DETAILED SUMMARY OF NON-P-GLYCOPROTEIN MULTIDRUG RESISTANCE IN THE OVERALL SCHEMA OF DRUG RESISTANCE.

- An intricate equilibrium of upstream factors, tumor factors and downstream factors causes drug resistance through their effect on the growth, proliferation and apoptosis of cancer cells. **Upstream factors** include drug sanctuaries (blood-brain barrier, blood-eye barrier, blood-testicle barrier), intrinsic tumor factors, and host factors that affect metabolism, distribution, and clearance of drugs. **Tumor factors** include non-ABC transporters that cause resistance to a single drug such as dihydrofolate reductase that mediates resistance to methotrexate. Tumor factors also include a variety of ATP-Binding Cassette (ABC) superfamily transporters that cause resistance to multiple drugs (increased expression of the cell membrane drug efflux transporters P-glycoprotein and BCRP, the cell membrane drug sequestrator MRP, the nuclear-cytoplasmic transporter LRP, the MRP-related Canalicular Multispecific Organic Anion Transporter c-MOAT, and the drug target-altering topoisomerase II enzyme; or decreased expression of drug-detoxifying glutathione enzyme GSH). **Downstream factors** include increased expression of the anti-apoptotic bcl-2 oncogene, amplification or increased expression of MYCN oncogene or other oncogenes, and amplification of the Murine Double Minute-2 (MDM2) cellular proliferation-promoting gene; or decreased expression of the p53 tumor suppressor gene or other tumor suppressor genes.

  o Classical MDR to chemotherapy drugs is caused by increased expression of the transmembrane transporter P-glycoprotein, first described by Ling et al in 1974. Ling et al had extended the original observations of the phenomenon by in 1970 by Biedler and Riehm, and by Danø in 1973.
    - Classical MDR broadly affects drug classes that include the hydrophobic and amphipathic natural product drugs such as the epipodophyllotoxins (etoposide, teniposide), taxanes (taxol), vinca alkaloids (vincristine, vinblastine), antibiotics (dactinomycin), and anthracyclines (doxorubicin, daunorubicin).
    - Non-natural product drugs are unaffected by MDR and they include the alkylators (cyclophosphamide, ifosfamide), platinums (cisplatin, carboplatin), antimetabolites (cytarabine, methotrexate, mercaptopurine), and steroids (prednisone).

  o The first form of non-P-glycoprotein MDR was described in 1992 by Cole and Deeley et al, who characterized the Multidrug Resistance-Associated Protein (now abbreviated as Multidrug Resistance Protein or MRP) in a multidrug-resistant human lung cancer cell line.
    - The human MRP family consists of at least 6 members: MRP1 that is the MRP prototype, and at least five homologues, MRP2, MRP3,
MRP4, MRP5, and MRP6.

- Many non-P-glycoprotein drug efflux pumps have been proposed but MRP is the most fully confirmed mechanism so far. The MRP gene is distantly related to the multidrug resistance MDR1 gene.
- The cloning of the MRP gene, and the MRP cDNA transfection experiments, proved unequivocally that overexpression of MRP confers MDR in mammalian cells.
- The MRP gene is localized to chromosome 16p13.1, and encodes a 7.8-8.2 kb mRNA transcript, which produces the 190-kDa membrane-bound MRP protein.
- MRP confers MDR to the same natural product drugs that are substrates of the P-glycoprotein drug efflux pump, and additionally exhibits resistance to heavy metal anion substrates, arsenite, arsenate, and trivalent antimony.
- The MRP protein is localized to the plasma membrane and Golgi region. It is N-glycosylated. It undergoes phosphorylation only at the serine residues due to the action of protein kinase C. The regulation of MRP is complex and occurs at multiple sites and is subjected to numerous environmental stimuli.
- The physiologic role of MRP appears to be acting as the ATP-dependent export pump for substrates that include the conjugates for lipophilic compounds, glutathione, leukotriene C4, LTD4, LTE4, and S-(2,4-dinitrophenyl)-glutathione, and sequestering these compounds into protective MRP-expressing vesicles to prevent cytotoxicity to normal cells.
- MRP protein is expressed in human malignancies that include acute lymphoblastic and myelogenous leukemia, B-cell chronic lymphocytic and promyelocytic leukemia, hairy cell leukemia, non-Hodgkin lymphoma, multiple myeloma, and neuroblastoma. It is also expressed in normal cells such as peripheral blood mononuclear cells (the protein immunostains with anti-MRP monoclonal antibodies; the mRNA is shown by reverse transcriptase polymerase chain reaction analysis, RT-PCR).
- Definitive correlation of MRP overexpression with poor outcome has not been fully defined in human malignancies.
  - Non-P-glycoprotein MDR could also be caused by the Atypical Multidrug Resistance Protein (AT-MDR), first described in 1987 by Beck et al and Danks et al, in T-cell acute lymphoblastic leukemia sublines selected with teniposide:
    - AT-MDR displays a cross-resistance profile very similar to the phenotypes of the MDR1 P-glycoprotein and MRP1, with a few exceptions. It does not confer resistance to vinca alkaloids, and drug accumulation is no different between drug-resistant and drug-
sensitive cell lines.

- AT-MDR apparently is mediated by the altered expression of the drug target-altering topoisomerase II enzyme.
- The expression and clinical significance of AT-MDR in human malignancies remain undefined.

- Non-P-glycoprotein MDR could also be caused by the Lung Cancer Resistance Protein (now abbreviated as Lung Resistance Protein or LRP or LRP-56 after the anti-LRP monoclonal antibody LRP-56), first described in 1993 by Scheper et al in lung cancer cell lines that exhibited decreased drug accumulation:
  - LRP is a 110-kDa vesicular protein overexpressed in several MDR cell lines, in normal epithelial cells, and in tissues (bronchi, intestine, renal tubules) exposed chronically to xenobiotics and potentially toxic agents.
  - The LRP gene has been cloned and demonstrates 60% homology (sequence identity) with the 104-kDa major vault protein from *Dictyostelium discoideum*.
  - Vault proteins are large cytoplasmic ribonucleoprotein structures found in eukaryotic cells. In rat liver, for example, there is one 104-kDa major vault protein and three 210-kDa, 192-kDa, and 54-kDa minor vault proteins that are hollow barrel-like multiunit organelles (two identical cup-like halves, each made of eight petal-like structures, joined at the open end) associated with cytoplasmic vesicle structures and nuclear pore complexes.
  - Vault proteins are highly conserved through the species.
  - LRP protein is overexpressed in human malignancies that include acute myelogenous leukemia. The clinical significance of LRP protein in human malignancies is not fully defined.

- Non-P-glycoprotein MDR could also be caused by the Mitoxantrone Resistance Protein MXR or Placenta-Specific ABC Protein ABCP1 or ABCG2 (now called Breast Cancer Resistance Protein or BCRP), first described in 1995 by Doyle and Ross et al:
  - The *BCRP* gene encodes a 2.4-kb mRNA transcript that translates into the 655-amino acid BCRP protein. BCRP is a 95 kDa N-linked membrane sialoglycoprotein that is differentially glycosylated (72 kDa when unglycosylated).
  - The BCRP protein is identified in a number of cell lines.
  - The BCRP protein is identified as an ABC half-transporter member of the ATP-binding cassette (ABC) transporter superfamily. It is unique among the ABC half-transporters in being localized to the plasma membrane rather than to the intracellular membranes.
  - The BCRP drug efflux pump/xenobiotic transporter confers high-
level resistance to mitoxantrone, and resistance to other anthracyclines (doxorubicin, daunorubicin), and to camptothecins.

- In normal human tissues, the BCRP protein is found in the placental syncytiotrophoblasts, small intestine and colon epithelium, liver canalicular membrane, breast ducts and lobules, and in the venous and capillary endothelium but not in the arterial endothelium.
- The apical localization of the BCRP protein in the small intestine and colon epithelium suggests a possible role in regulation of the back-transport of BCRP substrates that enter epithelial cells from the gut lumen.
- The placental localization of the BCRP protein suggests a possible protective role for the fetus.
- The BCRP protein is expressed in acute myeloid leukemia but its clinical relevance is not fully defined.

- P-glycoprotein remains the paradigm for understanding the subsequently described forms of multidrug resistance in the clinical and laboratory setting. The clinical relevance of non-P-glycoprotein MDR proteins has to be fully defined in clinical correlative studies. Merely identifying their presence in clinical samples is not sufficient; it requires:
  - The non-P-glycoprotein MDR protein should be detected before treatment in non-responsive tumors, and conversely, not found in cured tumors.
  - Prospective pretreatment evaluation of the tumor MDR protein should accurately predict the outcome of chemotherapy.
  - Cures should be achievable by treating the tumors expressing the MDR protein with agents that could reverse MDR in vitro.
  - Chemosensitizing agents that improve the response to chemotherapy drugs should specifically work by reversing MDR, rather than work indirectly by reducing the metabolic clearance of chemotherapy drugs.

- Some experimental studies show the reversal of non-P-glycoprotein MDR in vitro and in vivo.
- Effective but nontoxic MDR-reversal trials are not yet available in the clinic.

**PDF File #2: DETAILED DESCRIPTION OF THE MULTIDRUG RESISTANCE PROTEIN MRP GENE FAMILY.**

- **MRP IN THE OVERALL SCHEMA OF THE ATP-BINDING CASSETTE (ABC) TRANSPORTER SUPERFAMILY:**
  - MRP and P-glycoprotein belong to the same large ATP-dependent transmembrane transporters superfamily, the ATP-Binding Cassette
(ABC) transporters superfamily.

