	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			1	
	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			1	
	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			1	
	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			1	
	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			1	
	TT	1/12	0.1 (0.0, 0.5)	0.02		0.1 (0.0, 0.4)	0.01
ABCB1 haplotyp e ^c	Other	38/79	1			1	
	TTT/TTT	1/8	0.2 (0.0, 0.9)	0.09		0.1 (0.0, 0.5)	0.03
Diarrhea (n=95)							
Age		20/95	1.0 (1.0, 1.0)	0.62			
Gender	Male	15/74	1				
	Female	5/21	1.2 (0.4, 3.7)	0.73			
Baseline ECOG	0	6/27	1				
	1	9/50	0.8 (0.2, 2.6)	0.66			
	2	4/12		0.47			
	3	1/6	1.8 (0.4, 7.9)	0.76			
			0.7 (0.0, 5.6)				

Starting dose (mg)	<37.5 37.5 50	2/5 15/78 3/12	1 0.4 (0.1, 2.9) 0.5 (0.1, 5.2)	0.28 0.54		
ABCB1 3435 T/C	CC TT+CT	11/34 9/60	1 0.4 (0.1, 1.0)	0.05	0.3 (0.1, 0.8)	0.02
ABCB1 1236 T/C	CC TT+CT	7/9 13/82	1 0.1 (0.0, 0.3)	0.00 0.06	0.04 (0.0, 0.2)	0.00 0.05
Toxicity-necessitated treatment termination before completing the first two cycles (n= 87)						
Age		13/87	1.1 (1.0, 1.2)	0.01		
Gender	Male Female	10/66 3/21	0.9 (0.2, 3.5)	0.92		
Baseline ECOG	0 1 2 3	2/26 7/44 2/12 2/5	1 2.3 (0.5, 16.1) 2.4 (0.3, 22.4) 8.0 (0.8, 93.2)	0.33 0.41 0.08		
Starting dose (mg)	<37.5 37.5 50	3/4 8/71 2/12	1 0.0 (0.0, 0.4) 0.1 (0.0, 0.8)	0.01 0.05		
ABCB1 1236 T/C	CC+CT TT	10/53 2/30	1 0.3 (0.0, 1.3)	0.15	0.1 (0.0, 0.7)	0.04
Abbreviations: OR, ratio of the odds that the event occurs; CI, confidence interval; NR, not reached.						
^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 ABCB1 3435C/T, 1236C/T, 2677G/TA haplotype.						

[0068] Correlation of genotypes to tumor response and patient survival

[0069] Primary sunitinib resistance, defined as progressive disease as the best 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 7).

Median progression-free survival of the 81 patients who received sunitinib as the first-line therapy was 8.1 months and median overall survival was 19.5 months. As in Table 8, after including starting dose as covariate based on univariate $P < 0.05$, the ABCB1 3435, 1236, 2677 TTT haplotype was correlated with inferior progression-free survival ($P = 0.001$) and overall survival ($P = 0.005$). Survival curves depicting the correlations are provided in Figure 1.

[0070] Table 7. Factors associated with the clinical benefit of sunitinib (best response being partial response or stable disease) (n=90). Only those with multivariate $P \leq 0.05$ are listed.

Group		Prevalence ^b	Univariate		Multivariate ^a	
			OR (95% CI)	P	OR (95% CI)	P
Age		59/90	1.0 (0.9, 1.0)	0.38		
Gender	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		
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

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.

[0071] Table 8. Survival analyses in mRCC patients receiving sunitinib as first-line treatment (n=81). Only those with multivariate $P \leq 0.05$ are listed.

Factor		No	Median (months)	Univariate		Multivariate ^a	
				HR (95% CI)	P	HR (95% CI)	P
Progression-free survival							
Age		81	8.1	1.0 (1.0, 1.0)	0.56		
Gender	Female	20	10.0	1	0.69		
	Male	61	8.1	1.1 (0.6, 2.1)			
Baseline ECOG	0	21	16.1	1	0.08		
	1	43	6.9	2.2 (1.1, 4.4)			
	2	11	3.3				
	3	6	12.9	2.8 (1.1, 7.0)			
				2.8 (0.8, 10.3)			
Starting dose (mg)	≤37.5	5	1.8	1	0.01		
	37.5	71	8.3	0.2 (0.1, 0.8)			
	50	5	17.3	0.1 (0.0, 0.6)			
MSKCC	Good	7	12.6	1	0.14		
	Intermediate	33	10.0	1.3 (0.5, 3.6)			
	Poor	23	5.5	2.3 (0.8, 6.4)			
ABCB1 1236 T/C	CC+CT	51	11.7	1	0.09	1	0.09
	TT	28	3.6	1.7 (0.9, 3.0)		1.7 (0.9, 3.2)	
ABCB1 2677 G/TA	Other	71	8.4	1	0.09	1	0.44
	TT	10	2.7	2.3 (0.9, 6.0)		1.5 (0.5, 4.7)	
ABCB1 3435 C/T	CC+CT	70	8.4	1	0.19	1	0.19
	TT	10	2.7	1.7 (0.8, 3.9)		1.7 (0.8, 3.9)	
Haplotype ^b	Other	74	8.4	1	0.0006	1	0.001
	TTT/TTT	6	2.4	4.9 (1.8, 13.6)		5.5 (2.0, 15.4)	
Overall survival							
Age		81	19.5	1.0 (1.0, 1.0)	0.56		

