USE OF MDR-1 INDUCERS FOR TREATING OR PREVENTING DISEASES

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Renal cell carcinoma (RCC) is the third most frequent urological tumor, accounting in the United States for 28,000 cases in the year 1995 (Wingo et al.1995). About 11,000 patients die each year in the US from metastatic RCC (Wingo et al.1995). This disease can only be cured if it is limited to the kidney, which allows surgical removal by radical nephrectomy. If the tumor has spread to distant organs, the prognosis is poor with 5 year survival rates of at most 20% (Guinan et al. 1995, Dinney et al. 1992). Therefore, to achieve high survival rates, it is necessary to detect the disease at an early stage, where it is curable by surgery. Ultrasound screening is a suitable and widespread, albeit relatively expensive and investigator dependent, method. Risk factors (e.g. mutations in the von Hippel-Lindau gene, familiar predisposition, or polycystic kidney disease Linehan et al. 1995, Schlehofer et al.1996, Levine 1996) can be used to define risk groups that should be periodically examined by ultrasound.

The incidence of RCC has been increasing steadily by 2.3 to 4.3 % per year in the United States and other industrialized countries in the Central and Northern regions of Europe depending upon race and gender (Chow et al.1999). Wunderlich et al. (1999) showed that this increase can not be fully explained by the widespread use of ultrasound since the percentage of clinically recognized tumors on the total of all found RCCs in autopsy was nearly constant over a period of 12 years. The reason for this increase, especially in industrialized nations, has not yet been fully defined. However, a positive correlation between smoking, fat and meat consumption, obesity and hypertension with the occurrence of RCC has been shown (Benichou et al. 1998, Schlehofer et al. 1996, Lindblad et al. 1997,
Heath et al. 1997), whereas the intake of fruits, especially citrus fruits, vitamine C and E and carotene reduces the risk. It is feasible to assume that factors or genes that play a role in the defense of kidney cells against dietary and environmental toxins or metabolites may influence the individual susceptibility towards RCC.

It is common knowledge that environmental toxins and carcinogens not only may induce the development of malignant transformation of cells, but they are frequently also causative for direct cell damage. In term, acute or chronic cell damage can lead to inflammatory reactions and inflammatory disease of various tissues, such as kidney (glomerulonephritis), liver (hepatitis) or intestine (colitis).

The human multidrug resistance (MDR-1) gene is expressed in kidney cells in the proximal tubuli (Ambudkar et al. 1999, Gottesman et al. 1996) and its gene product, P-glycoprotein, is directly involved in the protection of cells against many toxic substances and metabolites. The P-glycoprotein is hereinafter also referred to as MDR-1 protein. MDR-1 encodes an integral membrane protein which pumps substances from the inside of cells and from membranes to the outside. The physiological role of this energy-dependent export mechanism is the protection of cells, although it may also play a role in steroid metabolism (Meda et al. 1987, Chen et al. 1990). MDR-1 is expressed in various organs, e.g. in the intestine, bladder, prostate or in leucocytes as well as in the blood/brain barrier, where it controls the adsorption and penetration of substances (Schinkel et al. 1999, Rao et al. 1999). For example, in the kidney, located in proximal tubuli, it likely contributes to the efficient excretion of substances from the tubuli cells into the urine. MDR-1 expression correlates directly with the "detoxification" capacity of cells in any of the above cited organs or tissues. This is of particular importance in cancer therapy, where high MDR-1 expression causes cancer cells to become refractory to treatment (Ambudkar et al. 1999). Likewise, it can be assumed that in nonmalignant tissues or organs the degree of MDR-1 expression influences the capacity of the cells to remove damaging agents. Thus, the tubular cells of low expressors of MDR-1 are most likely more exposed to damaging substances compared to inherently high expressors.
Recently, several naturally occurring MDR-1 polymorphisms have been described (Hoffmeyer et al. 2000, Mickley et al. 1999). One of these polymorphisms (C3435T in exon 26) correlates with the expression levels and function of MDR-1 protein in the intestine. Individuals homozygous for this polymorphism displayed low duodenal MDR-1 expression, and – in perfect agreement with that – high intestinal uptake of orally delivered MDR-1 protein substrate digoxin. Homozygosity for this MDR-1 variant, associated with low intestinal MDR-1 protein levels, was observed in 22-24% of normal caucasians (Hoffmeyer et al. 2000). If this MDR-1 variant correlates also with low renal expression, it could define a population whose tubular cells carry an increased risk of being damaged by toxic agents or metabolites. Consequently, since the development of cancer, inflammatory disease or tissue/organ damage might at least partly be promoted by dietary or environmental factors, this population with lower renal MDR-1 activity might be at a higher risk to develop said diseases.

