# Learning Some New Tricks From a Multidrug Transporter

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## Estimated New Cancer Cases & Deaths, 2008

| Sites | New Cases | Deaths* | % |
|---|---|---|---|
| All Sites | 1,437,180 | 565,650 | 39% |
| Prostate | 186,320 | 28,660 | 15% |
| Breast | 184,450 | 40,930 | 22% |
| Digestive System | 202,720 | 79,090 | 39% |
| Pancreas, Liver & Gall Bladder | 68,570 | 56,040 | 82% |
| Lung & Bronchus | 215,020 | 161,840 | 75% |
| Bladder | 68,810 | 14,100 | 20% |
| Kidney & Renal Pelvis | 54,390 | 13,010 | 24% |
| Ovary | 21,650 | 15,520 | 72% |
| Cervical Cancer | 11,070 | 3,870 | 35% |
| Lymphoma & Leukemia | 118,610 | 42,220 | 36% |
| Brain & Nervous System | 21,810 | 13,070 | 60% |

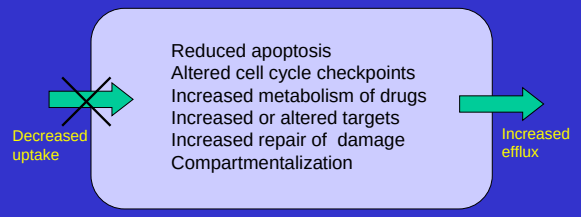
*Virtually all deaths are due to chemotherapy resistance

CA Cancer J Clin, 2008

## Drug Resistance in Cancer

- May affect multiple drugs used simultaneously: known as multidrug resistance (MDR)
- Affects all classes of drugs, including newly designed targeted drugs
- Just as oncogene targets have been catalogued, we need to enumerate all mechanisms of drug resistance in cancer to solve this problem and circumvent resistance

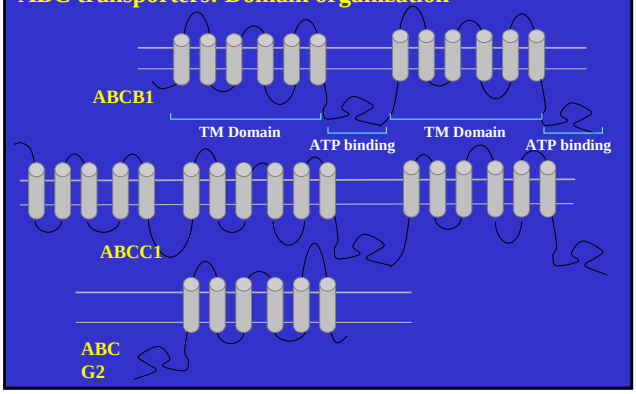
## Mechanisms of resistance to anti-cancer drugs




## Ultimate Goals

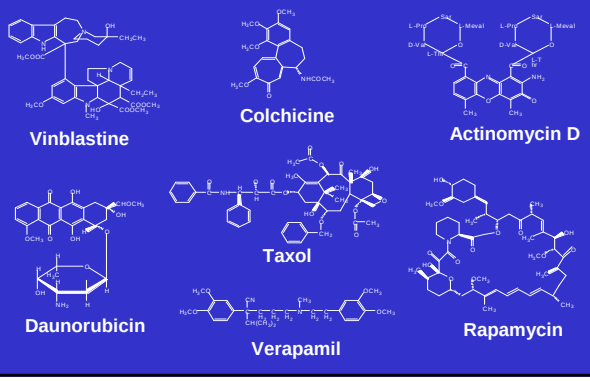
1. Molecular analysis of human cancers to predict response to therapy
2. Use this information to develop novel drugs to treat cancer and new imaging modalities for cancer
3. To learn more about cellular pharmacology and pharmacokinetics of drugs, including drug uptake, distribution, and excretion

