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(54) Title: MEANS AND METHODS FOR IMPROVED TREATMENT OF CANCER BASED ON CYP3A5

(57) Abstract: The present invention relates to the use of irinotecan or a derivative thereof for the preparation of a pharmaceutical composition for treating cancer, especially, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a patient having a genotype with a variant allele which comprises a polynucleotide in accordance with the present invention. Preferably, a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of variant allele compared to the corresponding wild type allele or an altered activity of the polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele. Finally, the present invention relates to a method for selecting a suitable therapy for a subject suffering from cancer, especially, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer.

Means and methods for improved treatment of cancer based on CYP3A5

The present invention relates to the use of camptothecin drugs, such as irinotecan (CPT-11) or a derivative thereof for the preparation of a pharmaceutical composition for treating colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a patient having a genotype with a variant allele which comprises a polynucleotide in accordance with the present invention. Preferably, a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of variant allele compared to the corresponding wild type allele or an altered activity of the polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele. Finally, the present invention relates to a method for selecting a suitable therapy for a subject suffering from cancer, especially, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer or pancreatic cancer.

Irinotecan is a semisynthetic analog of the cytotoxic alkaloid camptothecin (CPT), which is obtained from the oriental tree, Camptotheca acuminata Camptothecins demonstrate anti-neoplastic activities by inhibiting specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks [D'Arpa, *et al.*, 1989, Biochim Biophys Acta 989:163-77, Horwitz, *et al.*, 1973, Cancer Res 33:2834-6]. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks [Kawato, *et al.*, 1991, Cancer Res 51:4187-91]. Irinotecan serves as a water-soluble prodrug of the lipophilic metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) which is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain [Tsuji, *et al.*, 1991, J Pharmacobiodyn 14:341-9]. Carboxylesterase-2 is the primary enzyme involved in this hydrolysis at at pharmacological concentrations [Humerickhouse, *et al.*, 2000, Cancer Res 60:1189-92]. Topoisomerase inhibition and irinotecan-related single strand breaks

are caused primarily by SN-38 [Kawato, *et al.*, 1991, Cancer Res 51:4187-91]. Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma xenografts of various histological types [Furuta, *et al.*, 1988, Gan To Kagaku Ryoho 15:2757-60, Giovanella, *et al.*, 1989, Science 246:1046-8, Giovanella, *et al.*, 1991, Cancer Res 51:3052-5, Hawkins, 1992, Oncology (Huntingt) 6:17-23, Kunimoto, *et al.*, 1987, Cancer Res 47:5944-7].

Irinotecan is also oxidized by CYP3A4 and CYP3A5 [Haaz, *et al.*, 1998, Drug Metab Dispos 26:769-74, Kuhn, 1998, Oncology (Huntingt) 12:39-42, Santos, *et al.*, 2000, Clin Cancer Res 6:2012-20, Rivory, *et al.*, 1996, Cancer Res 56:3689-94]. The major elimination pathway of SN-38 is conjugation with glucuronic acid to form the corresponding glucuronide (SN-38G) [Atsumi, *et al.*, 1991, Xenobiotica 21:1159-69.]. SN-38G is reported to be deconjugated by the intestinal microflora to form SN-38 [Kaneda, *et al.*, 1990, Cancer Res 50:1715-20]. Glucuronidation of SN-38 is mediated by UGT1A1 and UGT1A7 [Iyer, *et al.*, 1998, J Clin Invest 101:847-54, Ciotti, *et al.*, 1999, Biochem Biophys Res Commun 260:199-202]. Mass balance studies have demonstrated that 64% of the total dose is excreted in the feces, confirming the important role of biliary excretion [Slatter, *et al.*, 2000, Drug Metab Dispos 28:423-33]. Studies suggest that the multidrug resistance protein 1 (MRP1) is a major transporter of irinotecan and its metabolites [Kuhn, 1998, Oncology (Huntingt) 12:39-42, Chen, *et al.*, 1999, Mol Pharmacol 55:921-8, Chu, *et al.*, 1997, Cancer Res 57:1934-8, Chu, *et al.*, 1997, J Pharmacol Exp Ther 281:304-14] and facilitate their biliary excretion, where they cause side effects, although P-glycoprotein also participates in irinotecan excretion [Chu, *et al.*, 1998, Cancer Res 58:5137-43, Chu, *et al.*, 1999, Drug Metab Dispos 27:440-1, Chu, *et al.*, 1999, J Pharmacol Exp Ther 288:735-41, Mattern, *et al.*, 1993, Oncol Res 5:467-74, Hoki, *et al.*, 1997, Cancer Chemother Pharmacol 40:433-8, Sugiyama, *et al.*, 1998, Cancer Chemother Pharmacol 42:S44-9].

Cellular resistance to camptothecins and thus, therapeutic response of irinotecan has been related to intracellular carboxylesterase activity and cleavage activity of topoisomerase I [van Ark-Otte, *et al.*, 1998, Br J Cancer 77:2171-6, Guichard, *et al.*, 1999, Br J Cancer 80:364-70].

The use of such camptothecin drugs, e.g. irinotecan, is limited by clearly dose-dependent myelosuppression and gastrointestinal toxicities, including nausea,

vomiting, abdominal pain, and diarrhea which side effects can prove fatal. The major dose-limiting toxicity of irinotecan therapy is diarrhea, which occurs in up to 88% of patients and which depends on intestinal SN-38 accumulation [van Ark-Otte, *et al.*, 1998, Br J Cancer 77:2171-6, Guichard, *et al.*, 1999, Br J Cancer 80:364-70, Araki, *et al.*, 1993, Jpn J Cancer Res 84:697-702] secondary to the biliary excretion of SN-38, the extent of which is determined by SN-38 glucuronidation [Gupta, *et al.*, 1994, Cancer Res 54:3723-5, Gupta, *et al.*, 1997, J Clin Oncol 15:1502-10]. Myelosuppression has been correlated with the area under the concentration-time curve of both irinotecan and SN-38 [Sasaki, *et al.*, 1995, Jpn J Cancer Res 86:101-10]. Despite the approval of irinotecan for patients with metastatic colorectal cancer refractory to 5-fluorouracil therapy in 1997, the therapeutic benefit remains questionable. Recently two large clinical trials on colorectal cancer involving more than 2000 patients had to be canceled by the National Institute of Cancer (NCI) due to an almost 3-times increase of irinotecan toxicity-related mortality within the first 60 days of treatment. Causes of death were diarrhea- and vomiting-related dehydratation and neutropenia-related sepsis [2001, arznei-telegramm 32:58]. Although irinotecan was proven to be effective against cancer itself, not all patients could benefit from longterm survival due to short term toxicity. Thus, it is highly desirable to identify those patients who will most likely suffer from irinotecan toxicity.

Currently, patients are treated according to most treatment schedules with a standard dose of initially 60 to 125 mg/m^2 irinotecan in combination with other anti-neoplastic drugs administered several courses of 3 to 4 weekly dosings, and subsequent doses are adjusted in 25 to 50 mg/m^2 increments based upon individual patient tolerance to treatment. Treatment may be delayed 1 to 2 weeks to allow for recovery from irinotecan-related toxicity and if the patient has not recovered, therapy has to be discontinued. Provided intolerable toxicity does not develop, treatment with additional courses are continued indefinitely as long as the patient continues to experience clinical benefit. Response rates varies depending from tumor type from less than 10 % to almost 90 %. However, it takes at least 6 to 8 weeks to evaluate therapeutic response and to consider alternatives. Thus, finding the right dosage for the patient is tedious, time-consuming and takes the risk of life threatening adverse effects. Patients might be unnecessary put to this risk who do not benefit from treatment and

additionally, worthwhile time is wasted before these patients receive their suitable treatment.

Furthermore, as observed for many chemotherapeutic agents, the risk to develop cellular resistances against therapy is increased upon suboptimal exposure of cells to chemotherapeutic agents, such as irinotecan.

Pharmacokinetic modulation with inhibitors of biliary excretion (*e. g.,* MRP and P-glycoprotein) and inducers of UGT1A1 have been suggested as a tool to reduce camptothecin-related toxicity [Gupta, *et al.*, 1996, Cancer Res 56:1309-14, Gupta, *et al.*, 1997, Cancer Chemother Pharmacol 39:440-4]. Although preliminary data of a clinical study of irinotecan in combination with cyclosporine A, and phenobarbital show some promising results in respect to limit camptothecin-related diarrhea [Ratain, 2000, Clin Cancer Res 6:3393-4], cotreatment with drugs such as cyclosporine A, and phenobarbital takes the additional risk of adverse events and drug interactions.

Large interpatient variability exist for both SN-38 and SN-38G pharmacokinetics [Canal, *et al.*, 1996, J Clin Oncol 14:2688-95], which is likely to be due to interpatient differences in the metabolism pathways of irinotecan [Rivory, *et al.*, 1997, Clin Cancer Res 3:1261-6]. Furthermore, severe irinotecan toxicity has been reported in patients with Gilbert syndrome [Wasserman, *et al.*, 1997, Ann Oncol 8:1049-51]. Consequently, a genetic predisposition to the metabolism of irinotecan, that patients with low UGT1A1 activity are at increased risk for irinotecan toxicity has been suggested [Iyer, *et al.*, 1998, J Clin Invest 101:847-54, Ando, *et al.*, 1998, Ann Oncol 9:845-7]. A common polymorphism in the UGT1A1 promoter [Monaghan, *et al.*, 1996, Lancet 347:578-81] has been correlated with *in vitro* glucuronidation of SN-38 [Iyer, *et al.*, 1999, Clin Pharmacol Ther 65:576-82], and its possible clinical use has been suggested from a case control study [Ando, *et al.*, 2000, Cancer Res 60:6921-6]. However, irinotecan-related toxicity was predicted by UGT1A1 genotype only in the minority of affected patients (< 15 %).

In conclusion, it would be highly desirable to significantly improve therapeutic efficacy and safety of camptothecin-based therapies and to avoid therapy-caused fatalities, to avoid unnecessary development of resistances, and to reduce adverse events- and therapeutic delay-related hospitalization costs. However, no accepted mechanism for reducing irinotecan toxicity or to improve therapeutic efficacy are currently available.

Thus, the technical problem underlying the present invention is to provide improved means and methods for the efficient treatment of cancer, preferably, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer, whereby the aforementioned undesirable side effects are to be avoided.
The technical problem underlying the present invention is solved by the embodiments characterized in the claims.

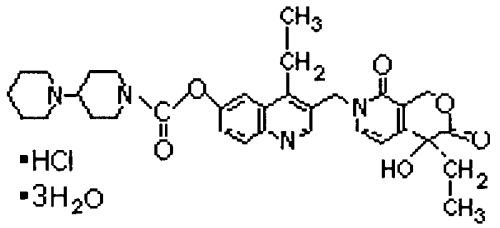
Accordingly, the present invention relates to the use of irinotecan or a derivative thereof for the preparation of a pharmaceutical composition for treating cancer, preferably, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a subject having a genome with a variant allele which comprises a polynucleotide selected from the group consisting of:

(a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 137, 138, 141, 142, 145, 146, 149 and/or 150;

(b) a polynucleotide capable of hybridizing to a Cytochrome P450, subfamily IIIA (nifedipine oxidase), polypeptide 5 (CYP3A5) gene, wherein said polynucleotide is having at a position corresponding to positions 47518 and/or 9736 of the CYP3A5 gene (Accession No: GI:10281451), a substitution of at least one nucleotide or at a position corresponding to positions 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452), a substitution of at least one nucleotide;

(c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) a C, at a position corresponding to position 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452) a G or at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451) a G.

The term "irinotecan or a derivative thereof" as used in accordance with the present invention preferably refers to a substance which is characterized by the general structural formula



$C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$

further described in US patents US05106742, US05340817, US05364858, US05401747, US05468754, US05559235 and US05663177. Moreover, also comprised by the term "irinotecan or a derivative thereof" are analogues and derivatives of camptothecin. The types and ranges of camptothecin analogues available are well known to those of skill in the art and described in numerous texts, e.g. [Hawkins, 1992, Oncology (Huntingt) 6:17-23, Burris, *et al.*, 1994, Hematol Oncol Clin North Am 8:333-55, Slichenmyer, *et al.*, 1993, J Natl Cancer Inst 85:271-91, Slichenmyer, *et al.*, 1994, Cancer Chemother Pharmacol 34:S53-7]. Specific examples of active camptothecin analogues are hexacyclic camptothecin analogues, 9-nitro-camptothecin, camptothecin analogues with 20S configuration with 9- or 10-substituted amino, halogen, or hydroxyl groups, seven-substituted water-soluble camptothecins, 9-substituted camptothecins, E-ring-modified camptothecins such as (RS)-20-deoxyamino-7-ethyl-10-methoxycamptothecin, and 10-substituted camptothecin analogues [Emerson, *et al.*, 1995, Cancer Res 55:603-9, Ejima, *et al.*, 1992, Chem Pharm Bull (Tokyo) 40:683-8, Sugimori, *et al.*, 1994, J Med Chem 37:3033-9, Wall, *et al.*, 1993, J Med Chem 36:2689-700, Wani, *et al.*, 1980, J Med Chem 23:554-60, Kingsbury, *et al.*, 1991, J Med Chem 34:98-107]. Various other camptothecin analogues with similar therapeutic activity are described [Hawkins, 1992, Oncology (Huntingt) 6:17-23, Burris and Fields, 1994, Hematol Oncol Clin North Am 8:333-55,

Slichenmyer, *et al.*, 1993, J Natl Cancer Inst 85:271-91, Slichenmyer, *et al.*, 1994, Cancer Chemother Pharmacol 34:S53-7]. Suitable methods for synthezising camptothecin analogues are described [Emerson, *et al.*, 1995, Cancer Res 55:603-9, Ejima, *et al.*, 1992, Chem Pharm Bull (Tokyo) 40:683-8, Sugimori, *et al.*, 1994, J Med Chem 37:3033-9, Wall, *et al.*, 1993, J Med Chem 36:2689-700, Wani, *et al.*, 1980, J Med Chem 23:554-60, Kingsbury, *et al.*, 1991, J Med Chem 34:98-107, Sugasawa, *et al.*, 1976, J Med Chem 19:675-9].

Said substances are known to be therapeutically useful as described, e.g., in colorectal cancer, non-small cell and small cell lung cancer, oesophageal cancer, renal cell carcinoma, ovarian cancer, breast cancer, pancreatic cancer, squamous cell cancer, leukemias and lymphomas [Kawato, *et al.*, 1991, Cancer Res 51:4187-91, Furuta, *et al.*, 1988, Gan To Kagaku Ryoho 15:2757-60, Hawkins, 1992, Oncology (Huntingt) 6:17-23, Slichenmyer, *et al.*, 1993, J Natl Cancer Inst 85:271-91, Slichenmyer, *et al.*, 1994, Cancer Chemother Pharmacol 34:S53-7, Tsuruo, *et al.*, 1988, Cancer Chemother Pharmacol 21:71-4, Wiseman, *et al.*, 1996, Drugs 52:606-23, Gottlieb, *et al.*, 1970, Cancer Chemother Rep 54:461-70, Negoro, *et al.*, 1991, J Natl Cancer Inst 83:1164-8, Rowinsky, *et al.*, 1994, Cancer Res 54:427-36]. Also encompassed by the use of the present invention are derivatives of those substances which are obtainable by way of any chemical modification, wherein said derivatives are equally well therapeutically suited for the use of the present invention. To determine whether a derivative of the substances of the invention is equally well therapeutically suited for the use of the invention biological assays well known in the art can be performed. Such assays are described , e.g., in [Kawato, *et al.*, 1991, Cancer Res 51:4187-91, Furuta, *et al.*, 1988, Gan To Kagaku Ryoho 15:2757-60, Giovanella, *et al.*, 1989, Science 246:1046-8, Giovanella, *et al.*, 1991, Cancer Res 51:3052-5, Kunimoto, *et al.*, 1987, Cancer Res 47:5944-7, Mattern, *et al.*, 1993, Oncol Res 5:467-74, Tsuruo, *et al.*, 1988, Cancer Chemother Pharmacol 21:71-4, Burris, *et al.*, 1992, J Natl Cancer Inst 84:1816-20, Friedman, *et al.*, 1994, Cancer Chemother Pharmacol 34:171-4].

It is contemplated that any of the compounds described in the above publications may be used in this invention.

It has been show that irinotecan is particularly well suited for the treatment of colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer,

and pancreatic cancer. Thus, most preferably the substance used according to the present invention is irinotecan.

The term "pharmaceutical composition" as used herein comprises the substances of the present invention and optionally one or more pharmaceutically acceptable carrier. The substances of the present invention may be formulated as pharmaceutically acceptable salts. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The pharmaceutical compositions can be conveniently administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The substances may be administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax. The substance according to the present invention can be administered in various manners to achieve the desired effect. Said substance can be administered either alone or in the formulated as pharmaceutical preparations to the subject being treated either orally, topically, parenterally or by inhalation. Moreover, the substance can be administered in combination with other substances either in a common pharmaceutical composition or as separated pharmaceutical compositions.

The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. A therapeutically effective dose refers to that amount of the substance according to the invention which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

A typical dose can be, for example, in the range of 5 to 100 mg however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 μg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1 μg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. However, depending on the subject and the mode of administration, the quantity of substance administration may vary over a wide range to provide from about 1 mg per m^2 body surface to about 500 mg per m^2 body surface, usually 20 to 200 mg per m^2 body surface.

The pharmaceutical compositions and formulations referred to herein are administered at least once in accordance with the use of the present invention. However, the said pharmaceutical compositions and formulations may be administered more than one time, for example once weekly every other week up to a non-li mited number of weeks.

Specific formulations of the substance according to the invention are prepared in a manner well known in the pharmaceutical art and usually comprise at least one active substance referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent thereof. For making those formulations the active substance(s) will usually be mixed with a carrier or diluted by a diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. A carrier may be solid, semisolid, gel-based or liquid material which serves as a vehicle, excipient or medium for the active ingredients. Said suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington´s Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania. The formulations can be adopted to the mode of administration comprising the forms of tablets, capsules, suppositories, solutions, suspensions or the like.

The dosing recommendations will be indicated in product labeling by allowing the prescriber to anticipate dose adjustments depending on the considered patient group, with information that avoids prescribing the wrong drug to the wrong patients at the wrong dose.

The term "treating" means alleviation of the disease symptomes, i.e. regression of symptomes or inhibited progression of such symptomes, in subjects or disease populations which have been treated. Said alleviation of disease can be monitored by the degree of the clinical symptomes (e.g. tumor size) accompanied with the disease. While the invention may not be effective in 100% of patients treated, it is effective in treating statistically significant (p value equal or less than 0.05) number of patients. Whether said number of subjects is significant can be determined by statistical tests such as the Student´s t-test, the chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis-test (H-Test), Jonckheere-Terpstra-test or the Wilcoxon-test.

The present invention also encompasses all embodiments described in connection with pharmaceutical compositions in US patents US05106742, US05340817, US05364858, US05401747, US05468754, US05559235 and US05663177.

The terms "colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer" comprise diseases and dysregulations related to cancer. Preferred diseases encompassed by the use of the present invention are colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer. Said diseases and dysregulations are well known in the art and the accompanied symptoms are described, e.g., in standard text books such as Stedman.

The term "subject" as used in the sense of the present invention comprises animals, preferably those specified herein after, and humans.

The term " variant allele" as used herein refers to a polynucleotide comprising one or more of the polynucleotides described herein below corresponding to a CYP3A5 gene. Each individual subject carries at least two alleles of the CYP3A5 gene, wherein said alleles are distinguishable or identical. In accordance with the use of the present invention a variant allele comprises at least one or more of the polynucleotides specified herein below. Said polynucleotides may have a synergistic influence on the regulation or function of the first variant allele. Preferably, a variant allele in accordance with the use of the present invention comprises at least two of the polynucleotides specified herein.

In the context of the present invention the term "polynucleotides" or "polypeptides" refers to different variants of a polynucleotide or a polypeptide specified in accordance with the uses of the present invention. Said variants comprise a reference or wild type sequence of the polynucleotides or polypeptides specified herein as well as variants which differ therefrom in structure or composition. Reference or wild type sequences for the polynucleotides and polypeptides have been defined by Genbank accession numbers above. The differences in structure or composition usually occur by way of nucleotide or amino acid substitution(s), addition(s) and/or deletion(s).

Preferably, said nucleotide substitution(s), addition(s) or deletion(s) referred to in accordance with the use of the present invention result(s) in one or more changes of

the corresponding amino acid(s) of the polynucleotide. The variant polynucleotides also comprise fragments of said polynucleotides. The polynucleotides as well as the aforementioned fragments thereof are characterized as being associated with a CYP3A5 dysfunction or dysregulation comprising, e.g., insufficient and/or altered drug metabolism and protein expression level.

The present invention also encompasses all embodiments described in connection with polynucleotides in WO9957322, WO0109183 or US5786344.

The term "hybridizing" as used herein refers to polynucleotides which are capable of hybridizing to the above polynucleotides or parts thereof which are associated with a CYP3A5 dysfunction or dysregulation. Thus, said hybridizing polynucleotides are also associated with said dysfunctions and dysregulations. Preferably, said polynucleotides capable of hybridizing to the aforementioned polynucleotides or parts thereof which are associated with CYP3A5 dysfunctions or dysregulations are at least 70%, at least 80%, at least 95% or at least 100% identical to the polynucleotides or parts thereof which are associated with CYP3A5 dysfunctions or dysregulations. Therefore, said polynucleotides may be useful as probes in Northern or Southern Blot analysis of RNA or DNA preparations, respectively, or can be used as oligonucleotide primers in PCR analysis dependent on their respective size. Also comprised in accordance with the use of the invention are hybridizing polynucleotides which are useful for analyzing DNA-Protein interactions via, e.g., electrophoretic mobility shift analysis (EMSA). Preferably, said hybridizing polynucleotides comprise at least 10, more preferably at least 15 nucleotides in length while a hybridizing polynucleotide to be used as a probe preferably comprises at least 100, more preferably at least 200, or most preferably at least 500 nucleotides in length.

It is well known in the art how to perform hybridization experiments with nucleic acid molecules, i.e. the person skilled in the art knows what hybridization conditions s/he has to use in accordance with the present invention. Such hybridization conditions are referred to in standard text books, such as Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. Preferred in accordance with the use of the present inventions are polynucleotides which are capable of hybridizing to the above

polynucleotides or parts thereof which are associated with a CYP3A5 dysfunction or dysregulation under stringent hybridization conditions, i.e. which do not cross hybridize to unrelated polynucleotides such as polynucleotides encoding a polypeptide different from the CYP3A5 polypeptides of the invention.