Gender	Female	20	19.9	1	0.87		
	Male	61	16.3	1.1 (0.6, 2.0)			
Baseline ECOG	0	21	32.9	1	0.06		
	1	43	19.6	1.9 (0.9, 3.9)			
	2	11	5.7	3.0 (1.3, 7.1)			
	3	6	15.7	2.7 (0.9, 8.0)			
Starting dose (mg)	≤37.5	5	4.6	1	<0.0001		
	37.5	71	19.5	0.2 (0.1, 0.4)			
	50	5	47.4	0.1 (0.0, 0.3)			
MSKCC	Good	7	41.4	1	0.10		
	Intermediate	33	19.6	2.2 (0.8, 6.5)			
	Poor	23	14	3.1 (1.0, 9.1)			
ABCB1 1236 T/C	CC+CT	51	20	1	0.09	1	0.07
	TT	28	10.4	1.7 (0.9, 2.9)		1.7 (1.0, 3.1)	
ABCB1 2677 G/TA	Other	71	19.6	1	0.01	1	0.12
	TT	10	5.9	2.9 (1.3, 6.7)		2.0 (0.8, 5.0)	
ABCB1 3435 C/T	CC+CT	70	19.5	1	0.25	1	0.21
	TT	10	7.2	1.6 (0.7, 3.6)		1.7 (0.7, 3.8)	
Haplotype ^b	Other	74	19.6	1	0.00	1	0.00
	TTT/TTT	6	4.6	3.9 (1.3, 11.7)	8	5.0 (1.6, 15.2)	5

Abbreviations: HR, hazard ratio; CI, confidence interval.

^a Including starting dose as covariate.

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

CLAIMS

1. A method for determining the sensitivity of a patient with cancer to receptor tyrosine kinase inhibitor therapy, comprising:
 - a) isolating a nucleic acid sample from a biological sample obtained from the said patient;
 - b) screening said nucleic acid sample to determine the identity of at least one single nucleotide polymorphism (SNP) genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof; and
 - c) determining the sensitivity of a patient with renal cancer to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP genotype selected from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof,wherein said sensitivity is selected from one or more of neutropenia, diarrhea, tumor response, early toxicity-necessitated treatment termination, overall survival and progression-free survival.
2. The method according to claim 1, wherein the cancer is renal cancer, neuroendocrine cancer, breast cancer, prostate cancer, cervical cancer, ovarian cancer, gastric cancer, colorectal cancer, pancreatic cancer, liver cancer, brain cancer, lung cancer, hematological malignancies, melanoma and sarcomas.
3. The method according to claim 2, wherein the renal cancer is metastatic renal cell carcinoma (mRCC).
4. The method according to claim 2, wherein the patient suffers from mRCC or wherein the patient suffers from mRCC and undergoes receptor tyrosine kinase inhibitor treatment.