Thus, the technical problem of the present invention is to provide means and methods to prevent or treat the above-described diseases. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to the use of a compound which induces or activates MDR-1, a nutrient which induces or activates MDR-1 for the preparation of a pharmaceutical composition for treating or preventing a risk factor associated disease.

The terms "treating", "preventing" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be
predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e. arresting its development; or (c) relieving the disease, i.e. causing regression of the disease. The present invention is directed towards treating patients with medical conditions relating to the above-described disease. Accordingly, a treatment of the invention would involve preventing, inhibiting or relieving any medical condition related to said disease.

The term "compound" in a use or method of the invention includes a single substance or a plurality of substances which may or may not be identical. Said compound(s) may be chemically synthesized or produced via microbial fermentation but can also be comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms. Furthermore, said compounds may be known in the art but hitherto not known to be useful as an inhibitor, respectively. The plurality of compounds may be, e.g., added to the culture medium or injected into a cell or non-human animal of the invention. If a sample containing (a) compound(s) is identified in the method of the invention, described infra, then it is either possible to isolate the compound from the original sample identified as containing the compound, in question or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample. It can then be determined whether said sample or compound displays the desired properties, for example, by the methods described herein or in the literature (Spector et al., Cells manual; see supra). Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. The methods of the present invention can be easily performed and designed by the person skilled in the art, for example in accordance with other cell based assays described in the prior art or by using and modifying the methods as described herein. Furthermore, the person skilled in the art will readily recognize which further compounds and/or enzymes may be used in
order to perform the methods of the invention, for example, enzymes, if necessary, that convert a certain compound into the precursor which in turn represents a substrate for the MDR-1 protein. Such adaptation of the method of the invention is well within the skill of the person skilled in the art and can be performed without undue experimentation.

Compounds which can be used in accordance with the present invention include peptides, proteins, nucleic acids, antibodies, small organic compounds, ligands, peptidomimetics, PNA and the like. Said compounds can also be functional derivatives or analogues of known drugs such as verapamil or cyclosporin.

Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art or as described. Furthermore, peptide mimetics and/or computer aided design of appropriate drug derivatives and analogues can be used, for example, according to the methods described below. Such analogs comprise molecules having as the basis structure of known MDR-substrates and/or inhibitors and/or modulators; see infra.

Appropriate computer programs can be used for the identification of interactive sites of a putative inhibitor and the MDR-1 protein of the invention by computer assistant searches for complementary structural motifs (Fassina, Immunomethods 5 (1994), 114-120). Further appropriate computer systems for the computer aided design of protein and peptides are described in the prior art, for example, in Berry, Biochem. Soc. Trans. 22 (1994), 1033-1036; Wodak, Ann. N. Y. Acad. Sci. 501 (1987), 1-13; Pabo, Biochemistry 25 (1986), 5987-5991. The results obtained from the above-described computer analysis can be used in combination with the method of the invention for, e.g., optimizing known inhibitors. Appropriate peptidomimetics and other inhibitors can also be identified by the synthesis of peptidomimetic combinatorial libraries through successive chemical modification and testing the resulting compounds, e.g., according to the methods described
herein. Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh, Methods in Enzymology 267 (1996), 220-234 and Dorner, Bioorg. Med. Chem. 4 (1996), 709-715. Furthermore, the three-dimensional and/or crystallographic structure of inhibitors and the MDR-1 protein of the invention can be used for the design of peptidomimetic drugs (Rose, Biochemistry 35 (1996), 12933-12944; Rutenber, Bioorg. Med. Chem. 4 (1996), 1545-1558).