## ABC transporters: Domain organization

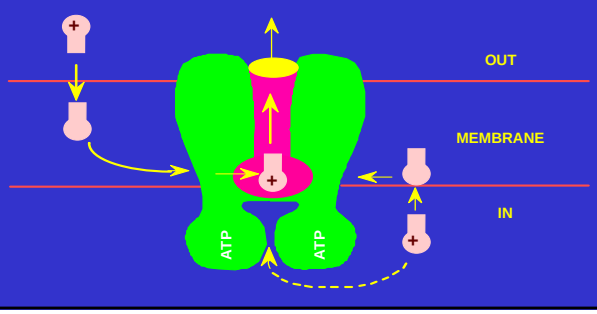


## Hypothetical Model of Human P-glycoprotein

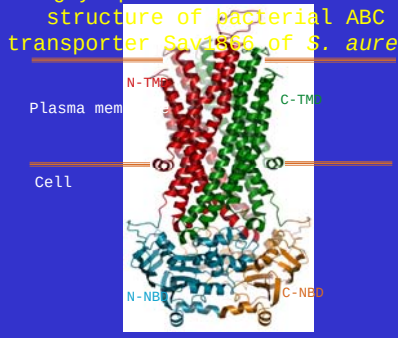

## Substrates and Reversing Agents of Pgp




## P-glycoprotein removes hydrophobic substrates directly from the plasma membrane



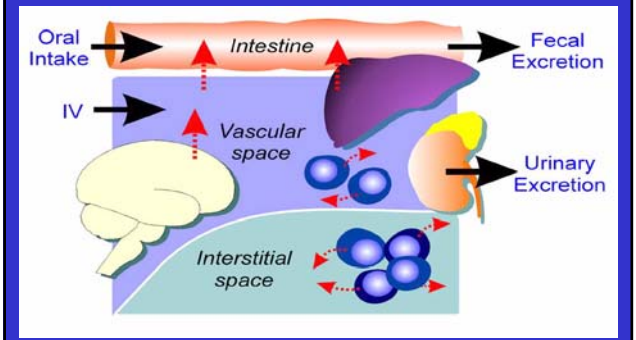
## Homology model of human P-glycoprotein based on the structure of bacterial ABC transporter Sav1866 of *S. aureus*



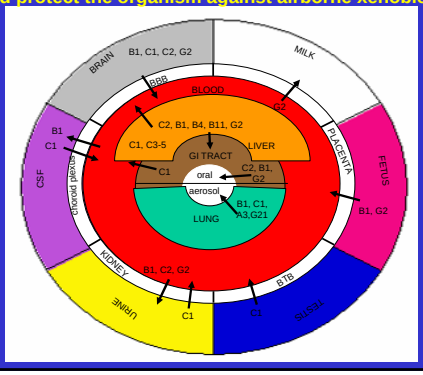

## Role of P-glycoprotein in cancer

- Approximately 50% of human cancers express P-glycoprotein at levels sufficient to confer MDR
- Cancers which acquire expression of P-gp following treatment of the patient include leukemias, myeloma, lymphomas, breast, ovarian cancer; preliminary results with P-gp inhibitors suggest improved response to chemotherapy in some of these patients
- Cancers which express P-gp at time of diagnosis include colon, kidney, pancreas, liver; these do not respond to P-gp inhibitors alone and have other mechanisms of resistance
- Being able to image P-gp in cancer (and ultimately other transporters that contribute to resistance) could help guide therapy

## Physiologic Role of P-glycoprotein



## ABC transporters determine oral bioavailability, excretion, penetration and protect the organism against airborne xenobiotics




## Polymorphisms in the human *MDR*1 gene

1. **More than 50 SNPs have been reported in the MDR1 gene. 14 of them are silent polymorphisms.**

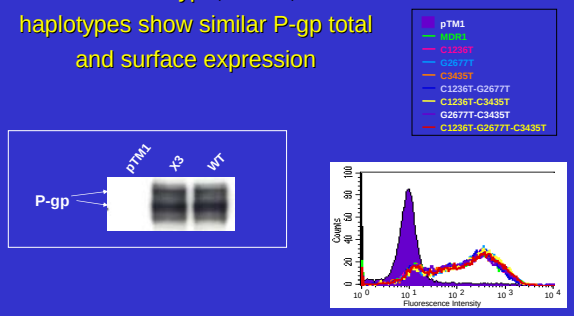
2. **5 common coding (non-synonymous) polymorphisms have no demonstrable effect on drug transport function.**

3. **The synonymous SNP in exon 26 (C3435T) was the first associated with altered MDR1 function and is often part of a haplotype including another synonymous (C1236T) and a nonsynonymous SNP (G2677T).**

## The C1236T, G2677T, C3435T haplotype has been linked to several different phenotypes

- Altered digoxin and fexofenadine pharmacokinetics
- Altered toxicity in transplant patients from cyclosporine A, tacrilimus
- Altered incidence of Crohn's disease, colon cancer, and Parkinson's disease

