Title: MUTATIONS OF THE MDR1 P-GLYCOPROTEIN THAT IMPROVE ITS ABILITY TO CONFER RESISTANCE TO CHEMOTHERAPEUTIC DRUGS

Abstract: The present invention provides mutant P-glycoprotein polypeptides that confer increased resistance to certain chemotherapeutic drugs relative to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185, and nucleic acid molecules encoding the same. The invention also provides antibodies that specifically bind mutant P-glycoproteins. The invention further provides methods for the diagnosis and treatment of conditions associated with P-glycoprotein-mediated multidrug resistance.
MUTATIONS OF THE MDR1 P-GLYCOPROTEIN THAT IMPROVE ITS ABILITY TO CONFER RESISTANCE TO CHEMOTHERAPEUTIC DRUGS

This application claims the benefit of priority from U.S. Provisional Patent Application No. 60/222,313, filed on August 1, 2000, the disclosure of which is explicitly incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to mutant human P-glycoprotein polypeptides that confer increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185, and nucleic acid molecules encoding the same. The invention also relates to antibodies that specifically bind mutant human P-glycoproteins. The invention further relates to methods for diagnosis and treatment of conditions associated with P-glycoprotein-mediated multidrug resistance.

2. Background of the Invention

Multidrug resistance (MDR) is a phenomenon in which a cell becomes resistant to a large group of structurally diverse chemotherapeutic drugs that act at different intracellular targets. One mechanism of MDR1 in clinical cancer is active drug efflux mediated by a cellular protein termed P-glycoprotein. P-glycoprotein is an integral membrane protein having six transmembrane domains and consisting of 1280 amino acids (SEQ ID NO: 2), which functions as a broad specificity efflux pump. The protein is composed of two structurally similar portions, of approximately 600 amino acids each, which are separated by a linker region. Human P-glycoprotein is encoded by the MDR1 gene (Chen et al., 1986, Cell 47:381-89), the wild-type cDNA sequence of which is identified herein as SEQ ID NO: 1.

A number of mutant P-glycoproteins, possessing an altered ability to transport different drugs, have been isolated. Most of these mutants show decreased drug efflux, while only a small number of mutants exhibit increased drug transport. Examples of the latter group are the MDR1-G185V (Choi et al., 1988, Cell 53:519-29; Safi et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:7225-29) and MDR1-H61F mutants (Taguchi et al., 1997, Biochemistry 36:8883-89). Mutant P-glycoproteins
showing increased resistance to certain chemotherapeutic drugs also show decreased resistance to other drugs.

Efforts to treat clinical cancer with cytotoxic drugs are often hampered by sensitivity of non-cancerous proliferating cells, such as hematopoietic cells, to the cytotoxic effects of these drugs. One approach to overcoming this shortcoming is to genetically engineer hematopoietic cells, particularly precursor cells such as stem cells, to express elevated levels of P-glycoprotein in order to make these cells more resistant to the cytotoxic effects of chemotherapeutic drugs, enabling higher doses of such drugs to be administered for more effective anticancer treatment. These efforts would be even more effective if the non-cancerous cells could be made preferentially resistant to a chemotherapeutic drug that was particularly appropriate for treatment of a specific cancer.

Additionally, expression of different mutant P-glycoproteins in clinical cancer can make inappropriate a specific choice of chemotherapeutic drug in an individual patient having a neoplastic disease conventionally treated with said specific drug. Identification of specific mutants in clinical cancer would provide clinicians with information enabling rational, individualized drug treatment choices in anticancer treatment.

Thus, there remains a need in the art for mutant P-glycoproteins that confer increased resistance to certain chemotherapeutic drugs relative to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185. The development of such mutants would have wide application in the treatment and diagnosis of cancer.

SUMMARY OF THE INVENTION

The present invention provides nucleic acid molecules encoding mutant human P-glycoprotein polypeptides that confer increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

Specifically, the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a mutant human P-glycoprotein having either an asparagine substitution at position 186, a phenylalanine substitution at position 56, phenylalanine substitutions at positions 190 and 197, a tryptophan substitution at position 763, a glutamic acid substitution at position 844, lysine
substitutions at positions 634 and 636, lysine substitutions at positions 643, 647, and 652, or lysine substitutions at positions 634, 636, 643, 647, and 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention also provides a nucleic acid molecule comprising a nucleotide sequence encoding a mutant human P-glycoprotein having either a phenylalanine substitution at position 190; a phenylalanine substitution at position 197; a lysine substitution at position 634; a lysine substitution at position 636; a lysine substitution at position 643; a lysine substitution at position 647; or a lysine substitution at position 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention further provides a nucleic acid molecule comprising a nucleotide sequence encoding a mutant human P-glycoprotein having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186; a phenylalanine substitution at position 56; phenylalanine substitutions at positions 190 and 197; a tryptophan substitution at position 763; a glutamic acid substitution at position 844; lysine substitutions at positions 634 and 636; and lysine substitutions at positions 643, 647, and 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention still further provides a nucleic acid molecule comprising a nucleotide sequence encoding a mutant human P-glycoprotein having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186, a phenylalanine substitution at position 56; a phenylalanine substitution at position 190; a phenylalanine substitution at position 197; a tryptophan substitution at position 763, a glutamic acid substitution at position 844, a lysine substitution at position 634; a lysine substitution at position 636; a lysine substitution at position 643; a lysine substitution at position 647; or a lysine substitution at position 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain
chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention also provides a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

The present invention further provides a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186; a phenylalanine substitution at position 56; phenylalanine substitutions at positions 190 and 197; a tryptophan substitution at position 763; a glutamic acid substitution at position 844; lysine substitutions at positions 634 and 636; and lysine substitutions at positions 643, 647, and 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention still further provides a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186, a phenylalanine substitution at position 56; a phenylalanine substitution at position 190; a phenylalanine substitution at position 197; a tryptophan substitution at position 763, a glutamic acid substitution at position 844, a lysine substitution at position 634; a lysine substitution at position 636; a lysine substitution at position 643; a lysine substitution at position 647; or a lysine substitution at position 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The nucleic acid molecules of the present invention may also encode mutant P-glycoproteins that have a valine substitution at position 185 in addition to the one or multiplicity of the specific amino acid substitutions disclosed herein.
The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein and recombinant host cells comprising the recombinant nucleic acid molecules as set forth herein.