A "nutrient" in accordance with the present invention may be a compound or a plurality of compounds which are physiologically required by a cell, a tissue, an organ or the active organism. The term "nutrient" refers to essential and non-essential nutrients. A nutrient of the present invention may be comprised in a particular food ingredient or a combination of food ingredients.

The term "risk factor" as used herein refers to a compound which may either promote or induce a disease in accordance with the invention. For example, carcinogenic or toxic compounds are risk factors for cancer or tissue/organ damage. Usually, said risk factors have to be present in a cell in a certain concentration to be harmful. Said concentration may vary between different risk factors. Usually, said risk factors may be prevented from accumulation in a cell by drug transporters such as MDR-1 protein.

Means and methods for determining whether said compound or nutrient induces or activates MDR-1 are well-known in the art and comprise, e.g., standard molecular biology techniques for determining the RNA or protein level present in the cell upon contacting of said cell with said compound or nutrient suspected to be an inducer. Preferably said standard methods may comprise Northern blot analysis, PCR or immunological techniques such as Immunoassays or FACS analysis. Moreover, assays for determining the activity of MDR-1 protein are well-known in the art and may comprise methods for measuring the enzymatic activity of MDR-1.

Preferably, a labeled substrate may be applied to a cell which comprises MDR-1 protein and the activity of said MDR-1 protein may be determined based on the
labeled substrate upon contacting of said cell with a compound or nutrient suspected to be an inducer or activator of the MDR-1 gene. A variety of techniques are available for labeling biomolecules, are well known to the person skilled in the art and are considered to be within the scope of the present invention. Such techniques are, e.g., described in Tijssen, "Practice and theory of enzyme immuno assays", Burden, RH and von Knippenburg (Eds), Volume 15 (1985), "Basic methods in molecular biology"; Davis LG, Dibmer MD; Battey Elsevier (1990), Mayer et al., (Eds) "Immunochemical methods in cell and molecular biology" Academic Press, London (1987), or in the series "Methods in Enzymology", Academic Press, Inc. There are many different labels and methods of labeling known to those of ordinary skill in the art. Commonly used labels comprise, inter alia, fluorochromes (like fluorescein, rhodamine, Texas Red, etc.), enzymes (like horse radish peroxidase, β-galactosidase, alkaline phosphatase), radioactive isotopes (like $^{32}$P or $^{125}$I), biotin, digoxygenin, colloidal metals, chemi- or bioluminescent compounds (like dioxetanes, luminol or acridiniums). Labeling procedures, like covalent coupling of enzymes or biotinyl groups, iodinations, phosphorylations, biotinylations, random priming, nick-translations, tailing (using terminal transferases) are well known in the art. Detection methods comprise, but are not limited to, autoradiography, fluorescence microscopy, direct and indirect enzymatic reactions, etc. Commonly used detection assays can comprise radioisotopic or non-radioisotopic methods. These comprise, inter alia, RIA (Radioisotopic Assay) and IRMA (Immune Radioimmunometric Assay), EIA (Enzym Immuno Assay), ELISA (Enzyme Linked Immuno Assay), FIA (Fluorescent Immuno Assay), and CLIA (Chemiluminescent Immune Assay). Other detection methods that are used in the art are those that do not utilize tracer molecules. Suitable assays measure e.g. increased RNA, protein or activity levels which may be indicative for a compound to be an inducer or activator of MDR-1. Further, said assays may comprise the measurement of RNA or activity of a reporter gene product. For such a reporter gene assay, a reporter gene, which has been operatively linked to a suitable promoter could be introduced in a suitable host cell. In a further step, said cell being contacted with the compound suspected to be an inducer prior to the measurement of the RNA levels or activity of the reporter gene RNA or protein product. The term "suitable promoter" as used
herein refers to the promoter or functional promoter elements of the MDR-1 gene. Suitable reporter genes may comprise, e.g., luciferase, green fluorescent protein and variants thereof, β-galactosidase, Renilla luciferase or Chloramphenicol transferase. Dependent on the choice of the reporter, the person skilled in the art will know how to perform said reporter gene assays.