Moreover, methods for determining whether a subject comprises a polynucleotide referred to herein above are well known in the art. To carry out said methods, it might be necessary to take a sample comprising biological material, such as isolated cells or tissue, from said subject. Further, the methods known in the art could comprise for example, PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphidm (SSCP), denaturating gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analyis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques. A preferred and convenient method to be used in order to determine the presence or absence of one or more of the above specified polynucleotides is to isolate blood cells from a subject and to perform a PCR based assay on genomic DNA isolated from those blood cells, whereby the PCR is used to determine whether said polynucleotides specified herein above or parts thereof are present or absent. Said method is described in more detail below and in the Examples.

The term "corresponding" as used herein means that a position is not only determined by the number of the preceding nucleotides and amino acids, respectively. The position of a given nucleotide or amino acid in accordance with the use of the present invention which may be deleted, substituted or comprise one or more additional nucleotide(s) may vary due to deletions or additional nucleotides or amino acids elsewhere in the gene or the polypeptide. Thus, under a "corresponding position" in accordance with the present invention it is to be understood that nucleotides or amino acids may differ in the indicated number but may still have similar neighboring nucleotides or amino acids. Said nucleotides or amino acids which may be exchanged, deleted or comprise additional nucleotides or amino acids are also comprised by the term "corresponding position". Said nucleotides or amino acids may for instance together with their neighbors form sequences which may be involved in the regulation

of gene expression, stability of the corresponding RNA or RNA editing, as well as encode functional domains or motifs of the protein of the invention.

By, e.g., "position 17970 to 17970" it is meant that said polynucleotide comprises one or more deleted nucleotides which are deleted between positions 17970 and position 17970 of the corresponding wild type version of said polynucleotide. The same applies mutatis mutandis to all other position numbers referred to in the above embodiment which are drafted in the same format.

By, e.g., "position 1222/1223" it is meant that said polynucleotide comprises one or more additional nucleotide(s) which are inserted between positions 1222 and position 1223 of the corresponding wild type version of said polynucleotide. The same applies mutatis mutandis to all other position numbers referred to in the above embodiment which are drafted in the same format, i.e. two consecutive position numbers separated by a slash (/).

In accordance with the present invention, the mode and population distribution of genetic variations in the CYP3A5 gene - the different alleles of the CYP3A5 gene - have been analyzed by sequence analysis of relevant regions of the human said gene from many different individuals. It is a well known fact that genomic DNA of individuals, which harbor the individual genetic makeup of all genes, including the CYP3A51 gene, can easily be purified from individual blood samples. These individual DNA samples are then used for the analysis of the sequence composition of the alleles of the CYP3A5 gene that are present in the individual which provided the blood sample. The sequence analysis was carried out by PCR amplification of relevant regions of said genes, subsequent purification of the PCR products, followed by automated DNA sequencing with established methods (e.g. ABI dyeterminator cycle sequencing).
One important parameter that has to be considered in the attempt to determine the individual genotypes and identify novel variants of the CYP3A5 gene by direct DNA-sequencing of PCR-products from human blood genomic DNA is the fact that each human harbors (usually, with very few abnormal exceptions) two gene copies of each autosomal gene (diploidy). Because of that, great care has to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous

sequence variations but also heterozygous variations. The details of the different steps in the identification and characterization of the polymorphisms in the CYP3A5 gene (homozygous and heterozygous) are described in the Examples below.

Over the past 20 years, genetic heterogeneity has been increasingly recognized as a significant source of variation in drug response. Many scientific communications (Meyer, Ann. Rev. Pharmacol. Toxicol. 37 (1997), 269-296 and West, J. Clin. Pharmacol. 37 (1997), 635-648) have clearly shown that some drugs work better in some patients than in others or may even be highly toxic and that such variations in patients´ responses to drugs can be correlated to a molecular basis. This "pharmacogenomic" concept spots correlations between responses to drugs and genetic profiles of patient's (Marshall, Nature Biotechnology, 15 (1997), 954-957; Marshall, Nature Biotechnology, 15 (1997), 1249-1252). In this context of population variability with regard to drug therapy, pharmacogenomics has been proposed as a tool useful in the identification and selection of patients which can respond to a particular drug without side effects. This identification/selection can be based upon molecular diagnosis of genetic polymorphisms by genotyping DNA from leukocytes in the blood of a patient, for example, and characterization of disease (Bertz, Clin. Pharmacokinet. 32 (1997), 210-256; Engel, J. Chromatogra. B. Biomed. Appl. 678 (1996), 93-103). For the founders of health care, such as health maintenance organizations in the US and government public health services in many European countries, this pharmacogenomics approach can represent a way of both improving health care and reducing costs related to health care caused by the development of unnecessary drugs, by ineffective drugs and by side effects due to drug administration.

The mutations in the CYP3A5 gene detected in accordance with the present invention are listed in Tables 1. As is evident to the person skilled in the art, the genetic knowledge of the polynucleotides specified herein above can be used to exactly and reliably characterize the genotype of a patient.

Advantageously, preventive or therapeutical measures which are based on irinotecan or a derivative thereof can be more efficiently applied when taking into consideration

said genetic knowledge. Undesirable side effects of said substances can be avoided and an effective but not harmful dosage can be calculated individually due the knowledge of the genetic makeup of the subject. Moreover in accordance with the foregoing, in cases where a given drug causes an unusual effect, a suitable individual therapy can be designed based on the knowledge of the individual genetic makeup of a subject. This tailored therapy will also be suitable to avoid the occurance of therapy resistances. Said resistances are one major problem in cancer chemotherapy with various chemotherapeutic agents, this fact being well known in the art. The use of the present invention, therefore, provides an improvement of the therapeutic applications which are based on the known therapeutically desirable effects of the substances referred to herein above since it is possible to individually treat the subject with an appropriate dosage and/or an appropriate derivative of said substances. Thereby, undesirable, harmful or toxic effects are efficiently avoided. Furthermore, the use of the present invention provides an improvement of the therapeutic applications which are based on the known therapeutically desirable effects of the substances referred to herein above since it is possible to identify those subject prior to onset of drug therapy and treat only those subjects with an appropriate dosage and/or an appropriate derivative of said substances who are most likely to benefit from therapy with said substances. Thereby, the unnecessary and potentially harmful treatment of those subjects who do not respond to the treatment with said substances (nonresponders), as well as the development of drug resistances due to suboptimal drug dosing can be avoided.

In a preferred embodiment of the use of the present invention said variant allele comprises a polynucleotide selected from the group consisting of:

(a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 137, 141, 145 or 149:

(b) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 47518 or 9736 of the CYP3A5 gene (Accession No: GI:10281451) or 145601 or 145929 of the CYP3A5 gene (Accession No: GI:11177452);

(c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a C at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) or a G at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451), or 145601 or 145929 of the CYP3A5 gene (Accession No: GI:11177452).

More preferably, said variant allele comprises a polynucleotide selected from the group consisting of:

(a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 137, 145 and/or 149;

(b) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 47518 or 9736 of the CYP3A5 gene (Accession No: GI:10281451) or 145929 of the CYP3A5 gene (Accession No: GI:11177452);

(c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a C at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) or a G at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451), or 145929 of the CYP3A5 gene (Accession No: GI:11177452).

The present invention also relates to a method of treating colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer comprising:

(a) determining the presence or absence of a variant allele comprising a polynucleotide referred to herein; and

(b) administering to a subject a therapeutically effective dosage of irinotecan.

The definitions used in accordance with the use of the present invention apply mutatis mutandis to the above method. Further, all embodiments described in accordance with the use of the present invention can be applied mutatis mutandis to the method of the

present invention. Moreover, also encompassed by the method of the present invention are any further developments of said method which the person skilled in the art can make without undue burden based on its knowledge and the prior art, such as those documents referred to throughout this specification.

In a preferred embodiment of the use of the present invention a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of the variant allele compared to the corresponding wild type allele.

As discussed above, the alleles referred to in accordance with the use of the present invention correspond to the CYP3A5 gene. It is well known in the art that genes comprise structural elements which encode an amino acid sequence as well as regulatory elements which are involved in the regulation of the expression of said genes. Structural elements are represented by exons which may either encode an amino acid sequence or which may code for RNA which is not encoding an amino acid sequence but is nevertheless involved in RNA function, e.g. by regulating the stability of the RNA or the nuclear export of the RNA.

Regulatory elements of a gene may comprise promoter elements or enhancer elements both of which could be involved in transcriptional control of gene expression. It is very well known in the art that a promoter is to be found upstream of the structural elements of a gene. Regulatory elements such as enhancer elements, however, can be found distributed over the entire locus of a gene. Said elements could reside, e.g., in introns, regions of genomic DNA which separate the exons of a gene. Promoter or enhancer elements correspond to polynucleotide fragments which are capable of attracting or binding polypeptides involved in the regulation of the gene comprising said promoter or enhancer elements. For example, polypeptides involved in regulation of said gene comprise the so called transcription factors.

Said introns may comprise further regulatory elements which are required for proper gene expression. Introns are usually transcribed together with the exons of a gene resulting in a nascent RNA transcript which contains both, exon and intron sequences. The intron encoded RNA sequences are usually removed by a process known as RNA splicing. However, said process also requires regulatory sequences present on a RNA transcript said regulatory sequences may be encoded by the introns.

In addition, besides their function in transcriptional control and control of proper RNA processing and/or stability, regulatory elements of a gene could be also involved in the control of genetic stability of a gene locus. Said elements control, e.g., recombination events or serve to maintain a certain structure of the DNA or the arrangement of DNA in a chromosome.

Therefore, single nucleotide polymorphisms can occur in exons of an allele of a gene which encode an amino acid sequence as discussed supra as well as in regulatory regions which are involved in the above discussed process. The polymorphisms comprised by the polynucleotides referred to in accordance with the use of the present invention can influence the expression level of CYP3A5A1 protein via mechanisms involving enhanced or reduced transcription of the CYP3A5 gene, stabilization of the gene's RNA transcripts and alteration of the processing of the primary RNA transcripts. Methods for the determination of an altered expression of a variant allele when compared to its wild type counterpart are well known in the art and comprise inter alia those referred to herein above, e.g., PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphism (SSCP), denaturating gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analysis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques. It might be necessary to obtain a sample comprising biological material, such as isolated cells or tissue from the subject prior to perform said methods for determination of the expression levels of the wild type and the variant alleles, respectively. An altered expression in accordance with the use of the present invention means that the expression of the wild type allele differs significantly from the expression of the variant allele. A significant difference can be determined by standard statistical methods, such as Student´s t-test, chi^2-test or the U-test according to Mann and Whitney. Moreover, the person skilled in the art can adopt these and other statistical method known in the art individually without an undue burden.

In a more preferred embodiment of the use of the invention said altered expression is due to an alteration of the processing of the primary RNA transcripts.

To determine whether the expression of an allele referred to in accordance to the present invention is increased or decreased in comparison to the corresponding wild type allele well known methods such as PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphism (SSCP), denaturating gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analysis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques can be applied. As discussed above, it might be necessary to obtain a sample comprising cells or tissue from the subject in order to determine the expression level of the variant allele referred to in the use of the invention. A decrease or increase of the expression is characterized by a significant difference in the expression level of the variant versus the wild type allele in those assays. Also encompassed by decreased expression is the absence detectable expression of a variant allele.

As discussed supra, the variant alleles comprising those polynucleotides specified herein which correspond to noncoding regions of the CYP3A5 gene that have an influence on the expression level of the polypeptides encoded by said variant alleles. The CYP3A5 protein, therefore, exhibit increased biological and/or immunological properties compared to those subjects with the corresponding wild type counterpart. It might be necessary to obtain a sample comprising biological material such as isolated cells or tissue from the subject prior to perform said methods for determination of the protein level and/or activities of the wild type and the variant polypeptides, respectively. Whether a variant polypeptide has an altered activity or level of expression compared to its wild type corresponding counterpart can be determined by standard techniques well known in the art. Such standard techniques may comprise, e.g., ELISA based assays, RIA based assays, HPLC-based assays, mass spectroscopy-based assays, western blot analysis or assays which are known in the art and described in [Janardan, *et al.*, 1996, Pharmacogenetics 6:379-85, Kivisto, *et al.*, 1996, Br J Clin Pharmacol 42:387-9, Lown, *et al.*, 1994, Drug Metab Dispos 22:947-55, Anttila, *et al.*, 1997, Am J Respir Cell Mol Biol 16:242-9, Tateishi, *et al.*, 1999, Biochem Pharmacol 57:935-9,

Gibbs, *et al.*, 1999, Drug Metab Dispos 27:180-7, Maenpaa, *et al.*, 1998, Pharmacogenetics 8:137-55, Haehner, *et al.*, 1996, Mol Pharmacol 50:52-9, Lown, *et al.*, 1994, Drug Metab Dispos 22:947-55] for CYP3A5.

An altered expression in accordance with the use of the present invention means that the protein level of the CYP3A5 gene differs significantly in subjects with polynucleotides as described in the present invention. A significant difference can be determined by standard statistical methods referred to herein above.

Moreover, in a further preferred embodiment of the use of the present invention said subject is an animal.
As described supra, the subject in accordance with the use of the present invention encompasses animals. The term "animal" as used herein encompasses all animals, preferably animals belonging to the vertebrate family, more preferably mammals. Moreover, the animals can be genetically engineered by well known techniques comprising transgenesis and homologous recombination in order to incorporate one or more of the polynucleotides referred to supra into the genome of said animals. Said animals comprising the genetically engineered animals can be used to study the pharmacological effects of drugs or pro-drugs which are based on the substances or derivatives thereof referred to herein, preferably irinotecan.

In accordance with the foregoing, most preferably, said animal is a mouse or rat.
Said animals are particularly well suited for assaying the pharmacological properties of the substances or derivatives referred to in accordance with the use of the present invention as described in detail in Giovanella, *et al.*, 1991, Cancer Res 51:3052-5, Kunimoto, *et al.*, 1987, Cancer Res 47:5944-7, Kaneda, *et al.*, 1990, Cancer Res 50:1715-20.
Preferably, said mouse is lacking functional cytochrome P450, MRP1, or MDR1. It is well known in the art how said mice lacking functional cytochrome P450, MRP1 or MDR1 can be obtained. For instance said mice might be generated by homologous recombination as described for cytochrome P450 in Pineau, *et al.*, 1998, Toxicol Lett

103:459-64, MRP1 in Rappa, *et al.*, 2000, Biochemistry 39:3304-10, and MDR1 in Schinkel, 1998, Int J Clin Pharmacol Ther 36:9-13, Schinkel, *et al.*, 2000, Pharmacogenetics 10:583-90.

Moreover, in another preferred embodiment of the use of the present invention said subject is a human.

In particular, the present invention is applicable to humans as is evident from the above. The use of the present invention is to be applied in order to treat or prevent side effects in patients which suffer from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer. The pharmacological effects of the above substances or derivatives thereof are well described in humans. However, the conventional therapies do not take into account the individual genetic makeup of the patient. Ethnical populations have different genetic backgrounds, which can also influence the function or regulation of a variant allele and thereby alter the pharmacological response of a patient to a substance or derivative used as a basis for a drug or pro-drug in accordance with the invention.

In light of the foregoing, most carefully, said human is selected from the African population who shows compared to Caucasians (approx. 10 %) a higher frequency (approx. 40%) of the CYP3A5 high expresser allele (nucleotide C at a position corresponding to position 47518 of the CYP3A5 GenBank accession No. GI: 10281451, nucleotide G at a position corresponding to position 145929 of the CYP3A5 gene, GenBank accession No. GI: 11177452 and 9736 of the CYP3A5 gene, GenBank accession No. 10281451) and are therefore more likely to altered metabolism of drugs such as irinotecan.

In light of the foregoing, most preferably, said human is African or Asian.

The present invention also relates to a method for selecting a suitable therapy for a subject suffering from cancer, especially, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer, wherein said method comprises:

(a) determining the presence or absence of a variant allele referred to above in the genome of a subject in a sample obtained from said subject; and

(b) selecting a suitable therapy for said subject based on the results obtained in (a).

The definitions and explanations of the terms made above apply mutatis mutandis to the above method.

The term "suitable therapy" as used herein means that a substance according to the invention is selected and said substance being administered in a certain dosage to a subject, wherein said substance and said dosage are selected based on the knowledge of the presence or absence of a first, second, third and/or fourth variant allele referred to in accordance with the use of the invention. Said substance and said dosage of the substance are selected in a way that on one hand they are most effective in treating cancer, especially, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer on the other hand they do not cause toxic or undesirable side effects.

As is evident from the above, a prerequisite for selecting a suitable therapy is the knowledge of the presence or absence of a variant allele referred to in accordance with the use of the invention. Therefore, the method of the present invention encompasses the determination of the presence or absence of said variant alleles in a sample which has been obtained from said subject. The sample which is obtained by the subject comprises biological material which is suitable for the determination of the presence or absence of said variant alleles, such as isolated cells or tissue. Methods for the determination of the presence or absence of the variant alleles of the method of the invention comprise those methods referred to herein above.

Thanks to the method of the present invention, it is possible to efficiently select a suitable therapy for a subject, preferably a human, suffering from cancer, especially, colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer Thereby, mistreatment of patients based on wrong medications and the results thereof, such as development of resistance towards cancer therapy, and subsequent increased costs in health care, can be efficiently avoided. Furthermore, patients that are at high risk can be excluded from therapy prior to the first dose and/or dosage can be adjusted according to the individual's genetic

makeup prior to the onset of drug therapy. Also, inhibitors for the mentioned metabolizing genes (e.g. CYP3A5) can be applied in genetically defined patient subpopulations. Thus, adverse effects can be avoided and the optimal drug level can be reached faster without time-consuming and expensive drug monitoring-based dose finding. This can reduce costs of medical treatment and indirect costs of disease (e.g. shorter time and less frequent hospitalization of patients).

The following 24 items are also encompassed by the present invention. The definitions and explanations made supra apply mutatis mutandis to the terms used to characterize the claims.

1. A method of using irinotecan to treat a patient suffering from cancer which comprises:
   (a) determining if the patient has one or more variant alleles of the CYP3A5 gene;
   (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene.

2. The method of item 1 wherein the cancer is colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, or pancreatic cancer.

3. The method of item 2 in which:
   (a) the one or more variant alleles result in the patient expressing low amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is decreased to avoid toxicity; or
   (b) the one or more variant alleles result in the patient expressing high amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is increased to enhance efficacy.

4. The method of item 3 wherein the one or more variant alleles are in the promoter region of the CYP3A5 gene.

5. The method of item 3 wherein the one or more variant alleles are in the coding region of the CYP3A5 gene.

6. The method of item 3 wherein the one or more variant alleles are not in either the promoter region or the coding region of the CYP3A5 gene.

7. The method of item 3 wherein the one or more variant alleles are in both the promoter region and the coding region of the CYP3A5 gene.

8. The method of item 3 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:

(a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 137, 138, 141, 142, 145, 146, 149 and/or 150;

(b) a polynucleotide capable of hybridizing to a Cytochrome P450, subfamily IIIA (nifedipine oxidase), polypeptide 5 (CYP3A5) gene, wherein said polynucleotide is having at a position corresponding to positions 47518 and/or 9736 of the CYP3A5 gene (Accession No: GI:10281451), a substitution of at least one nucleotide or at a position corresponding to positions 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452), a substitution of at least one nucleotide;

(c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) a C, at a position corresponding to position 145601 and/or 145929 of the CYP3A5 gene (Accession No:

GI:11177452) a G or at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451) a G.

9. The method of item 8 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:
   (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 137, 141, 145 or 149:
   (b) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 47518 or 9736 of the CYP3A5 gene (Accession No: GI:10281451) or 145601 or 145929 of the CYP3A5 gene (Accession No: GI:11177452);
   (c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a C at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) or a G at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451), or 145601 or 145929 of the CYP3A5 gene (Accession No: GI:11177452).
10. The method of item 8 in which the one or more variant alleles results in the patient expressing low amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is decreased.

11. The method of item 8 in which the one or more variant alleles results in the patient expressing high amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is increased.

12. The method of item 9 in which the one or more variant alleles results in the patient expressing low amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is decreased.

13. The method of item 9 in which the one or more variant alleles results in the patient expressing high amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is increased.

14. A method for determining whether a patient is at risk for a toxic reaction to treatment with irinotecan which comprises determining if the patient has one or more variant alleles of the CYP3A5 gene.

15. The method of item 14 which further comprises administering to the patient reduced amounts of irinotecan if the patient has one or more variant alleles that result in decreased expression of the CYP3A5 gene.

16. A method for determining the optimum treatment regimen for administering irinotecan to a patient suffering from cancer which comprises:
    (a) determining if the patient has one or more variant alleles of the CYP3A5 gene;
    (b) in a patient having one or more of such alleles increasing or decreasing the amount of irinotecan in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene.

17. A method of treating cancer in a patient having one or more variant alleles of the CYP3A5 gene such that expression levels of the CYP3A5 gene product are lower than in the general population and so indicates high sensitivity to irinotecan which comprises administering to the patient a decreased amount of irinotecan.

18. A method of treating cancer in a patient having one or more variant alleles of the CYP3A5 gene such that expression levels of the CYP3A5 gene product are higher than in the and so indicates resistance or predisposition to resistance to irinotecan which comprises administering to the patient an increased amount of irinotecan.

19. A method of treating cancer in a patient which comprises internally administering to the patient an effective amount of irinotecan, wherein the treatment regimen is modified based upon the genotype of the patient's CYP3A5 gene.

20. A method of treating a population of patients suffering from cancer which comprises:
    (a) determining, on a patient by patient basis, if the patient has one or more variant alleles of the CYP3A5 gene;
    (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene.

21. A method for predicting sensitivity to irinotecan in a patient suffering from cancer which comprises determining if the patient has one or more variant alleles of the CYP3A5 gene, which alleles indicate that the cancerous cells express low or high amounts of the CYP3A5 gene product, whereby low expression indicates high sensitivity to irinotecan and high expression indicates resistance or predisposition to resistance to irinotecan.

22. The method of item 21 in which patients that have a genotype that indicates resistance or predisposition to resistance are treated with a CYP3A5 inhibitor.

23. The method of item 22 wherein the CYP3A5 inhibitor is selected from the group consisting of: Clarithromycin, Erythromycin, Diltiazem, Mibefradil, grapefuit juice, Cimetidine, Ciprofloxacin, Norfloxacin, Fluconazole, Itraconazole, Ketoconazole, Fluvoxamine, Norfluoxetine, Nefazodone, Troleandomycin, Delaviridine, Indinavir, Nelfinavir, Ritonavir, Saquinavir, Mifepristone, and gestodene

24. The method of item 21 wherein the patients that have a genotype that indicates resistance or predisposition to resistance are monitored during treatment by assaying for expression levels of the CYP3A5 gene product in the cancerous cells.

The decreased expression as referred to herein above includes in addition to a significantly decreased amount of transcripts encoding a functional gene product also a normal or even elevated amount of transcripts encoding a gene product which has no activity or a significantly decreased activity.