The present invention also provides mutant human P-glycoproteins that confer increased resistance to certain chemotherapeutic drugs relative to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

Specifically, the present invention provides a mutant human P-glycoprotein having either an asparagine substitution at position 186, a phenylalanine substitution at position 56, phenylalanine substitutions at positions 190 and 197, a tryptophan substitution at position 763, a glutamic acid substitution at position 844, lysine substitutions at positions 634 and 636, lysine substitutions at positions 643, 647, and 652, or lysine substitutions at positions 634, 636, 643, 647, and 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention also provides a mutant human P-glycoprotein having either a phenylalanine substitution at position 190; a phenylalanine substitution at position 197; a lysine substitution at position 634; a lysine substitution at position 636; a lysine substitution at position 643; a lysine substitution at position 647; or a lysine substitution at position 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention further provides a mutant human P-glycoprotein having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186; a phenylalanine substitution at position 56; phenylalanine substitutions at positions 190 and 197; a tryptophan substitution at position 763; a glutamic acid substitution at position 844; lysine substitutions at positions 634 and 636; and lysine substitutions at positions 643, 647, and 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.
The present invention still further provides a mutant human P-glycoprotein having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186; a phenylalanine substitution at position 56; a phenylalanine substitution at position 190; a phenylalanine substitution at position 197; a tryptophan substitution at position 763; a glutamic acid substitution at position 844; a lysine substitution at position 634; a lysine substitution at position 636; a lysine substitution at position 643; a lysine substitution at position 647; or a lysine substitution at position 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention also provides a polypeptide as set forth in any of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

The present invention further provides a polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186; a phenylalanine substitution at position 56; phenylalanine substitutions at positions 190 and 197; a tryptophan substitution at position 763; a glutamic acid substitution at position 844; lysine substitutions at positions 634 and 636; and lysine substitutions at positions 643, 647, and 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The present invention still further provides a polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid substitution selected from the group consisting of: an asparagine substitution at position 186, a phenylalanine substitution at position 56; a phenylalanine substitution at position 190; a phenylalanine substitution at position 197; a tryptophan substitution at position 763, a glutamic acid substitution at position 844, a lysine substitution at position 634; a lysine substitution at position 636; a lysine substitution at position 643; a lysine substitution at position 647; or a lysine substitution at position 652, wherein expression of each of these mutant P-glycoproteins in mammalian cells confers increased resistance to certain
chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The mutant P-glycoproteins of the present invention may also have a valine substitution at position 185 in addition to the one or multiplicity of the specific amino acid substitutions disclosed herein.

The molecules of the present invention are advantageously used for the treatment and diagnosis of cancer. In a preferred embodiment, P-glycoprotein mutants occurring in a clinical tumor sample are identified by immunological assay using an antibody of the invention or antigen-binding fragment thereof specific for said mutant, or by nucleic acid hybridization using a nucleic acid probe specific for said mutant, or by in vitro amplification of mutant-encoding nucleic acid. In further preferred embodiments, a P-glycoprotein mutant of the invention is advantageously introduced into a non-cancerous cell, most preferably a hematopoietic cell, of a human cancer patient, and reintroduced into said patient, thereby permitting increased levels of a particular chemotherapeutic drug (to which the P-glycoprotein mutant specifically confers increased resistance compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185) to be administered to said patient.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

**DESCRIPTION OF THE DRAWINGS**

Figure 1 illustrates the selections that were used to isolate P-glycoprotein mutants conferring enhanced etoposide;

Figure 2 illustrates the array selection scheme that was used to isolate P-glycoprotein mutants conferring etoposide hyper-resistance;

Figures 3A through 3G illustrate an amino acid sequence alignment of human wild-type P-glycoprotein (wt P-gp; SEQ ID NO: 2) and P-glycoprotein mutants encoded by MDR1-G185V (G185V; SEQ ID NO: 3), MDR1-I186N (I186N; SEQ ID NO: 4), MDR1-L56P (8-A4; SEQ ID NO: 5), MDR1-I190F/M197F (5-C3; SEQ ID NO: 6), MDR1-G763W (2-B2; SEQ ID NO: 7), MDR1-G844E (2-B1; SEQ ID NO: 8),
MDR1-E634K/E636K (6-C1; SEQ ID NO: 9), MDR1-E643K/E647K/E652K (8-B4; SEQ ID NO: 10) and MDR1-E634K/E636K/E643K/E647K/ E652K (EK5; SEQ ID NO: 11);

Figures 4A through 4E illustrate the results of clonogenic assays for resistance to etoposide (panel A), colchicine (panel B), doxorubicin (panel C), vinblastine (panel D), and taxol (panel E) for NIH 3T3 cells transduced with LXSN (closed squares), wild-type MDR1 (closed circles), MDR1-G185V (open circles), MDR1-I186N (closed triangles), or MDR1-G185V/I186N (open triangles);

Figures 5A through 5C illustrate the results of clonogenic assays for resistance to etoposide (panel A), doxorubicin (panel B), and colchicine (panel C) for NIH 3T3 cells transduced with LMDR1-G185V (open circles), LMDR1-G185V-EK2 (closed triangles), LMDR1-G185V-EK3 (open triangles), or LMDR1-G185V-EK5 (closed diamonds);

Figures 6A through 6E illustrate the results of clonogenic assays for resistance to etoposide (panel A), doxorubicin (panel B), colchicine (panel C), vinblastine (panel D), and taxol (panel E) for NIH 3T3 cells transduced with LMDR1-G185V (closed circles), LMDR1-G185V-L56F (open circles), LMDR1-G185V-I190F/M197F (closed triangles), LMDR1-G185V-G763W (open triangles), or LMDR1-G185V-G844Q (closed squares).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides nucleic acid molecules encoding mutant human P-glycoprotein polypeptides that confer increased resistance to certain chemotherapeutic drugs relative to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.

The production of proteins such as the mutant P-glycoproteins of the invention from cloned genes by genetic engineering means is well known in this art. The discussion that follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

For the purpose of this invention, “P-glycoprotein” refers to the polypeptide encoded by the human MDR1 gene.
Nucleic acid encoding the mutant P-glycoproteins of this invention can be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from tumor cells resistant to MDR-type drugs or cultured cell lines derived therefrom, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of cDNA prepared from cellular mRNA or genomic DNA may be carried out with full-length, partial or oligonucleotide probes generated from mutant P-glycoprotein nucleotide sequence information provided herein, as discussed above. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, mutant P-glycoprotein encoding nucleic acid can be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the mutant P-glycoprotein nucleotide sequences provided herein. See U.S. Patent Nos. 4,683,195 to Mullis et al. and 4,683,202 to Mullis. In preferred embodiments, in vitro mutagenesis techniques known in the art and as described herein can be used to produce any of the mutant P-glycoproteins of the invention using cloned human P-glycoprotein-encoding nucleic acid identified herein as SEQ ID NO: 1.

Nucleic acids and oligonucleotides of the present invention are useful as probes for screening DNA libraries to isolate and characterize additional mutant P-glycoprotein-encoding nucleic acids of the invention. For the purposes of this invention, these probes are used under high stringency hybridization conditions, defined herein as follows. The probes are labeled with 150 μCi $^{32}$P-ATP and hybridized overnight at 42°C in a hybridization buffer comprising 6X SSC (0.9M NaCl, 0.09M sodium citrate dihydrate, pH 7.0), 2X Denhardt’s solution (0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin), and 0.25% sodium dodecyl sulfate (SDS). After hybridization, filters are washed four or five times for ten minutes apiece in an excess volume of 6X SSC (0.9 M NaCl, 0.09M sodium citrate dihydride, pH 7.0) and 0.05% SDS at room temperature. Thereafter, three ten-minute washes are performed one after another in this buffer at temperatures of 55°C, 60°C and 63°C.
Mutant P-glycoproteins may be synthesized in cells, most preferably cells comprising exogenously-added, recombinant DNA, most preferably cDNA, encoding said proteins. In particular, cells transformed with a recombinant expression construct comprising a nucleic acid encoding a mutant P-glycoprotein of the invention can be used to provide a homogeneous culture of mutant P-glycoprotein expressing cells. Recombinant expression constructs comprising the mutant P-glycoprotein coding sequences as disclosed herein can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding a mutant P-glycoprotein and/or to express DNA that encodes a mutant P-glycoprotein. For the purposes of this invention, a recombinant expression construct is a replicable DNA construct in which a DNA sequence encoding mutant P-glycoprotein is operably linked to suitable control sequences capable of effecting the expression of the receptor in a suitable host cell. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator or enhancer sequence to control or regulate transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation, and in mammalian cells, sequences that direct 5’ terminal capping and 3’ terminal polyadenylation of the primary transcript. Amplification vectors, on the other hand, do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. See, Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press: New York).

Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and particularly integratable DNA fragments (i.e., fragments integratable into the host genome by recombination). The vector may replicate and function independently of the host genome, or more preferably, may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. Transformed host cells are preferably prokaryotic or eukaryotic cells that have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising mutant P-glycoprotein-encoding sequences. Preferred host cells are bacterial cells, most preferably Escherichia coli cells, mammalian cells, most preferably mammalian
cells such as 293 cells, HeLa cells, COS, and CHO cells, and insect cells, most preferably Sf9 cells (using baculoviral vectors). Transformed host cells are chosen that are capable of expressing functional mutant P-glycoproteins introduced using the recombinant expression construct. See, Sambrook et al., ibid.

Homogeneous compositions of the mutant P-glycoproteins are provided by the invention. As used herein, the term “homogeneous” is intended to encompass preparations wherein the mutant P-glycoprotein of the invention is the predominant protein species in the preparation, preferably wherein the mutant P-glycoprotein comprises at least 70%, more preferably wherein the mutant P-glycoprotein comprises from 80-90%, and most preferably where the mutant P-glycoprotein comprises greater than 90% of the protein in the preparation. In preferred embodiments, said preparations have one or a multiplicity of additional protein components wherein no one component comprises more than about 5% and more preferably about 1% of the protein in the preparation.

The invention also provides antibodies that are immunologically reactive to a mutant P-glycoprotein, most preferably human mutant P-glycoproteins explicitly disclosed herein. The antibodies provided by the invention may be raised as follows. First, peptide fragments of the mutant P-glycoproteins of the invention can be chemically synthesized or isolated from the full-length sequence, covalently attached to a carrier molecule such as keyhole limpet hemocyanin, and injected into an appropriate experimental animal. Animals that are used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals for inoculation are rodents (including mice, rats, and hamsters) and rabbits. For preparing polyclonal antisera, the most preferred animal is rabbit. For preparing monoclonal antibodies, which are preferred, the most preferred animal is the mouse. Antibodies isolated in this manner are tested for binding specificity to identify antibodies that specifically bind mutant P-glycoproteins and do not bind wild-type P-glycoprotein or P-glycoprotein having only a glycine to valine substitution at position 185.

Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell, clonal isolate, or cell line that expresses a mutant P-glycoprotein or any epitope thereof, most preferably as a result of molecular or genetic engineering. In one embodiment, said recombinant protein is prepared in E. coli cells, most preferably having been transformed with a recombinant expression
construct comprising a nucleic acid encoding a human mutant P-glycoprotein or epitopes thereof, and that express the mammalian gene product, wherein it is understood that such preparations are sufficiently purified from pyrogenic or other bacterial toxins or contaminants as to be suitable for injection into an animal. In another embodiment, said recombinant protein is prepared in insect cells, preferably Sf9 cells, most preferably having been transformed with a recombinant baculovirus construct comprising a nucleic acid encoding a human mutant P-glycoprotein or epitopes thereof, and that express the mammalian gene product. Additional preferred cells are mammalian cells expressing a mutant P-glycoprotein of the invention or epitope thereof, most preferably a cell that is syngeneic or congenic with the animal in which the antibody is raised, so as to minimize non-mutant P-glycoprotein-specific immunological responses in said animal.

The present invention provides polyclonal antisera and monoclonal antibodies that are immunologically reactive with an epitope that is a mutant P-glycoprotein or fragment thereof. Monoclonal antibodies are the most preferred. These antibodies are made using methods and techniques well known to those of skill in the art.

Monoclonal antibodies provided by the present invention are produced by hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art. Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with mutant P-glycoprotein, or cells expressing such a mutant P-glycoprotein, as described above. The myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates, and humans. Preferred myeloma cell lines are from mouse, and the most preferred mouse myeloma cell line is P3X63-Ag8.653. The animals from which spleens are obtained after immunization are rats, mice, and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from in vitro cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most
preferably a mouse, and the monoclonal antibodies obtained from blood and/or ascites fluid.

Monoclonal antibodies provided by the present invention are also produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are immunologically reactive with an epitope of a mutant P-glycoprotein.

The present invention encompasses fragments of said antibody that are immunologically reactive with an epitope of a mutant P-glycoprotein. Such fragments are produced by any number of methods known in the art, including but not limited to proteolytic cleavage, chemical synthesis, or preparation of such fragments by means of genetic engineering technology, and include Fab fragments, Fab' fragments, F(ab)₂ fragments, and Fv fragments. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mutant P-glycoprotein made by methods known to those of skill in the art.

The present invention also encompasses an epitope of a mutant P-glycoprotein that is comprised of sequences and/or a conformation of sequences present in the mutant P-glycoprotein molecule. Most preferably, the epitope comprises a peptide comprising at least one mutated amino acid residue compared with wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mutant P-glycoprotein molecule and isolation of an epitope-containing peptide or may be obtained by chemical and more preferably automated chemical synthesis of an epitope-containing peptide using methods well known to those skilled in the art.

The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered cells, preferably prokaryotic cells and most preferably E. coli cells, or by mammalian cells.

The invention also provides methods for screening compounds for the capacity to disrupt binding of cytotoxic drugs with the mutant P-glycoprotein or to disrupt drug efflux from cells expressing said mutant P-glycoprotein. In these methods, drug efflux from cells expressing wild-type P-glycoprotein, P-glycoprotein having a glycine to valine substitution at position 185, and a mutant P-glycoprotein of the invention is compared in the presence and absence of a test compound. In preferred embodiments, drug efflux is determined by cytotoxicity, whereby decreased drug
efflux in the presence of the compound is detected by increased cytotoxicity in the presence of a constant concentration of cytotoxic drug. In alternative embodiments, drug efflux is determined using non-toxic compounds that are effluxed by P-glycoprotein, most preferably detectable compounds such as rhodamine.