Thanks to the present invention it will be possible to efficiently treat diseases which are associated with either low expression or function of MDR-1 by e.g. administration of compounds or nutrients identified to be inducers or activators of MDR-1. Moreover, compounds or nutrients identified by the above described method to be inducers or activators may be used to prevent the above described diseases in particular in subjects having a susceptibility thereto.

In a preferred embodiment of the use of the invention said disease is cancer.

In a most preferred embodiment of the use of the invention said cancer is derived from a MDR-1 expressing tissue.

In a particularly preferred embodiment of the use of the invention said cancer is renal cell carcinoma (RCC), liver cancer, colon cancer, bladder cancer, prostate cancer or leukemia.

In another preferred embodiment of the use of the invention said disease is an inflammatory disease.

In still another preferred embodiment of the use of the invention said disease is tissue/organ damage.

In a most preferred embodiment said tissue/organ is kidney, liver, colon, bladder, prostate or blood.

Also preferred is the use of the present invention wherein said risk factor can be identified by a method described herein after.
Moreover, in a preferred embodiment of the use of the present invention wherein said risk factor is absence or reduced levels of the MDR-1 polypeptide.

In a furthermore preferred embodiment of the use of the present invention said risk factor is

(a) a nucleotide substitution, addition or deletion at a position corresponding to position 3435 of exon 26 of the MDR-1 gene (Accession No: AF016535);
(b) a C to T substitution at a position corresponding to position 3435 of exon 26 of the MDR-1 gene (Accession No: AF016535); or
(c) a C to T substitution at position 3435 of exon 26 of the MDR-1 gene (Accession No: AF016535).

A cell comprising a nucleotide exchange of C to T at a position corresponding to position 3435 of exon 26 of the MDR-1 gene, as discussed above, has been shown to have reduced levels of MDR-1 expression and thus a reduced level of MDR-1 protein. Due to the reduced level of MDR-1 protein said cell has a reduced drug transporter activity and a reduced ability to prevent risk factors from accumulation in said cells.

The MDR-1 C3435T polymorphism, which influences intestinal expression and uptake of MDR-1 substrates, also correlates with renal expression of MDR-1 protein. For example, individuals homozygous for the T-allele display significantly lower expression of MDR-1 in the proximal tubuli of the kidney compared to heterozygous and homozygous C-allele carriers (p=0.06, N=19CC and 28TT). Since MDR-1 protein plays a role in the excretion of toxic substances, the tubular cells of individuals with homozygous low expressor (T) alleles are more exposed to toxic agents. In agreement with the hypothesis that exposure to noxic or toxic agents promotes the development of deficiencies such as renal cell carcinoma (RCC), it was found that the MDR-1 3435T allele is a susceptibility factor for e.g. RCC: Individuals that are homozygous T-allele carriers (24 % of the normal population, N>400 but 39 % in RCC, N=87) have an increased risk to develop
deficiencies such as RCC compared to heterozygous or homozygous carriers of the C-allele.

The above definitions for the terms used to describe the use of the present invention apply mutatis mutandis to the method described hereinafter.

Moreover, the present invention relates to a method for identifying and obtaining a compound or nutrient which is an inducer or activator of MDR-1 comprising the steps of:
(a) contacting a cell capable of expressing MDR-1 with said compound or nutrient suspected to be an inducer or activator, and
(b) determining whether said compound or nutrient induces or activates MDR-1.

A cell which is capable of expressing MDR-1 may be a cell of a tissue or organ known to express MDR-1, such as kidney, liver, intestine, bladder, prostate or blood.