By "in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene" a standard dose is meant which is routinely administered to patients in need thereof without regarding the genotype. Such a general population of patients is considered as having the normal genotype, i.e. wildtype genotype.

Further, the present invention encompasses a method for improving and/or modifying a therapy comprising determining the expression level of CYP3A5, hereinafter referred to as expression profile or the protein level of the CYP3A5 protein, hereinafter referred to as the protein profile, or the activity level of the said protein, hereinafter referred to as the activity profile.

The term "expression level" as referred to in the context of the present invention means the detectable amount of transcripts of the CYP3A5 gene relative to the amount of transcripts for a housekeeping gene, such as PLA2. The amount of transcripts can be determined by standard molecular biology techniques including Northern analysis, RNAse protection assays, PCR based techniques encompassing Taq-Man analysis. Preferably, the determination can be carried out as described in the accompanied Examples 4 and 5. The term "expression profile" means that the expression level of the CYP3A5 gene is determined and the expression level is compared to a reference standard. As a reference standard, preferably transcripts are obtained from cells or tissues of a subject having the aforementioned wildtype alleles of the respective genes in their genomes.

The term "protein level" refers to the detectable amount of CYP3A5 relative to the amount of a protein encoded by a housekeeping gene, such as PLA2. The amount of

proteins can be determined by standard biochemical techniques, such as Western analysis, ELISA, RIA or other antibody based techniques known in the art. The term "protein profile" means that the protein level of a panel of the aforementioned proteins is determined and the protein levels are compared to a reference standard. As a reference standard, preferably proteins are obtained from cells or tissues of a subject having the aforementioned wildtype alleles of the respective gene in their genomes.

The term "activity level" means the detectable biological activity of CYP3A5 relative to the activity or amount of a encoded by the allellic variants of these gene as disclosed in the present invention relative to the activity of the protein encoded by the corresponding wild-type allele of the gene. Biological assays for the aforementioned proteins are well known in the art and described in Gorski *et al.*, 1994, Biochemical Pharmacoloy 40:1643-53. As a reference standard, preferably proteins are obtained from cells or tissues of a subject having the aforementioned wildtype alleles of the respective genes in their genomes.

The aforementioned methods, preferably, comprise the steps (i) obtaining a tumor sample from a patient during specific stages of a tumor therapy; and (ii) determining the expression profile, protein profile or activity profile for CYP3A5. Based on the expression profiles a clinician can efficiently adapt the therapy. This comprises inter alia dosage adjustment and/or including administration of an CYP3A5 inhibitor. Preferably, said inhibitor is selected from the following group of inhibitors: Clarithromycin, Erythromycin, Diltiazem, Mibefradil, grapefuit juice, Cimetidine, Ciprofloxacin, Norfloxacin, Fluconazole, Itraconazole, Ketoconazole, Fluvoxamine, Norfluoxetine, Nefazodone, Troleandomycin, Delaviridine, Indinavir, Nelfinavir, Ritonavir, Saquinavir, Mifepristone, gestodene (http://medicine.iupui.edu/flockhart).

The term inhibitor as used herein encompasses competitive and non-competitive inhibitors. Preferably competitive inhibitors are substrates such as (GF120918, LY335979, XR 9576, XR 9051, flavonoids). Preferably non-competitive inhibitors are substrates such as (SDZ PSC 833, SDZ 280-446, B669, B-859-35, Verapamil, MS-209, PAK-104p).

Finally, the present invention encompasses a method for determining whether a patient has developed a resistance against the drugs referred to in the context of the

present invention. Said method comprising the steps of (i) obtaining a tumor sample from a patient during specific stages of a tumor therapy; and (ii) determining the expression level of CYP3A5. The expression of the respective genes can be determined as described in Examples 4 and 5 or as described above. Based on the evaluation of said expression profile, a clinician can more efficiently adapt the therapy. This comprises inter alia dosage adjustment and/or including administration of a CYP3A5 inhibitor as defined supra.

Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference.

The nucleic acid and amino acid sequences referred to in this application by sequence identification numbers (SEQ ID NOs.) are listed in the following Tables 1. For positions of polymorphic nucleotides, the following substitute letters are used in the nucleic acid sequences: R, G or A; Y, T or C; M, A or C; K, G or T; S, G or C; W, A or T.

Amino acid sequences are shown in the one letter code. The letter X at polymorphic amino acid positions represents the modified amino acid or its corresponding wild type amino acid (see accession numbers).

Moreover, all nucleic acid and amino acid sequences referred to herein by making reference to GenBank accession numbers are shown in Figures 4 to 29 below.

Table 1: The nucleic acid and amino acid sequences referred to in this application

Gene	Variation	SNP	Acc.no.	SEQ ID No	Sequence forward	SEQ ID NO	Sequence reverse	SEQ ID NO	Sequence wt>mut forward	SEQ ID NO	Sequence wt>mut reverse
UGT1A1	T>G	59	GI:8850235	001	GTCCTGGGCC**G** GCTGCTGTGT	002	ACACAGCAGC**C** GGCCCAGGAC	003	GTCCTGGGCC**K** GCTGCTGTGT	004	ACACAGCAGC**M** GGCCCAGGAC
UGT1A1	C>T	160	GI:8850235	005	GGCCATCCAG**T** AGCTGCAGCA	006	TGCTGCAGCT**A** CTGGATGGCC	007	GGCCATCCAG**Y** AGCTGCAGCA	008	TGCTGCAGCT**R** AGCTGCAGCA
UGT1A1	G>A	226	GI:8850235	009	CATCAGAGAC**A** GAGCATTTTA	010	TAAAATGCTC**T**G TCTCTGATG	011	CATCAGAGAC**R** GAGCATTTTA	012	TAAAATGCTC**Y**G TCTCTGATG
UGT1A1	T>A	539	GI:8850235	013	TTGCATGCAC**A** GCCATGCAGC	014	GCTGCATGGC**T** GTGCATGCAA	015	TTGCATGCAC**W** GCCATGCAGC	016	GCTGCATGGC**K** GTGCATGCAA
UGT1A1	T>C	544	GI:8850235	017	TGCACTGCCA**C** GCAGCCTGGA	018	TCCAGGCTGC**G** GTGCATGCAA	019	TGCACTGCCA**Y** GCAGCCTGGA	020	TCCAGGCTGC**R** GTGCATGCAA
UGT1A1	C>T	640	GI:8850235	021	CTTCCTGCAG**T** GGGTGAAGAA	022	TTCTTCACCC**A**C TGCAGGAAG	023	CTTCCTGCAG**Y** GGGTGAAGAA	024	TTCTTCACCC**R**C TGCAGGAAG

UGT1A1	C>A	701	GI:8850235	025	GTTTATTCCC**A**G	026	GGTTGCATAC**T**	027	GTTTATTCCC**M**	028	GGTTGCATAC**K**
					TATGCAACC		GGGAATAAAC		GTATGCAACC		GGGAATAAAC
UGT1A1	G>C	841	GI:8850235	029	GGTTTTTGTT**C**G	030	TTGATTCCAC**G**A	031	GGTTTTTGTT**S**G	032	TTGATTCCAC**S**A
					TGGAATCAA		ACAAAAACC		TGGAATCAA		ACAAAAACC
UGT1A1	C>A	855	GI:8850235	033	GAATCAACTG**A**	034	TTTGGTGAAG**T**	035	GAATCAACTG**M**	036	TTTGGTGAAG**K**
					CTTCACCAAA		CAGTTGATTC		CTTCACCAAA		CAGTTGATTC
UGT1A1	C>T	890	GI:8850235	037	GAATTTGAAG**T**C	038	ATTAATGTAG**A**C	039	GAATTTGAAG**Y**	040	ATTAATGTAG**R**C
					TACATTAAT		TTCAAATTC		CTACATTAAT		TTCAAATTC
UGT1A1	G>A	938	GI:8850235	041	TTCTCTTTGG**A**A	042	GACCATTGAT**T**C	043	TTCTCTTTGG**R**A	044	GACCATTGAT**Y**
					TCAATGGTC		CAAAGAGAA		TCAATGGTC		CCAAAGAGAA
UGT1A1	C>T	1006	GI:8850235	045	CAAAATCCCT**T**A	046	AGGACTGTCT**A**	047	CAAAATCCCT**Y**A	048	AGGACTGTCT**R**
					GACAGTCCT		AGGGATTTTG		GACAGTCCT		AGGGATTTTG
UGT1A1	A>G	1007	GI:8850235	049	AAAATCCCTC**G**	050	CAGGACTGTC**C**	051	AAAATCCCTC**R**	052	CAGGACTGTC**Y**
					GACAGTCCTG		GAGGGATTTT		GACAGTCCTG		GAGGGATTTT
UGT1A1	G>A	1020	GI:8850235	053	CAGTCCTGTG**A**	054	CAGTGTACCG**T**	055	CAGTCCTGTG**R**	056	CAGTGTACCG**Y**
					CGGTACACTG		CACAGGACTG		CGGTACACTG		CACAGGACTG
UGT1A1	C>T	1084	GI:8850235	057	GTGGCTACCC**T**	058	AGATCGTTT**A**G	059	GTGGCTACCC**Y**	060	AGATCGTTT**R**G
					AAACGATCT		GGTAGCCAC		AAACGATCT		GGTAGCCAC
UGT1A1	A>G	1085	GI:8850235	061	TGGCTACCCC**G**	062	CAGATCGTTT**C**	063	TGGCTACCCC**R**	064	CAGATCGTTT**Y**

				AAACGATCTG		GGGGTAGCCA	AAACGATCTG		GGGGTAGCCA
UGT1A1	C>G	1114	GI:8850235	065	CCCGATGACC**G**	066	ATAAAGGCAC**C**	067	CCCGATGACC**S**	068	ATAAAGGCAC**S**
					GTGCCTTTAT		GGTCATCGGG		GTGCCTTTAT		GGTCATCGGG

UGT1A1	G>A	1117	GI:8850235	069	GATGACCCGT**A** CCTTTATCAC	070	GTGATAAAGG**T**A CGGGTCATC	071	GATGACCCGT**R** CCTTTATCAC	072	GTGATAAAGG**Y** ACGGGTCATC
UGT1A1	C>T	1139	GI:8850235	073	CATGCTGGTT**T** CCATGGTGTT	074	AACACCATGG**A**A ACCAGCATG	075	CATGCTGGTT**Y** CCATGGTGTT	076	AACACCATGG**R** AACCAGCATG
UGT1A1	C>G	1158	GI:8850235	077	TTTATGAAAG**G**A TATGCAATG	078	CATTGCATAT**C**CT TTCATAAA	079	TTTATGAAAG**S**A TATGCAATG	080	CATTGCATAT**S**C TTTCATAAA
UGT1A1	CC> GT	1175 to 1176	GI:8850235	081	AATGGCGTTC**G** **T**ATGGTGATGA	082	TCATCACCAT**AC** GAACGCCATT	083	AATGGCGTTC**Y** ATGGTGATGA	084	TCATCACCAT**SR** GAACGCCATT
UGT1A1	G>C	1216	GI:8850235	085	GATGGACAAT**C** CAAAGCGCAT	086	ATGCGCTTTG**G**A TTGTCCATC	087	GATGGACAAT**S** CAAAGCGCAT	088	ATGCGCTTTG**S** ATTGTCCATC
UGT1A1	A>G	1297	GI:8850235	089	AAATGCTCTA**G**A AGCAGTCAT	090	ATGACTGCTT**C**TA GAGCATTT	091	AAATGCTCTA**R**A AGCAGTCAT	092	ATGACTGCTT**Y**T AGAGCATTT
UGT1A1	A>T	1324	GI:8850235	093	CAAAAGTTAC**T**A GGAGAACAT	094	ATGTTCTCCT**A**GT AACTTTTG	095	CAAAAGTTAC**W** AGGAGAACAT	096	ATGTTCTCCT**W** GTAACTTTTG
UGT1A1	T>G	1471	GI:8850235	097	CTGGTACCAG**G** ACCATTCCTT	098	AAGGAATGGT**C**C TGGTACCAG	099	CTGGTACCAG**K** ACCATTCCTT	100	AAGGAATGGT**M** CTGGTACCAG
UGT1A1	C>T	1478	GI:8850235	101	CAGTACCATT**T**C TTGGACGTG	102	CACGTCCAAG**A**A ATGGTACTG	103	CAGTACCATT**Y**C TTGGACGTG	104	CACGTCCAAG**R** AATGGTACTG

UGT1A1	*del*CT	372 to 373	GI:8850235	105	TAAAAAAGG**AC**	106	AGCATAGCA**GT**C	107	TAAAAAAGGA**n**C	108	AGCATAGCAG**n**
					TGCTATGCT		CTTTTTTA		TGCTATGCT		TCCTTTTTTA
UGT1A1	*del.*	523 to 525	GI:8850235	109	GCCCACTGT**AT**	110	CATGCAAGA**AT**A	111	GCCCACTGTA**n**	112	CATGCAAGAA**n**T
	TTC				TCTTGCATG		CAGTGGGC		TTCTTGCATG		ACAGTGGGC
UGT1A1	*del*	892 to 905	GI:8850235	113	ATTTGAAGC**CT**	114	ATGTTCTCC**AG**G	115	ATTTGAAGCC**n**T	116	ATGTTCTCCA**n**G
	TACA				GGAGAACAT		CTTCAAAT		GGAGAACAT		GCTTCAAAT
	TTA										
	ATGC										
	TTC										
UGT1A1	*ins*T	470/ 471	GI:8850235	129	CTGACGGACC**C**	130	AAGGAAGGAA**AT**	131	CTGACGGACCC	132	AAGGAAGGAAA
					TTTTCCTTCCTT		**G**GGTCCGTCAG		**n**TTTCCTTCCTT		**n**GGGTCCGTCA
											G
UGT1A1	*ins*G	1222/ 1223	GI:8850235	133	CAATGCAAAG**C**	134	AGTCTCCATG**CC**	135	CAATGCAAAGC	136	AGTCTCCATGC
					GGCATGGAGAC		**G**CTTTGCATTG		**n**GCATGGAGAC		**n**GCTTTGCATTG
					T				T		
Cyp3A5	T>C	47518	GI:10281451	137	AAGGA**C**TTCTA	138	TAGAA**G**TCCTT	139	AAGGA**Y**TTCTA	140	TAGAA**R**TCCTT
Cyp3A5	T>G	145601	GI:11177452	141	TGGGC**G**TGCAA	142	TTGCA**C**GCCCA	143	TGGGC**K**TGCAA	144	TTGCA**M**GCCCA
Cyp3A5	A>G	145929	GI:11177452	145	GCCCC**G**CCTCC	146	GGAGG**C**GGGGC	147	GCCCC**R**CCTCC	148	GGAGG**Y**GGGG

													C
Cyp3A5	A>G	9736	GI:10281451	149	CTCAC**G**CTGGG	150	CCCAG**C**GTGAG	151	CTCAC**R**CTGGG	152	CCCAG**Y**GTCTC
MRP1	G>A	21133	U91318	169	CCCAAAACAC**A**	170	GCAGGGTGTGT**G**	171	CCCAAAACAC**R**	172	GCAGGGTGTG**Y**
					CACACCCTGC		TGTTTTGGG		CACACCCTGC		GTGTTTTGGG
MRP1	G>T	57998	GI:7209451	173	ACGCTCAGAG**T**	174	AGTCCATGAA**C**	175	ACGCTCAGAG**K**	176	AGTCCATGAA**M**
					TTCATGGACT		TCTGAGCGT		TTCATGGACT		CTCTGAGCGT
MRP1	C>T	137667	AC026452	177	GCAGGTGGCC**T**	178	AATGTGCAC**A**G	179	GCAGGTGGCC**Y**	180	AATGTGCAC**R**
					TGTGCACATT		GCCACCTGC		TGTGCACATT		GGCCACCTGC
MRP1	C>T	137647	AC026452	181	TTGCCGTCTA**T**	182	CAATGGTCAC**A**T	183	TTGCCGTCTA**Y**	184	CAATGGTCAC**R**
					GTGACCATTG		AGACGGCAA		GTGACCATTG		TAGACGGCAA
MRP1	G>A	27258	AC003026	185	GATTCTCTCC**A**A	186	GATGTTTTCT**T**GG	187	GATTCTCTCC**R**A	188	GATGTTTTCT**Y**G
					GAAAACATC		AGAGAATC		GAAAACATC		GAGAGAATC
MRP1	G>A	14008	U91318	189	CTGGGAAGTC**A**	190	GGGTCAGGGA**T**G	191	CTGGGAAGTC**R**	192	GGGTCAGGGA**Y**
					TCCCTGACCC		ACTTCCCAG		TCCCTGACCC		GACTTCCCAG
MRP1	C>T	18067	U91318	193	CCACGGCAGC**T**	194	CCAGGTCCAC**A**G	195	CCACGGCAGC**Y**	196	CCAGGTCCAC**R**
					GTGGACCTGG		CTGCCGTGG		GTGGACCTGG		GCTGCCGTGG
MRP1	G>A	79	AF022830	197	CCAGGCAGCC**A**	198	CAACCTTCAC**T**G	199	CCAGGCAGCC**R**	200	CAACCTTCAC**Y**
					GTGAAGGTTG		GCTGCCTGG		GTGAAGGTTG		GGCTGCCTGG
MRP1	T>C	88	AF022830	201	CGGTGAAGGT**C**	202	AGGAGTACAC**G**A	203	CGGTGAAGGT**Y**	204	AGGAGTACAC**R**
					GTGTACTCCT		CCTTCACCG		GTGTACTCCT		ACCTTCACCG

MRP1	T>G	249	AF022830	205	CTCATGAGCT**G** CTTCTTCAAG	206	CTTGAAGAAG**C**A GCTCATGAG	207	CTCATGAGCT**K** CTTCTTCAAG	208	CTTGAAGAAG**M** AGCTCATGAG
MRP1	T>C	95	AF022831	209	AGTTCGTGAA**C** GACACGAAGG	210	CCTTCGTGTC**G**T TCACGAACT	211	AGTTCGTGAA**Y** GACACGAAGG	212	CCTTCGTGTC**R** TTCACGAACT
MRP1	C>T	57853	GI:7209451	213	GGCAGTGGGC**T** GAGGGAGTGG	214	CCACTCCCTC**A**G CCCACTGCC	215	GGCAGTGGGC**Y** GAGGGAGTGG	216	CCACTCCCTC**R** GCCCACTGCC
MRP1	C>G	53282	GI:7209451	217	GCCAGTTGGA**G** TCACTTGGGG	218	CCCCAAGTGA**C**T CCAACTGGC	219	GCCAGTTGGA**S** TCACTTGGGG	220	CCCCAAGTGA**S** TCCAACTGGC
MRP1	A>G	137710	AC026452	221	ACTCTCACTC**G** GGGCACAGCA	222	TGCTGTGCCC**C**G AGTGAGAGT	223	ACTCTCACTC**R** GGGCACAGCA	224	TGCTGTGCCC**Y** GAGTGAGAGT
MRP1	G>C	27159	AC003026	225	TCGTTGATCA**C**A TCTGTCTGT	226	ACAGACAGAT**G**T GATCAACGA	227	TCGTTGATCA**S**A TCTGTCTGT	228	ACAGACAGAT**S** TGATCAACGA
MRP1	G>A	34218	AC003026	229	GTGCACTCAC**A** TGGCCGGGTG	230	CACCCGGCCA**T**G TGAGTGCAC	231	GTGCACTCAC**R** TGGCCGGGTG	232	CACCCGGCCA**Y** GTGAGTGCAC
MRP1	G>C	34215	AC003026	233	CATGTGCACT**C** ACGTGGCCGG	234	CCGGCCACGT**G**A GTGCACATG	235	CATGTGCACT**S** ACGTGGCCGG	236	CCGGCCACGT**S** AGTGCACATG
MRP1	G>A	39508	GI:7209451	237	GTTTCGTTGT**A** GGGGGTGGGA	238	TCCCACCCCC**T**A CAACGAAAC	239	GTTTCGTTGT**R** GGGGGTGGGA	240	TCCCACCCCC**Y** ACAACGAAAC
MRP1	T>C	55472	AC003026	241	TGTCTAATTA**C**A	242	ATCCATTTCT**G**TA	243	TGTCTAATTA**Y**A	244	ATCCATTTCT**R**T

					GAAATGGAT		ATTAGACA		GAAATGGAT		AATTAGACA	
MRP1	G>A	150727	AC025277	245	CCATGTCAGC**A** TGACACAGGT	246	ACCTGTGTCA**T**G CTGACATGG	247	CCATGTCAGC**R** TGACACAGGT	248	ACCTGTGTCA**Y** GCTGACATGG
MRP1	*del*T	17970	U91318	249	CTGGTTTTT**TC**T TCCGGTCA	250	TGACCGGAA**GA**A AAAACCAG	251	CTGGTTTTTT**n**C TTCCGGTCA	252	TGACCGGAAG**n** AAAAAAACCAG
MRP1	C>T	17900	U91318	253	TGTCTCCTTT**T**G CTTCTCCCA	254	TGGGAGAAGC**A**A AAGGAGACA	255	TGTCTCCTTT**Y**G CTTCTCCCA	256	TGGGAGAAGC**R** AAAGGAGACA
MRP1	G>A	18195	U91318	257	CACTGGCACA**A** TGGCCTCTAG	258	CTAGAGGCCA**T**T GTGCCAGTG	259	CACTGGCACA**R** TGGCCTCTAG	260	CTAGAGGCCA**Y** TGTGCCAGTG
MRP1	G>A	33551	AC025277	261	TGTGACCACA**A** ATGAGTGTGT	262	ACACACTCAT**T**TG TGGTCACA	263	TGTGACCACA**R** ATGAGTGTGT	264	ACACACTCAT**Y**T GTGGTCACA
MRP1	C>T	174	AF022828	265	CCAGGCCCCC**T** AGACCTCAGG	266	CCTGAGGTCT**A**G GGGGCCTGG	267	CCAGGCCCCC**Y** AGACCTCAGG	268	CCTGAGGTCT**R** GGGGGCCTGG
MRP1	C>A	248	AF022829	269	CCTTTCCACT**A**C TGTGGCCTC	270	GAGGCCACAG**T**A GTGGAAAGG	271	CCTTTCCACT**M** CTGTGGCCTC	272	GAGGCCACAG**K** AGTGGAAAGG
MRP1	C>G	258	AF022829	273	CCTGTGGCCT**G** AATCCAGGAT	274	ATCCTGGATT**C**A GGCCACAGG	275	CCTGTGGCCT**S** AATCCAGGAT	276	ATCCTGGATT**S**A GGCCACAGG
MRP1	A>G	259	AF022831	277	AAGGTAGGGG**G** CGCTGTGCCA	278	TGGCACAGCG**C**C CCCTACCTT	279	AAGGTAGGGG**R** CGCTGTGCCA	280	TGGCACAGCG**Y** CCCCTACCTT