- ABC transporters family members are prominent in many diseases including malaria, leishmaniasis and cancer.
- Several mammalian ABC transporters family members are involved in essential physiologic processes such as the sulfonylurea receptor SUR1 subunit of the ATP-sensitive potassium channel regulator of pancreatic beta cells in modulation of insulin secretion, the CFTR CAMP-regulated chloride channel of cystic fibrosis, and the TAP1 and TAP2 peptide transporters associated with antigen presentation by the major histocompatibility Class I molecules.
- Mutations of several ABC genes cause genetic disorders such as persistent hyperinsulinemia hypoglycemia of infancy (SUR1 mutation), Dubin-Johnson congenital hyperbilirubinemia (MOAT mutation), cystic fibrosis (CFTR mutation), and Stargardt’s disease with retina rod photoreceptor-related loss central vision of childhood (RIM or ABCR mutation).

- **PHYLOGENETIC TREE SHOWING THE RELATIONSHIP BETWEEN MRP AND OTHER ABC TRANSPORTERS:**

  - This phylogenetic tree shows the close relationship between the human MRP homologues (MRP1, MRP2, MRP3, MRP4, MRP4, MRP5, MRP6, MRP7), human SUR1 (pancreatic beta cell sulfonylurea receptor/potassium channel regulator), murine SUR2 (brain and heart sulfonylurea receptor/potassium channel regulator), and human CFTR (cystic fibrosis receptor); whereas the human MDR1 and MDR3 homologues are more closely related to BSEP.

<table>
<thead>
<tr>
<th>TABLE #1: THE PHYLOGENETIC TREE GENERATED USING THE CARBOXYL-TERMINUS OF EACH PROTEIN TO ALIGN WITH THAT OF MRP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR</td>
</tr>
<tr>
<td>SUR2</td>
</tr>
<tr>
<td>MRP6</td>
</tr>
</tbody>
</table>

- **STRUCTURAL FEATURES COMMON TO MRP AND OTHER ABC TRANSPORTERS:**

  - More than 200 prokaryotic and eukaryotic ABC proteins have been cloned and characterized; their common structural features include:
A hydrophobic polytopic membrane-spanning domain with up to six transmembrane helices.

A hydrophilic cytosolic nucleotide-binding domain containing three highly conserved sequence motifs, the Walker A motif, the Walker B motif, and the active transport family signature.

- In bacterial ABC proteins, these structural domains are usually encoded as separate polypeptides; they associate with one another to form an active transporter.
- In eukaryotic ABC protein transporters, there are usually four domains configured either as two alternating membrane-spanning domains and nucleotide-binding domains encoded as a single large polypeptide (eg, CFTR), or as two independently encoded half-molecules which then associate to function as a heterodimer (eg, TAP1/TAP2).
- The closest ABC proteins that share 30-90% identity and 48-96% similarity to MRP have similar but not identical functions to MRP.

### TABLE #2: THE CLOSEST ABC PROTEINS THAT SHARE A HIGH DEGREE OF HOMOLOGY WITH MRP HAVE SIMILAR BUT NOT IDENTICAL FUNCTIONS TO MRP

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>MRP1</td>
<td>GS-X pump; Anionic conjugate transporter; MDR</td>
</tr>
<tr>
<td>Mouse</td>
<td>mrp</td>
<td>GS-X pump; Anionic conjugate transporter; MDR</td>
</tr>
<tr>
<td>Human</td>
<td>MOAT/MRP2</td>
<td>GS-X pump; Liver anionic conjugate transporter</td>
</tr>
<tr>
<td>Rabbit</td>
<td>EBCR</td>
<td>MOAT ortholog</td>
</tr>
<tr>
<td>C. elegans</td>
<td>mrp1</td>
<td>Heavy metal resistance</td>
</tr>
<tr>
<td>C. elegans</td>
<td>mrp2</td>
<td>Unknown</td>
</tr>
<tr>
<td>Human</td>
<td>MRP6</td>
<td>Unknown</td>
</tr>
<tr>
<td>Yeast</td>
<td>YCF1</td>
<td>Cadmium resistance; Vacuolar GS-X pump</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>AtMRP1</td>
<td>GS-X pump</td>
</tr>
<tr>
<td>Human</td>
<td>SUR1</td>
<td>Sulfonylurea receptor; Pancreas K+ channel regulator</td>
</tr>
<tr>
<td>Murine</td>
<td>sur1</td>
<td>Sulfonylurea receptor; Brain, heart K+ channel regulator</td>
</tr>
<tr>
<td>Yeast</td>
<td>YOR1/YRS1</td>
<td>Oligomycin resistance</td>
</tr>
<tr>
<td>Leishmania</td>
<td>LtpgpA</td>
<td>Antimony and arsenic resistance</td>
</tr>
</tbody>
</table>

**DRUG RESISTANCE IN HUMAN CANCER:**

- For the first ABC protein transporter, the 170 kDa P-glycoprotein (Ling et al, 1976) described, the human *MDR1* gene has been cloned (Roninson et al, 1986), as has the mouse *mdr1* gene (Gros et al, 1986), one hamster *pgp* gene (Gros et al, 1986), and the other 5 hamster *pgp* genes (Van der Bliek et al, 1986).
- For the second ABC protein transporter, the 190 kDa MRP protein (Cole and Deeley, 1992), the human *MRP1* gene has been cloned (Cole and
Deeley, 1992).

- These two mammalian ABC proteins differ substantially in a number of important structural, mechanistic and pharmacological properties.

**THE MAMMALIAN P-GLYCOPROTEIN FAMILY:**
- Only the Class I protein and Class II protein confer multidrug resistance by acting as a drug efflux pump in cancer cells.
- The physiologic function of Class I P-glycoprotein appears to protect against toxins at the blood-brain barrier.
- Class III P-glycoprotein does not confer multidrug resistance.
- The physiologic function of Class III P-glycoprotein has not been fully defined but it appears to function as the phosphatidylcholine translocator that regulates the secretion of phosphatidylcholine into bile.

**TABLE #3: THE NOMENCLATURE FOR THE MAMMALIAN P-GLYCOPROTEIN GENES**

<table>
<thead>
<tr>
<th>Mammal</th>
<th>Class I P-glycoprotein</th>
<th>Class II P-glycoprotein</th>
<th>Class III P-glycoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human gene</td>
<td>MDR1</td>
<td>—</td>
<td>MDR3 (also called MDR2)</td>
</tr>
<tr>
<td>Mouse gene</td>
<td>mdr3 (also called mdr1a)</td>
<td>mdr1 (also called mdr1b)</td>
<td>mdr2</td>
</tr>
<tr>
<td>Rat gene</td>
<td>mdr1a</td>
<td>mdr1b</td>
<td>mdr2</td>
</tr>
<tr>
<td>Hamster gene</td>
<td>pgp1</td>
<td>pgp2</td>
<td>pgp3</td>
</tr>
</tbody>
</table>

**THE MAMMALIAN MULTIDRUG RESISTANCE PROTEIN MRP FAMILY:**
- The Multidrug Resistance Protein, first cloned by Cole and Deeley in 1992, is now known as MRP1.
- The MRP2 gene has been cloned in 1996 by Flens et al; by Buchler et al; and by Taniguchi et al.
- In the 1997 Gosau ABC transporters meeting, Marcel Kool introduced the concept of a MRP family with 5 members.
- A seventh family member of amphipathic anion transporters, MRP7 (ABCC10), was predicted from a database search. The MRP7 gene maps to chromosome 6p12-21, in proximity to several genes associated with glutathione conjugation and synthesis. It encodes a 158 kDa protein comprised of 1492 amino acids. The MRP7 protein resembles MRP1, MRP2, MRP3, and MRP6 structurally. It is expressed at low levels in
normal tissues.

- In 1996, Allikmets et al. identified 21 other new ABC transporters on the basis of conserved sequences.
- The MRP family group is clearly demarcated from the P-glycoprotein family group, CFTR, and the sulfonylurea receptor family group.
- The MRP family members fall into two groups – a group consisting of a larger number of amino acids, MRP1 (1531), MRP2 (1545), MRP3 (1527), and MRP6 (1503), and a group consisting of fewer amino acids, MRP4 (1325), and MRP5 (1437):
  - MRP1, MRP2, MRP3, and MRP6, have the highest homology, and possess the extra N-terminus domain lacking in MDR1.
  - The characteristic extra N-terminus extension of MRP1, MRP2, MRP3, and MRP6, with 5 transmembrane regions connected to a P-glycoprotein-like core by a cytoplasmic linker, is also seen in the other glutathione-drug conjugate (GS-X) pumps from simple eukaryotes like yeast and leishmania.
  - MRP4 and MRP5, which only have 40% identity with MRP1 and are smaller than MRP1, lack the extra N-terminus domain, but are still more homologous to the other MRP family members than to MDR1 or other ABC transporters.
  - The extra N-terminus domain is not necessary for transport activity.
  - The essential domain in MRP1, the linker domain zero, is conserved in the long N-terminus intracellular parts of MRP4 and MRP5.
- The Anthracycline-Associated Protein, ARA, is almost identical to the 3’ end of the MRP6 protein and found in epirubicin-selected leukemia cells.
- MRP2, characterized by Jansen et al as the Canalicular Multispecific Organic Anion Transporter (cMOAT), is missing in rats with an inborn error of metabolism in the biliary secretion of anions that include conjugated bilirubin. This is the origin of the alternative MOAT nomenclature for MRP family members.
- The sMRP is a cloning artefact, and represents the 3’ end of the MRP5 gene that is incidentally co-amplified with MRP1.