By "a cell capable of expressing MDR-1" it is meant that said cell may also comprise further genes which are required to control the expression of MDR-1, such as genes encoding transcription factors which may be required to express MDR-1 in a cell or genes encoding proteins which are involved in transport or translation of the MDR-1 mRNA. In addition, genes which are required for expression of MDR-1 might be genes encoding proteins which are involved in transport or processing of the MDR-1 protein. A cell capable of expressing MDR-1 may be also genetically engineered as described above with at least one of said further genes.

Other genes, proteins or compounds are, however, also comprised and well-known in the art. In the context of the present invention the term "expressing" comprises transcription as well as translation. Preferably, said cell is a cell cultured in vitro.

In a preferred embodiment the present invention relates to a method for producing a pharmaceutical composition comprising the steps of the method of the present
invention and the further step of formulating the compound or nutrient identified in step (b), above, in a pharmaceutically acceptable form.

The therapeutically useful compounds identified in accordance with the method of the invention may be formulated and administered to a patient having one of the above-described diseases. The therapeutic uses and doses have to be determined to be appropriated for said patients. Means and methods therefor are known in the art. A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors 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 pharmaceutical composition produced by the method of the invention may be conveniently administered by any route known in the art and being appropriate therefor, e.g. as a pharmaceutically acceptable salt. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The compounds 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, enulsions, 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 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.

In another embodiment the present invention relates to a method for identifying
and obtaining a risk factor for a disease comprising the steps of:
(a) contacting a cell comprising a nucleotide exchange of C to T at a position
corresponding to position 3435 of exon 26 of the MDR-1 gene (Accession
No: AF016535) with a compound suspected to be a risk factor for a disease;
and
(b) measuring the physiological status of said cell, wherein an altered status is
indicative for a compound which is a risk factor.

By "physiological status of said cell" the cellular functions, such as metabolism,
gene expression, differentiation, signal transduction process, proliferation, vitality
and virability inducing apoptosis are comprised. Said cellular functions are usually
influenced by a risk factor to be determined by the method of the invention. A risk
factor in accordance with the invention may influence such functions by different
ways, e.g., by introducing mutations in genes which may encode proteins which
are involved in said cellular functions, by modifying said proteins involved in said
cellular functions or by modifying the activity of said proteins involved in said
cellular functions.
Also comprised by the invention is measuring of the physiological status by
analysis of more than one of the above cellular functions in combination. Methods
for measuring said physiological status are known in the art and comprise
standard molecular biology techniques.
Advantageously, the above-described method, wherein the reduced ability to prevent risk factors from accumulation in said cells is used to efficiently and reliably identify said risk factors for a disease by altering the normal physiological status of said cells.

In a preferred embodiment of the method of the invention said measuring the physiological status comprises analysis of gene expression, analysis of phosphorylation, analysis of metabolism, analysis of the cell cycle, analysis of proliferation, analysis of apoptosis.

In a furthermore preferred embodiment of the method of the invention said disease is cancer.

In a more preferred embodiment of the method of the invention said cancer is derived from MDR-1 expressing tissues.

In a most preferred embodiment of the method of the invention said cancer is renal cell carcinoma (RCC), liver cancer, colon cancer, bladder cancer, prostate cancer or leukemia.

In another preferred embodiment of the method of the invention said disease is a inflammatory disease.

In still another preferred embodiment of the method of the invention said disease is tissue/organ damage.

In a most preferred embodiment of the method of the invention said tissue/organ is kidney, liver, colon, bladder, prostate or blood.

In a further preferred embodiment of the method of the invention said cell is a renal cell, a liver cell, a colon cell, a bladder cell, a prostate cell or a blood cell.
In accordance with the method of the present invention said cells may be obtained from said tissues by suitable methods known in the art. Said cell may comprise primary cells or cells of cell lines derived from said tissues by standard techniques known in the art.

The compositions, methods and uses of the present invention may be desirably employed in humans, although animal treatment is also encompassed by the methods and uses described herein.