MRP1	T>C	124667	AC026452	281	GCGTGCCCAG**C** CCTGGGGTTT	282	AAACCCCAGG**G**C TGGGCACGC	283	GCGTGCCCAG**Y** CCTGGGGTTT	284	AAACCCCAGG**R** CTGGGCACGC
MRP1	G>A	1884	U07050	285	AGCCTTGGAG**A** ATCTGGGGTG	286	CACCCCAGAT**T**C TCCAAGGCT	287	AGCCTTGGAG**R** ATCTGGGGTG	288	CACCCCAGAT**Y** CTCCAAGGCT
MRP1	G>C	38646	AC026452	289	CCTTAAACAG**C** ATTTGAAAAG	290	CTTTTCAAAT**G**CT GTTTAAGG	291	CCTTAAACAG**S**A TTTGAAAAG	292	CTTTTCAAAT**S**C TGTTTAAGG
MRP1	C>A	1625	U07050	293	GGGAATCACT**A** AACCTCTCTG	294	CAGAGAGGTT**T**A GTGATTCCC	295	GGGAATCACT**M** AACCTCTCTG	296	CAGAGAGGTT**K** AGTGATTCCC
MRP1	C>T	1163	U07050	297	TGTGATCGGC**T** CGCCTCGGCT	298	AGCCGAGGCG**A** GCCGATCACA	299	TGTGATCGGC**Y** CGCCTCGGCT	300	AGCCGAGGCG**R** GCCGATCACA
MRP1	A>G	381	U07050	301	TGGGGGACCC**G** GGCCAATAAA	302	TTTATTGGCC**C**G GGTCCCCCA	303	TGGGGGACCC**R** GGCCAATAAA	304	TTTATTGGCC**Y**G GGTCCCCCA
MRP1	G>A	233	U07050	305	AAGAGTAGCA**A** TTTTATCTTG	306	CAAGATAAAA**T**TG CTACTCTT	307	AAGAGTAGCA**R** TTTTATCTTG	308	CAAGATAAAA**Y**T GCTACTCTT
MRP1	C>A	189	U07050	309	AAAAAAATCC**A**A ATCCAAAAA	310	TTTTTGGATT**T**GG ATTTTTTT	311	AAAAAAATCC**M** AATCCAAAAA	312	TTTTTGGATT**K**G GATTTTTTT
MRP1	C>T	440	U07050	313	CTCCTTCCCT**T**G CTAGGTCCT	314	AGGACCTAGC**A**A GGGAAGGAG	315	CTCCTTCCCT**Y** GCTAGGTCCT	316	AGGACCTAGC**R** AGGGAAGGAG
MRP1	*del*AT	34206	AC003026	317	AGTCTCACA**CG**	318	GTGAGTGCA**CG**T	319	AGTCTCACAC**n**	320	GTGAGTGCAC**n**

		to 34207			TGCACTCAC		GTGAGACT		GTGCACTCAC		GTGTGAGACT
MRP1	*del* GGTA	1720 to 1723	U07050	321	ACTCCAGGC**AG** GCTCCGTTC	322	GAACGGAGC**CT**G CCTGGAGT	323	ACTCCAGGCA**n** GGCTCCGTTC	324	GAACGGAGCC**n** TGCCTGGAGT
MRP1	*ins*T	926/ 927	U07050	325	TTAATTTTTTT**TT** **A**TTATTATTT	326	AAATAATAA**TAA**A AAAAAATTAA	327	TTAATTTTTTT**n** ATTATTATTT	328	AAATAATAAT**n**A AAAAAAATTAA
MRP1	*Ins*TC CTTC C	437/ 438	U07050	329	TTCCTCCTTC**CT** **CCTTCCC**TCGC TAGGT	330	ACCTAGCGA**GGG** **AAGGAG**GAAGG AGGAA	331	TTCCTCCTTCC**n** CTCGCTAGGT	332	ACCTAGCGAG**A** GGAAGGAGGAA
MRP1	*ins*TG GGG C	55156/ 55157	AC003026	333	GGGGCTGGGG **CTGGGGCT**GGG TGCGTG	334	CACGCACCCG**AC** **CCCGA**CCCAGCC CC	335	GGGGCTGGGG C**n**TGGGTGCGT G	336	CACGCACCCG**n** ACCCAGCCCC
MDR1	T>C	140837	AC002457	337	GCTCATTCGAG **C**AGCGGCTCT	338	AGAGCCGCT**G**CT CGAATGAG	339	CTCATTCGAG**Y** AGCGGCTCTT	340	AGAGCCGCT**R**C TCGAATGAG
MDR1	G>A	84701	AC005068	341	AAAATTGCT**A**TC ACTATCT	342	AGATAGTGA**T**AG CAATTTT	343	AAAATTGCT**R**TC ACTATCT	344	AGATAGTGA**Y**A GCAATTTT
MDR1	G>A	101	M29432	345	TTCACTTCA**A**TT ACCCATC	346	ATGGGTAA**T**TGA AGTGAA	347	TCACTTCA**R**TTA CCCATC	348	GATGGGTAA**Y**T GAAGTGAA
MDR1	C>T	308	M29432	349	CTTGAAGGG**T**C TGAACCTGA	350	TCAGGTTCAG**A**C CCTTCAAGA	351	TCTTGAAGGG**Y** CTGAACCTG	352	TCAGGTTCAG**R** CCCTTCAAGA

MDR1	C>T	83946	AC005068	353	TCAGCAGT**T**AC ATTGCA	354	TGCAATGT**A**ACT GCTGA	355	CAGCAGT**Y**ACA TTGCAC	356	TGCAATGT**R**ACT GCTGA
MDR1	G>A	83973	AC005068	357	GACCCATGC**A**A GCTAGACC	358	GGTCTAGCT**T**GC ATGGGTC	359	GACCCATGC**R**A GCTAGACC	360	GGTCTAGCT**Y**G CATGGGTC
MDR1	A>G	84032	AC005068	361	GAGCACAAC**G**G TCCAGCTG	362	CAGCTGGAC**C**GT TGTGCTC	363	GAGCACAAC**R**G TCCAGCTG	364	CAGCTGGAC**Y**G TTGTGCTC
MDR1	G>A	84074	AC005068	365	TGGGCAGAC**A**G TGGCCCTG	366	CAGGGCCAC**T**GT CTGCCCA	367	TGGGCAGAC**R**G TGGCCCTG	368	CAGGGCCAC**Y**G TCTGCCCA
MDR1	G>A	84119	AC005068	369	CTCGTCCTG**A**T AGATCTTG	370	CAAGATCTA**T**CA GGACGAG	371	CTCGTCCTG**R**T AGATCTTG	372	CAAGATCTA**Y**CA GGACGAG
MDR1	A>G	77811	AC005068	373	GGCTTGAAG**G**T GTAAGAAT	374	ATTCTTACA**C**CTT CAAGCC	375	GGCTTGAAG**R**T GTAAGAAT	376	ATTCTTACA**Y**CT TCAAGCC
MDR1	T>A	78170	AC005068	377	TATTCCTTTAC**A** AATTTTTG	378	CAAAAATT**T**GTAA AGGAATA	379	TATTCCTTTAC**W** AATTTTTG	380	ACAAAAATT**W**G TAAAGGAAT
MDR1	A>G	73252	AC005068	381	ACTTTGTCT**G**AT CTCCTGC	382	GCAGGAGAT**C**AG ACAAAGT	383	ACTTTGTCT**R**AT CTCCTGC	384	GCAGGAGAT**Y**A GACAAAGT
MDR1	G>A	141529	AC002457	385	CTTCAGGTCGG **A**ATGGATCTTG	386	CAAGATCCAT**T**C CGACCTGA	387	CTTCAGGTCGG **R**ATGGATCTTG	388	CAAGATCCAT**Y** CCGACCTGAAG
MDR1	A>G	141590	AC002457	389	AAACTGAAC**G**A TAAAAGGTA	390	TACCTTTTAT**C**GT TCAGTTTAA	391	AAACTGAAC**R**AT AAAAGGTA	392	TACCTTTTAT**Y**G TTCAGTTTAA

MDR1	C>T	70200	AC005068	393	TTCTCCTTA**T**GG	394	CTAACACCC**A**TA	395	TTCTCCTTA**Y**GG	396	CTAACACCC**R**T
					GTGTTAG		AGGAGAA		GTGTTAG		AAGGAGAA
MDR1	C>A	70204	AC005068	397	AATTTTCTC**A**TT	398	CACCCGTAA**T**GA	399	AATTTTCTC**M**TT	400	CACCCGTAA**K**G
					ACGGGTG		GAAAATT		ACGGGTG		AGAAAATT
MDR1	C>T	70237	AC005068	401	TTAATTGGC**T**AT	402	GTCCAAAAT**A**GC	403	TTAATTGGC**Y**AT	404	GTCCAAAAT**R**G
					TTTGGAC		CAATTAA		TTTGGAC		CCAATTAA
MDR1	G>A	70253	AC005068	405	TCTACTGGT**A**TT	406	TAAGACAAA**T**AC	407	TCTACTGGT**R**TT	408	TAAGACAAA**Y**AC
					TGTCTTA		CAGTAGA		TGTCTTA		CAGTAGA
MDR1	C>A	70371	AC005068	409	AATCATTTT**A**TG	410	TGTGGCACA**T**AA	411	AATCATTTT**M**TG	412	TGTGGCACA**K**A
					TGCCACA		AATGATT		TGCCACA		AAATGATT
MDR1	C>T	137	M29445	413	GAACATTGC**T**TA	414	GTCTCCATA**A**GC	415	GAACATTGC**Y**TA	416	GTCTCCATA**R**G
					TGGAGAC		AATGTTC		TGGAGAC		CAATGTTC
MDR1	C>T	176	M29445	417	GAAGAGAT**T**GT	418	CCCTCAC**A**ATCT	419	GAAGAGAT**Y**GT	420	CCCTCAC**R**ATC
					GAGGG		CTTC		GAGGGC		TCTTC
MDR1	A>C	43263	AC005068	421	TGAATGTTC**C**GT	422	CGGAGCCAC**G**GA	423	TGAATGTTC**M**G	424	CGGAGCCAC**K**G
					GGCTCCG		ACATTCA		TGGCTCCG		AACATTCA
MDR1	T>A	43162	AC005068	425	CGGGTGGT**G**AC	426	CTTCCTGTG**T**CA	427	CGGGTGGTG**W**	428	CTTCCTGTG**W**C
					ACAGGAAG		CCACCCG		CACAGGAAG		ACCACCCG
MDR1	C>T	145984	AC002457	429	AAAATACTT**T**GG	430	CAAATTTCC**A**AA	431	AAAATACTT**Y**GG	432	CAAATTTCC**R**AA
					AAATTTG		GTATTTT		AAATTTG		GTATTTT

MDR1	T>C	171404	AC002457	433	ATCATTAAA**C**GA AATGAGT	434	ACTCATTTC**G**TTT AATGAT	435	ATCATTAAA**Y**GA AATGAGT	436	ACTCATTTC**R**TT TAATGAT
MDR1	G>C	171456	AC002457	437	GACTAAAGA**C**A CATAAATG	438	CATTTATGT**G**TCT TTAGTC	439	GACTAAAGA**S**A CATAAATG	440	CATTTATGT**S**TC TTTAGTC
MDR1	G>T	171466	AC002457	441	GACATAAATG**T**T ATGTTTGTTT	442	AAACAAACATA**A** CATTTATGTCT	443	AGACATAAATG **K**TATGTTTGT	444	AAACAAACATA **M**CATTTATGTC
MDR1	T>C	171511	AC002457	445	GATACAGGG**C**T CTTCATGA	446	TCATGAAGA**G**CC CTGTATC	447	GATACAGGG**Y**T CTTCATGA	448	TCATGAAGA**R**C CCTGTATC
MDR1	T>C	171512	AC002457	449	GATACAGGGT**C** CTTCATGAAT	450	ATTCATGAAG**G**A CCCTGTATC	451	GATACAGGGT**Y** CTTCATGAAT	452	ATTCATGAAG**R**A CCCTGTATC
MDR1	G>A	174901	AC002457	453	GTGCACGAT**A**T TGGGGAGC	454	GCTCCCCAA**T**AT CGTGCAC	455	GTGCACGAT**R**T TGGGGAGC	456	GCTCCCCAA**Y**A TCGTGCAC
MDR1	C>T	175068	AC002457	457	TAAGCAGCAA**T** AATGTCGTGT	458	ACACGACATT**A**TT GCTGCTTA	459	TAAGCAGCAA**Y** AATGTCGTGT	460	ACACGACATT**R**T TGCTGCTTA
MDR1	C>T	175074	AC002457	461	CAACAATGT**T**GT GTGCATC	462	GATGCACAC**A**AC ATTGTTG	463	CAACAATGT**Y**GT GTGCATC	464	GATGCACAC**R**A CATTGTTG
MDR1	A>G	175142	AC002457	465	CATTAAATG**G**A GGACTGGG	466	CCCAGTCCT**C**CA TTTAATG	467	CATTAAATG**R**AG GACTGGG	468	CCCAGTCCT**Y**C ATTTAATG
MDR1	A>G	175180	AC002457	469	TCCTCTGAG**G**A TGTGCAGT	470	ACTGCACAT**C**CT CAGAGGA	471	TCCTCTGAG**R**A TGTGCAGT	472	ACTGCACAT**Y**CT CAGAGGA

MDR1	A>G	139015	AC002457	473	AACTTACTT**G**TA	474	TCAAAGATA**C**AA	475	AACTTACTT**R**TA	476	TCAAAGATA**Y**AA
					TCTTTGA		GTAAGTT		TCTTTGA		GTAAGTT
MDR1	A>T	139064	AC002457	477	AGAAATAGT**T**TA	478	TGTTGATTA**A**ACT	479	AGAAATAGT**W**T	480	TGTTGATTA**W**A
					ATCAACA		ATTTCT		AATCAACA		CTATTTCT
MDR1	T>C	139119	AC002457	481	TAGGGAGGG**C**T	482	TGGCCTTAA**G**CC	483	TAGGGAGGG**Y**T	484	TGGCCTTAA**R**C
					TAAGGCCA		CTCCCTA		TAAGGCCA		CCTCCCTA
MDR1	G>A	139177	AC002457	485	GAAAGGTGA**A**A	486	TTGCTTTAT**T**TCA	487	GAAAGGTGA**R**A	488	TTGCTTTAT**Y**TC
					TAAAGCAA		CCTTTC		TAAAGCAA		ACCTTTC
MDR1	C>T	139276	AC002457	489	CATTTACCC**T**AG	490	GGTCCATCT**A**GG	491	CATTTACCC**Y**AG	492	GGTCCATCT**R**G
					ATGGACC		GTAAATG		ATGGACC		GGTAAATG
MDR1	G>A	140118	AC002457	493	ATATGGAAG**A**A	494	TTGTAATTT**T**CTT	495	ATATGGAAG**R**A	496	TTGTAATTT**Y**CT
					AATTACAA		CCATAT		AATTACAA		TCCATAT
MDR1	A>G	140216	AC002457	497	AACACGGGC**G**T	498	TCAGATCAA**C**GC	499	AACACGGGC**R**T	500	TCAGATCAA**Y**G
					TGATCTGA		CCGTGTT		TGATCTGA		CCCGTGTT
MDR1	T>C	140490	AC002457	501	TGTATTAAA**C**GC	502	GGGATTCGC**G**TT	503	TGTATTAAA**Y**GC	504	GGGATTCGC**R**T
					GAATCCC		TAATACA		GAATCCC		TTAATACA
MDR1	G>A	140568	AC002457	505	TTGAAAGAC**A**T	506	ATGTAGACA**T**GT	507	TTGAAAGAC**R**T	508	ATGTAGACA**Y**G
					GTCTACAT		CTTTCAA		GTCTACAT		TCTTTCAA
MDR1	A>T	140576	AC002457	509	CGTGTCTAC**T**TA	510	TTCAACTTA**A**GTA	511	CGTGTCTAC**W**T	512	TTCAACTTA**W**G

					AGTTGAA		GACACG		AAGTTGAA		TAGACACG
MDR1	A>G	140595	AC002457	513	ATGTCCCCA**G**T	514	GCTGAATCA**C**TG	515	ATGTCCCCA**R**T	516	GCTGAATCA**Y**T
					GATTCAGC		GGGACAT		GATTCAGC		GGGGACAT
MDR1	G>A	140727	AC002457	517	CCGGGCCGG**A**A	518	ATGACTGCT**T**CC	519	CCGGGCCGG**R**A	520	ATGACTGCT**Y**C
					GCAGTCAT		GGCCCGG		GCAGTCAT		CGGCCCGG
MDR1	G>A	139479	AC002457	521	GAGGCGGGC**A**	522	CTCGTGATC**T**GC	523	GAGGCGGGC**R**	524	CTCGTGATC**Y**G
					GATCACGAG		CCGCCTC		GATCACGAG		CCCGCCTC
MDR1	T>C	139619	AC002457	525	GGAGAATGG**C**G	526	CGGGTTCAC**G**CC	527	GGAGAATGG**Y**G	528	CGGGTTCAC**R**C
					TGAACCCG		ATTCTCC		TGAACCCG		CATTCTCC
MDR1	G>T	65241	AC005068	636	ACTAGAAGGT**T**	637	ACCTTCCCAG**A**A	638	ACTAGAAGGT**K**	639	ACCTTCCCAG**M**
					CTGGGAAGGT		CCTTCTAGT		CTGGGAAGGT		ACCTTCTAGT
MDR1	G>A	50537	AC005068	640	TCCTGACTAT**A**C	641	TTGGCTTTGG**T**AT	642	TCCTGACTAT**R**C	643	TTGGCTTTGG**Y**
					CAAAGCCAA		AGTCAGGA		CAAAGCCAA		ATAGTCAGGA
TOP1	1334 G>T	133418 45	GI:11225259	529	ACTTTTCCGT**T**G	530	TTGCCGCGGC**A**A	531	ACTTTTCCGT**K**G	532	TTGCCGCGGC**M**
					CCGCGGCAACT		CGGAAAAGTTC		CCGCGGCAACT		ACGGAAAAGTT C
TOP1	1845 A>G	1845	GI:11225259	533	CTCGGGAAGG**G**	534	TCTGATGGAG**C**C	535	CTCGGGAAGG**R**	536	TCTGATGGAG**Y**
					CTCCATCAGA		CTTCCCGAG		CTCCATCAGA		CCTTCCCGAG

Table 2: The nucleic acid and amino acid sequences referred to in this application

Gene	AS change	Protein No	Acc SEQ ID NO	**Protein**	SEQ ID N=	Protein wt>mut
UGT1A1	L15R	G8850236	538	PLVLG**R**LLCVL	539	PLVLG**X**LLCVL
UGT1A1	G71R	G8850236	540	LYIRD**R**AFYTL	541	LYIRD**X**AFYTL
UGT1A1	D119Dframeshift	G8850236	542	KKIKKD**CYAFC**	543	KKIKK**DX**
UGT1A1	P152Pframeshift	G8850236	544	VMLTDPF**PSLQ**	545	VMLTD**PX**
UGT1A1	F170del	G8850236	546	LSLPTV**FLHAL**	547	LSLPTV**FX**
UGT1A1	L175Q	G8850236	548	FFLHA**Q**PCSLE	549	FFLHA**X**PCSLE
UGT1A1	C177R	G8850236	550	LHALP**R**SLEFE	551	LHALP**X**SLEFE
UGT1A1	R209W	G8850236	552	MTFLQ**W**VKNML	553	MTFLQ**X**VKNML
UGT1A1	P229Q	G8850236	554	DVVYS**Q**YATLA	555	DVVYS**X**YATLA
UGT1A1	G276R	G8850236	556	NMVFV**R**GINCL	557	NMVFV**X**GINCL

UGT1A1	A292V	G8850236	558	SQEFE**V**YINAS	559	SQEFE**X**YINAS
UGT1A1	Y293Wframeshift	G8850236	560	QEFEA**W**RTWN	561	QEFEA**X**INASG
UGT1A1	G308E	G8850236	562	VVFSL**E**SMVSE	563	VVFSL**X**SMVSE
UGT1A1	Q331R	G8850236	564	LGKIP**R**TVLWR	565	LGKIP**X**TVLWR
UGT1A1	Q357R	G8850236	566	VKWLP**R**NDLLG	567	VKWLP**X**NDLLG
UGT1A1	R367G	G8850236	568	GHPMT**G**AFITH	569	GHPMT**X**AFITH
UGT1A1	A368T	G8850236	570	HPMTR**T**FITHA	571	HPMTR**X**FITHA
UGT1A1	P387R	G8850236	572	ICNGV**R**MVMMP	573	ICNGV**X**MVMMP
UGT1A1	S375F	G8850236	574	ITHAG**F**HGVYE	575	ITHAG**X**HGVYE
UGT1A1	S381R	G8850236	576	HGVYE**R**ICNGV	577	HGVYE**X**ICNGV
UGT1A1	A401P	G8850236	578	DQMDN**P**KRMET	579	DQMDN**X**KRMET
UGT1A1	R403Rframeshift	G8850236	580	MDNAK**RHGD.**	581	MDNAK**X**
UGT1A1	K428E	G8850236	582	LENAL**E**AVIND	583	LENAL**X**AVIND
UGT1A1	Y486D	G8850236	584	LTWYQ**D**HSLDV	585	LTWYQ**X**HSLDV
UGT1A1	S488F	G8850236	586	WYQYH**F**LDVIG	587	WYQYH**X**LDVIG

UGT1A1	Q49stop	G8850236	588	LGAIQ.	589	LGAIQ.
UGT1A1	C280stop	G8850236	590	VGGIN.	591	VGGIN.
UGT1A1	Q331stop	G8850236	592	LGKIP.	593	LGKIP.
UGT1A1	W335stop	G8850236	594	PQTVL.	595	PQTVL.
UGT1A1	Q357stop	G8850236	596	VKWLP.	597	VKWLP.
UGT1A1	K437stop	G8850236	598	NDKSY.	599	NDKSY.
MRP1	F329C	G2828206	600	YFLMSCFFKAI	601	YFLMSXFFKAI
MRP1	R433S	G2828206	602	SVDAQSFMDLA	603	SVDAQXFMDLA
MRP1	R723Q	G2828206	604	QNDSLQENILF	605	QNDSLXENILF
MDR1	N21D	G2506118	606	FFKLNDKSEKD	607	FFKLNXKSEKD
MDR1	F103L	G2506118	608	INDTGLFMNLE	609	INDTGXFMNLE
MDR1	V168I	G2506118	610	FDVHDIGELNT	611	FDVHDXGELNT
MDR1	S400N	G2506118	612	RNVHFNYPSRK	613	RNVHFXYPSRK
MDR1	G412G	G2506118	614	VKILKGLNLKV	615	VKILKXLNLKV
MDR1	T436T	G2506118	616	CGKSTTVQLMQ	617	CGKSTXVQLMQ

MDR1	A893S	G2506118	618	KELEG**S**GKIAT	619	KELEG**X**GKIAT
MDR1	A999T	G2506118	620	FAPDY**T**KAKIS	621	FAPDY**X**KAKIS
MDR1	A1001T	G2506118	622	PDYAK**T**KISAA	623	PDYAK**X**KISAA
MDR1	Q1107P	G2506118	624	KRLNV**P**WLRAH	625	KRLNV**X**WLRAH
MDR1	A1132A	G2506118	626	IAENI**A**YGDNS	627	IAENI**X**YGDNS
MDR1	S1141T	G2506118	628	NSRVV**T**QEEIV	629	NSRVV**X**QEEIV
MDR1	I1145I	G2506118	630	VSQEE**I**VRAAK	631	VSQEE**X**VRAAK
TOP1	G363C	G12644118	632	PGLFR**C**RGNHP	633	PGLFR**X**RGNHP
TOP1	D533G	G12644118	634	DFLGK**G**SIRYY	635	DFLGK**X**SIRYY

The figure show:

Figure 1 shows the correlation of the exon 26 SNP with inestinal MDR1 expression in 21 volunteres determined by Western blot analyses. The box plot shows the distribution of MDR1 expression clustered according to the MDR1 3435C>T genotype at position corresponding to position 176 of the MDR1 gene (GenBank Acc. No. M29445). The T allele was associated with a lower expression of p-glycoprotein.