5. The method according to claim 1, wherein the biological sample is obtained from the group consisting of frozen tissue, tissue biopsies, circulating cells, bodily fluids and cheek swab.
6. The method according to claim 5, wherein the biological sample is non-neoplastic.
7. The method according to claim 5, wherein the biological sample is neoplastic.
8. The method according to claim 4, wherein the bodily fluids are selected from the group consisting of blood, saliva, ascites, effusions and urine.
9. The method according to claim 1, wherein the receptor tyrosine kinase inhibitor is a multikinase inhibitor for receptor tyrosine kinase including but not limited to sunitinib, pazopanib, axitinib, sorafenib, regorafenib, tivozanitinib or combinations thereof.
10. The method according to claim 1, wherein the patient is a mammal or a human.
11. The method according claim 10, wherein the human is an ethnic Asian, or an ethnic Caucasian, or an ethnic African.
12. The method according to claim 1, for determining the risk of diarrhea during receptor tyrosine kinase inhibitor treatment, the method further comprising: (a) screening for at least one SNP genotype selected from the group consisting of rs1128503 and rs1045642, and (b) determining the risk of diarrhea based on the screening result obtained in step (a).
13. The method according to claim 12, wherein in step (b), with respect to rs1128503, the risk of diarrhea during receptor tyrosine kinase inhibitor treatment is higher for a CC genotype than a CT or TT genotype.
14. The method according to claim 12, wherein in step (b), with respect to rs1045642, the risk of diarrhea during receptor tyrosine kinase inhibitor treatment is higher for a CC genotype than a CT or TT genotype.

15. The method according to claim 1, for determining the risk of neutropenia during receptor tyrosine kinase inhibitor treatment, the method further comprising: (a) screening for at least one SNP genotype selected from the group consisting of rs1933437, rs2032582, rs1045642, rs1128503 and rs2231142, and (b) determining the risk of neutropenia based on the screening result obtained in step (a).
16. The method according to claim 15, wherein in step (b), with respect to rs1933437, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment is higher for a TT genotype than a CC or CT genotype.
17. The method according to claim 15, wherein in step (b), with respect to rs2032582, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment is higher for a AA or AG or GG genotype than a AT or GT or TT genotype.
18. The method according to claim 15, wherein in step (b), with respect to rs1045642, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment is higher for a CC or CT genotype than a TT genotype.
19. The method according to claim 15, wherein in step (b), with respect to rs1128503, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment is higher for a CC or CT genotype than a TT genotype.
20. The method according to claim 15, wherein in step (b), with respect to rs2231142, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment is higher for a CC or AC genotype than a AA genotype.
21. The method according to claim 15, wherein in step (b), with respect to the three SNPs including rs1045642, rs2032582 and rs1128503, the risk of neutropenia during receptor tyrosine kinase inhibitor treatment is higher for allele combinations other than T allele homozygosity at all of the three SNP sites than for a TTT/TTT genotype.

22. The method according to claim 1, for determining the risk of early toxicity-necessitated treatment termination during receptor tyrosine kinase inhibitor treatment, the method comprising: (a) screening for the SNP genotype of rs1128503, and (b) determining the risk of early toxicity-necessitated treatment termination based on the genotyping result obtained in step (a).
23. The method according to claim 22, wherein in step (b), with respect to rs1128503, the risk of early toxicity-necessitated treatment termination during receptor tyrosine kinase inhibitor treatment is higher for a CC or CT genotype than a TT genotype.
24. The method according to claim 1, for determining the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment, the method comprising: (a) screening for at least one SNP genotype selected from the group consisting of rs1045642, rs2032582 and rs1128503, and (b) determining the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment based on the genotyping result obtained in step (a).
25. The method according to claim 24, wherein in step (b), with respect to rs1045642, the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment is higher for a TT genotype than a CC or CT genotype.
26. The method according to claim 24, wherein in step (b), with respect to rs2032582, the risk of primary tumour resistance to receptor tyrosine kinase inhibitor treatment is higher for a TT genotype than a AA or AG or GG or AT or GT genotype.
27. The method according to claim 24, wherein in step (b), with respect to the three SNPs including rs1045642, rs2032582 and rs1128503, the risk of primary tumour resistance to receptor tyrosine kinase inhibitor therapy is higher for a TTT/TTT

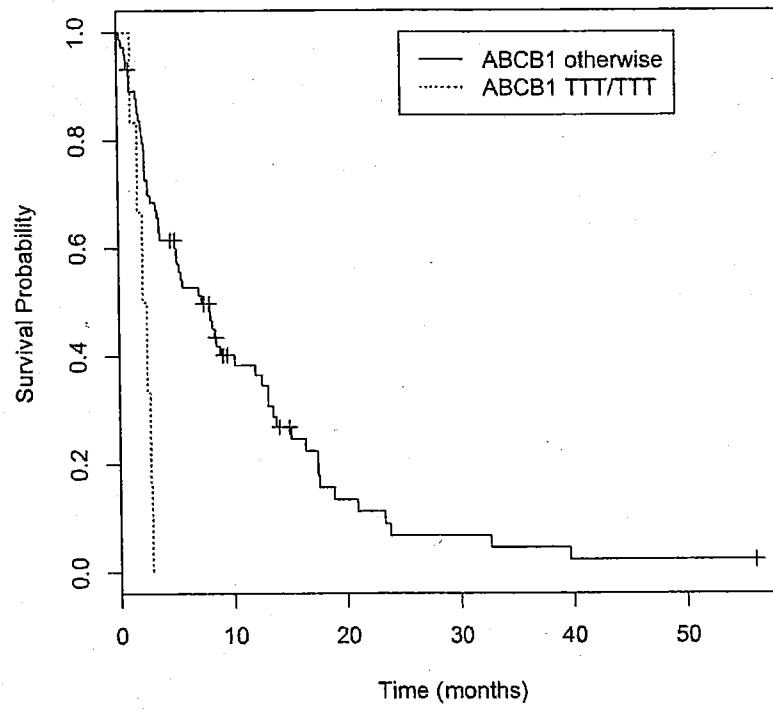
genotype than for allele combinations other than T allele homozygosity at all of the three SNP sites.