<p>| TABLE #4: THE DIFFERENT NOMENCLATURE OF MAMMALIAN MRP GENES IN THE LITERATURE |
|----------------------------------------|-----------------|------------------|------------------|------------------|
| MRP Nomenclature | ABC Nomenclature | Alternative Names | Alternative Names | Alternative Names |
| MRP1       | ABCC1          | MRP              |                 |                 |
| MRP2       | ABCC2          | cMOAT            | cMRP            | EBCR (rabbits) |</p>
<table>
<thead>
<tr>
<th>MRP3</th>
<th>ABCC3</th>
<th>MOAT-D</th>
<th>CMOAT-2</th>
<th>MLP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP4</td>
<td>ABCC4</td>
<td>MOAT-B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP5</td>
<td>ABCC5</td>
<td>MOAT-C</td>
<td>PABC11</td>
<td>sMRP  (only the 3’ end)</td>
</tr>
<tr>
<td>MRP6</td>
<td>ABCC6</td>
<td>MLP-1</td>
<td>ARA (only the 3’ end)</td>
<td></td>
</tr>
<tr>
<td>MRP7</td>
<td>ABCC10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TRANSPORT FUNCTIONS OF THE MRP FAMILY MEMBERS:**

- Up to now, 7 MRP family members are described that act as transporters of substances including:
  - organic anions and anionic drugs like methotrexate;
  - compounds converted to organic anions by conjugation with glutathione, glucuronide, and sulfate (which MDR1 has low-affinity for because of their negative charge);
  - neutral drugs conjugated to acidic ligands such as glutathione, glucuronide, and sulfate;
  - neutral organic drugs transported together with free glutathione, but not conjugated to the glutathione, glucuronide or sulfate acidic ligands.

- MRP family members act as GS-X pumps to cause resistance:
  - MRP1 transports drugs conjugated to glutathione out of cells, and possibly arsenite and antimony in complexes formed by combining with 3 glutathione molecules.
  - MRP2, MRP3, and MRP5 transport glutathione conjugates.
  - MRP1 and MRP2 transport anionic conjugates.

- MRP family members also transport other compounds to cause resistance:
  - MRP2/cMOAT probably transports cisplatin and carboplatin in complexes with glutathione.
  - MRP4 transports the nucleoside analogues 9-(2-phosphonylmethoxyethyl) adenine and azidothymidine monophosphate (anti-human immunodeficiency virus [HIV] drugs).
  - MRP family members also transport the nucleoside analogue anticancer drugs.

- The physiologic roles of MRP family members are related to their transporter functions:
  - MRP1 provides high-affinity transport for leukotriene C4 that
mediates response to inflammatory stimuli (eg, MRP1 knock-out mice without leukotriene C4 have an altered response to inflammatory stimuli).

- MRP2 provides transport for the secretion of bilirubin glucuronide into bile (eg, humans without MRP2 develop a mild liver disease, Dubin-Johnson syndrome).
- Other physiologic functions of the MRP family members are not known.
  - The transporter functions of MRP family members are related to their intracellular locations:
    - Most of the MRP1 is not found in the plasma membrane but in intracellular vesicles.
    - MRP2 is routed to the apical membrane of kidney and other cells (supporting a protective function against toxins and drugs by efflux pump activity as in the case of MDR1).
    - MRP1, MRP3, and MRP5 are located in the basolateral membrane (indicating that the drugs tend to be pumped into cells rather than out of cells).

<p>| TABLE #5: THE TRANSPORTER FUNCTIONS OF MRP FAMILY MEMBERS &amp; TISSUE LOCATIONS |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>MRP Family</th>
<th>GS-X Pump</th>
<th>Methotrexate Transporter</th>
<th>MDR Drugs Transporter</th>
<th>Tissue Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Ubiquitous but low in liver</td>
</tr>
<tr>
<td>MRP2 (cMOAT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Liver, kidney, gut</td>
</tr>
<tr>
<td>MRP3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Liver, adrenals, pancreas, kidney, gut</td>
</tr>
<tr>
<td>MRP4</td>
<td>?</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Prostate, lung, muscle, pancreas, testis, ovary, bladder, gallbladder</td>
</tr>
<tr>
<td>MRP5</td>
<td>+</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>MRP6</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Liver, kidney</td>
</tr>
</tbody>
</table>

- SUMMARY OF MRP1 TRANSPORT FUNCTION:
  - MRP1 is an organic anion transporter for anionic drugs such as
MRP1 transports compounds conjugated with glutathione, glucuronide and sulfate.

MRP1 confers resistance to vincristine, vinblastine, etoposide, doxorubicin, epirubicin, mitoxanthrone, and camptothecins CPT-11 and SN38, but not cisplatin.

MRP1 transports neutral drugs conjugated to the acidic glutathione, glucuronide and sulfate ligands.

MRP1 also co-transport together with free glutathione those neutral organic drugs that cannot be conjugated by glutathione, glucuronide and sulfate.

MRP1 is a GS-X pump that transports drugs conjugated to glutathione out of cells.

MRP1 confers resistance to arsenite and antimony by transporting them as complexes with three glutathione molecules.

MRP1 has a role in resistance against nucleoside analogues used as anticancer drugs.

The physiologic role of MRP1 is high-affinity transport of leukotriene C4, but its absence only alters the response to inflammatory stimuli without affecting the health otherwise.

**SUMMARY OF MRP2/cMOAT TRANSPORT FUNCTION:**

- MRP2 handles a similar range of drugs like MRP1:
  - These drugs include methotrexate, cisplatin, vincristine, vinblastine, doxorubicin, epirubicin, mitoxanthrone, and the camptothecins CPT-11 and SN38.
  - The situation with etoposide remains indeterminate. MRP2 antisense does not enhance sensitivity to etoposide, but transfection of the MRP2 gene does cause resistance to etoposide.
  - When the results of the transfection experiments are completed, the spectrum of drug resistance for MRP2 may turn out to be identical to that of MRP1, with the exception that MRP2 induces cisplatin resistance that is not seen in MRP1-expressing cells.
  - MRP2/cMOAT (like MRP5) functions much better at the intracellular membrane than the plasma membrane.

- MRP1 and MRP2 transport of vinca alkaloids and anthracyclines that are weak organic bases not conjugated to acidic ligands.
  - Therefore, it is puzzling that MRP1 and MRP2 could confer vinca alkaloid and anthracycline resistance.
  - These drugs are probably co-transported with glutathione because:
    - (1) intracellular depletion of glutathione abolishes MRP-mediated
vinca alkaloid and anthracycline resistance; (2) vesicular transport experiments of vincristine and doxorubicin work only in the presence of glutathione; (3) in MRP2-transfected polarized kidney cells, the increased transport of vinblastine is associated with a stoichiometrically increased export of glutathione.

- It looks like MRP1-mediated MDR and MRP2-mediated MDR require a continuous supply of glutathione to allow the export of unconjugated drugs.
- Indeed, there is often a simultaneous increase in the tumor cells of the expression of MRP1 and that of the glutathione synthetic enzyme, g-glutamylcysteine synthetase.

**SUMMARY OF MRP3 TRANSPORT FUNCTION:**
- MRP3 is also an organic anion transporter:
  - MRP3 prefers glucuronide conjugates as substrates; in contrast, MRP1 and MRP2 prefer glutathione conjugates as substrates.
  - In transfection experiments, MRP3 confers resistance to epipodophyllotoxins but not the other drugs.
  - MRP3 also confers resistance to short term exposure of methotrexate.
  - Technical transfection difficulties preclude the elucidation of the full range of MRP3 resistance.
  - The physiologic function of MRP3 remains to be established, but MRP3 may allow the efflux of organic anions from the liver into the blood when secretion into the bile is blocked. MRP3 may also have a role in the normal uptake of bile salts from the gut. This physiologic function is suggested by the basolateral location of MRP3 in the hepatic canalicular membrane, and the massive increase in liver expression of MRP3 in the liver during cholestasis.
  - It is also unknown why MRP3 expression is high in the adrenal cortex.

**SUMMARY OF MRP4 TRANSPORT FUNCTION:**
- The function of MRP4 is less well defined compared to other family members. It has been shown to be an organic anion transporter and a nucleotide analogue pump.
  - MRP4 is recently found to function as a high-affinity cellular pump for the anti-human immunodeficiency virus drugs, 9-(2-phosphonylmethoxyethyl) adenine (PMEA) and azidothymidine monophosphate (AZT), which are organic anions.
  - MRP4 also causes resistance to several nucleoside analogues including 9-(2-phosphonylmethoxyethyl) guanine that has antineoplastic activity. MRP4 (like MRP5) appears to be a
nucleotide analogue pump.

- MRP4 may confer resistance to the anticancer base analogues such as mercaptopurine and thioguanine.
- It still remains to be determined whether MRP4, like MRP5, causes clinical drug resistance in acute lymphoblastic leukemia and acute myelogenous leukemia that do not have the hypoxanthine phosphoribosyl transferase gene mutation.
- MRP4 might be specific for phosphate conjugates.
- It remains to be shown if MRP4 can also transport glutathione, glucuronide and sulfate conjugates.
- MRP4’s physiologic function is unknown.