Figure 2 shows the correlation of MDR1 3435C>T genotype and digoxin uptake in 14 healthy volunteers who participated in a clinical study that addresses peak plama levels of digoxin at steady state [Johne et al., 1999, Clin. Pharmacol. Ther 66:338-345]. Maximum digoxin levels were statistically significantly different ($p=0.006$, Mann Whitney U test) between the two groups which were homozygous for the T and C allele, respectively.

Figure 3 represent the correlation of the genotype (wt/wt: 1; wt/mut and mut/mut:2) with MRP1 mRNA content in duodenal biopsies from healthy volunteers derived from two independent experiments, before and after application of rifampicin. Treatment with rifampicin had no effect on MRP1 mRNA expression ($p<0.001$, paired t-test). A strong trend of an association of MRP1 genotype with MRP1 mRNA levels was detected ($p=0.086$, Kruskal-Wallis test).

Figures 4 to 28 show the nucleic acid and amino acid sequences referred to herein.

Figure 29 shows the expression profile of genes relevant to Irinotecan metabolism in carcinoma cell lines. This semiquantitativ RT-PCR shows amounts of transcripts for the genes indicated right to the amplicons. PCR products were analyzed by agarose electrophoresis, stained with ethidium bromid. The respective fragment sizes are indicated on the left in basepaires (bp).

Figure 30 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with epithelial carcinoma cell lines LS174T (colon), KB 3-1 (cervix) and RT112 (bladder).

Concentrations of CPT-11 ranged from 0 to 200 µg/ml and of SN-38 from 0 to 200 ng/ml. Cells were treated for three days. The data for each concentration are mean values of at least three wells.

Figure 31 growth inhibition curves for CPT-11 (A) and SN-38 (B) with a epithelial cervix carcinoma cell line KB 3-1 and two subclones expressing high amounts of MDR1, KB 3-1 (MDR1) and KB 3-1 (MDR1, CYP3A5). Concentrations of CPT-11 ranged from 0 to 200 µg/ml and of SN-38 from 0 to 200 ng/ml. Cells were treated for three days. The data for each concentration are mean values and standard deviation of at least three wells.

Figure 32 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with the bladdercancer cell line RT112 and and its subclones RT112 (MDR1, UGT1A1) expressing MDR1 and higher amounts of UGT1A1. Concentrations of CPT-11 ranged from 0 to 200 µg/ml and of SN-38 from 0 to 200 ng/ml. Cells were treated for three days. The data for each concentration are mean values and standard deviation of at least three wells.

Figure 33 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with inhibition of MDR1 by R-Verapamil. The epithelial cervix carcinoma cell line KB 3-1 and the two subclones KB 3-1 (MDR1) and KB 3-1 (MDR1, CYP3A5), with high MDR1 expression, were tested for the influence of MDR1 inhibition by R-Verapamil on drug sensitivity. Concentrations of CPT-11 ranged from 0 to 200 µg/ml and of SN-38 from 0 to 200 ng/ml and R-Verapamil was added to 10 µg/ml final concentration(+V). Cells were treated for three days. The data for each concentration are mean values of two wells.

Figure 34 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with inhibition of MDR1 by R-Verapamil. To circumvent the MDR1 effect on drug resistance cells were treated in parallel with R-Verapamil. The KB 3-1 (MDR1) and KB 3-1 (MDR1, CYP3A5), which differ in their CYP3A5 expression, were tested for remaining resistance after inhibition of MDR1. Concentrations of CPT-11 ranged

from 0 to 200 μg/ml and of SN-38 from 0 to 200 ng/ml and R-Verapamil was added to 10 μg/ml final concentration(+V). Cells were treated for three days. The data for each concentration are mean values of two wells.

The present invention is illustrated by reference to the following biological Examples which are merely illustrative and are not to be constructed as a limitation of the scope of the present invention.

Example 1: Phenotypically impact of the C to T substitution at position corresponding to position 176 of the MDR1 gene (Acc. No. M29445).

To investigate the influence of the single nucleotide C to T substitution at position corresponding to position 176 of the MDR1 gene (Acc. No. M29445) also referred to as MDR1 exon 26 SNP C3435T on intestinal P-glycoprotein (PGP) expression, samples from biopsies and duodenal enterocyte preparations from 21 were investigated at the Dr. Margarete Fischer-Bosch-Institute for Clinical Pharmacology in Stuttgart by quantitative immunohistochemistry and Western blots. The results are shown in Figure 1. Homozygous carriers of the T allele (having at a position corresponding to position 176 of the MDR1 gene (Accession No: M29445) a T) demonstrated significantly higher PGP levels compared to homozygous carriers of the C allele (having at a position corresponding to position 176 of the MDR1 gene (Accession No: M29445) a C). Individuals with heterozygous genotype showed an intermediate level of PGP expression.

Furthermore, the influence of the MDR1 genotype on intestinal uptake-related pharmacokinetics of digoxin was investigated in a clinical study at the University Medical Center, Charite in Berlin. Maximal digoxin blood levels (Cmax) at steady state were correlated with the MDR1 3435C>T genotype 14 healthy volunteers after oral application of digoxin. Figure 2 shows, volunteers homozygous for the T allele show statistically significantly lower digoxin levels than volunteers with a C/C genotype. (p=0.006, Mann Whitney U test) and reflects the impact of this polymorphism on digoxin pharmacokinetics.

Example 2: Correlation of MRP1 polymorphisms with MRP1 expression and side effects during therapy with MRP1 substrates

Functional polymorphisms in the MRP1 gene affect the transport activity which in consequence modulates plasma levels and/or intracellular concentrations of MRP1 substrate drugs. Increased levels of such drugs can lead to side effects whereas decreased levels may result in subtherapeutical drug levels and therapy failure. MRP1 polymorphisms were correlated with the occurence of drug-related adverse effects and therapeutic efficacy in patients treated with MRP1 substrate drugs. In a case-control study, the frequency distribution of MRP1 SNPs was compared between a group of patients who suffered from cisplatin-related nephrotoxicity and a group of patients with nephro- and hepatotoxicities caused from anti-cancer drugs with a group of healthy controls. Furthermore, samples of known MRP1 mRNA levels were screened for MRP1 genotype. The results in the group of patients demonstrating nephro- and hepatotoxicity during anti-cancer treatment, are listed in the following table for one MRP1 SNP:

SNP	group	Allele frequency [%]		Genotype frequency [%]		
		G allele	A allele	*G/A	*A/A	*A/A expected[2]
150727G>A[1]	Controls	66.7	33.3	50	8.3	10.9
	Cases	50.0	50.0	14.3	42.9	25.0

[1]according to Acc. No. AC025277

[2] calculated according to Hardy-Weinberg

In contrast to control samples, the A allele (substitution of G to A at position according to position 150727 of the MRP1 gene, Acc. No. AC025277) was statistically significantly overrepresented in patients suffering from drug-related kidney- and liver side effects compared to healthy controls ($p=0.044$, Chi^2 test) and was thus predictive for these side effects.

Furthermore, an association of MRP1 genotype with mRNA expression before and after rifampicin application was detected for two MRP1 SNP's, 95T>C (SEQ ID NOs. 209, 210, 211, and 212, nucleotide substitution of T to C at a position corresponding to position 95 of the MRP1 gene, Acc. No. AF022831) and 259A>G (SEQ ID NOs. 277, 278, 279, and 280, nucleotide substitution of A to G at a position

corresponding to position 259 of the MRP1 gene, Acc. No. AF022831). These SNPs are linked and form one allele. The mutant allele (MRP1mut, C at position 95 and G at position 259 of the MRP1 gene, Acc. No. AF022831) is statistically significantly correlated with decreased MRP1 mRNA expression and the wildtype allele (MRP1wt, T at position 95 and A at position 259 of the MRP1 gene, Acc. No. AF022831) with increased MRP1 expression in two independent experiments (with and without rifampicin induction), as illustrated in figure 3.

The differences in the MRP1 mRNA content are based on MRP1 genotype-related interindividual differences and the analysis of these SNP's is of high diagnostic and prognostic value for MRP1 expression levels and to predict the therapeutic outcome and adverse effects of MRP1 substrate drugs.

Example 3: Dosage calculation

Therapeutic efficacy ans adverse effects of irinotecan depend on plasma levels and intracellular concentrations of the parent compound and the active metabolites (e.g. SN-38), processes which are controlled by CYP3A5- and UGT1A1-related metabolism and MRP1- and MDR1-related transport processes [Atsumi, *et al.*, 1991, Xenobiotica 21:1159-69, Iyer, *et al.*, 1998, J Clin Invest 101:847-54, Ciotti, *et al.*, 1999, Biochem Biophys Res Commun 260:199-202, Santos, *et al.*, 2000, Clin Cancer Res 6:2012-20, Kuhn, 1998, Oncology (Huntingt) 12:39-42, Chen, *et al.*, 1999, Mol Pharmacol 55:921-8, Chu, *et al.*, 1997, Cancer Res 57:1934-8, Chu, *et al.*, 1997, J Pharmacol Exp Ther 281:304-14; Chu, *et al.*, 1998, Cancer Res 58:5137-43, Chu, *et al.*, 1999, Drug Metab Dispos 27:440-1, Chu, *et al.*, 1999, J Pharmacol Exp Ther 288:735-41, Mattern, *et al.*, 1993, Oncol Res 5:467-74, Hoki, *et al.*, 1997, Cancer Chemother Pharmacol 40:433-8, Sugiyama, *et al.*, 1998, Cancer Chemother Pharmacol 42:S44-9]. For example, MRP1 works in close connection with glucuronosyltransferases as part of the cellular detoxification system and is known to transport glucuronosyl conjugates such as SN-38G [König et al., 1999, Biochim Biophys Acta 1461:377-394, Kerb et al., 2001, Pharmacogenomics 2:51-64]. For example, the extend to which SN-38G is exported from the cell into bile greatly influences the rate of its formation. For an efficient

detoxification of SN-38 both processes are necessary, conjugation by UGT1A1 and export of the glucuronide.

The 47518T>C (SEQ ID NOs.137, 138, 139, and 140) and 9736A>G (SEQ ID NOs. 149, 150, 151, 152) nucleotide substitutions of the CYP3A5 gene (Acc. No. GI:10281451), and the 145601T>G (SEQ ID NOs. 141, 142, 143, 144) and 145929A>G (SEQ ID NOs. 145, 146, 147, and 148) nucleotide substitutions of the CYP3A5 gene (Acc. No. GI:11177452) form an high CYP3A5 expression-related allele and are therefore associated with a higher metabolic inactivation of irinotecan. Individuals with this allele are extensive metabolizers (EMs) and are therefore in contrast the reminder poor metabolizers (PMs) less likely to suffer from irinotecan toxicity. Those with one high expresser and one low expresser-related allele are regarded as intermediate metabolizers (IMs).

The 176C>T nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MDR1 gene (Accession No: M29445) is associated with low PGP expression-related low drug efflux, and the 95T>C (SEQ ID NOs. 209, 210, 211, and 212) and the 259A>G (SEQ ID NOs. 277, 278, 279, and 280) nucleotide substitutions of the MRP1 gene (Acc. No. AF022831) are associated with low mRNA expression and the 150727G>A nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MRP1 gene (Accession No: M29445) is associated with low PGP expression-related low drug efflux and the 150727G>A nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MRP1 gene (Accession No: AC025277) is associated with adverse effects. Individuals carrying low transporter expression-related alleles are therefore less capable to clear cells from toxic compounds. Both, transport and metabolism are affected in a gene-dose dependant manner. According to the number of low expression-related alleles of the respective transport protein, individuals can be classified as having either extensive (ET), intermediate (IT) or poor transporter capacity (PT) of the respective gene.

By genetic testing prior to onset of treatment with irinotecan, the MDR1- and MRP1-related transport capacity of the patients can be predicted. The individual risk to adverse effects depends on the number of PM and/or PT alleles Individuals with PM-related alleles of CYP3A5 and UGT1A1 and PT-related alleles of MDR1 and MRP1 are at the highest risk to suffer from irinotecan toxicity.

Based on this knowledge, the initial dose can be adjusted prior to the first dose as shown by Brockmöller et al. (2000, Pharmacogenomics 1:125) for substrate drugs of CYP2D6, CYP2C9, and CYP2C19.

Dose adjustment can be achieved using a scoring system. For each PM- or PT-related allele a certain score is assigned e.g. a score of 2 is assigned to UGT1A1 PM alleles 226A, (SEQ ID NOs 9, 10, 11, 12, 540, 541) and 701A (SEQ ID NOs. 25, 26, 27, 28, 554, 555), and a score of 1 is assigned to the CYP3A5 PM-related alleles (47523T plus 35649A plus 145601T plus 145929A, 47523T plus 35649G plus 145601G plus 145929G, and 47523C plus 35649A plus 145601T plus 145929A), to the MDR1 low expression allele 176T (SEQ ID NOs.: 417, 418, 419, and 420), to the MRP1 low expression alleles 150727A (SEQ ID NOs. 217, 218, 219, and 220) and 259G (SEQ ID NOs. 277, 278, 279, and 280), to the MRP1 150727A allele (SEQ ID NOs. 217, 218, 219, and 220). After genotyping the scores are summarized and irinotecan dosage is adjusted according to the sum. Each single score corresponds to a dose reduction of 10%, i.e. a score of one corresponds to a 10% dose reduction, a score of two to 20%, a score of 3 to 30%, etc.

Example 4: Culture conditions and biological assays

The human epithelial cervical cancer cell line KB 3-1 with two subclones (KB 3-1 ($MDR1^{+++}$) and KB 3-1 ($MDR1^{+++}$, CYP3A5)) and the bladder cancer cell line RT112, also with subclone (RT112 ($MDR1^{+}$, UGT1A1)), were cultured in Dulbecco's Modified Eagle Medium (DMEM) including 3.7 g/l $NaHCO_3$, 4.5 g/l D-Glucose, 1.028 g/l N-Acetyl-L-Alanyl-L-glutamine and supplemented with 10% fetal bovine, 1 mM Na-pyruvate and 1% non-essential amino acids. The human colon cancer cell line LS174T was cultured in Dulbecco's modified Eagle medium containing L-glutamine, pyridoxine hydrochloride and 25 mM Hepes buffer without phenol red, supplemented with 10% fetal bovine, 1 mM Na-pyruvate and 1% non-essential amino acids. All cells were incubated at 37°C with 5% CO_2 in a humidified atmosphere.

Drugs

Irinotecan (CPT-11) and its active metabolite SN-38 were provided by Pharmacia. For preparation of stock solutions the substances were dissolved in methanol, 10 mg/ml for CPT-11 and 1 mg/ml for SN-38 and stored at 4°C protected from light. Lower concentrated dilutions were prepared in PBS and cell culture medium. R-Verapamil was applied from SIGMA, dissolved in DMSO to 50 mg/ml and further diluted in PBS.

Treatment of cells with drugs

Cells were seeded in 96-well culture plates 24 h prior to treatment. With respect to differential growth rates KB 3-1 and RT112 cells were seeded at 700 cells/well, RT112 ($MDR1^{+}$, UGT1A1) at 1000 cells/well and KB 3-1 ($MDR1^{+++}$) and KB 3-1 ($MDR1^{+++}$, CYP3A5) at 1200 cells/well. LS174T were seeded at 1.0×10^4 cells/well. Cells were treated with freshly prepared serial dilutions in culture medium, 0, 0.5, 1, 2.5, 5, 7.5, 10, 25, 50, 75, 100 and 200 µg/ml for CPT-11, and 0, 0.1, 0.25, 0.5, 1, 5, 10, 25, 50, 75, 100 and 200 ng/ml for SN-38. Four well were treated with the same drug dilution. Cells were incubated for 3 days at 37°C in a humidified 5% CO_2 atmosphere.

For MDR1 inhibition experiments R-Verapamil was added to 10 µg/ml final concentration in two wells of each drug dilution.

Cytotoxicity assay

A commercially available MTS assay system (Promega, Madison, USA) was used to determine growth inhibition and cell death according to the instructions of the manufacturer. Three days after adding the drugs, 20 µl of the combined MTS/PMS solution was added to each well of the 96-well culture plate. The plate was incubated for at least 45 min at 37°C in a humidified 5% CO_2 atmosphere and the absorbance at 492 nm was measured. The absorbance values of untreated control cells on each plate were set as 100% growth and used to calculate the remaining growth of drug treated cells. Untreated cells on the culture plates served as controls for unaffected growth and survival.

The drug concentration effecting a 50% inhibition of cell growth was defined as the IC_{50}.

RNA preparation and cDNA synthesis

From each cell batch used in these experiments messenger RNA was isolated from cell lysates by oligo-dT magnet beads (μMACS mRNA Isolation Kit; Miltenyi Biotech) following the instructions of the manufacturer. 250 ng mRNA of each cell line was applied in a 20 μl cDNA synthesis reaction with Superscript II reverse transcriptase (Gibco BRL). Dilutions of this cDNAs served as template in transcript specific amplification reactions.

PCR primers and reaction conditions

PCRs were set up in 25 μl reactions with 0.5 units Taq Polymerase (Qiagen), 200 μM nucleotide mix, 5 μl cDNA template dilution and 0.2 μM gene specific primers, as indicated in Table 3. All reactions were run under the same amplification conditions, differing only in number of cycles (table), 2 min pre-denaturation at 94°C, than for amplification: 45 sec denaturation at 94°C, 45 sec annealing at 62°C and 45 sec elongation at 72°C, except for UGT1A1 which needed longer elongation of 2 min.

Table 3: Sequences of gene specific primers and conditions for PCR reactions. F: forward primer; R: reverse primer for mRNA sequences.

Gene	Primer sequence (5'-3')	cDNA dilution	cycle number
MDR1	F: TGCCTTCATCGAGTCACTGCC R: TCACTGGCGCTTTGTTCCAGC	1:100	26
MRP1	F: TCTCCAAGGAGCTGGACACA R: CGTGGTGACCTGCAATGAGT	1:10	30
UGT1A	F: GATGATGCCCTTGTTTGGTG R: TGTTTTCAAGTTTGGAAATGACTAGGG	1:100	30
UGT1A1	F: AACCTCTGGCAGGAGCAAAGG R: TGTTTTCAAGTTTGGAAATGACTAGGG	1:10	34

CYP3A4	F: TCAGCCTGGTGCTCCTCTATCTAT R: AAGCCCTTATGGTAGGACAAAATATTT	1:10	34
CYP3A5	F: TTGTTGGGAAATGTTTTGTCCTATC R: ACAGGGAGTTGACCTTCATACGTT	1:10	34
PLA2 (house keeping gene)	F: GCTGGTTCAGAAGGCCAAAC R: GGGCCAGACCCAGTCTGATA	1:100	26

Example 5: Expression of genes involved in irinotecan metabolism

Messenger RNA was isolated from the human bladder cancer cell line RT112, its subclone RT112 (MDR1, UGT1A1), the human epithelial cervical cancer cell line KB 3-1 and two subclones KB 3-1 ($MDR1^{+++}$) and KB 3-1 ($MDR1^{+++}$, CYP3A5), and the colon carcinoma cell line LS174T (ATCC CL-188). These mRNAs were reverse transcribed into cDNA and applied as templates in transcript-specific amplification reactions to determine the expression levels of genes involved in irinotecan transport and metabolism (MDR1, MRP1, UGT1A, UGT1A1, CYP3A4, CYP3A5). Amplification of the house keeping gene phospholipase A2 (PLA2) was used as a control for comparable cDNA amounts in the reactions.

The amplification reactions in figure 29 show that the carcinoma cell lines RT112, KB 3-1, and LS174T have no or very low expression of MDR1, respectively. RT112 (MDR1, UGT1A1) is a subclone of RT112, which was selected for resistance to cytotoxic drugs as described in Seemann et al. (Urol Res 1995; 22:353-360), and is characterised by a moderately increased MDR1 expression. The drug resistant subclones KB 3-1 ($MDR1^{+++}$) and KB 3-1 ($MDR1^{+++}$, CYP3A5) were derived similarly from the original KB 3-1 cell line by exposure to MDR1 substrates. These subclones are characterized by highly increased MDR1 expression. They show >20-times more transcripts than the original KB 3-1 cells, implicating a very high MDR1 activity. MRP1 is expressed at the same level in all cell lines. Transcripts of UGT1A enzymes are present only in RT112, RT112 (MDR1, UGT1A1), and LS174T cells. UGT1A1 is only weakly expressed in RT112, stronger expressed in RT112 (MDR1, UGT1A1) and shows highest expression in LS174T cells. CYP3A4 was solely detected in very small amounts in LS174T. RT112 cells, RT112 (MDR1,

UGT1A1), and LS174T show a heterozygous expression of the functionally inactive splice variant and the functionally active transcript of CYP3A5. In contrast, KB 3-1 and KB 3-1 ($MDR1^{+++}$) cells have only the active CYP3A5 transcript and the KB 3-1 ($MDR1^{+++}$, CYP3A5) showed the highest expression of the active CYP3A5 transcript, implicating that the latter have the highest CYP3A5 activity.

Example 6: Colon and other epidermal cancer cell lines with no or low MDR1 and CYP3A5 activity are sensitive to CPT-11 and SN-38.