The mutant P-glycoprotein molecules of the present invention are advantageous for use in treating cancer. One aspect of this advantageous use is in diagnosis, particularly in determining, prior to administration of a chemotherapeutic drug, whether the tumor cells express a mutant P-glycoprotein having increased resistance to a particular chemotherapeutic drug. In the art, clinical trials have developed specific chemotherapeutic drug regimens wherein certain types of clinical cancer are treated with particular chemotherapeutic drugs, often in combination. The disclosure herein that certain P-glycoprotein mutants are preferentially resistant to certain such chemotherapeutic drugs makes it advantageous to know prior to initiating treatment with a particular drug whether an individual patient’s tumor expresses a mutant P-glycoprotein that has increased resistance to said drug. In preferred embodiments, a tumor sample such as a biopsy sample is contacted with an immunological reagent such as an antibody that is specific for the particular mutant P-glycoprotein of the invention having increased resistance to a particular chemotherapeutic drug. In alternative embodiments, the sample is tested using nucleic acid hybridization techniques using probes specific for the particular mutant P-glycoprotein of the invention having increased resistance to a particular chemotherapeutic drug. In a particularly preferred alternative embodiment, the sample is tested using in vitro amplification techniques such as the polymerase chain reaction, using amplification oligonucleotide primers and conditions specific for the particular mutant P-glycoprotein of the invention having increased resistance to a particular chemotherapeutic drug.

Another aspect of the advantageous uses of this invention is in therapy, particularly with regard to protecting non-cancerous proliferating cells in a patient’s body, most preferably hematopoietic cells, hair follicles, and epithelial cells of the gastrointestinal tract, from the cytotoxic effects of chemotherapeutic drug administration. As is well appreciated in the art, a consequence of chemotherapeutic drug treatment is the destruction of several classes of non-cancerous proliferating cells, most importantly hematopoietic cells. Frequently the amount of duration of chemotherapeutic drug treatment is limited by the deleterious effects of
chemotherapeutic drugs on hematopoietic cells, resulting *inter alia* in acute, potentially life-threatening conditions such as leukocytopenia. In one preferred embodiment, the innate resistance of hematopoietic cells to chemotherapeutic drugs is augmented by transducing hematopoietic cells, most preferably hematopoietic stem cells, with a recombinant expression construct encoding a mutant P-glycoprotein of the invention. In this aspect, the mutant P-glycoprotein is chosen to confer on the cell increased resistance to the cytotoxic drug understood and accepted in the art as an appropriate chemotherapeutic agent for the particular cancer. In this aspect, the hematopoietic cells are transduced *ex vivo* and then returned to the patient, followed thereafter by treatment with greater than conventional amounts of the chemotherapeutic drug to which the hematopoietic cells have been made preferentially resistant by expression of the mutant P-glycoprotein. In another embodiment, homogeneous preparations of mutant P-glycoproteins are delivered to normal cells as polypeptides rather than via gene therapy, for example in liposome-encapsulated form.

The Examples, which follow, are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

**EXAMPLE 1**

**Isolation of the mutant P-glycoprotein encoded by MDR1-I186N**

A population of NIH 3T3 cells expressing a randomly mutagenized human MDR1-G185V gene was prepared as previously described (Roninson *et al.*, 1998, *Methods Enzymol.* 292: 225-48). This population was used to select MDR1-G185V mutants that confer hyper-resistance to etoposide (using the selection scheme shown in Figures 1A and 1B).

A portion of the 3T3 cell population covering the complexity of the randomly mutagenized human MDR1-G185V library by approximately one-fold (1.2 x 10^5 cells) was initially selected in 2 μg/mL etoposide. Approximately 1 in 1000 cells (0.1%) survived this selection. Surviving colonies were expanded and pooled, and MDR1 cDNA was rescued and recloned back into the parental vector LXSN as previously described (Roninson *et al.*, 1998, *ibid*.). The library of rescued MDR1 cDNA, designated Mut II, was then used to transduce naïve NIH 3T3 cells.
The population of NIH 3T3 cells transduced with Mut II was then selected in 10 μg/mL etoposide. The 131 colonies obtained from this selection were expanded and genomic DNA extracted from the clonal lines as previously described (Roninson et al., 1998, *ibid*). Mutant MDR1-G185V cDNA prepared using mRNA from eighteen clonal lines was also isolated, sequenced, and analyzed for mutations. A mixture of MDR1 cDNA from all 131 clonal lines was sequenced in both directions, and compared with unmutagenized MDR1-G185V, in order to determine whether particular mutations were heavily represented among the surviving MDR1-G185V mutants.

Mutant MDR1-G185V cDNA isolated from eleven clonal lines was tested for the ability to confer elevated resistance to etoposide as compared to unmutagenized MDR1-G185V. Fragments of MDR1 cDNA containing mutations were first excised from the MDR1 cDNA using restriction endonucleases that recognize appropriate sites flanking the mutation, and then the fragments were recloned into the parental LMDR1-G185V vector. Naïve NIH 3T3 cells were individually transduced with the eleven LMDR1-G185V mutants, wild-type LMDR1-G185V, or LXSN, and then analyzed by FACS for MDR1 expression. Each of the populations containing LMDR1-G185V (either mutant or wild-type) showed a similar level of MDR1 expression, as indicated by staining with the P-glycoprotein-specific antibody UIC2.

The cell populations were then assayed for etoposide resistance. Only one of the eleven mutants showed an increased resistance to etoposide as compared to the wild-type MDR1-G185V. Sequence analysis revealed that the P-glycoprotein molecule encoded by this mutant MDR1-G185V possessed an asparagine residue (rather than the wild-type isoleucine residue) at position 186, and was thus termed MDR1-I186N.

**EXAMPLE 2**

**Isolation of mutant P-glycoproteins by array selection**

An array selection strategy was employed to isolate additional MDR1 mutants conferring hyper-resistance to etoposide from the cells transduced with Mut II and selected in 10 μg/mL etoposide, as described in Example 1 (this selection scheme is shown in Figure 1C and in Figure 2). The 131 clonal lines from Example 1 were divided into four pools. MDR1 cDNA was recovered by PCR from each of the four pools, recloned back into LXSN, and used to transform bacterial cells to generate four MDR1 mutant libraries. These libraries were plated; individual bacterial colonies
were picked and tested by array analysis. For this analysis, each well of a 96-well plate was seeded with four randomly selected colonies from each MDR1 mutant library (for a total of 16 colonies per well), and 8 wells, comprising a total of 128 clones (array α), were tested. Plasmid was recovered from bacteria grown in each well (a 16-clone group) and used to transduce NIH 3T3 cells, and the transduced NIH 3T3 cells were selected in 2 μg/ml etoposide. The cDNA from each of the 16-clone groups that showed survival (groups 2, 5, 6 and 8) was then put into array β. The groups were subdivided into eight overlapping pools, each containing four mutant MDR1 cDNA (Figure 2; pools A-D and pools 1-4). Each pool was then transduced into NIH 3T3 cells that were then tested for survival in 2 μg/mL etoposide. The shaded boxes in Figure 2 denote pools that showed survival. The boldfaced numbers in Figure 2 indicate individual mutant MDR1 cDNA that confer hyper-resistance to etoposide. Six mutants that confer increased etoposide resistance relative to parental MDR1-G185V cDNA were identified in the array selection.

These mutants, designated as 2-B1, 2-B2, 5-C3, 6-C1, 8-A4, and 8-B4, were sequenced in both directions. The mutations that were identified in the MDR1-G185V mutants by sequence analysis are shown in Table I. Additional mutations, which were either silent or very conservative, were also found in most of the MDR1-G185V mutants. Four non-conservative mutations were also identified in the MDR1-G185V mutants, but these mutations, when introduced into the MDR1 cDNA as described in Example 3, conferred no greater etoposide resistance than parental MDR1-G185V cDNA.