**SUMMARY OF MRP5 TRANSPORT FUNCTION:**

- MRP5 is an organic anion transporter of glutathione conjugates and a nucleotide analogue pump (like MRP4).
- MRP5 confers low-level resistance to the thiopurines, 6-mercaptopurine, and 6-thioguanine.
- MRP5 confers resistance to the anti-human immunodeficiency virus drug PMEA.
- MRP5-expressing cells tend to accumulate less 6-mercaptopurine and less PMEA, and to extrude more 6-thioinosinemonophosphate and PMEA.
- MRP5 does not appear to confer resistance to the other anticancer drugs, anthracyclines, vinca alkaloids, epipodophyllotoxins or methotrexate.
- Some investigators, but not others, have reported that MRP5 confers resistance to the heavy metals cadmium chloride and potassium antimonyl tartrate.
- MRP5 (like MRP2/cMOAT) functions better at the intracellular membrane rather than the plasma membrane.
- It still remains to be determined whether MRP5, like MRP4, causes clinical drug resistance in acute lymphoblastic leukemia and acute myelogenous leukemia that do not have the hypoxanthine phosphoribosyl transferase gene mutation.
- MRP5’s physiologic function is unknown.

**SUMMARY OF MRP6 TRANSPORT FUNCTION:**

- The transporter function of MRP6 is undefined. The physiologic function is unclear. The role in multidrug resistance is unclear.
- Recently, the 3’ end of the MRP6 protein is found to be almost identical to the anthracycline-associated protein, ARA, which is
- MRP6 is highly expressed in the liver and kidney, but in low levels in other tissues.
- Overexpression and amplification of the MRP6 gene are only found in resistant tumor cells that express concurrently high levels of MRP1 and showing MRP1 gene amplification. It is likely that the MRP6 gene is co-amplified with the MRP1 gene, because the location of the MRP6 gene is immediately adjacent to that of the MRP1 gene on 16p13.1.

**TABLE #6: THE DRUG & PHYSIOLOGIC SUBSTRATES OF MDR1, MRP1 and cMOAT**

<table>
<thead>
<tr>
<th>ABC Transporter</th>
<th>Drugs</th>
<th>Physiologic Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRP1</strong></td>
<td>Vinca alkaloid Anthracycline Epipodophylotoxin Taxane: poorly transported Others</td>
<td>Leukotriene C4 17 beta-estradiol 17-(beta-D-glucuronide) Bilirubin glucuronide Sulfated bile salts Oxidized glutathione Prostaglandin A Arsenite Antimony Aflatoxin B1</td>
</tr>
<tr>
<td><strong>cMOAT/MRP2</strong></td>
<td>Vinca alkaloid Camptothesin: CPT-11, SN-38 Platinum: cisplatin, carboplatin</td>
<td>Leukotriene C4 S-(2,4-dinitrophenyl)-glutathione Oxidized glutathione Bilirubin glucuronide Organic anions</td>
</tr>
</tbody>
</table>

**TABLE #7: THE COMPARISON OF MDR1, MRP1, cMOAT/MRP2 AND MRP3**

<table>
<thead>
<tr>
<th>GENE</th>
<th>MDR1</th>
<th>MRP1</th>
<th>cMOAT/MRP2</th>
<th>MRP3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KNOCK-OUTS</strong></td>
<td>mdr1a-/- &amp; mdr1b-/- drug sensitive especially at blood-brain barrier; mdr2 phosphatidylcholine untranslocated</td>
<td>mpr1-/- high tissue glutathione.</td>
<td>No, but there are mutant rats naturally without MRP2.</td>
<td>Yes, technical problems, and still under study.</td>
</tr>
<tr>
<td>CHROMOSOME</td>
<td>7q21.1</td>
<td>16p13.1</td>
<td>10q24</td>
<td>17q22</td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>TRANSCRIPT</td>
<td>4.5 kb</td>
<td>6.5 kb</td>
<td>6.5-7 kb</td>
<td>6.6 kb</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>P-glycoprotein</td>
<td>Multidrug resistance protein</td>
<td>Canalicular multispecific organic anion transporter</td>
<td>MRP3</td>
</tr>
<tr>
<td>MOLE WEIGHT</td>
<td>170 kDa</td>
<td>190 kDa</td>
<td>190 kDa</td>
<td>190 kDa</td>
</tr>
<tr>
<td>AMINO ACIDS</td>
<td>1280</td>
<td>1531</td>
<td>1545</td>
<td>1527</td>
</tr>
<tr>
<td>TISSUES</td>
<td>Adrenal, kidney, liver (canalicular membrane), colon, brain (endothelial cells/blood-brain barrier), uterus (pregnancy)</td>
<td>Placenta, testis, lung, skeletal muscle, heart, monocyte, liver (sinusoidal membrane)</td>
<td>Liver (canalicular membrane), duodenum, kidney</td>
<td>Liver, duodenum, adrenal, pancreas</td>
</tr>
<tr>
<td>EPITHELIAL LOCATION</td>
<td>Apical membrane</td>
<td>Basolateral membrane</td>
<td>Apical canalicular membrane of liver and kidney cells</td>
<td>Basolateral membrane</td>
</tr>
<tr>
<td>CANCERS</td>
<td>Neuroblastoma, retinoblastoma, soft tissue and bone sarcoma, leukemia, lymphoma; breast, pancreas, colon, adrenal, liver, renal, ovarian, non-small cell lung carcinoma</td>
<td>Neuroblastoma, retinoblastoma, leukemia, lymphoma; non-small cell lung, anaplastic thyroid, esophageal, gastric, breast, bladder carcinoma</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>CLINICAL CORRELATIVE STUDIES SHOWING SIGNIFICANCE</td>
<td>Neuroblastoma, retinoblastoma, soft tissue and bone sarcoma, leukemia, lymphoma, breast carcinoma</td>
<td>Neuroblast-oma retinoblast-oma</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>MULTIDRUG RESISTANCE PROFILE</td>
<td>Vinca alkaloid, Anthracycline, Epipodophytoxin, Taxane, Antibiotic</td>
<td>Vinca alkaloid, Anthra-cycline, Podophytoxin</td>
<td>Vinca alkaloid, Anthracycline, Camptothesin, Platinum</td>
<td>Vinca alkaloid, Anthracycline, Podophytoxin, Platinum</td>
</tr>
<tr>
<td>METHOTREXATE RESISTANCE</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>INHIBITORS</td>
<td>Verapamil, Cyclosporines, Carboxamides</td>
<td>High affinity substrates, Organic acids</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
</tbody>
</table>

**TABLE #8: THE COMPARISON OF THE OTHER MRP FAMILY MEMBERS**
<table>
<thead>
<tr>
<th>GENE</th>
<th>MRP4</th>
<th>MRP5</th>
<th>MRP6</th>
<th>EST182763</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNOCK-OUTS MICE</td>
<td>No</td>
<td>Yes but still under study</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CHROMOSOME</td>
<td>13q32</td>
<td>3q25-26</td>
<td>Immediately next to MRP1 on 16p13.1</td>
<td>6p21</td>
</tr>
<tr>
<td>TRANSCRIPT</td>
<td>6.5 kb</td>
<td>7 kb</td>
<td>6.5-7 kb</td>
<td>5.5 kb</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>MRP4</td>
<td>MRP5</td>
<td>MRP6</td>
<td>EST182763</td>
</tr>
<tr>
<td>MOLE WEIGHT</td>
<td>190 kDA</td>
<td>190 kDA</td>
<td>190 kDA</td>
<td>190 kDA</td>
</tr>
<tr>
<td>AMINO ACIDS</td>
<td>1325</td>
<td>1437</td>
<td>1503</td>
<td>Undefined</td>
</tr>
<tr>
<td>TISSUES</td>
<td>Pancreas, skeletal muscle, lung, kidney, liver, prostate testis, ovary, bladder, gallbladder</td>
<td>Breast, skeletal muscle, pancreas, hippocampus</td>
<td>Pancreas, liver, kidney</td>
<td>Lung, liver, skeletal muscle</td>
</tr>
<tr>
<td>EPITHELIAL LOCATION</td>
<td>Undefined</td>
<td>Basolateral membrane</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>CANCERS</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>CLINICAL SIGNIFICANCE</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>MDR PROFILE</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>METHOTREXATE RESISTANCE</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
<tr>
<td>INHIBITORS</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
<td>Undefined</td>
</tr>
</tbody>
</table>

- **TOPOLOGY & HIGHER ORDER STRUCTURE OF THE MRP FAMILY:**
  - The membrane topology and higher order structure of the MRP proteins illustrate how MRP proteins bind to substrates and hydrolyze ATP. Membrane topology and higher order structure of MRP proteins are studied using:
    - Monoclonal antibodies directed against known regions of the protein and limited proteolysis to map the location of specific peptide sequences on either side of the plasma membrane;
    - Site-directed mutagenesis to determine which potential N-glycosylation sites are used;
    - Immunostaining of permeabilized cells with antisera and monoclonal antibodies to confirm the cytoplasmic location of hydrophilic nucleotide-binding domains 1 and 2;
    - Expression of individual and combinations of structural domains of MRP in a baculovirus system to measure the interactions in
functional assays;

- Detergent-solubilized and lipid-reconstructed MRP protein to investigate the physical interaction of various MRP domains in electron microscopy and image analysis.