28. The method according to claim 1, for determining overall survival, during receptor tyrosine kinase inhibitor treatment, the method comprising: (a) screening for at least one SNP genotype selected from the group consisting of rs1045642, rs2032582 and rs1128503, and (b) determining overall survival, based on the genotyping result obtained in step (a).
29. The method according to claim 28, wherein in step (b), with respect to the three SNPs including rs1045642, rs2032582 and rs1128503, overall survival during receptor tyrosine kinase inhibitor treatment is lower for a TTT/TTT genotype than for allele combinations other than T allele homozygosity at all of the three SNPs.
30. The method according to claim 1, for determining progression-free survival, during receptor tyrosine kinase inhibitor treatment, the method comprising: (a) screening for at least one SNP genotype selected from the group consisting of rs1045642, rs2032582 and rs1128503, and (b) determining progression-free survival, based on the genotyping result obtained in step (a).
31. The method according to claim 30, wherein in step (b), with respect to the three SNP genotypes rs1045642, rs2032582 and rs1128503, progression-free survival during receptor tyrosine kinase inhibitor treatment is lower for a TTT/TTT genotype than for allele combinations other than T allele homozygosity at all of the three SNPs.
32. Use of the method according to claim 1 to identify the most appropriate receptor tyrosine kinase inhibitor therapy regimen for a patient with renal cancer.
33. A kit for use according to the method of claim 1, comprising components for the screening of said nucleic acid sample to determine the identity of at least one SNP genotype selected

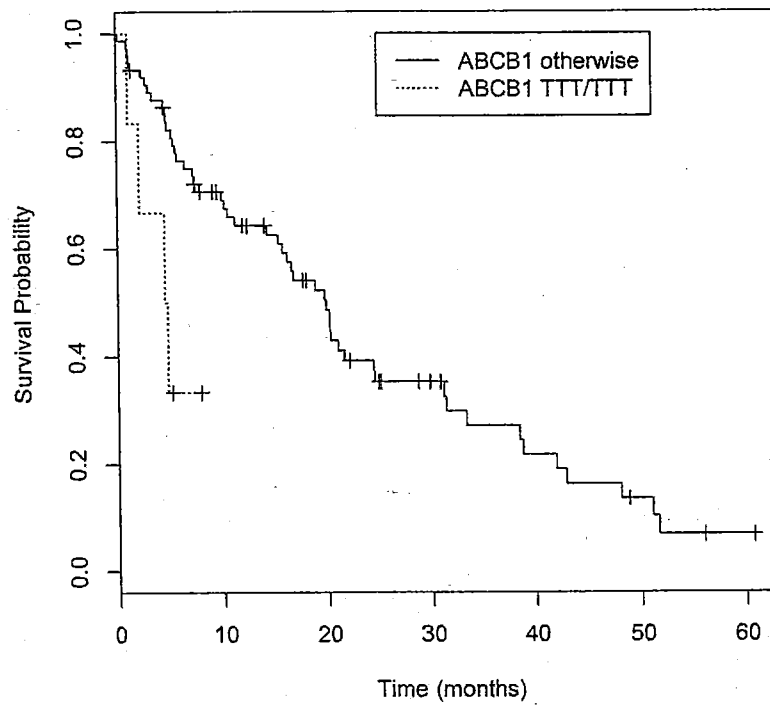
from the group consisting of rs1933437, rs1045642, rs1128503, rs2032582 and rs2231142 and any combination thereof.