The colon cancer cell line LS174T, the cervical cancer cell line KB 3-1 and the bladder cancer cell line RT112 were seeded in 96-well culture plates 24 h prior to treatment. Four wells of each cell line were incubated with serial dilutions of CPT-11 and SN-38 and analysed as described above. Figure 30 shows that all three epidermal cancer cell lines stop proliferation and die upon treatment with CPT-11 and SN-38. The concentrations resulting in 50% inhibition (IC_{50}) for CPT-11 are 1.5 μg/ml for LS174T, 2.5 μg/ml for RT112 and 5 μg/ml for KB 3-1 cells. The active metabolite of CPT-11, SN-38 shows a 1000-fold higher efficacy than CPT-11, since 10^3-times lower concentrations cause the same degree of growth inhibition and cell death. The IC_{50} of SN-38 is 5 ng/ml for LS174T cells, 4 ng/ml for RT112 cells and 25 ng/ml for KB 3-1 cells.

These results show that all three epidermal cancer cell lines although derived from different tissues are similarly sensitive to CPT-11 and SN-38 treatment. This also indicates that cancer cells expressing no or only low levels of MDR1 (Figure 29) can be efficiently killed by CPT-11 and SN-38 (Figure 30).

Example 7: MDR1 activity correlates with resistance of cancer cells toward CPT-11 and SN-38

Cells of KB 3-1 and its strongly MDR1 expressing subclones KB 3-1 ($MDR1^{+++}$) and the KB 3-1 ($MDR1^{+++}$, CYP3A5) were seeded in 96-well culture 24 h prior to treatment. Four wells of each cell line were incubated with serial dilutions of CPT-11 and SN-38 and treated as described above. The inhibition curves (Figure 31) of the MDR1 high expresser KB 3-1 subclones (KB 3-1 ($MDR1^{+++}$) and KB 3-1 ($MDR1^{+++}$,

CYP3A5)) (Figure 29) demonstrate a significant higher resistance to CPT-11 and SN-38 compared to the MDR1 low expresser KB 3-1 cell line (KB 3-1). The IC_{50} for CPT-11 increases 17 to 40 fold from 5 μg/ml in KB 3-1 to 85 μg/ml in KB 3-1 ($MDR1^{+++}$) and 200 μg/ml in KB 3-1 ($MDR1^{+++}$, CYP3A5) cells. The IC_{50} for SN-38 increases at least 8 times from 25 ng/ml in KB 3-1 to 200 ng/ml in KB 3-1 ($MDR1^{+++}$) and >200 ng/ml in KB 3-1($MDR1^{+++}$, CYP3A5).

CPT-11 and SN-38 are substrates of MDR1, and are therefore removed from the cells by MDR1 activity. The MDR1 expression level correlates inversely with the sensitivity of tumor cells towards CPT-11 and SN-38. Subsequently, the killing of cells with high MDR1 expresser phenotype requires much higher concentrations of CPT-11.

Example 8: UGT1A1 activity correlates with sensitivity towards SN-38 and not towards CPT-11

CPT-11 and SN-38 sensitivity was compared between RT112 cells and its subclone RT112 (MDR1, UGT1A1). Four wells of each cell line were incubated with serial dilutions of CPT-11 and SN-38 and treated as described above.

The difference in sensitivity against CPT-11 is only small as shown in Figure 32A. The IC_{50} of RT112(MDR1, UGT1A1) cells of 4 μg/ml CPT-11 is two-times higher compared to RT112 cells (IC_{50} of 2.5 μg/ml). In contrast to RT112 cells which express no MDR1, RT112 MDR1, UGT1A1) cells express an intermediate amount of MDR1 which can explain the small though significant increase of CPT-11 sensitivity. A much stronger difference exists between RT112 (IC_{50} of 4 ng/ml) and RT112 (MDR1, UGT1A1) cells (IC_{50} of 75 ng/ml) after treatment with SN-38 (Figure 32B). This 19-fold higher resistance of the RT112 (MDR1, UGT1A1) cell line can be explained by the additional detoxifying effect of UGT1A1 which is expressed at a higher level in RT112 (MDR1, UGT1A1) than in RT112 cells (Figure 29). In contrast to SN-38, CPT-11 is not metabolized by UGTs. Therefore, CPT-11-related toxicity is not affected by UGT1A1 expression and the resistance-enhancing capabilitiy of UGTs in RT112(MDR1, UGT1A1) cells is only detected by application of SN-38.

Example 9: MDR1 inhibition serves as sensitizer towards CPT-11 and SN-38 in MDR1 high expressing but not low expressing cancer cells.

The sensitivity of KB 3-1 cells and its subclones KB 3-1 (MDR1^{+++}) and KB 3-1 (MDR1^{+++}, CYP3A5) against CPT-11 and SN-38 was assessed after blocking MDR1 function using the specific inhibitor R-Verapamil. Four wells of each cell line were incubated with serial dilutions of CPT-11, SN-38 and analysed as described above. Two wells were additionally treated with the MDR1 inhibitor R-Verapamil. Figure 33 shows that addition of R-Verapamil has only marginal effects on the CPT-11 and SN-38 sensitivity of MDR1 low expresser KB 3-1 cells (CPT-11 and SN-38 IC50s of 5 μg/ml and 25 ng/ml without R-Verapamil versus 4.5 μg/ml and 15 ng/m with R-Verapamil, respectively). In contrast, the sensitivity of the MDR1 expressing cells KB 3-1(MDR1^{+++}) and KB 3-1(MDR1^{+++}, CYP3A5) towards CPT-11 and SN-38 was 8-fold and 10-fold higher after inhibition of MDR1 transport function with R-Verapamil. The IC_{50} of KB 3-1(MDR1^{+++}) cells for CPT-11 decreased from 85 μg/ml without to 10 μg/ml with R-Verapamil and from 200 μg/ml without to 25 μg/ml with R-Verapamil in KB 3-1 (MDR1^{+++}, CYP3A5) cells. The effect of MDR1 inhibition during SN-38 treatment is even stronger in these MDR1 high expresser cells, R-Verapamil blocked the MDR1 transport completely and they become as sensitive as KB 3-1 cells.

These results demonstrate that the MDR1 activity is relevant for resistance of cancer cells to CPT-11 and SN-38 and that inhibition of MDR1 sensitises the cells, so that they are more efficiently killed at lower drug concentrations.

Example 10: CYP3A5 activity influences resistance to CPT-11

KB 3-1 (MDR1^{+++}) and KB 3-1 (MDR1^{+++}, CYP3A5) cells which differ by their amounts of CYP3A5 (Figure 29). Four wells of each cell line were incubated with serial dilutions of CPT-11, SN-38 and analyzed as described above. Two wells were additionally treated with the MDR1 inhibitor R-Verapamil.

Because MDR1 activity is a major determinant of cellular sensitivity toward CPT11 and SN-38, the MDR1 activity in these MDR1 high expresser cell lines was completely blocked using an excess of the specific MDR1 inhibitor R-Verapamil to analyze the impact of CYP3A5 on CPT-11 and SN-38 sensitivity without interference of MDR1.

The high CYP3A5 expresser cell line KB 3-1 (MDR1^{+++}, CYP3A5) is with an IC_{50} of 25 μg/ml 2.5-times more resistant to CPT-11 than KB 3-1 (MDR1^{+++}) showing an

IC_{50} of 10 μg/ml (Figure 34). No difference between these two cell lines can be observed regarding their sensitivity towards SN-38.

These experiments demonstrate a significant impact of CYP3A5 expression on the resistance to CPT-11 in contrast to SN-38. The fact that CYP3A5 activity had no influence on SN-38 toxicity further confirms the CYP3A5 effect, because CPT-11 but not SN-38 is metabolized by CYP3A5.

Example 11: MDR1 genotyping improves therapeutic efficacy of irinotecan by genotype-based prediction and monitoring of drug resistance.

Therapeutic efficacy and adverse effects of irinotecan depend on plasma levels and on intracellular tumor concentrations of the parent compound and the active metabolites (e.g. SN-38). The MDR1 gene controls the PGP-dependent penetration of irinotecan across membranes [Luo et al., Drug Metab Dispos 2002, 30:763-770; Jansen et al., Br J Cancer 1998, 77:359-65 Chu et al., J Pharmacol Exp Ther 1999; 288, 735-41; Sugiyama et al., Cancer Chemother Pharmacol 1998, 42 Suppl:S44-9] and is therefore an important determinant for its systemic availability and intracellular accumulation. The 176C>T nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MDR1 gene (Accession No: M29445) is associated with low PGP expression-related low drug efflux and patient carrying this substitution are more likely to respond to irinotecan treatment for two reasons: 1) Due to the lower amount of PGP in enterocytes more irinotecan can enter the body across the intestinal barrier causing more irinotecan to reach its site of action, the tumor. 2) Due to the lower amount of PGP in the tumor cell membranes more irinotecan can penetrate into the tumor cells to deploy its cytotoxic effects. The currently used standard dose of irinotecan kills highly effective most tumor cells within the first cycles of chemotherapy with only very few surviving drug-resistant tumor cells and tolerable adverse events. Independently from the mechanisms of drug resistance, in these patients, the number of surviving cells is to small to develop into a drug-resistant tumor which does not respond any longer to irinotecan therapy.

Patients with the high expresser MDR1 genotype (nucleotide C at position 176 of the MDR1 gene, Accession No: M29445) are less likely to respond to irinotecan treatment. Higher doses would be necessary to achieve a sufficiently efficient killing

of tumor cells in order to prevent the development of a drug-resistant tumor. However, elevation of irinotecan dosage is limited due to the occurrence of intolerable adverse events (e.g. diarrhea, neutropenia, or thromboembolic complications). Alternatively, efficacy of irinotecan treatment can be improved by addition of a PGP inhibitor. A PGP inhibitor blocks efficiently the PGP function in MDR1 high expresser patients in such a way as to enable irinotecan to concentrate in the tumor cells for exerting its cytotoxicity as effective as in MDR1 low expresser patients. Consequently, genotypically MDR1 high expresser patients become phenotypically comparable to MDR1 low expressers.

According to the number of low or high expresser alleles of the MDR1 gene, individuals can be classified as having either extensive (ET, two high expresser alleles), intermediate (IT, one high expresser, one low expresser allele) or poor transport capacity (PT, two low expresser alleles). By genetic testing prior to onset of treatment with irinotecan, patients can be classified as ET, IT, or PT and the MDR1-related transport capacity of the patients can be predicted. The individual risk of an insufficient anticancer treatment increases with the number of MDR1 high expresser alleles. Individuals with ET genotype are at the highest risk to suffer from insufficient response to irinotecan and are at the highest risk to develop a drug resistant tumor. ET patients should be treated with a PGP-inhibitor in addition to irinotecan and more closely monitored for adverse events and for the development of chemotherapy-related drug-resistance. Furthermore, these patients, who are at high risk for developing a drug-resistant tumor, can particularly benefit from taking a tumor biopsy between each cycle of chemotherapy with subsequent individual profiling of tumor cells for drug resistance.

Example 12: Identification of genetic determinants of CYPA5 protein expression

Protein expression of CYP3A5 was determined in 186 Caucasian liver samples by Western blotting using CYP3A5-specific antibodies (Gentest). Liver microsomes were prepared as previously described (Zanger, Biochemistry 27 (1988), 5447-54). To obtain total protein homogenate, powdered liver tissue was homogenised in 0.1 M Tris-Cl pH 7.4, 1 mM EDTA, 1 mM Pefa Bloc SC, 1 μg/ml leupeptin, 1 μg/ml

pepstatin with a Potter Elvehjem homogenisator (glass/Teflon) for 2 min at 1000 rpm. Homogenates were then sonified with a Bandelin Sonoplus HD 200 and stored at –80°C.

For Western blotting, 12.5 μg microsomal protein homogenate or 40 μg total protein homogenate were separated in a 10 % SDS-polyacrylamide gel. Electrophoretic transfer onto PVDF membranes was carried out in a TankBlot Cell (BioRad) for 1.5 hours at constant voltage (100 V) and at 10 °C. Following the transfer, the membranes were incubated for 60 min in 5 % milk, TBS, 0.1 % Tween 20 to reduce the unspecific antibody binding. Incubations with either primary antibody (Gentest, dilution 1:500) were performed in 1 % milk, TBS, 0.1 % Tween 20 for 60 min, those with the secondary antibody (anti-rabbit IgG-POD Fab-fragments, Dianova, dilution 1:10000 in the same solution for 30 min. CYP3A5 protein bands were detected with Supersignal Dura (Pierce) and a digital CCD-camera (LAS-1000, Fuji). Signal quantification was performed with AIDA (Raytest). Protein expression levels were calculated based on calibration curves obtained with microsomes expressing recombinant CYP3A5 proteins (Gentest).

Homogenates or microsomal fractions were prepared from 186 human livers and investigated by Western blotting using a CYP3A5-specific antibody. CYP3A5 protein was detected in all samples analysed and its expression showed a bimodalar distribution . 168 livers (~ 90 %), further referred to as LE (low-expressing), showed expression close to or below the lower limit of quantification (LLOQ) of the assay (0.3 pmol/mg homogenate protein and 1.0 pmol/mg microsomal protein) whereas 18 samples (~ 10 %), further referred to as HE (high-expressing), could be destinguished by significantly higher CYP3A5 expression levels. The expression was in the range between 1.6 and 2.9 pmol/mg homogenate protein (2.3 ± 0.5; n = 6) and between 3.9 and 15.5 pmol/mg microsomal protein (9.7 ± 4.1; n = 12). Taking the LLOQ of the assay as the expression level of CYP3A5 in LE livers, HE livers express on average 8 to 10 times more CYP3A5 protein than LE livers.

The frequencies of Caucasian CYP3A5 gene variants were analyzed in 186 liver samples from Caucasian origin and correlated with CYP3A5 protein expression. The frequencies of variants (SEQ IDs 137, 141, 145, and 149) were significantly

increased in HE livers (all $\chi^2 > 13.3$, df = 1, $p < 0.01$, Bonferroni corrected). Except one, all tested HE livers (17/18, 94 %) were heterozygous for three variants (SEQ IDs 145, 149 and 137). 16 of those samples were heterozygous for ch-v-020 as well. One HE sample could not be genotyped for this variant. In contrast, LE livers were either wildtype (155/168, 92.3 %), heterozygous for SEQ IDs 145 and 149 (9/168, 5.4 %) or heterozygous for the SEQ ID 137 (4/168, 2.4 %) only. However, in LE livers all three variants never occurred simultaneously. These results defined either of the three variants as a useful but imperfect marker of increased CYP3A5 expression.

The distribution of SEQ IDs 145, 149, and 137 in the samples screened strongly suggest that they constitute a haplotype. In the following, the hypotheses whether these three variants recombine independently or not has been tested. Assuming their independent inheritance, the expected 3-loci-genotype frequencies for all combinations of variants and compared them with the observed frequencies have been calculated. The difference is highly significant ($\chi^2 = 93.6$; classes 'all wildtype', 'single variant hetero- or homozygous', 'two or three variants hetero- or homozygous'; df = 1; $p << 0.001$). There were more individuals with two or three of the variants than expected and less individuals with only one of the variants. This result suggests linkage among the three variants. The degree of linkage with the linkage disequilibrium parameter D for the three pairs of variants was estimated. Using maximum likelihood estimates for haplotype frequencies, D was calculated to be 0.041 for the variant pairs with the SEQ IDs 145/137 and 149/137, which is 80 % of its theoretical maximum, and 0.065 for the variants with the SEQ IDa 145 and 149 which corresponds to 100 % of its theoretical maximum.

The probability that individuals showing the respective variant genotype are HE (positive predictive value) is estimated to be 65 % for SEQ IDs 145 and 149, respectively, and 81 % for the SEQ ID 137 variant. For the combination of all three variants the positive predictive value is 100 % in our sample set. However, assuming that these variants need to be located in *cis* for increased protein expression, it is clear that there is some probability for individuals showing all three variants to be LE. The results show that at least the allele comprising SEQ IDs 145/149 and the allele from SEQ ID 137 actually exist and therefore the existence of a genotype with a combination of these two alleles has to be postulated. The

maximum likelihood estimate for the frequency of these 3-fold heterozygotes having not all three variants in *cis* is 0.05 % of all samples screened or 0.61 % of samples hetero- or homozygote for all three variants. In other words, of 100 Caucasians screened statistically about 9 of them will be hetero- or homozygous for all three variants and about 0.05 of these will have not all three variants in *cis*. Therefore, it can be expected that the positive predictive power of the 3-variant genotype to be about 99.95 %. Of course, the same values would be achieved for a combination of only two variants, the SEQ IDs 145/137 or 149/137.

Claims

1. A method of using irinotecan to treat a patient suffering from cancer which comprises:
   (a) determining if the patient has one or more variant alleles of the CYP3A5 gene;
   (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene.

2. The method of claim 1 wherein the cancer is colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, or pancreatic cancer.

3. The method of claim 2 in which:
   (a) the one or more variant alleles result in the patient expressing low amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is decreased to avoid toxicity; or
   (b) the one or more variant alleles result in the patient expressing high amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is increased to enhance efficacy.

4. The method of claim 3 wherein the one or more variant alleles are in the promoter region of the CYP3A5 gene.

5. The method of claim 3 wherein the one or more variant alleles are in the coding region of the CYP3A5 gene.

6. The method of claim 3 wherein the one or more variant alleles are not in either the promoter region or the coding region of the CYP3A5 gene.

7. The method of claim 3 wherein the one or more variant alleles are in both the promoter region and the coding region of the CYP3A5 gene.

8. The method of claim 3 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:
   (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 137, 138, 141, 142, 145, 146, 149 and/or 150;
   (b) a polynucleotide capable of hybridizing to a Cytochrome P450, subfamily IIIA (nifedipine oxidase), polypeptide 5 (CYP3A5) gene, wherein said polynucleotide is having at a position corresponding to positions 47518 and/or 9736 of the CYP3A5 gene (Accession No: GI:10281451), a substitution of at least one nucleotide or at a position corresponding to positions 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452), a substitution of at least one nucleotide;
   (c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) a C, at a position corresponding to position 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452) a G or at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451) a G.

9. The method of claim 8 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:
   (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 137, 141, 145 or 149:

(b) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 47518 or 9736 of the CYP3A5 gene (Accession No: GI:10281451) or 145601 or 145929 of the CYP3A5 gene (Accession No: GI:11177452);

(c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having a C at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) or a G at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451), or 145601 or 145929 of the CYP3A5 gene (Accession No: GI:11177452).

10. The method of claim 8 in which the one or more variant alleles results in the patient expressing low amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is decreased.

11. The method of claim 8 in which the one or more variant alleles results in the patient expressing high amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is increased.

12. The method of claim 9 in which the one or more variant alleles results in the patient expressing low amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is decreased.

13. The method of claim 9 in which the one or more variant alleles results in the patient expressing high amounts of the CYP3A5 gene product, whereby the amount of irinotecan administered to the patient is increased.

14. A method for determining whether a patient is at risk for a toxic reaction to treatment with irinotecan which comprises determining if the patient has one or more variant alleles of the CYP3A5 gene.

15. The method of claim 14 which further comprises administering to the patient reduced amounts of irinotecan if the patient has one or more variant alleles that result in decreased expression of the CYP3A5 gene.

16. A method for determining the optimum treatment regimen for administering irinotecan to a patient suffering from cancer which comprises:
    (a) determining if the patient has one or more variant alleles of the CYP3A5 gene;
    (b) in a patient having one or more of such alleles increasing or decreasing the amount of irinotecan in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene.

17. A method of treating cancer in a patient having one or more variant alleles of the CYP3A5 gene such that expression levels of the CYP3A5 gene product are lower than in the general population and so indicates high sensitivity to irinotecan which comprises administering to the patient a decreased amount of irinotecan.

18. A method of treating cancer in a patient having one or more variant alleles of the CYP3A5 gene such that expression levels of the CYP3A5 gene product are higher than in the and so indicates resistance or predisposition to resistance to irinotecan which comprises administering to the patient an increased amount of irinotecan.

19. A method of treating cancer in a patient which comprises internally administering to the patient an effective amount of irinotecan, wherein the treatment regimen is modified based upon the genotype of the patient's CYP3A5 gene.

20. A method of treating a population of patients suffering from cancer which comprises:

(a) determining, on a patient by patient basis, if the patient has one or more variant alleles of the CYP3A5 gene;

(b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the CYP3A5 gene.

21. A method for predicting sensitivity to irinotecan in a patient suffering from cancer which comprises determining if the patient has one or more variant alleles of the CYP3A5 gene, which alleles indicate that the cancerous cells express low or high amounts of the CYP3A5 gene product, whereby low expression indicates high sensitivity to irinotecan and high expression indicates resistance or predisposition to resistance to irinotecan.

22. The method of claim 21 in which patients that have a genotype that indicates resistance or predisposition to resistance are treated with a CYP3A5 inhibitor.

23. The method of claim 22 wherein the CYP3A5 inhibitor is selected from the group consisting of: Clarithromycin, Erythromycin, Diltiazem, Mibefradil, grapefuit juice, Cimetidine, Ciprofloxacin, Norfloxacin, Fluconazole, Itraconazole, Ketoconazole, Fluvoxamine, Norfluoxetine, Nefazodone, Troleandomycin, Delaviridine, Indinavir, Nelfinavir, Ritonavir, Saquinavir, Mifepristone, and gestodene

24. The method of claim 21 wherein the patients that have a genotype that indicates resistance or predisposition to resistance are monitored during treatment by assaying for expression levels of the CYP3A5 gene product in the cancerous cells.

25. Use of irinotecan or a derivative thereof for the preparation of a pharmaceutical composition for treating cancer, especially, colorectal cancer,

cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a subject having a genome with a first variant allele which comprises a polynucleotide selected from the group consisting of:

(a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 137, 138, 141, 142, 145, 146, 149 and/or 150;

(b) a polynucleotide capable of hybridizing to a Cytochrome P450, subfamily IIIA (nifedipine oxidase), polypeptide 5 (CYP3A5) gene, wherein said polynucleotide is having at a position corresponding to positions 47518 and/or 9736 of the CYP3A5 gene (Accession No: GI:10281451), a substitution of at least one nucleotide or at a position corresponding to positions 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452), a substitution of at least one nucleotide;

(c) a polynucleotide capable of hybridizing to a CYP3A5 gene, wherein said polynucleotide is having at a position corresponding to position 47518 of the CYP3A5 gene (Accession No: GI:10281451) a C, at a position corresponding to position 145601 and/or 145929 of the CYP3A5 gene (Accession No: GI:11177452) a G or at a position corresponding to position 9736 of the CYP3A5 gene (Accession No: GI:10281451) a G.

26. The use of claim 25, wherein a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of the variant allele compared to the corresponding wild type alleles.

27. The use of claim 26, wherein said altered expression is decreased or increased expression.

28. The use of claim 25, wherein a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered activity of the

polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele.

29. The use of claim 28, wherein said altered activity is decreased or increased activity.

30. The use of any one of claims 25 to 29, wherein said subject is an animal.

31. The use of any one of claim 30, wherein said subject is a mouse.

32. The use of any one of claims 25 to 29, wherein said subject is a human.

33. The use of claim 32, wherein said human is African or Asian.

34. A method for selecting a suitable therapy for a subject suffering from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer, wherein said method comprises:
    (a) determining the presence or absence of a variant allele as specified in claim 25 in the genome of a subject in a sample obtained from said subject; and
    (b) selecting a suitable therapy for said subject based on the results obtained in (a).