The invention will now be described by reference to the following biological examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

**Example 1**

**Identification of candidate substances that are responsible for inflicting damage to and subsequently inducing malignant transformation of tubular cells**

An important aspect for cancer prevention is the identification of candidate substances that are responsible for inflicting damage to and subsequently inducing malignant transformation of tubular cells. With the exception of the solvent trichlorethylen, specific nephrocarcinogenic substances have not yet been identified. Because the low PGP expressor genotype serves as risk factor for RCC, it is feasible to assume that PGP substrates play a significant role. This is furthermore corroborated by our observation that TT frequency was highest among the >50 yrs RCC group (higher lifetime exposure to damaging substances) and in the group of RCC with somatic VHL alterations. This suggests, that DNA-damaging effects in renal tubular cells may be promoted by continued carcinogen exposure due to impaired efflux. Many PGP substrates inflict cell damage or are cytoto- or genotoxy. In patients with benign kidney disease, the MDR1 T allele was not overrepresented. This may indicate that the responsible agents do not inflict general cell damage or chronic stress to cause kidney failure. Thus, environmental components of RCC are likely PGP substrates with genotoxic or mutagenic action.
Example 2

Influence of MRP-1 genotype and expression on kidney cancer and the potential of protecting from RCC by increasing the levels of MDR-1 gene encoded P-glycoprotein

The discovery that low expression of PGP (T allele) serves as a risk factor for renal cell carcinoma is documented by the prevalence of the MDR1 TT genotype in patients suffering from RCC. Likewise, individuals with the CC genotype should profit from normal or even increased PGP expression and function. Considering all age groups, the MDR TT genotype contributed to an 1.5-fold elevated risk, and in the >50 yr group (which accounts for most RCCs) the OR increased to 2.1. On the first view, this OR seems to be minor when compared to other (highly penetrant) genetic risk factors e.g. germline mutations in the VHL tumor suppressor gene, where individuals carrying a germline mutation in the VHL gene have a greater than 95% chance to develop Hippel-Lindau syndrome, that is frequently associated with RCC. However, in contrast to such mutations which are rare and therefore their contribution to the overall numbers of RCC is small, the low expressor MDR1 allele is a frequent allele. Due to its overall high prevalence of 26% in the caucasian population, it contributes to 15% of all RCC as displayed by an ethiological fraction of 0.15.

The discovery of PGP substrates as environmental components of RCC (see Example 1) may allow to ameliorate their impact: Expression of MDR-1 is inducible by a variety of substances, including dietary ingredients. This induction phenomenon allows individuals with a low expressor genotype to reach protective PGP concentrations that are comparable to those of individuals that carry the MDR-1 C-allele (normal to high PGP expression). Therefore, individuals which are at higher risk to develop kidney cancer due to their MDR-1 genotype can achieve relative protection levels similar to genotypically 'normal' individuals by application of MDR-1 inducing substances. These may be nonharmful substances, such as hypericum derived extracts, or other natural compounds. It is also feasible to identify, extract or concentrate such substances from various sources and formulate them to pharmaceutical products for easier and more convenient regular application.
The high prevalence of 26% of the low expressor allele in the caucasian population, leads to it contributing to 15% of all RCC as displayed by an ethiological fraction of 0.15. In Europe and the US alone, this accounts for 10,000 cases per year. It can be assumed that artificial elevation of PGP levels in these individuals, as described in this example, will directly influence their cancer risk to the reduced levels that are observed in the MDR-1 high expressor group. Thus, artificial elevation of MDR-1 encoded PGP is a powerful means in cancer prevention.

Among the general population, some individuals and families carry a much greater risk to develop tumors than the remainder of the population. Examples are families with a mutated VHL gene. Because PGP levels serve as an independent risk factor (documented in Table 1), individuals that have low PGP expressor genes combined with other cancer susceptibility genes or defects will particularly benefit from diets or medications that induce MDR-1 expression to provide some protection against the development of kidney cancer.