- **THE PHYSIOLOGIC FUNCTIONS OF THE MRP PROTEIN FAMILY:**
  - Drug transport that includes: (1) cisplatin, vincristine, doxorubicin, and etoposide for MRP1; (2) cisplatin, vincristine, doxorubicin, but not etoposide for cMOAT/MRP2.
  - Defense against internal and external toxins and toxic drugs that includes: (1) *Caenorhabditis elegans* MRP proteins protecting against heavy metal ions and toxins of the bacteria it ingests; (2) murine MRP proteins protecting against etoposide toxicity to the bone marrow, testis, kidney and oral and gut mucosa, protection that is lost in MRP1 knock-out mice.
  - Different physiologic functions suggested by the different tissue distribution that includes: (1) placenta, testis, lung, skeletal muscle, heart, and monocytes for MRP1, but lower levels in the sinusoidal membrane of liver; (2) canalicular membrane of liver for cMOAT/MRP2, but lower levels in duodenum and kidney.
  - Anionic conjugates, that are physiologic substrates of MRP1 protein actively transported by MRP1 into cells unidirectionally across basolateral epithelial membranes, include: (1) leukotriene C4; (2) endogenously formed organic anion conjugates, 17 beta-estradiol 17-(beta-D-glucuronide), bilirubin glucuronide, sulfated bile salts, and oxidized glutathione.
  - Anionic conjugates, that are physiologic substrates of cMOAT/MRP2 protein actively transported by MRP2 unidirectionally across apical epithelial membranes out of cells into the extracellular space, include: (1) leukotriene C4; (2) glutathione, glucuronide and sulfate conjugates, glucuronosyl bilirubin, glucuronosyl estradiol, sulfatolithocholytaurine, and oxidized glutathione.
  - Different physiologic functions suggested by the subcellular localization that includes: (1) basolateral epithelial membranes for MRP1 to pump drugs and substrates into cells; (2) apical epithelial membranes for cMOAT/MRP2 (like P-glycoprotein) to pump drugs and substrates out of cells into the extracellular space, bile, urine, and gut.
  - MRP1 as a basolateral epithelial transporter with protective functions that include: (1) protects the basal stem cell layer of the oral mucosa from destruction; (2) protects germ cells by the Sertoli cell basement membrane of the testicular tubule pumping drugs out from the testicular tubule; (3) protects the brain by the epithelial layer of the choroid plexus in the blood-brain barrier regulating the exchange of metabolites between the blood and cerebrospinal fluid (eg, MRP1 knock-out mice accumulate...
considerable administered etoposide in the cerebrospinal fluid).

- **MRP1** as the major high affinity basolateral epithelial transporter of leukotriene C4 that includes: (1) no recognized human disease in the absence of this major leukotriene C4 excretion pathway; (2) MRP1 knock-out mice appear more resistant than wild-type mice against inflammatory stimuli and bacterial infections possibly due to decreased leukotriene C4 secretion.

- **MRP2** as the major apical epithelial transporter for the secretion of organic anions from the liver that includes: (1) humans without MRP2 develop liver disease due to the inability of the liver to excrete bilirubin-glucuronides; (2) there are no MRP2 knock-out mice but mutant rats without MRP2 are compromised in organic anionic excretion from the liver.

- **MRP3** as a basolateral epithelial transporter that includes: (1) uptake of organic anions into intestinal cells; (2) removal of organic acids from bile into liver cells during cholestasis; (3) probable role in secretion of hydrophilic steroid hormones of the adrenal cortex.

- **MRP5** transporter function is undefined but MRP5 knock-out mice have been generated that appear healthy and their pharmacology is under study.

- **HEREDITARY DEFICIENCIES OF MRP1 AND MRP2 PROTEINS:**
  - **Dubin-Johnson syndrome:** Patients have mutations localized mostly at the first ATP-binding site in the cMOAT/MRP2 gene and present with hereditary conjugated hyperbilirubinemia - an inherited defect in the secretion of amphiphilic anionic conjugates from hepatocytes into bile, associated with deposition of a dark pigment in the hepatocytes. They have no cMOAT/MRP2 protein in the hepatic apical canalicular membrane, but normal expression of the MRP1 protein in hepatic and erythrocytic basolateral membranes. They show prolonged retention of sulfobromophthalein but otherwise normal liver functions.

  - **Hyperbilirubinemic TR/GY mutant rats:** Rats have no mrp2 protein in the hepatic apical canalicular membrane and cannot transport leukotriene C4, bilirubin glucuronide, and thyroxine glucuronide conjugates, or amphiphilic anions across the canalicular membrane.

  - **Hereditary deficiency of MRP1:** Humans have no recognized disease in the absence of this major leukotriene C4 excretion pathway, but MRP1 knock-out mice with decreased leukotriene C4 secretion appear more resistant against inflammatory stimuli and bacterial infections.

- **GLUTATHIONE-DRUG CONJUGATE (GS-X) PUMP IN CELLS EXPRESSING MRP THAT EXCRETE GLUTATHIONE (GSH):**
  - The MRP1 and MRP2/cMOAT GS-X pumps are:
    - **Cisplatin transporters:** cisplatin forms a 1:2 complex with
glutathione so that the cisplatin-glutathione complex is removed by the GS-X pump.

- **Organic anion transporters**: for transport in liver membrane vesicles of leukotriene C4, dinitrophenyl glutathione, glutathione disulfide, glutathione conjugates of copper and zinc, bilirubin glucuronide, sulfobromophthalein, and other organic anions.

- **Non-anionic organic drug transporters**: for anthracyclines and vinca alkaloids by conjugation to an anionic ligand (eg, glutathione, glucuronic acid, sulfate). Glutathione is required for the vesicular transport of vincristine by MRP1, and conversely, for the inhibition of MRP1-mediated organic anion transport by vincristine. Glutathione only binds to MRP1 with low affinity. Glutathione is apparently transported by MRP1 and MRP2, and probably required as an activator or a co-transported substrate.

- **Working hypothesis**: MRP1 and MRP2 have 2 drug-binding sites for glutathione – the G-site (high affinity for glutathione but low affinity for drugs), and the D-site (high affinity for drugs but low affinity for glutathione). Without drugs, both sites are occupied by glutathione, exporting glutathione slowly. With low drug concentrations, the G-site is occupied by glutathione and the D-site, by drugs, co-transporting both compounds. With high drug concentrations, some negatively charged drugs occupy both sites, transporting only the drugs. Compounds and anticancer cells that are conjugated to glutathione, glucuronide and sulfate have high affinity for the D-site and G-site and are transported efficiently without requiring free glutathione or stimulating glutathione transport.

- The MRP3 GS-X pump has:
  - A low affinity for glutathione since cells with high expression of MRP3 do not secrete glutathione.

### MULTIDRUG TRANSPORTER IN CELLS EXPRESSING MRP:

- The MRP1 and MRP2/cMOAT multidrug transporters are:
  - **Natural product anticancer drug transporters**: transport indirectly some (vinca alkaloids, anthracyclines, epipodophyllotoxins), but not all, natural product anticancer drugs, by using a drug/glutathione co-transport mechanism. Glutathione appears more important for vinca alkaloid than anthracycline MRP1-mediated transport. High concentrations of etoposide appear to stimulate the drug/glutathione co-transport. It is not known precisely how MRP1-mediated transport is coupled to the energy providing ATP binding and hydrolysis. Glutathione by itself is not a substrate of MRP1, but MRP1 might mediate glutathione export in association with an as yet unidentified endogenous metabolite.
Methotrexate transporters: causing high level resistance to short methotrexate exposure, but do not transport polyglutamylated methotrexate during long methotrexate exposure.

High affinity leukotriene transporters: transport actively the cysteinyl leukotriene C4.

Glutathione-conjugated xenobiotic transporters: protect against chemical carcinogens such as glutathione-conjugated aflatoxin B1.

Protect against chemical carcinogenesis: transport actively other glutathione-conjugated xenobiotics including glutathione-conjugated aflatoxin B1.

- The MRP3 natural product anticancer drug transporter is:
  - A non-anionic organic drug transporter: for doxorubicin, etoposide, and vincristine.
  - A cisplatin transporter
  - An anionic organic drug transporter: but prefers glucuronides to glutathione as substrate.
  - A methotrexate transporter

- The MRP5 drug transporter is:
  - Probably thiopurine transporter

PDF File #3: DETAILED DESCRIPTION OF THE LUNG RESISTANCE PROTEIN LRP GENE FAMILY.

- The Lung Resistance Protein was first reported by Scheper et al in 1993:
  - It is also called LRP-56 since LRP was first immunostained with the anti-LRP monoclonal antibody LRP-56 in lung cancer cell lines exhibiting non-P-glycoprotein reduced drug accumulation.
  - The LRP gene has been cloned and demonstrates a 60% sequence identity (homology) with the 104-kDa major vault protein from Dictyostelium discoideum.
  - The LRP gene is localized to chromosome 16p11.2, approximately 27 cM proximal to the location of the MRP1 gene at band 16p13.1.
  - Vault proteins are large cytoplasmic ribonucleoprotein structures found in eukaryotic cells. For example, in rat liver, there is one major vault protein (104 kDa), and three minor vault proteins (210 kDa, 192 kDa, 54 kDa). Vault proteins are hollow barrel-like multunit organelles consisting of 2 identical cup-like halves, each made of 8 petal-like structures joined at the open end, that are associated with cytoplasmic vesicle structures and nuclear pore complexes. Vault proteins are highly conserved through the species.
Recently, two minor vault proteins have been identified, one of which is the telomerase-associated protein (TEP1), the other of which is the vault-poly (ADP-ribose) polymerase (VPARP).

LRP is a 110-kDa vesicular protein overexpressed in several MDR cell lines, in normal epithelial cells, and in tissues chronically exposed to xenobiotics and potentially toxic agents, such as bronchial cells, and cells lining the intestines and kidney tubules.

LRP protein is found in the kidney, adrenal, heart, lung, muscle, thyroid, prostate, bone marrow, and testis.