1/1
FIG. 1

A



B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2014/000467

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE, BIOSIS, HCAPLUS (Keywords including Receptor Tyrosine Kinase/RTK/sunitinib/afatinib/axitinib/cediranib/erlotinib/gefitinib/gradinin/lapatinib/lestaurtinib/neratinib/

pazopanib/quizartinib/regorafenib/semaxanib/sorafenib/tivozanib/toceranib/vandetanib, cancer/tumour, rs1933437, rs1045642, rs1128503, rs2032582, rs2231142 and like terms)

EPOQUE: WPI, EPODOC, TXTUS(0-5), TXTEP1, TXTGB1, TXTWO1, TXTAU1, TXTCA1, TXTSG1 (Keywords as for STN)

GOOGLE: (Keywords including TAN Min-Han, CHU Ying Hsia, LAI Johnathan, TAN Puah Hoon; then as for STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 8 January 2015	Date of mailing of the international search report 08 January 2015
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Rebecca Hinton AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 2194

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/SG2014/000467
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Garcia-Donas J <i>et al.</i> 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 Nov;12(12):1143-50. Epub 2011 Oct 17. Whole document, especially Abstract; 'Methods: Procedures'; 'Results'; Table 3	1-11, 28-33
X	Yuasa T <i>et al.</i> Biomarkers to predict response to sunitinib therapy and prognosis in metastatic renal cell cancer. Cancer Sci. 2011 Nov;102(11):1949-57. Epub 2011 Sep 14. Whole document, especially 'Results' p1952-1953; Table 2	1-11, 15, 16, 22, 24, 30-33
X	van Erp NP <i>et al.</i> Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity. J Clin Oncol. 2009 Sep 10;27(26):4406-12. Epub 2009 Aug 10. Erratum in: J Clin Oncol. 2010 Jun 1;28(16):2805. Whole document, especially 'Abstract'	1-11, 15, 16, 32, 33
X	Kim HR <i>et al.</i> Pharmacogenetic determinants associated with sunitinib-induced toxicity and ethnic difference in Korean metastatic renal cell carcinoma patients. Cancer Chemother Pharmacol. 2013 Oct;72(4):825-35. Epub 2013 Sep 8. Whole document, especially 'Abstract'	1-11, 15, 32, 33
X	Beuselinck B <i>et al.</i> Single-nucleotide polymorphisms associated with outcome in metastatic renal cell carcinoma treated with sunitinib. Br J Cancer. 2013 Mar 5;108(4):887-900. Whole document, especially 'Abstract'; 'Discussion' third paragraph	1-11, 24, 28-33
X	van der Veldt AA <i>et al.</i> Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. Clin Cancer Res. 2011 Feb 1;17(3):620-9. Epub 2010 Nov 19. Whole document, especially 'Abstract'; last paragraph of p625	1-11, 30-33
X	WO 2012/103060 A1 (GLAXO WELLCOME MFG PTE LTD) 02 August 2012 p32; claims 7-14	1-11, 28-33
A	Cusatis G <i>et al.</i> Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. J Natl Cancer Inst. 2006 Dec 6;98(23):1739-42. Abstract	

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-11, 15, 16, 32 and 33 (all in part) are directed to a method and a kit for determining the sensitivity of a patient to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP comprising rs1933437. The feature of the specific SNP 'rs1933437' is specific to this group of claims.
- Claims 1-12, 14, 15, 18, 21, 24, 25 and 27-33 (all in part) are directed to a method and a kit for determining the sensitivity of a patient to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP comprising rs1045642. The feature of the specific SNP 'rs1045642' is specific to this group of claims.
- Claims 1-12, 14, 15, 18, 21, 24, 25 and 27-33 (all in part) are directed to a method and a kit for determining the sensitivity of a patient to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP comprising rs1128503. The feature of the specific SNP 'rs1128503' is specific to this group of claims.
- Claims 1-11, 15, 17, 21, 24 and 26-33 (all in part) are directed to a method and a kit for determining the sensitivity of a patient to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP comprising rs2032582. The feature of the specific SNP 'rs2032582' is specific to this group of claims.
- Claims 1-11, 15, 20, 32 and 33 (all in part) are directed to a method and a kit for determining the sensitivity of a patient to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP comprising rs2231142. The feature of the specific SNP 'rs2231142' is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the determination of the sensitivity of a patient with renal cancer to receptor tyrosine kinase inhibitor therapy based on the identity of at least one SNP

However this feature does not make a contribution over the prior art because it is disclosed in:

D1

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

Despite the technical lack of unity, all inventions have been searched and commented upon in this report.

Supplemental Box

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/SG2014/000467	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2012/103060 A1	02 August 2012		
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			