Figure 1

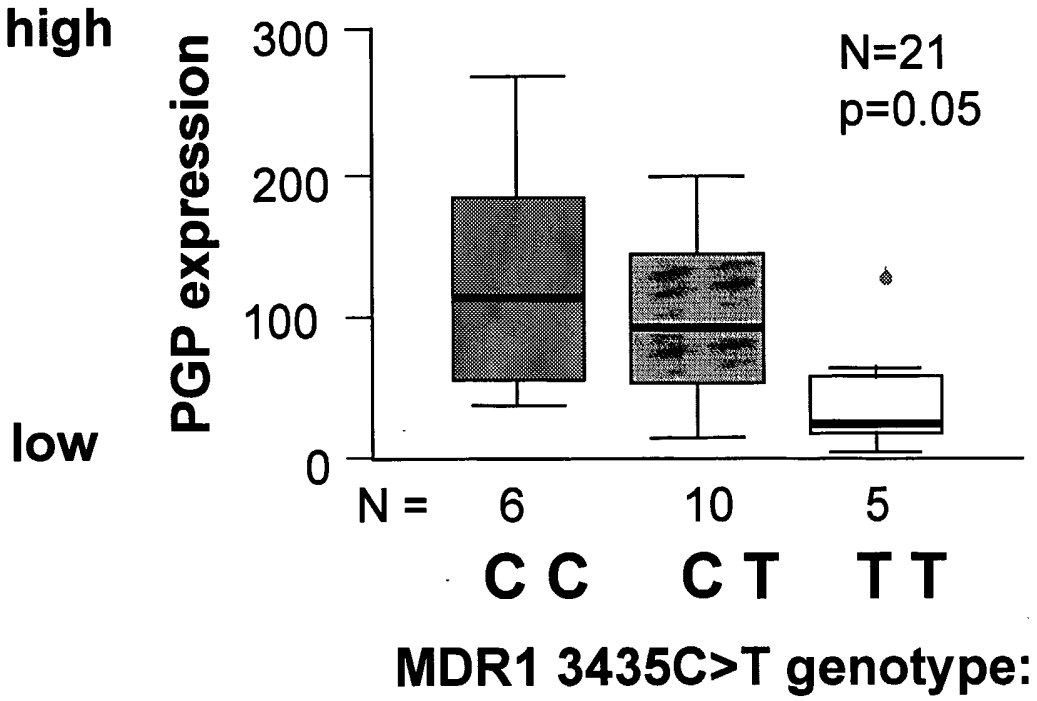

high
low
PGP expression
300
200
100
0
N=21
p=0.05
N = 6 10 5
C C C T T T
MDR1 3435C>T genotype:

Figure 1

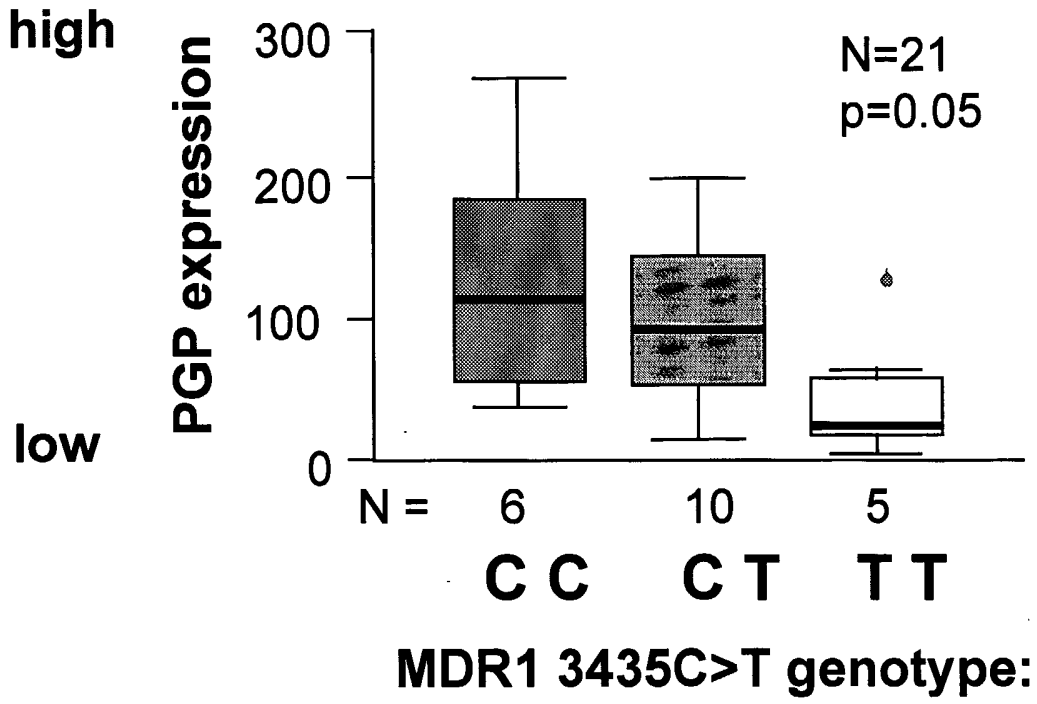
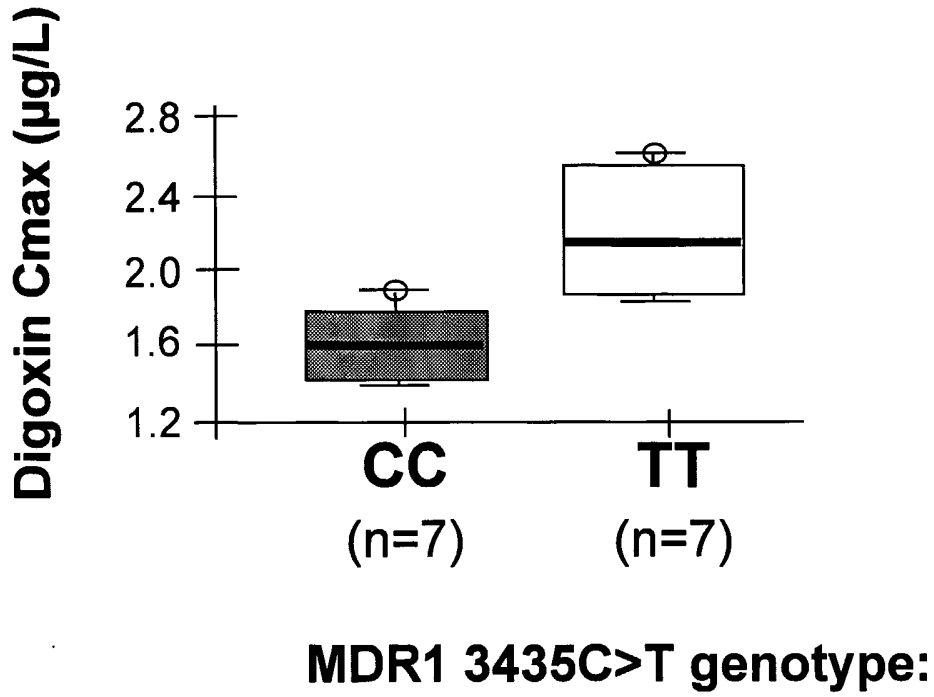

high
low
PGP expression
300
200
100
0
N=21
p=0.05
N = 6 10 5
C C C T T T
MDR1 3435C>T genotype:

Figure 2


Digoxin Cmax (µg/L)
2.8
2.4
2.0
1.6
1.2
CC
(n=7)
TT
(n=7)
MDR1 3435C>T genotype:

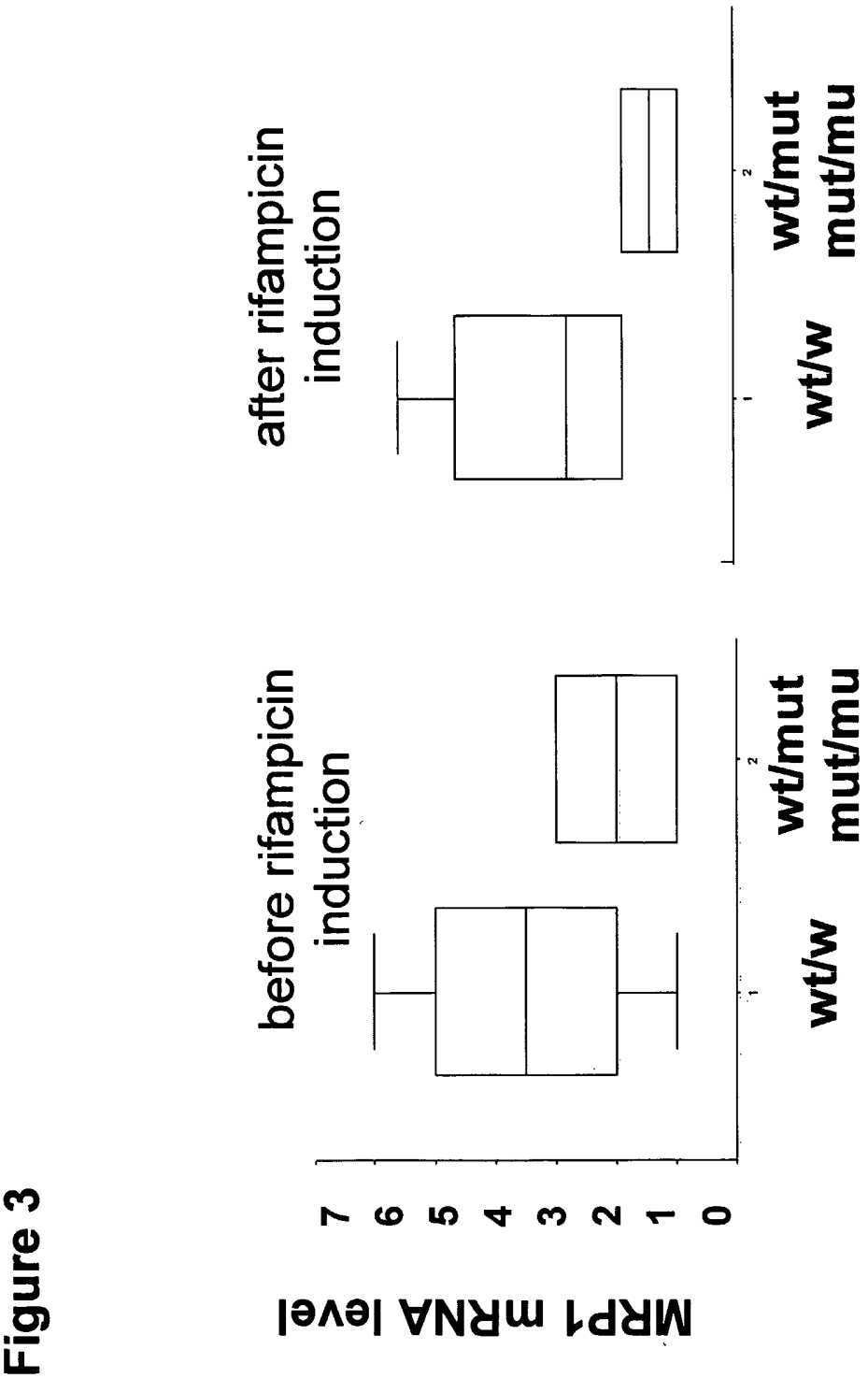

MRP1 mRNA level
7
6
5
4
3
2
1
0
before rifampicin induction
after rifampicin induction
wt/w
wt/mut
mut/mu
MRP1 Genotype:

Figure 3

Figure 29

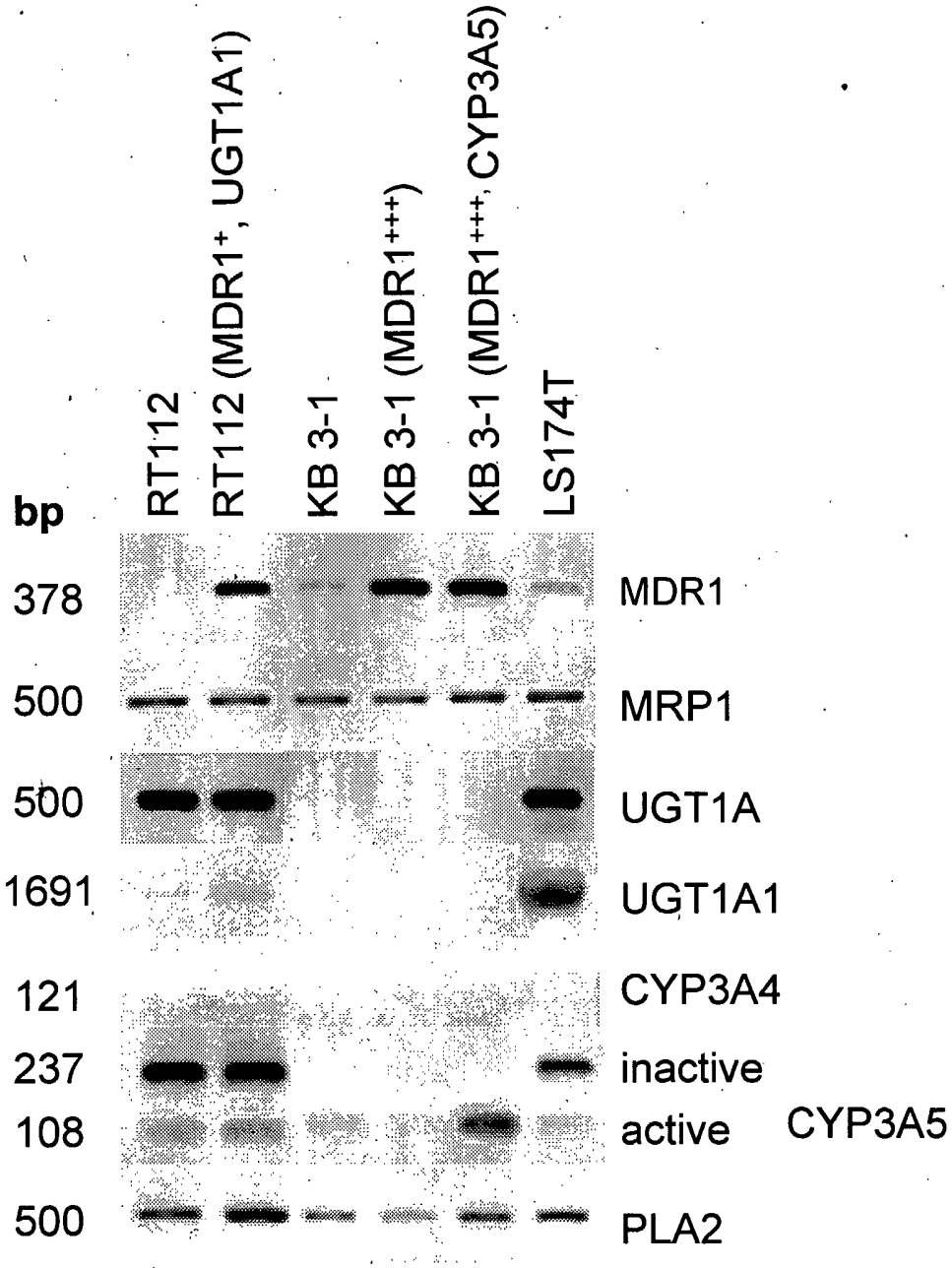
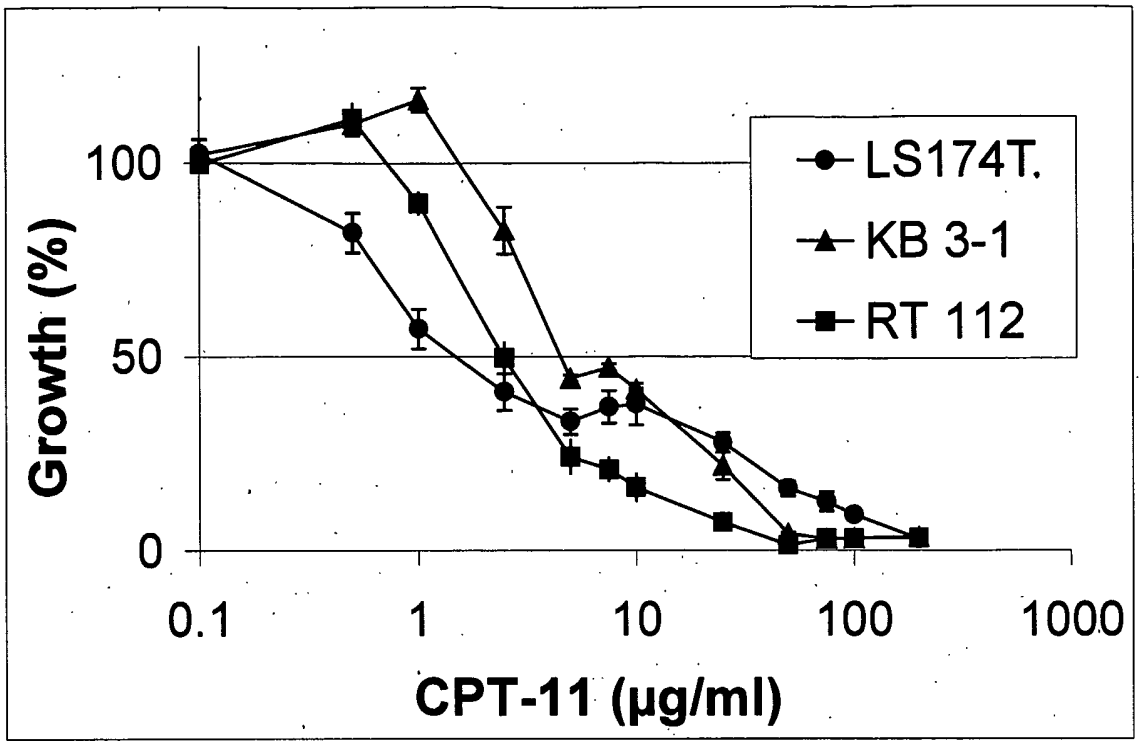

RT112
RT112 (MDR1+, UGT1A1)
KB 3-1
KB 3-1 (MDR1+++)
KB 3-1 (MDR1+++, CYP3A5)
LS174T
bp
378
MDR1
500
MRP1
500
UGT1A
1691
UGT1A1
121
CYP3A4
237
inactive
108
active
CYP3A5
500
PLA2

Figure 30


Growth (%)
100
50
0
0.1
1
10
100
1000
CPT-11 (µg/ml)
LS174T.
KB 3-1
RT 112

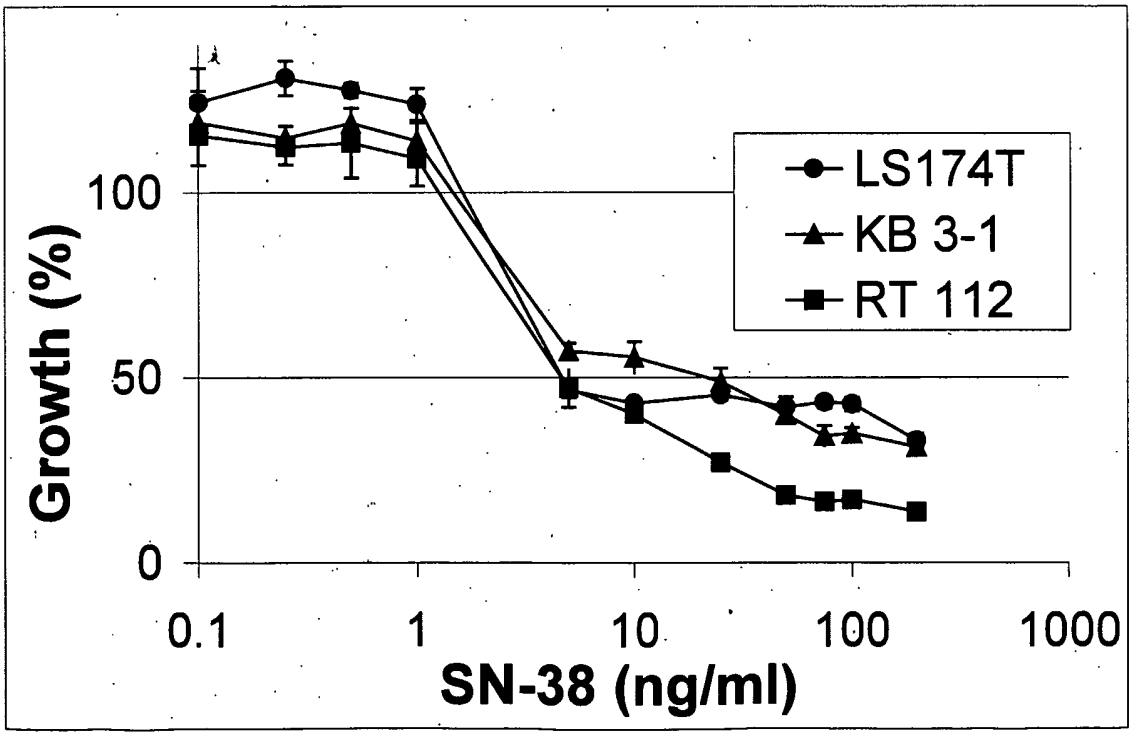
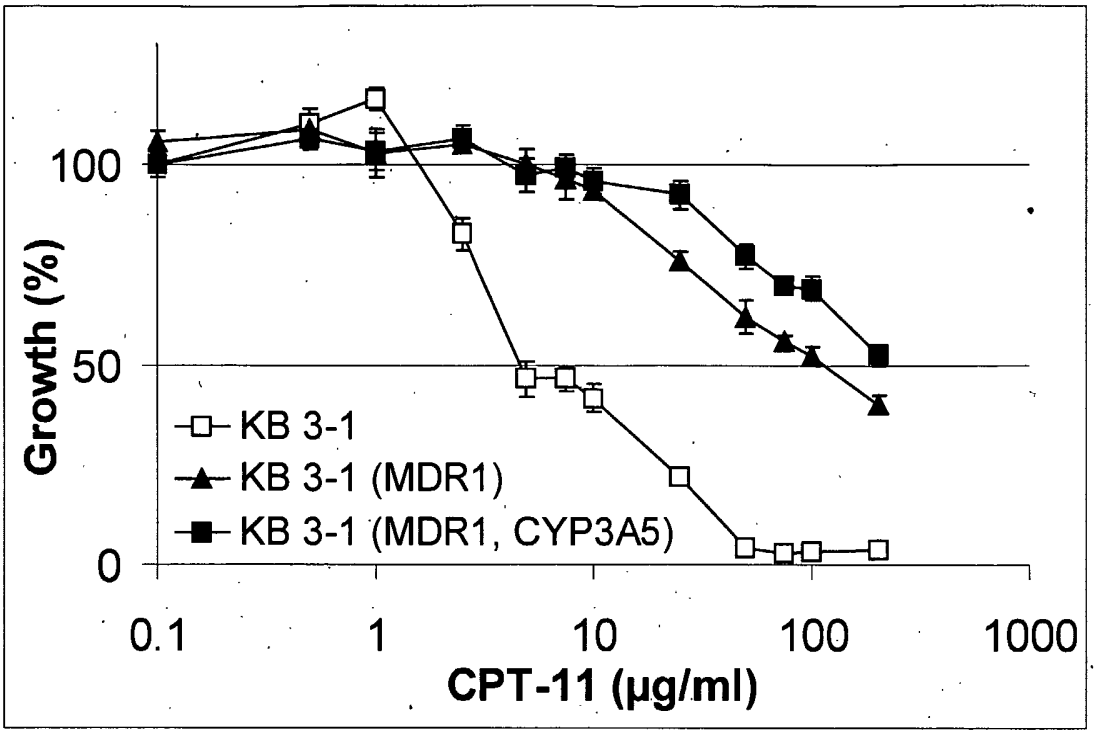