<table>
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<tr>
<th>Samples</th>
<th>N</th>
<th>% CC</th>
<th>% CT</th>
<th>% TT</th>
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<td>35.6</td>
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Table 1: MDR C_{3435}T genotype and VHL mutations
Claims

1. Use of a compound which induces or activates MDR-1, a nutrient which induces or activates MDR-1 for the preparation of a pharmaceutical composition for treating or preventing a risk factor associated disease.

2. The use of claim 1, wherein said disease is cancer.

3. The use of claim 2, wherein said cancer is derived from a MDR-1 expressing tissue.

4. The use of claim 2 or 3, wherein said cancer is renal cell carcinoma (RCC), liver cancer, colon cancer, bladder cancer, prostate cancer or leukemia.

5. The use of claim 1, wherein said disease is an inflammatory disease.

6. The use of claim 1, wherein said disease is tissue/organ damage.

7. The use of claim 6, wherein said tissue/organ is kidney, liver, colon, bladder, prostate or blood.

8. The use of any one of claims 1 to 7, wherein said risk factor is absence or reduced levels of the MDR-1 polypeptide.

9. The use of any one of claims 1 to 7, wherein said risk factor is
(a) a nucleotide substitution, addition or deletion at a position corresponding to position 3435 of exon 26 of the MDR-1 gene (Accession No: AF016535);
(b) a C to T substitution at a position corresponding to position 3435 of exon 26 of the MDR-1 gene (Accession No: AF016535); or
(c) a C to T substitution at position 3435 of exon 26 of the MDR-1 gene (Accession No: AF016535).
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/275 A61K38/13 A61P35/00 A61P29/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

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

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

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>WO 93 08797 A (UNIV NEW YORK) 13 May 1993 (1993-05-13) * see claim 1, pages 12 and 14 *</td>
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<td>WO 01 09183 A (EICHELBAUM MICHEL ;EPIDAURUS (DE); HOFFMEYER SVEN (DE); ROOTS IVAR) 8 February 2001 (2001-02-08) * see claims 24-28, 41-42, pages 27 last paragraph and page 29 *</td>
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<td>US 4 996 193 A (HEWITT CHARLES W ET AL) 26 February 1991 (1991-02-26) * see abstract and col. 3 lines 10-28 *</td>
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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search 15 May 2001

Date of mailing of the international search report 15, 06, 01

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Authorized officer Merckling, V
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<td>DATABASE WPI&lt;br&gt;Section Ch, Week 199518&lt;br&gt;Derwent Publications Ltd., London, GB;&lt;br&gt;Class B05, AN 1995-136798&lt;br&gt;XP002167388&lt;br&gt;&amp; JP 07 061937 A (HIRAKI S),&lt;br&gt;7 March 1995 (1995-03-07)&lt;br&gt;abstract</td>
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| A        | FROMM M.F.: "P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs."
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v. 38, February 2000 (2000-02), pages 69-74, XP000995816
* see abstract and page 73 * | 1-9 |
| A        | HAUSER I. ET AL.: "Role of reactive oxygen species in TNF-alpha-induced overexpression of multidrug resistance P-glycoprotein (MDR-1) in human endothelial cells."
J. AM. SOC. NEPHROLOGY, vol. 10, September 1999 (1999-09), page 528A XP000999060
* abstract * | 1-9 |
PROC. NAT. ACAD. SCI. USA, vol. 97, 28 March 2000 (2000-03-28), pages 3473-3478, XP000996164
* see abstract and page 3475 right col. * | 1-9 |
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

   see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.
Continuation of Box I.2

Present claims 1-9 relate to an extremely large number of possible compounds and medical uses. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds and uses claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been restricted to cyclosporin and verapamil (and derivatives thereof) as far as the compounds are concerned. These are the only examples of MDR-1 inducers cited in the application. Similarly, the expression "a risk factor associated disease" has no generally recognized meaning. The search was limited to the diseases/conditions listed in claims 2-7.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
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