Four of the mutants were found to have mutations that resulted in amino acid substitutions in the transmembrane (TM) domains (Figure 3A-G and Table I). A mutation in the 8-A4 mutant was found to generate a P-glycoprotein molecule having a phenylalanine residue (rather than the wild-type leucine residue) at position 56, which is located in TM1. Three mutations within close proximity of each other in the 5-C3 mutant were found to generate a P-glycoprotein having a phenylalanine residue (rather than the wild-type isoleucine residue) at position 190 and a phenylalanine (rather than the wild-type methionine residue) at position 197, both residues of which are located in TM3. Two mutations in the 2-B2 mutant were found to generate a P-glycoprotein having a tryptophan (rather than the wild-type glycine) at position 763, which is located in TM8. Finally, a mutation in the 2B-1 mutant was found to
generate a P-glycoprotein having a glutamic acid (rather than the wild-type glycine) at position 844, which is located in TM9.

Two of the mutants were found to have mutations that resulted in amino acid substitutions in the linker region (Figure 3A-G and Table I). Two mutations in the 6-C1 mutant were found to generate a P-glycoprotein molecule having lysine residues (rather than the wild-type glutamic acid) at positions 634 and 636. Three mutations in the 8-B4 mutant were found to generate a P-glycoprotein molecule having lysine residues (rather than the wild-type glycine) at positions 643, 647, and 652.

EXAMPLE 3

Construction and characterization of mutant P-glycoproteins having G185V and I186N substitutions

To confirm that the mutation in the MDR1-I186N mutant was indeed responsible for the elevated etoposide resistance observed in NIH 3T3 cells transduced with this mutant, and to investigate how this mutation interacts with the neighboring G185V mutation, wild-type MDR1 and MDR1-G185V cDNA was modified by site-directed mutagenesis (Quick-Change™ mutagenesis kit; Statagene) to introduce the I186N mutation directly into the P-glycoprotein sequence.

Retroviral vectors carrying LXSN (negative control carrying the neomycin/G418 resistance gene), wild-type MDR1, MDR1-G185V, MDR1-I186N, or MDR1-G185V/I186N were individually transduced into NIH 3T3 cells. Populations transduced with MDR1 containing vectors were stained with the P-glycoprotein-specific monoclonal antibody MRK16, and then sorted to obtain cell populations expressing a similar level of P-glycoprotein.

Figures 4A through 4E illustrate the effects of the different mutations on drug resistance conferred by MDR1. The G185V substitution (Figure 4A; open circles) was found to increase etoposide resistance relative to cells transduced with wild-type MDR1 (closed circles). This correlated with the results shown by Sañ et al., 1990, ibid. The I186N substitution (closed triangles) was found to increase etoposide resistance to a similar degree as the G185V substitution. However, cells transduced with the MDR1-G185V/I186N construct were found to exhibit a higher level of etoposide resistance than cells transduced with either the MDR1-G185V or MDR1-I186N constructs.
Similar results were obtained when the transduced cells were analyzed for doxorubicin resistance (Figure 4C). In cells transduced with either the MDR1-G185V or MDR1-I186N constructs, doxorubicin resistance was enhanced relative to cells transduced with wild-type MDR1. In cells transduced with the MDR1-G185V/I186N construct, an even higher level of doxorubicin resistance was observed.

While the I186N substitution, like the G185V substitution, was found to increase colchicine resistance relative to cells transduced with wild-type MDR1, the G185V substitution (which was originally identified in colchicine-selected cells) was found to confer an even higher level of colchicine resistance (Figure 4B). However, in contrast to the observations made with respect to etoposide and doxorubicin resistance, cells transduced with the MDR1-G185V/I186N construct exhibited a level of resistance that was indistinguishable from that obtained for cells transduced with the MDR1-G185V construct.

A major functional difference was observed in the resistance conferred by the MDR1 mutants to vinblastine (Figure 4D) and taxol (Figure 4E). In agreement with previous reports (Choi et al., 1988, ibid.; Saha et al., 1990, ibid.), the G185V substitution was found to decrease resistance levels to both of these drugs relative to cells transduced with wild-type MDR1. In contrast, the I186N substitution was found to increase resistance to both of these drugs relative to cells transduced with wild-type MDR1. In cells transduced with the MDR1-G185V/I186N construct, the opposite effects of the I186N and G185V substitutions appear to balance out, and therefore, the level of vinblastine and taxol resistance are similar to that observed in cells transduced with wild-type MDR1.

These results indicate that the I186N substitution alone (like the G185V substitution) can confer increased resistance to etoposide and doxorubicin, and that the I186N substitution, when present in combination with the G185V substitution can augment the effect of the latter substitution by conferring an even higher level of etoposide and doxorubicin resistance. These results suggest that a mutant human P-glycoprotein having only a single substitution (e.g., an individual lysine substitution at position 634), similarly to the mutant P-glycoproteins described herein that have multiple substitutions (e.g., lysine substitutions at positions 634 and 636 in the 6-C1 mutant), can confer increased resistance to certain chemotherapeutic drugs compared to wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185.
EXAMPLE 4

Construction and characterization of mutant P-glycoproteins
2-B1, 2-B2, 5-C3, 6-C1, 8-A4, and 8-B4

To investigate the mutants 6-C1 and 8-B4 disclosed in Example 2 above, which are located in the linker region separating the two homologous, membrane-spanning domains, MDR1-G185V cDNA was modified by site-directed mutagenesis to create a mutant construct termed LMDR1-G185V-EK2 (wherein “EK2” denoted that two glutamic acid to lysine substitutions had been introduced, at positions 634 and 636) and a mutant construct termed LMDR1-G185V-EK3 construct (wherein “EK3” denoted that three glutamic acid to lysine substitutions had been introduced, at positions 643, 647, and 652). In addition, to test whether an even greater change in the charge of the linker region would yield an even higher level of etoposide resistance, a third construct was created in which the amino acid substitutions of both the 6-C1 and 8-B4 mutants were introduced. This construct was designated LMDR1-G185V-EK5 (“EK5” denoting that glutamic acid to lysine substitutions had been introduced at positions 634, 636, 643, 647 and 652).

NIH 3T3 cells were transduced with the LMDR1-G185V, LMDR1-G185V-EK2, LMDR1-G185V-EK3, or LMDR1-G185V-EK5 constructs, and the transduced cells were then sorted by FACS to generate pure populations of P-glycoprotein positive cells having similar levels of P-glycoprotein. Following sorting, the cell populations were analyzed for their drug resistance. The results of this analysis are shown in Figures 5A through 5C. Cells transduced with the LMDR1-G185V-EK2 and LMDR1-G185V-EK3 constructs showed a 1.4 and 1.6-fold increase in etoposide resistance relative to the LMDR1-G185V construct (Figure 5A; Table II). The level of etoposide resistance obtained in cells transduced with the LMDR1-G185V-EK5 construct, however, indicated that the effect of the double and triple lysine mutants was not additive.