Growth (%)
100
50
0
0.1
1
10
100
1000
SN-38 (ng/ml)
LS174T
KB 3-1
RT 112

Figure 31


100
50
0
Growth (%)
KB 3-1
KB 3-1 (MDR1)
KB 3-1 (MDR1, CYP3A5)
0.1
1
10
100
1000
CPT-11 (µg/ml)

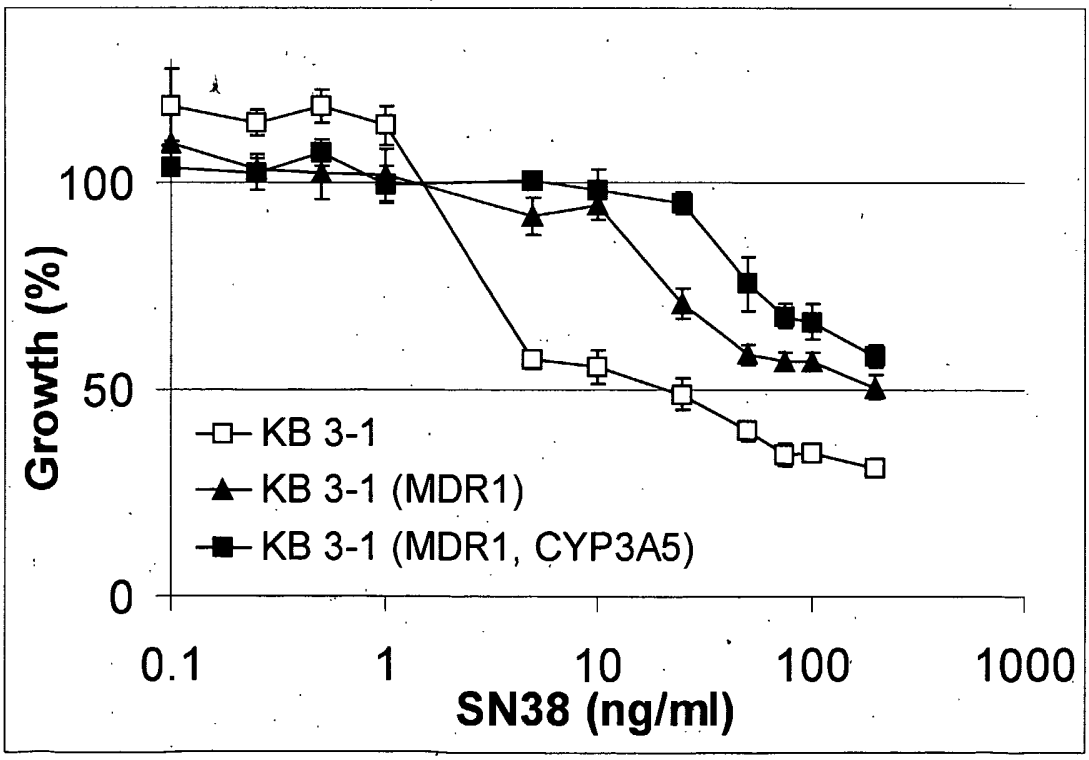
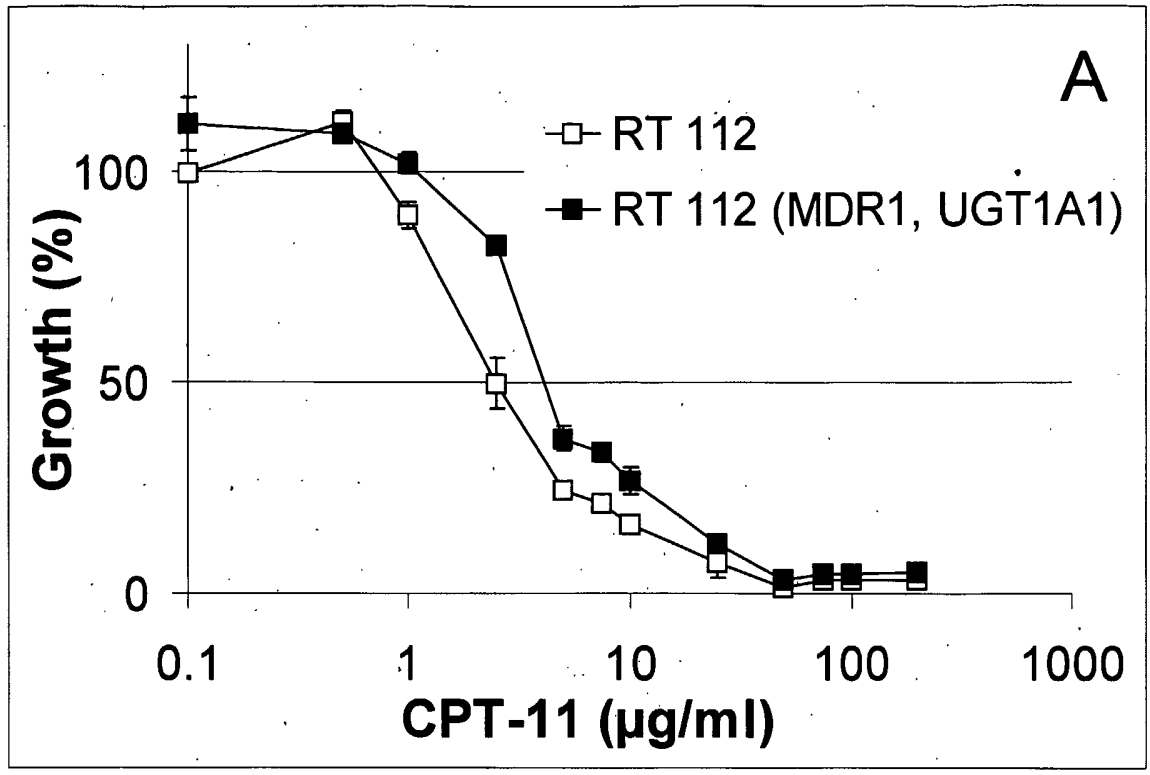

100
50
0
Growth (%)
KB 3-1
KB 3-1 (MDR1)
KB 3-1 (MDR1, CYP3A5)
0.1
1
10
100
1000
SN38 (ng/ml)

Figure 32


A
RT 112
RT 112 (MDR1, UGT1A1)
Growth (%)
100
50
0
0.1
1
10
100
1000
CPT-11 (µg/ml)

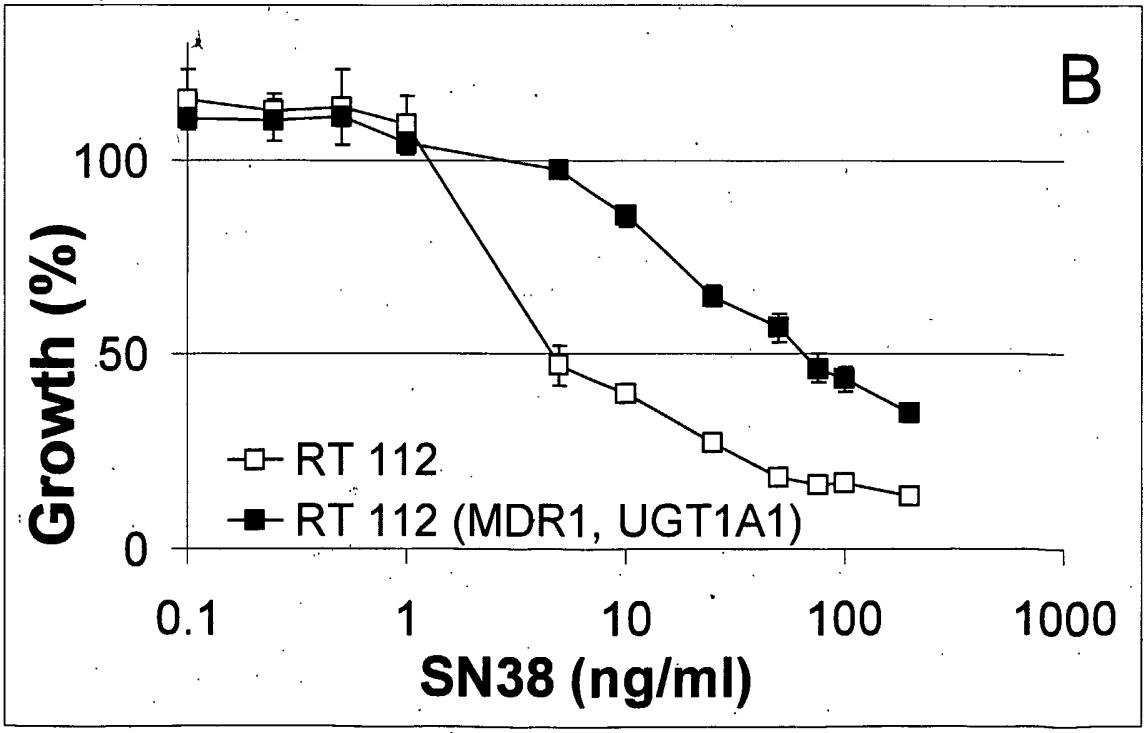
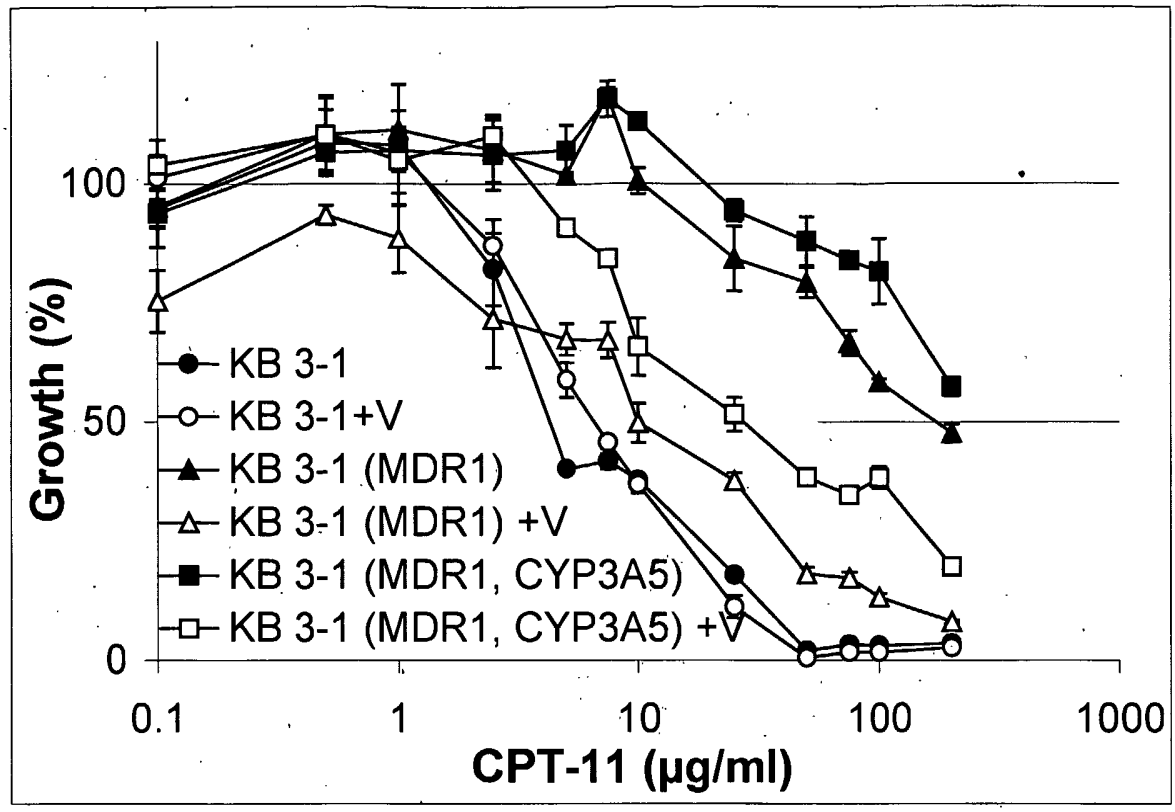

B
Growth (%)
100
50
0
RT 112
RT 112 (MDR1, UGT1A1)
0.1
1
10
100
1000
SN38 (ng/ml)

Figure 33


Growth (%)
100
50
0
KB 3-1
KB 3-1+V
KB 3-1 (MDR1)
KB 3-1 (MDR1) +V
KB 3-1 (MDR1, CYP3A5)
KB 3-1 (MDR1, CYP3A5) +V
0.1
1
10
100
1000
CPT-11 (µg/ml)

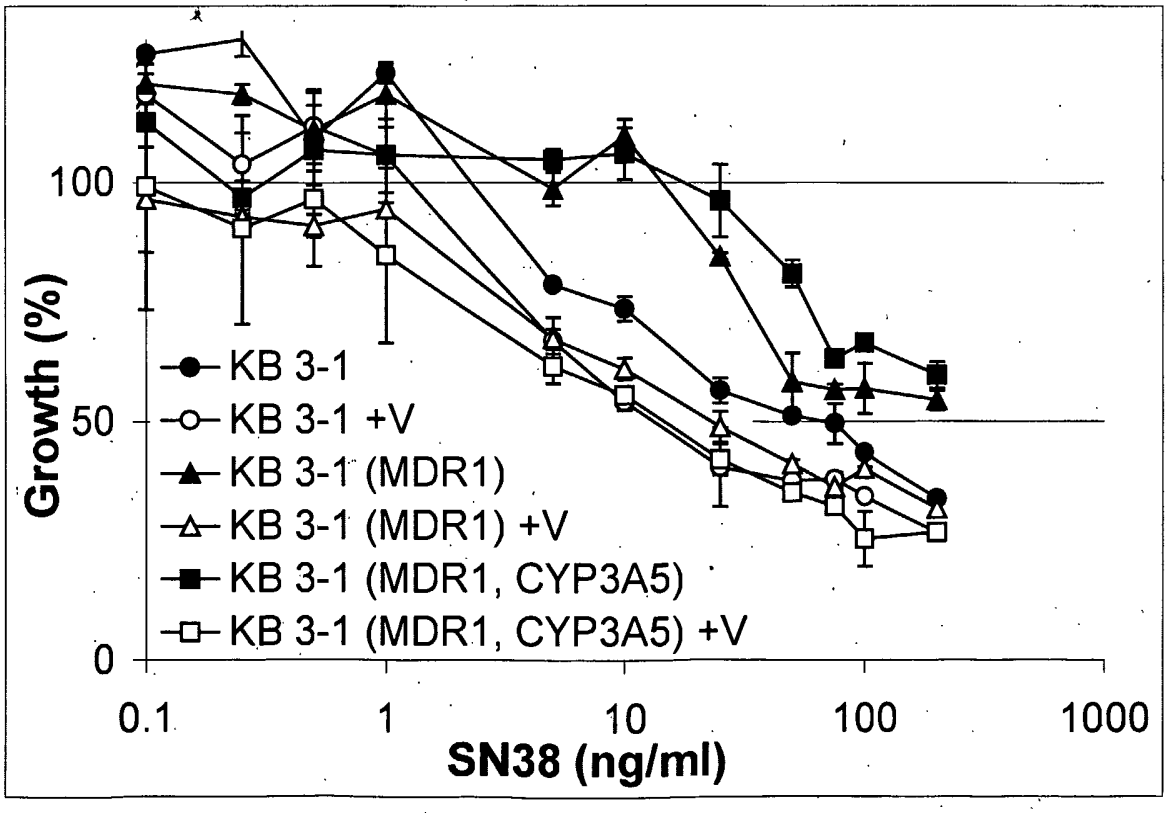
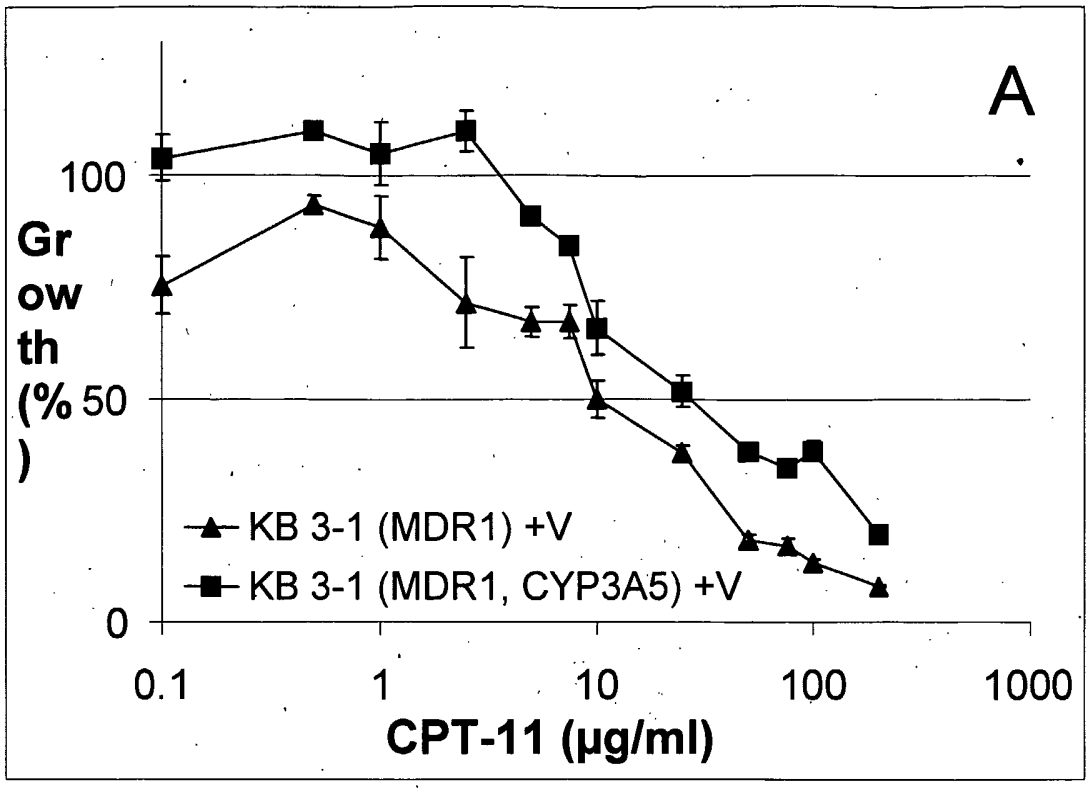
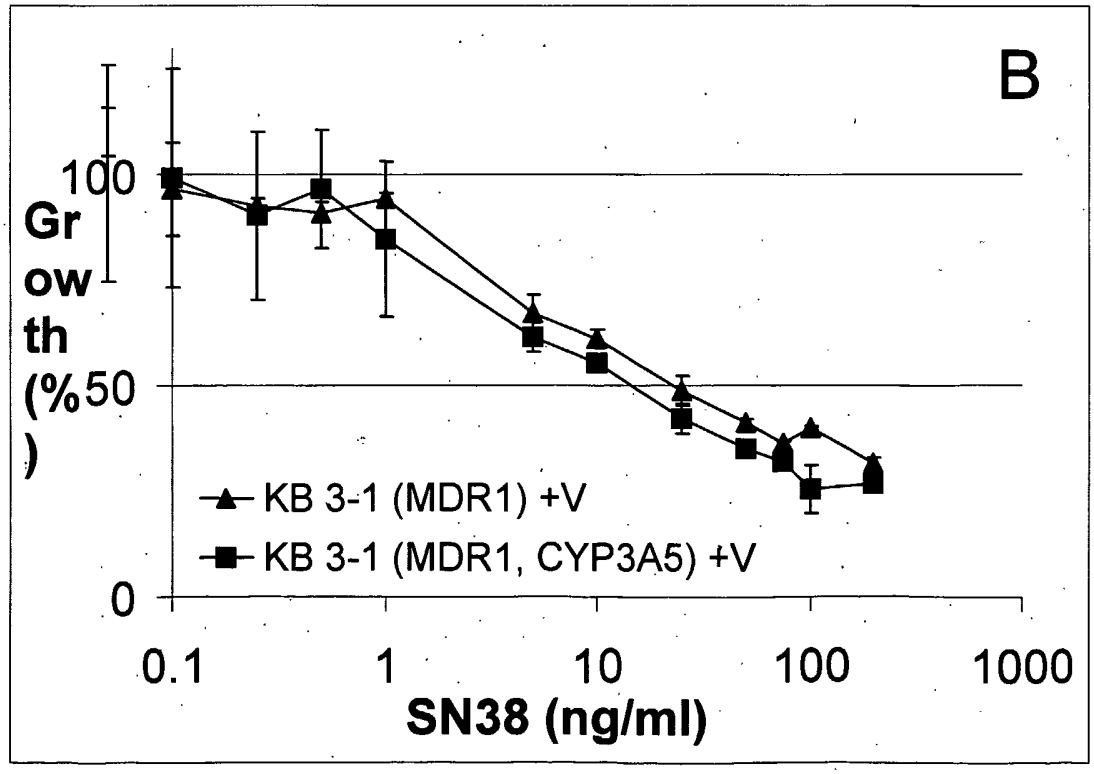

Growth (%)
100
50
0
KB 3-1
KB 3-1 +V
KB 3-1 (MDR1)
KB 3-1 (MDR1) +V
KB 3-1 (MDR1, CYP3A5)
KB 3-1 (MDR1, CYP3A5) +V
0.1
1
10
100
1000
SN38 (ng/ml)

Figure 34


A
100
Gr
ow
th
(%
)
50
0
KB 3-1 (MDR1) +V
KB 3-1 (MDR1, CYP3A5) +V
0.1
1
10
100
1000
CPT-11 (µg/ml)


B
100
Gr
ow
th
(%
)
50
0
KB 3-1 (MDR1) +V
KB 3-1 (MDR1, CYP3A5) +V
0.1
1
10
100
1000
SN38 (ng/ml)