LRP protein is overexpressed in human malignancies, including acute myelogenous leukemia, acute lymphoblastic leukemia, ovarian cancer, nonsmall-cell lung cancer, melanoma, neuroblastoma, osteosarcoma, and multiple myeloma, but a definitive clinical correlation with poor outcome of chemotherapy has not been not fully defined.

**PDF File #4: DETAILED DESCRIPTION OF THE ATYPICAL MULTIDRUG RESISTANCE PROTEIN.**

- The Atypical Multidrug Resistance Protein (Beck et al, 1987; Danks et al, 1987) was first described in CEM/VM-1, a non-P-glycoprotein multidrug-resistant, teniposide-selected T-cell acute lymphoblastic leukemia subline.
  - AT-MDR is apparently mediated by altered activity or amount of the drug target-altering topoisomerase II (Topo II) enzyme characterized by:
    - Decreased Topo II catalytic and cleavage activity.
    - Decreased drug sensitivity, activity, and amount of nuclear matrix Topo II.
    - Increased ATP requirement of Topo II.
    - A single base mutation in Topo II results in a change of Arg to Gln at position 449, at the start of the motif B/nucleotide binding site.
    - Decreased Topo II phosphorylation suggesting decreased kinase or increased phosphatase activity.
  - The DNA Topo II isozyme has a molecular weight of 170 kDa.
  - Cytogenetic analysis indicates that the CEM/VM-1 subline contains an abnormally banded region on chromosome 13q, suggesting the presence of non-MDR1 gene amplification.
  - AT-MDR displays a cross-resistant profile very similar to the MDR1 and MRP1 phenotypes:
    - Cross resistance to several drugs that interact with topoisomerase II, including teniposide, etoposide, amsacrine, doxorubicin, daunorubicin, and mitoxantrone.
- Lack of vinca alkaloid resistance, unlike MDR1 and MRP1.
- No cross resistance to dactinomycin or colchicine.
- Absence of a drug accumulation defect, unlike MDR1.
  - Altered expression of the topoisomerase II enzyme is described in relapsed acute lymphocytic leukemia, rhabdomyosarcoma, gastric carcinoma, and testicular teratoma.
  - The expression and clinical significance of AT-MDR in human malignancies are not fully defined.

**PDF File #5: DETAILED DESCRIPTION OF THE BREAST CANCER RESISTANCE PROTEIN (BCRP).**

- The Breast Cancer Resistance Protein (also called the Mitoxantrone Resistance Protein MXR or Placenta-Specific ABC Protein ABCP1 or ABCG2) is first described in 1995 by Doyle and Ross:
  - The BCRP gene has been cloned and sequenced by Doyle and Ross, and its transfection into drug-sensitive cells causes overexpression of the BCRP protein conferring multidrug resistance.
    - It encodes a 2.4-kb mRNA transcript that translates into the 655-amino acid BCRP protein.
    - BCRP is a 95 kDa N-linked membrane sialoglycoprotein that is differentially glycosylated (72 kDa when unglycosylated).
    - BCRP protein is detected by immunostaining with the BXP-34 and BXP-21 monoclonal antibodies.
  - BCRP is identified in a number of cell lines including the MCF-7/AdrVp breast cancer, NCI-H1688 small cell lung cancer, human ovarian tumor, resistant mouse fibroblast, EPF86-079 fibrosarcoma, S1 and HT29 human colon carcinoma, EPG85-257 gastric carcinoma, and human small-cell lung cancer cell lines.
    - The MCF-7/AdrVp cell line that shows particularly high resistance to mitoxantrone has amplification of BCRP genomic DNA.
  - BCRP has been identified as an ABC half-transporter member of the ATP-binding cassette transporter superfamily. Normally ABC full-transporters are expressed in the plasma membrane, whereas half-transporters are expressed in the intracellular membrane.
    - BCRP is unique among the ABC half-transporters in being localized to the plasma membrane rather than in the intracellular membrane.
    - The BCRP drug efflux pump/xenobiotic transporter confers high level resistance to mitoxantrone, and resistance to anthracyclines (doxorubicin, daunorubicin), and camptothecins.
In normal tissues, BCRP is found in placental syncytiotrophoblasts, small intestine and colon epithelium, liver canalicular membrane, breast ducts and lobules, and venous and capillary endothelium, but not arterial endothelium.

- The apical localization of BCRP in the small intestine and colon epithelium suggests a possible role in regulation of the uptake of orally administered substrate drugs by back-transport from the gut lumen.
- The placental localization of BCRP suggests a possible protective role for the fetus.

BCRP expression is associated with low daunorubicin accumulation in acute myeloid leukemic blasts but its clinical relevance is not fully defined.
DETAILED DESCRIPTION OF THE KEY CLINICAL CORRELATIVE STUDIES.

- MDR1 with MRP1 expression:
  - Zhou et al (1995) reported that the expression of MDR1 and MRP1 impacted negatively on the outcome of chemotherapy for acute myelogenous leukemia, and that there may be a common mechanism for the induction mechanism of MDR1 and MRP1 overexpression.
  - Schuurhuis et al (1995) reported that high expression of MDR1 and MRP1 in acute myelogenous leukemia correlates strongly with clinical resistance to combination chemotherapy.
  - Van der Kolk et al (2000) reported that there is no association between the expression of MRP1 and MDR1 and the overall survival of acute myelogenous leukemia patients.

- MRP1 expression:
  - Norris et al (1996) reported that high expression of the MRP gene in patients with neuroblastoma correlates strongly with poor outcome, suggesting that MRP accounts for the association between amplification of the MYCN oncogene and the reduced survival.
  - Chan et al (1997) reported that it is possible to detect low levels of MRP1 expression in clinical neuroblastoma samples.
  - Chan et al (1997) reported that it is the expression of MRP1, rather than the expression of MDR1, that accounts for the rare failures of chemotherapy in retinoblastoma patients treated with a cyclosporine MDR-reversal of multidrug resistance protocol. This may explain why cyclosporine is sometimes ineffective as a chemo sensitiz er for some cancers treated with chemotherapy.

- LRP expression:
  - Filipits et al (1998) reported that expression of the LRP protein predicts poor outcome in acute myelogenous leukemia. Outcome is best in patients lacking both LRP and MDR1 expression.

- MDR1, MRP1 with LRP expression:
  - Malayeri et al (1996) reported that the expression of MDR1, MRP1 and LRP mediate multifactorial MDR in acute myelogenous leukemia.
  - Broxterman et al (1999) reported that MDR1 function, but not the expression of LRP or MRP1, correlates with a low steady-
state accumulation of daunorubicin in acute myelogenous leukemia. MDR1 activity, however, does not predict daunorubicin sensitivity in the blasts measured by an MTT-based assay. The contribution of MRP1 expression to reduced accumulation of daunorubicin appears minor compared to that of MDR1 expression.

- **BCRP expression:**
  - Ross et al (2000) reported that high expression of BCRP mRNA is observed in 33% of acute myelogenous leukemia patients, suggesting that BCRP expression has a potential role in MDR.

PDF File #7: DETAILED DESCRIPTION OF THE CLINICAL RELEVANCE & INHIBITION OF NON-P-GLYCOPROTEIN MDR.

- **CLINICAL RELEVANCE OF MDR PROTEINS IN HUMAN CANCERS:**
  - **MRP1 expression:**
    - MRP1 is ubiquitous in human tissues and potentially could cause MDR in most tumors. MRP1 has been detected in every type of tumor but the correlation between MRP1 expression levels and clinical drug resistance is not strong. Since there are also no effective and specific inhibitors of MRP1, reversal studies could not be used to determine the role of MRP1 in causing clinical drug resistance.
  - **MRP2 expression:**
    - No association has been found between the expression of MRP2 and MDR in drug-selected resistant cell lines. MRP2 has been reported in most cases of renal cell, lung, gastric, colorectal and hepatocellular carcinoma but the clinical significance is not known.
  - **MRP3 expression:**
    - Initial studies have shown no association between the expression of MRP3 and MDR in in cell lines, but more recent studies of lung cancer cell lines show a strong correlation between the expression of MRP3 and resistance to doxorubicin, and a weaker correlation between the expression of MRP3 and resistance to vincristine, etoposide, and cisplatin.
  - **MRP and clinical methotrexate resistance:**
    - The MRP1, MRP2 and MRP3 GS-X pumps protect cells against 4-hour high-dose methotrexate exposure but not against 96-hour low-
dose methotrexate exposure. Apparently, the loss of resistance during longer methotrexate exposures may be due to the build up of methotrexate during its polyglutamylation. There is competition between the export of methotrexate by MRP and the polyglutamylation of methotrexate, and methotrexate glutamates are not transported by MRP. Polyglutamylation of folates may also prevent MRP depletion of cellular folate pools.

- **MRP and clinical cisplatin resistance:**
  - Cisplatin forms complexes with glutathione that are still toxic and require removal from the cells by a GS-X pump. MRP2, but not MRP1, is the GS-X pump that removes the cisplatin-glutathione complexes. It has not been determined whether increased tumor MRP2 expression is associated with clinical cisplatin resistance.

- **MRP and arsenite resistance:**
  - Arsenite therapy could promote apoptosis and induce remission in acute promyelocytic leukemia, but arsenite resistance occurs quickly probably because of the overexpression of MRP. Experimental data suggest that arsenite increases the efflux of glutathione from cells that overexpress MRP1, MRP2, and MRP3. These cell lines that overexpress MRP1 are resistant to arsenite, whereas embryonal stem cells in which the MRP1 gene is disrupted are hypersensitive to arsenite.