The introduction of the glutamic acid to lysine substitutions in the LMDR1-G185V-EK2 and LMDR1-G185V-EK3 constructs was also found to increase doxorubicin resistance relative to the LMDR1-G185V construct (Figure 5B). As indicated in Table II, the resistance of the double lysine mutant showed an almost two-fold increase in doxorubicin resistance, while the triple lysine mutant showed nearly a four-fold increase in doxorubicin resistance. As with etoposide resistance,
the effect of the double and triple lysine mutants was not additive. This lack of additivity suggests that the increase in drug resistance is likely to be conferred by any combination of one or more glutamic acid to lysine mutations in the linker region, rather than only by the specific EK2 and EK3 combinations tested here.

In contrast to the results obtained for etoposide and doxorubicin, introduction of the glutamic acid to lysine substitutions in the LMDR1-G185V-EK2 and LMDR1-G185V-EK3 constructs was found to decrease colchicine resistance relative to the LMDR1-G185V construct (Figure 5C). In addition, cells transduced with the LMDR1-G185V-EK5 construct exhibited the greatest decrease in colchicine resistance (Table II). The multiple glutamic acid to lysine substitutions did not seem to affect taxol and vinblastine resistance (Table II).

To investigate the mutants 8-A4, 5-C3, 2-B2, and 2-B1, having mutations in one of the transmembrane domains, MDR1-G185V cDNA was modified by site-directed mutagenesis to create the following constructs: LMDR1-G185V-L56F (leucine to phenylalanine mutation at residue 56), LMDR1-G185V-I190F/M197F (isoleucine to phenylalanine mutation at amino acid residue 190 and methionine to phenylalanine mutation at residue 197), LMDR1-G185V-G763W (glycine to tryptophan mutation at residue 763), and LMDR1-G185V-G844Q (glycine to glutamine mutation at residue 844). NIH 3T3 cells were transduced with either the LMDR1-G185V construct or one of the transmembrane mutant constructs, and the transduced cells were then sorted by FACS to generate pure populations of P-glycoprotein positive cells having similar levels of P-glycoprotein. Following sorting, the cell populations were analyzed for their drug resistance.

All of the mutants conferred increased resistance to etoposide with the I190F/M197F mutant yielding the greatest resistance (Figure 6A; Table II). While only the L56F mutant conferred increased colchicine resistance, the G844Q mutant was found to exhibit decreased colchicine resistance (Figure 6C; Table II). With the exception of the G844Q mutant, each of the mutants showed an increased doxorubicin resistance relative to the LMDR1-G185V construct (Figure 6B). While the L56F mutant conferred very strong resistance to doxorubicin, the I190F/M197F and G763W mutants only conferred moderate increases in doxorubicin resistance (Table II). Although the I190F/M197F and G763W mutants were both found to confer increased vinblastine resistance (the latter mutant showing only a moderate increase in vinblastine resistance), the L56F mutant exhibited decreased vinblastine resistance.
(Figure 6D; Table II). While the L56F mutant also exhibited decreased taxol resistance, the I190F/M196F mutant showed a significant increase in taxol resistance, and the G763W and G844Q mutants appeared to have no effect on taxol resistance (Figure 6E; Table II).

Since the I186N substitution was found to increase etoposide resistance even when introduced into wild-type MDR1 alone (i.e., not in tandem with the G185V substitution), it is likely that the substitutions described above would also confer increased resistance to etoposide when introduced into wild-type MDR1 alone or in combinations.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.
WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having an asparagine residue at position 186.

2. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a phenylalanine residue at position 56.

3. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a phenylalanine residue at positions 190 and 197.

4. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a tryptophan residue at position 763.

5. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a glutamic acid residue at position 844.

6. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at positions 634 and 636.

7. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at positions 643, 647, and 652.

8. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at positions 634, 636, 643, 647, and 652.

9. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a phenylalanine residue at position 190.

10. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a phenylalanine residue at position 197.
11. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at position 634.

12. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at position 636.

13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at position 643.

14. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at position 647.

15. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having a lysine residue at position 652.

16. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having at least one amino acid substitution selected from the group consisting of:

(a) an asparagine residue at position 186;
(b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at positions 190 and 197;
(d) a tryptophan residue at position 763;
(e) a glutamic acid residue at position 844;
(f) a lysine residue at positions 634 and 636; and
(g) a lysine residue at positions 643, 647, and 652.

17. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human P-glycoprotein and having at least one amino acid substitution selected from the group consisting of:

(a) an asparagine residue at position 186;
(b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at position 190;
(d) a phenylalanine residue at position 197;
(e) a tryptophan residue at position 763;
(f) a glutamic acid residue at position 844;
(g) a lysine residue at position 634;
(h) a lysine residue at position 636;
(i) a lysine residue at position 643;
(j) a lysine residue at position 647; and
(k) a lysine residue at position 652.

18. An isolated nucleic acid molecule comprising a nucleotide sequence
encoding a polypeptide as set forth in any of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID
NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID
NO: 11.

19. An isolated nucleic acid molecule comprising a nucleotide sequence
encoding a polypeptide as set forth in any of SEQ ID NO: 12, SEQ ID NO: 13, SEQ
ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

20. An isolated nucleic acid molecule comprising a nucleotide sequence
encoding a polypeptide as set forth in SEQ ID NO: 2 and having at least one amino
acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at positions 190 and 197;
   (d) a tryptophan residue at position 763;
   (e) a glutamic acid residue at position 844;
   (f) a lysine residue at positions 634 and 636; and
   (g) a lysine residue at positions 643, 647, and 652.

21. An isolated nucleic acid molecule comprising a nucleotide sequence
encoding a polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid
substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at position 190;
(d) a phenylalanine residue at position 197;
(e) a tryptophan residue at position 763;
(f) a glutamic acid residue at position 844;
(g) a lysine residue at position 634;
(h) a lysine residue at position 636;
(i) a lysine residue at position 643;
(j) a lysine residue at position 647; and
(k) a lysine residue at position 652.

22. The isolated nucleic acid molecule of any of Claims 1-21 having a valine at position 185.

23. A recombinant expression construct comprising the nucleic acid molecule of any of Claims 1-21.


27. An isolated human P-glycoprotein molecule having a phenylalanine residue at positions 190 and 197.


29. An isolated human P-glycoprotein molecule having a glutamic acid residue at position 844.
30. An isolated human P-glycoprotein molecule having a lysine residue at positions 634 and 636.

31. An isolated human P-glycoprotein molecule having a lysine residue at positions 643, 647, and 652.

32. An isolated human P-glycoprotein molecule having a lysine residue at positions 634, 636, 643, 647, and 652.

33. An isolated human P-glycoprotein molecule having a phenylalanine residue at position 190.

34. An isolated human P-glycoprotein molecule having a phenylalanine residue at position 197.

35. An isolated human P-glycoprotein molecule having a lysine residue at position 634.

36. An isolated human P-glycoprotein molecule having a lysine residue at position 636.

37. An isolated human P-glycoprotein molecule having a lysine residue at position 643.

38. An isolated human P-glycoprotein molecule having a lysine residue at position 647.


40. An isolated human P-glycoprotein molecule having at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at positions 190 and 197;
(d) a tryptophan residue at position 763;
(e) a glutamic acid residue at position 844;
(f) a lysine residue at positions 634 and 636; and
(g) a lysine residue at positions 643, 647, and 652.