- **Clinical correlative studies:** Most studies are descriptive and few clinical studies have shown a significant correlation between the expression of drug resistance proteins in clinical tumor samples and the outcome of the patients. Some clinical correlative studies have shown contradictory results. Clinical correlative studies include:
  - **MDR1 and MRP1 expression:**
    - Zhou et al (1995) reported that the expression of MDR1 and MRP1 impacted negatively on the outcome of chemotherapy for acute myelogenous leukemia, and that there may be a common mechanism for the induction mechanism of MDR1 and MRP1 overexpression.
    - Schuurhuis et al (1995) reported that high expression of MDR1 and MRP1 in acute myelogenous leukemia correlates strongly with clinical resistance to combination chemotherapy.
    - Van der Kolk et al (2000) reported that there is no association between the expression of MRP1 and MDR1 and the overall survival of acute myelogenous leukemia patients.
  - **MRP expression:**
    - Norris et al (1996) reported that high expression of the MRP gene in patients with neuroblastoma correlates strongly with
poor outcome, suggesting that MRP accounts for the association between amplification of the *MYCN* oncogene and the reduced survival.

- Chan et al (1997) reported that it is possible to detect low levels of MRP1 expression in clinical neuroblastoma samples.
- Chan et al (1997) reported that it is the expression of MRP1, rather than the expression of MDR1, that accounts for the rare failures of chemotherapy in retinoblastoma patients treated with a cyclosporine MDR-reversal of multidrug resistance protocol. This may explain why cyclosporine is sometimes ineffective as a chemosensitizer for some cancers treated with chemotherapy.

- **LRP expression:**
  - Filipits et al (1998) reported that expression of the LRP protein predicts poor outcome in acute myelogenous leukemia. Outcome is best in patients lacking both LRP and MDR1 expression.

- **MDR1, MRP1 and LRP expression:**
  - Malayeri et al (1996) reported that the expression of MDR1, MRP1 and LRP mediate multifactorial MDR in acute myelogenous leukemia.
  - Broxterman et al (1999) reported that MDR1 function, but not the expression of LRP or MRP1, correlates with a low steady-state accumulation of daunorubicin in acute myelogenous leukemia. MDR1 activity, however, does not predict daunorubicin sensitivity in the blasts measured by an MTT-based assay. The contribution of MRP1 expression to reduced accumulation of daunorubicin appears minor compared to that of MDR1 expression.

- **BCRP expression:**
  - Ross et al (2000) reported that high expression of BCRP mRNA is observed in 33% of acute myelogenous leukemia patients, suggesting that BCRP expression has a potential role in MDR.

- **MDR1, MRP1, Topoisomerase II, and BCL-2 expression:**
  - Lohri et al (1997) reported that the expression of MDR1 and MRP1 correlates with each other, with FAB M4 and M5 status, and with extramedullary disease in acute myelogenous leukemia, whereas low expression of BCL-2 correlates with a better outcome, low expression of topoisomerase II predicts with a poorer outcome, and lack of expression of MDR1 expression correlates with a better prognosis.
• LRP, Heat Shock Protein 27, Glutathione-S-Transferase pi:
  - Uozaki et al (1997) reported that overexpression of LRP, Glutathione-S-Transferase pi, and Heat Shock Protein 27 may be associated with failure of presurgery chemotherapy for osteosarcoma.

• PHYSIOLOGIC RELEVANCE OF THE PROTECTIVE FUNCTIONS OF MDR PROTEINS IN NORMAL HUMAN TISSUES:
  - The basolateral epithelial location of MRP1 allows drugs to be pumped into cells, in contrast to the apical epithelial location of MDR1 and MRP2 that facilitates the efflux of drugs from cells.
    - MRP1 knock-out mice show no decrease in disposal of drugs, but are hypersensitive to etoposide because MRP1 does have a non-redundant protective function against the toxic effects of etoposide on the bone marrow, oropharyngeal epithelium, testicular tubules, and renal collecting ducts.
    - The basolateral location of MRP1 in Sertoli cells protects testicular tubules and germ cells against damage from drugs.
    - The protective function of MRP1 in the choroid plexus prevents the entry of drugs and toxins into the cerebrospinal fluid.

• INHIBITION OF NON-P-GLYCOPROTEIN MDR IN VITRO:
  - The verapamil, cyclosporine A, the cyclosporine D analogue PSC 833, and the MS-209, GF120918 and GG918 carboxamide inhibitors that are effective in blocking MDR1 P-glycoprotein in vitro are ineffective for the MRP proteins, MRP1 and MRP2.
    - Most high affinity substrates of MRP1 and MRP2 are organic anions with a substantial hydrophobic moiety and at least one, but preferably two, negative changes.
    - The potent competitive inhibitors of MRP1 and MRP2 are their high affinity substrates, leukotriene C4, S-decylgluthione, and the leukotriene C4 antagonist MK571.
    - Other inhibitors of MRP1 are organic acids that are originally developed to inhibit the transport of uric acid, like sulfinpyrazone, benzbromarone, and probenecid.
    - Although MRP1 and MRP2 have similar substrate specificity, MRP1 inhibitors are not necessarily good MRP2 inhibitors. Sulfinpyrazone, for instance, does not inhibit the transport of the MRP2 substrate dinitrophenyl S-glutathione.
    - Negatively charged compounds do not enter cells readily, so there are no obvious lead compounds for the development of MRP1 and MRP2 inhibitors.
    - The primarily experimental and in vitro MRP inhibition studies:
Rhodes et al (1994) reported that the Golgi inhibitor brefeldin A, and the H⁺ ATPase inhibitors, bafilomycin A1 and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole, could inhibit the expression of MRP.

Versantvoort et al (1994) reported that the iso-flavonoid tyrosine kinase inhibitor genistein could inhibit the expression of MRP.

Versantvoort et al (1994) reported that the buthionine sulfoximine inhibitor of glutathione synthesis partially inhibited the expression of the MRP expression and reversed the daunorubicin accumulation deficit.

Takeda et al (1994) reported that the tyrosine kinase inhibitor genistein could inhibit the expression of MRP.

Feller et al (1995) reported that the buthionine sulfoximine inhibitor of glutathione synthesis depleted intracellular glutathione and inhibited the MRP-mediated cellular accumulation deficit of daunorubicin.

Abe et al (1995) reported that the 1,4-dihydropyridine verapamil analogue could inhibit the expression of MRP and MDR1.

Van der Graaf et al (1995) reported that the morpholino anthracyclines, methoxymorpholino anthracycline and cyanomorpholino anthracycline, could inhibit the expression of MRP and MDR1.

Gekeler et al (1995) reported that the leukotriene C4 receptor antagonist MK571 could inhibit the expression of MRP.

Gekeler et al (1995) reported that the specific bisindolylmaleimide protein kinase C inhibitor GF 109203II could inhibit the expression of MRP.

Chaturvedi et al (1996) reported that the amido-keto pipecolinate derivative VX-710 could inhibit the expression of MDR1, and perhaps also expression of MRP1.

Iersel et al (1996) reported that the alpha, beta-unsaturated carbonyl derivatives, curcumin, acrolein, cinnamaldehyde, citral, crotonaldehyde, ethacrynic acid, and trans-2-hexenal, could inhibit the expression of glutathione-S-transferase, by inhibition of the MRP1 glutathione conjugate GS-X export pump.

Stewart et al (1996) reported that MRP1 mRNA and protein expression are reduced by antisense eicosomeric phosphorothioate oligonucleotides complementary to different regions along the entire length of the MRP mRNA.

Germann et al (1997) reported that the pipecolinate derivative VX-710 is not only a potent modulator of MDR1-mediated multidrug resistance, but also affects multidrug resistance in
MRP-expressing cells perhaps in part by binding directly to the MRP protein.

- Chen et al (1997) reported that the pyridine analogue PAK-104P almost completely reverses the MRP1-mediated resistance to antimonials and arsenicals, and appears more effective than the gamma-glutamylcysteine synthetase inhibitor, buthione sulfoximine, which depletes intracellular glutathione.

- Sumizawa et al (1997) reported that the pyridine analogue PAK-104P completely reverses vincristine resistance by directly interacting with the MRP1 protein and inhibiting the MRP1 transporting activity.

- Vanhoefer et al (1997) reported that N,N-bis(2-chloroethyl)-N-nitrosourea carbamoylation of glutathione reductase inhibits MRP1 function.

- Soszynski and Bartosz (1997) reported that peroxynitrite inhibits glutathione conjugate-mediated transport and may affect MRP1 function.

- Gollapudi et al (1997) reported that probenecid reverses MDR in MRP1-expressing cell lines.

- Zhang et al (1997) reported that the New Caledonian sponge Neosiphonia superset cytotoxin, sphinxolide, depletes microfilament and circumvents MDR1 and MRP1 MDR.

- Martell et al (1997) reported that the glucose transport inhibitors, cytochalasin B and phloretin, block the active efflux of vincristine in MRP1-expressing cell lines.

- Draper et al (1997) reported that the cyclo-oxygenase inhibitor/glutathione-S-transferase inhibitor/anion transport modulator, indomethacin, mediates reversal of MRP1 function, but not MDR1 function, in human and murine cell lines.

- Jager et al (1997) reported that the tyrosine-kinase inhibitor genistein, a substrate of the uridindiphosphate-glucuronyltransferase isoenzymes, affects the cMOAT/MRP2 transport in liver.

- Chou et al (1998) reported that the hexahydropyrroloindole alkaloids, 5-N-acetylardeemin and 5-N-acetyl-8-demethylardeemin, reverse MRP1, MDR1 and LRP-associated MDR, increasing the intracellular accumulation of vinblastine and markedly decreased its efflux.