41. An isolated human P-glycoprotein molecule having at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at position 190;
   (d) a phenylalanine residue at position 197;
   (e) a tryptophan residue at position 763;
   (f) a glutamic acid residue at position 844;
   (g) a lysine residue at position 634;
   (h) a lysine residue at position 636;
   (i) a lysine residue at position 643;
   (j) a lysine residue at position 647; and
   (k) a lysine residue at position 652.

42. An isolated polypeptide as set forth in any of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.

43. An isolated polypeptide as set forth in any of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

44. An isolated polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at positions 190 and 197;
   (d) a tryptophan residue at position 763;
(e) a glutamic acid residue at position 844;
(f) a lysine residue at positions 634 and 636; and
(g) a lysine residue at positions 643, 647, and 652.

45. An isolated polypeptide as set forth in SEQ ID NO: 2 having at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at position 190;
   (d) a phenylalanine residue at position 197;
   (e) a tryptophan residue at position 763;
   (f) a glutamic acid residue at position 844;
   (g) a lysine residue at position 634;
   (h) a lysine residue at position 636;
   (i) a lysine residue at position 643;
   (j) a lysine residue at position 647; and
   (k) a lysine residue at position 652.

46. The isolated polypeptide of any of Claims 25-45 having a valine at position 185.

47. An antibody or fragment thereof that specifically binds the polypeptide of any of Claims 25-45.

48. An antibody or fragment thereof that specifically binds the polypeptide of Claim 46.

49. A mammalian cell culture comprising a recombinant expression construct according to claim 23.

50. A method for determining whether a human tumor sample comprises tumor cells expressing a mutant P-glycoprotein, the method comprising the steps of contacting cells comprising the human tumor sample with an antibody immunologically specific for said mutant P-glycoprotein.
51. The method of claim 50, wherein the mutant P-glycoprotein comprises at least one amino acid substitution selected from the group consisting of:

(a) an asparagine residue at position 186;
(b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at positions 190 and 197;
(d) a tryptophan residue at position 763;
(e) a glutamic acid residue at position 844;
(f) a lysine residue at positions 634 and 636; and
(g) a lysine residue at positions 643, 647, and 652.

52. The method of claim 50, wherein the mutant P-glycoprotein comprises at least one amino acid substitution selected from the group consisting of:

(a) an asparagine residue at position 186;
(b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at position 190;
(d) a phenylalanine residue at position 197;
(e) a tryptophan residue at position 763;
(f) a glutamic acid residue at position 844;
(g) a lysine residue at position 634;
(h) a lysine residue at position 636;
(i) a lysine residue at position 643;
(j) a lysine residue at position 647; and
(k) a lysine residue at position 652.

53. A method for determining whether a human tumor sample comprises tumor cells expressing a mutant P-glycoprotein, the method comprising the steps of hybridizing an mRNA or cDNA sample from said tumor cells comprising the human tumor sample with a nucleic acid probe specific for said mutant P-glycoprotein, wherein said nucleic acid probe comprises a nucleotide sequence encoding a mutant P-glycoprotein or fragment thereof having at least one mutated amino acid residue.
54. The method of claim 53, wherein the nucleic acid probe comprises a nucleotide sequence encoding a mutant P-glycoprotein or fragment thereof having at least one amino acid substitution selected from the group consisting of:

(a) an asparagine residue at position 186;
(b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at positions 190 and 197;
(d) a tryptophan residue at position 763;
(e) a glutamic acid residue at position 844;
(f) a lysine residue at positions 634 and 636; and
(g) a lysine residue at positions 643, 647, and 652.

55. The method of claim 53, wherein the nucleic acid probe comprises a nucleotide sequence encoding a mutant P-glycoprotein or fragment thereof having at least one amino acid substitution selected from the group consisting of:

(a) an asparagine residue at position 186;
(b) a phenylalanine residue at position 56;
(c) a phenylalanine residue at position 190;
(d) a phenylalanine residue at position 197;
(e) a tryptophan residue at position 763;
(f) a glutamic acid residue at position 844;
(g) a lysine residue at position 634;
(h) a lysine residue at position 636;
(i) a lysine residue at position 643;
(j) a lysine residue at position 647; and
(k) a lysine residue at position 652.

56. A method for determining whether a human tumor sample comprises tumor cells expressing a mutant P-glycoprotein, the method comprising the steps of amplifying in vitro a nucleic acid fragment comprising an mRNA or cDNA sample from said tumor cells, wherein said nucleic acid fragment comprises a nucleotide sequence encoding a mutant P-glycoprotein or fragment thereof having at least one mutated amino acid residue.
57. The method of claim 56, wherein the nucleic acid fragment comprises a nucleotide sequence encoding a mutant P-glycoprotein or fragment thereof having at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at positions 190 and 197;
   (d) a tryptophan residue at position 763;
   (e) a glutamic acid residue at position 844;
   (f) a lysine residue at positions 634 and 636; and
   (g) a lysine residue at positions 643, 647, and 652.

58. The method of claim 56, wherein the nucleic acid fragment comprises a nucleotide sequence encoding a mutant P-glycoprotein or fragment thereof having at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at position 190;
   (d) a phenylalanine residue at position 197;
   (e) a tryptophan residue at position 763;
   (f) a glutamic acid residue at position 844;
   (g) a lysine residue at position 634;
   (h) a lysine residue at position 636;
   (i) a lysine residue at position 643;
   (j) a lysine residue at position 647; and
   (k) a lysine residue at position 652.

59. A method for administering an increased amount of a chemotherapeutic agent to an individual with cancer in need thereof, the method comprising the steps of transducing ex vivo hematopoietic cells from said individual with a recombinant expression construct encoding a mutant P-glycoprotein that is expressed in said hematopoietic cells and confers increased resistance to said chemotherapeutic agent compared with resistance conferred by wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185, reintroducing said transduced hematopoietic cells into said individual, and
administering an increased amount of a chemotherapeutic agent without consequent hematopoietic cytotoxicity.

60. The method of claim 59, wherein the mutant P-glycoprotein comprises at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at positions 190 and 197;
   (d) a tryptophan residue at position 763;
   (e) a glutamic acid residue at position 844;
   (f) a lysine residue at positions 634 and 636; and
   (g) a lysine residue at positions 643, 647, and 652.

61. The method of claim 59, wherein the mutant P-glycoprotein comprises at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at position 190;
   (d) a phenylalanine residue at position 197;
   (e) a tryptophan residue at position 763;
   (f) a glutamic acid residue at position 844;
   (g) a lysine residue at position 634;
   (h) a lysine residue at position 636;
   (i) a lysine residue at position 643;
   (j) a lysine residue at position 647; and
   (k) a lysine residue at position 652.

62. A method for detecting a compound that reduces in a cell expressing a mutant P-glycoprotein an increased resistance to a chemotherapeutic agent compared with resistance conferred by wild-type P-glycoprotein or P-glycoprotein having a glycine to valine substitution at position 185, the method comprising the steps of culturing in the presence and absence of the compound a first cell expressing the mutant P-glycoprotein, a second cell expressing wild-type P-glycoprotein, and a third cell expressing P-glycoprotein having a glycine to valine substitution at position 185
with the compound in the presence of a cytotoxic drug for which the mutant P-glycoprotein confers increased resistance in the first cell, and detecting increased cytotoxicity in the cells expressing the mutant P-glycoprotein in the presence of the compound than in the absence of the compound.