- Chuman et al (1998) reported that the pyridine analogue, PAK-104P, combines with the gamma-glutamylcysteine synthetase inhibitor, buthione sulfoximine, and reverses MRP-mediated vincristine resistance.
Gao et al (1998) reported that the co-transfection of MDR1 antisense RNA and MRP antisense RNA abolishes MDR in a human lung cancer cell line that overexpresses both MDR1 and MRP1 proteins.

Yanagisawa et al (1999) reported that the pippecolinate derivative VX-710 might be a useful modulator of both MDR1 and MRP1 expression in neuroblastoma.

Roller et al (1999) reported that the inhibition by the nonsteroidal anti-inflammatory drugs (NSAID) of MRP1 expression in human glioma cells is at least in part responsible for the potentiation of doxorubicin and vincristine cytotoxicity.

Marbeuf-Gueye et al (2000) reported that the pyridine analogue, PAK-104P, inhibited the MDR1 and MRP1-mediated efflux of anthracyclines and calceinacetoxymethyl ester whose efflux depends on the presence of glutathione.

Okumura et al (2000) reported that a taxoid from the Japanese yew Taxus cuspidata, 5-O-benzoylated taxinine K, reverses MDR1 and MRP1-mediated MDR.

Payen et al (2001) reported that the diabetes mellitus therapy sulphonylurea glibenclamide could block the activity of ABC transporters, inhibit MRP1-mediated MDR in a human lung cancer cell line, and block organic anionic efflux from MRP2-expressing hepatocytes.

**INHIBITION OF NON-P-GLYCOPROTEIN MDR IN VIVO:**

- A number of compounds that showed in vitro inhibition of the function of MRP have also been tested in vivo.
  - None of these experimental compounds for inhibiting the function of MRP have translated to clinical usage so far.
    - Massart et al (1996) reported that S9788 inhibits MRP1 expression in medullary thyroid carcinoma xenografts in nude mice.
    - Vanhoefer et al (1996) reported that glutathione depletion by buthionine sulfoximine reverses mrp1 expression, but not mdr1 expression, in nude mice xenografts.

**INHIBITION OF NON-P-GLYCOPROTEIN MDR IN THE CLINIC:**

- Peck et al (1996) reported acceptable toxicity but no objective responses in a Phase I clinical trial of VX-710 aimed at blocking the expression of MRP1 and MDR1.
It remains to be determined whether long term inhibition of MRP would be tolerated because of the far-reaching physiologic functions of the MRP family members, assuming that suitably efficacious and non-toxic MRP inhibitors could be found.

**PDF File #8: DETAILED CONCLUSIONS.**

- Drug resistance is due to the cumulative endpoint, in a particular individual, of all the applicable and operative **UPSTREAM FACTORS** (drug sanctuaries, intrinsic tumor factors, drug metabolism, distribution and clearance factors), **TUMOR FACTORS** (ATP-Binding Cassette ABC superfamily transporters and non-ABC transporters), and **DOWNSTREAM FACTORS** (anti-apoptotic bcl-2 family of oncogenes, MYCN and other oncogenes, p53 and other tumor suppressor genes, and Murine Double Minute-2 MDM2 cellular proliferation-promoting gene).

- ATP-dependent transmembrane transporters-MDR mediators include, but are not limited to, P-glycoprotein (MDR1 and family members of cell membrane drug efflux transporters), Multidrug Resistance Protein (MRP1 and MRP2/cMOAT and family members of cell membrane drug sequestrator), Lung Resistance Protein (LRP nuclear-cytoplasmic transporter), Breast Cancer Resistance Protein (BCRP cell membrane drug efflux transporter), topoisomerase II (drug target-altering enzyme), and glutathione (GSH drug-detoxifying enzyme).

- MDR1/ P-glycoprotein MDR broadly affects many natural product drug classes including the epipodophyllotoxins (etoposide, teniposide), taxanes (taxol), vinca alkaloids (vincristine, vinblastine), antibiotics (dactinomycin), and anthracyclines (doxorubicin, daunorubicin).
  - Non-natural product drugs are not affected by MDR and include alkylators (cyclophosphamide, ifosfamide), platinum (cisplatin, carboplatin), antifolates (cytarabine, methotrexate, mercaptopurine), and steroids (prednisone).
  - Chemotherapy drugs and naturally occurring toxins are natural products that enter cells by passive diffusion through the lipid bilayer of the plasma membrane. The rate of entry takes minutes since drug entry is retarded by its hydrophilic components. These natural products are hydrophobic and could diffuse through the lipid bilayer, but are still hydrophilic enough to be water-soluble and reach the drug targets. They do not need carrier proteins that the cells can target as a protective mechanism, such as the reduced folate carrier, dihydrofolate reductase, for the water-soluble drug methotrexate.

- Genes encoding proteins that are drug pumps and drug-conjugate pumps are prominent in the genome across all species.
  - The three classes of human drug pumps are the P-glycoprotein family of
proteins, the MRP proteins, and the BCRP proteins, with 12 members presently identified.

- A large number of drug pumps are required because of the broad variety of substrates that they transported.
- The P-glycoprotein family of proteins could transport a wide range of neutral or slightly basic organic compounds.

- The MRP family proteins are more versatile transporters. The human MRP family consists of at least 6 members, MRP1 which is the MRP prototype, and at least five homologues, MRP2, MRP3, MRP4, MRP5, and MRP6.
  - MRP1, MRP2, and MRP3 transport the neutral or slightly basic organic drugs and compounds, organic anionic compounds, neutral drugs conjugated to acidic glutathione, glucuronide and sulfate ligands via the GS-X pump, anionic drugs such as methotrexate, arsenite and antimony complexed with glutathione, cisplatin complexed with glutathione (MRP2), the physiologic leukotriene C4, and possibly the nucleoside analogues.
  - MRP4 and MRP5 transport nucleotide analogues such as the anti-human immunodeficiency virus drugs phosphated PMEA and AZT nucleotide analogues, the antineoplastic mercaptopurine and thioguanine nucleotide analogues, organic anionic compounds and drugs, possibly the heavy metal conjugates, and possibly the glutathione, glucuronide and sulfate conjugates.
  - The transport function of MRP remains undefined.

- MRP1 is a 190-kDa membrane-bound encoded by the *MRP1* gene localized to chromosome 16p13.1, first described by Cole and Deeley and coworkers in 1992.
  - The physiologic role of MRP may be to function as the ATP-dependent export pump for substrates that include the conjugates of the lipophilic compounds, glutathione, leukotriene C4, LTD4, LTE4, and S-(2,4-dinitrophenyl)-glutathione, sequestering these compounds into MRP-expressing vesicles to prevent their cytotoxicity to normal cells.
  - MRP protein is expressed in human malignancies such as leukemia, lymphoma, myeloma, and neuroblastoma, and in normal peripheral blood mononuclear cells (MRP protein immunostained by the anti-MRP monoclonal antibodies, and MRP mRNA shown by the reverse transcriptase polymerase chain reaction analysis (RT-PCR).
  - The definitive clinical correlation of MRP overexpression with poor outcome of chemotherapy is not fully defined in malignancies.

- LRP is a 110-kDa vesicular protein for which the *LRP* gene has been cloned, first reported by Scheper et al in 1993.
  - LRP demonstrates 60% homology with the 104-kDa major vault protein of *Dictyostelium discoideum*. Vault proteins are large cytoplasmic
ribonucleoprotein structures found in eukaryotic cells. Vault proteins are highly conserved through the species.

- LRP protein is overexpressed in human malignancies such as acute myelogenous leukemia but a definitive clinical correlation with poor outcome of chemotherapy is not fully defined.

- AT-MDR was described by Beck et al in 1987, and by Danks et al in 1987, in non-P-glycoprotein multidrug-resistant T-cell acute lymphoblastic leukemia sublines selected with teniposide.
  - AT-MDR displays a cross-resistant profile very similar to the MDR1 and MRP1 phenotypes, except for the lack of vinca alkaloid resistance, and the absence of a drug accumulation deficit.
  - AT-MDR is apparently mediated by altered expression of the drug target-altering topoisomerase II enzyme.
  - The expression and clinical significance of AT-MDR in human malignancies are undefined.

- BCRP is also called Mitoxantrone Resistance Protein MXR or Placenta-Specific ABC Protein ABCP1 or ABCG2, described in 1995 by Doyle and Ross and coworkers.
  - BCRP is a 95 kDa N-linked membrane sialoglycoprotein. It is identified as an ABC half-transporter member of the ATP-binding cassette (ABC) transporter superfamily that is expressed in the plasma membrane unlike other half-transporters that are expressed in the intracellular membranes.
  - The BCRP confers a high level of resistance to mitoxantrone, and resistance to the other anthracyclines, doxorubicin and daunorubicin, and the camptothecins.
  - BCRP is found in the placental syncytiotrophoblasts most likely for protecting the fetus. The apical localization of BCRP in the small intestine and colon epithelium suggests possible regulation of the back-transport of the substrate drugs that enter into cells from the gut lumen. BCRP is also found in the liver canalicular membrane, breast ducts and lobules, and the venous and capillary endothelium, but not the arterial endothelium.
  - BCRP is expressed in acute myeloid leukemia but its clinical significance is not fully defined.

- Experimental studies could reverse multidrug resistance in vitro and in vivo, but MDR-reversal trials that are relatively nontoxic and yet efficacious are still not available in the clinical setting.


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