63. The method of claim 62, wherein the mutant P-glycoprotein comprises at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at positions 190 and 197;
   (d) a tryptophan residue at position 763;
   (e) a glutamic acid residue at position 844;
   (f) a lysine residue at positions 634 and 636; and
   (g) a lysine residue at positions 643, 647, and 652.

64. The method of claim 62, wherein the mutant P-glycoprotein comprises a nucleotide sequence encoding at least one amino acid substitution selected from the group consisting of:
   (a) an asparagine residue at position 186;
   (b) a phenylalanine residue at position 56;
   (c) a phenylalanine residue at position 190;
   (d) a phenylalanine residue at position 197;
   (e) a tryptophan residue at position 763;
   (f) a glutamic acid residue at position 844;
   (g) a lysine residue at position 634;
   (h) a lysine residue at position 636;
   (i) a lysine residue at position 643;
   (j) a lysine residue at position 647; and
   (k) a lysine residue at position 652.
4 bacterial clones per pool were inoculated into each well of array a and bacterial from each individual well was grown up and plated.

Genomic DNA of 131 clones from the 10k selection was divided into 4 pools, recombined back into LXS1, and transformed.

FIG. 2
### FIG. 3A

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<th>5-C3</th>
<th>2-B2</th>
<th>2-B1</th>
<th>6-C1</th>
<th>8-B4</th>
<th>EK5</th>
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FIG. 3B

wt P-gp  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
G185V  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
I186N  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
8-A4  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
5-C3  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
2-B2  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
2-B1  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
6-C1  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
8-B4  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250
EK5  201  FTGFIVGFTGRWKTLVLAILISPVGLGSAAVWAKISFFTDKELLAYAKA  250

wt P-gp  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
G185V  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
I186N  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
8-A4  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
5-C3  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
2-B2  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
2-B1  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
6-C1  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
8-B4  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300
EK5  251  GAVAEVLAAIRTVIAFGGQKKELEARYNKLEAEARKIIGKKAITANISIG  300

wt P-gp  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
G185V  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
I186N  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
8-A4  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
5-C3  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
2-B2  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
2-B1  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
6-C1  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
8-B4  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350
EK5  301  AAFLLISAYALAFWGGTLVLSYEYISGQVLTVFSSVLAGFSGQASP  350

wt P-gp  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
G185V  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
I186N  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
8-A4  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
5-C3  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
2-B2  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
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6-C1  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
8-B4  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
EK5  351  SIEAFANARGAYEFKIIDNKFSDSYSKSGHKPDNIGNLEFRNVFHS  400
FIG. 3D

wt P-gp  601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
G185V   601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
I186N   601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
8-A4    601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
5-C3    601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
2-B2    601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
2-B1    601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
6-C1    601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
8-B4    601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650
EK5     601 FDDGVIVEKGNDELMEKGIYFKLVTMQTAGNEVELENADKSEIDA  650

wt P-gp  651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
G185V   651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
I186N   651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
8-A4    651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
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2-B2    651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
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6-C1    651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
8-B4    651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700
EK5     651 LEMSNDSSRLIRKSTRSSVGRSQAQRKLSKTEDALDESIPVSVFWR1  700

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G185V   701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
I186N   701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
8-A4    701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
5-C3    701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
2-B2    701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
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6-C1    701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
8-B4    701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750
EK5     701 MKLNLTEWYPFVGVFCAINGGLQPAIIIFSKIIIGVFTRIDDPETKRQ  750

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G185V   751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
I186N   751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
8-A4    751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
5-C3    751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
2-B2    751 NSNLFSLLFLALWIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
2-B1    751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
6-C1    751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
8-B4    751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
EK5     751 NSNLFSLLFLALGIIISFIITFLQGFTFGEGETKRLRYMVFRSMRQD  800
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<td>8-B4</td>
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<td>FSYAGCFRGAYLVAHKLMSFEDVLLVFSAVVFAMAVQAQQSSFAPDYAK</td>
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<td>FSYAGCFRGAYLVAHKLMSFEDVLLVFSAVVFAMAVQAQQSSFAPDYAK</td>
<td>1000</td>
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<td>2-B2</td>
<td>FSYAGCFRGAYLVAHKLMSFEDVLLVFSAVVFAMAVQAQQSSFAPDYAK</td>
<td>1000</td>
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<tr>
<td>2-B1</td>
<td>FSYAGCFRGAYLVAHKLMSFEDVLLVFSAVVFAMAVQAQQSSFAPDYAK</td>
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<td>6-C1</td>
<td>FSYAGCFRGAYLVAHKLMSFEDVLLVFSAVVFAMAVQAQQSSFAPDYAK</td>
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<td>FSYAGCFRGAYLVAHKLMSFEDVLLVFSAVVFAMAVQAQQSSFAPDYAK</td>
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FIG. 3F

wt P-gp 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
G185V 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
I186N 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
8-A4 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
5-C3 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
2-B2 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
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6-C1 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
8-B4 1001 AKISAAHIMIIIEKTPLIDSYESTEGLMFTLESNGTVFGEVFVNYFPTRPD1 1050
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G185V 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
I186N 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
8-A4 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
5-C3 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
2-B2 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
2-B1 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
6-C1 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
8-B4 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100
EK5 1051 PVQLQGSLQVKGQLTLALVGSSGCGLSCKSTTVQLVERFYDPLAGKVLLDGKE 1100

wt P-gp 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
G185V 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
I186N 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
8-A4 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
5-C3 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
2-B2 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
2-B1 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
6-C1 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
8-B4 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150
EK5 1101 IKLRNQWLRQLAHGVQEPILFDCSIAENIAYGDSNVSVSQQEEIVRAAK 1150

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G185V 1151 EANIHAFIESLPKYSTKVGDPKGTKQLSGQQKQRIAIARALVRQPHHLLLD 1200
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2-B1 1151 EANIHAFIESLPKYSTKVGDPKGTKQLSGQQKQRIAIARALVRQPHHLLLD 1200
6-C1 1151 EANIHAFIESLPKYSTKVGDPKGTKQLSGQQKQRIAIARALVRQPHHLLLD 1200
8-B4 1151 EANIHAFIESLPKYSTKVGDPKGTKQLSGQQKQRIAIARALVRQPHHLLLD 1200
EK5 1151 EANIHAFIESLPKYSTKVGDPKGTKQLSGQQKQRIAIARALVRQPHHLLLD 1200
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FIG. 4

A

Percent Survival

100
80
60
40
20
0
100
1000
Etoposide [ng/ml]

B

Percent Survival

100
80
60
40
20
0
100
Colchicine [ng/ml]

C

Percent Survival

100
80
60
40
20
0
10
100
Doxorubicin [ng/ml]

D

Percent Survival

100
80
60
40
20
0
10
Vinblastine [ng/ml]

E

Percent Survival

100
80
60
40
20
0
100
Taxol [ng/ml]
FIG. 5

A

Percent Survival

100
80
60
40
20
0
1000

Etoposide [ng/ml]

B

Percent Survival

120
100
80
60
40
20
0
10
100

Doxorubicin [ng/ml]

C

Percent Survival

100
80
60
40
20
0
100

Colchicine [ng/ml]