## *MDR*1 wild-type, SNPs, and haplotypes show similar P-gp total and surface expression

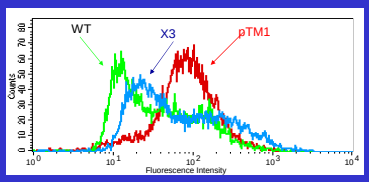




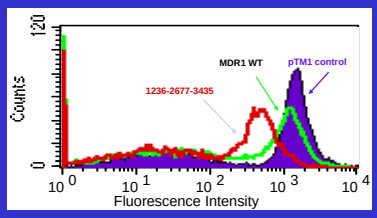





## *MDR*1 wild-type and the haplotype (1236-2677-3435) do not exhibit similar Bodipy-verapamil accumulation

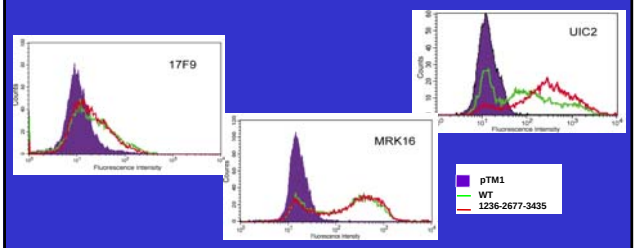



## *MDR*1 wild-type and the haplotype exhibit different patterns using rhodamine 123 efflux with cyclosporin A reversing agent

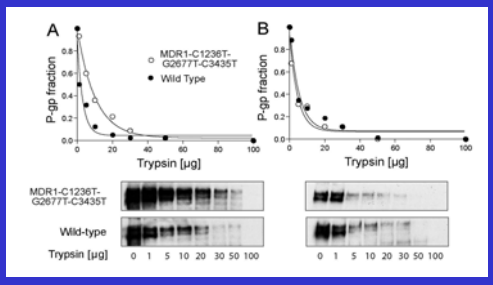



5 mM CsA

## *MDR*1 wild-type and haplotype show the same P-gp cell surface expression using MRK16 and 17F9, but not UIC2 - a conformational sensitive antibody



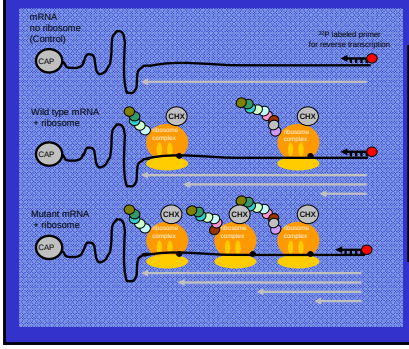

## *MDR*1 wild-type and haplotype show different trypsinization patterns confirming altered conformation

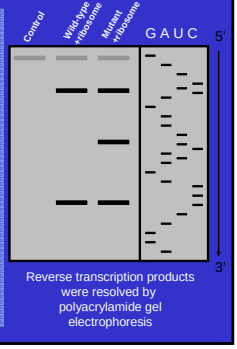



## Polymorphic forms of P-gp with alleles that don't change amino acid sequence change the conformation of P-gp

1. In transient transfection experiments, the amount of P-gp mRNA, protein, and protein localization on the cell surface is unchanged.
2. The conformation of polymorphic P-gp is altered as shown by tryptic peptide analysis and conformation-specific MoAbs.
3. Translational toeprint experiments show a major delay in translation at the site of the "silent" polymorphism.

## Primer extension inhibition (toeprint) assay – to detect presence of ribosome stalling sites

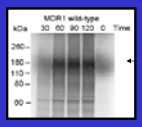



Reverse transcription products were resolved by polyacrylamide gel electrophoresis

## Toeprint analysis of *MDR1* mRNA around the 3435 SNP

A

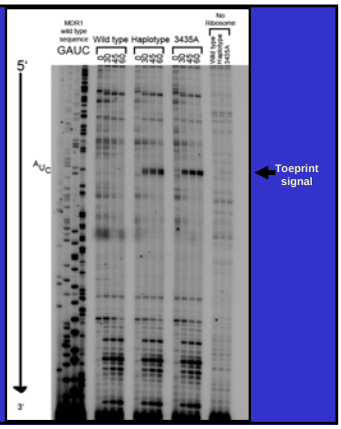



*In vitro* translated MDR1

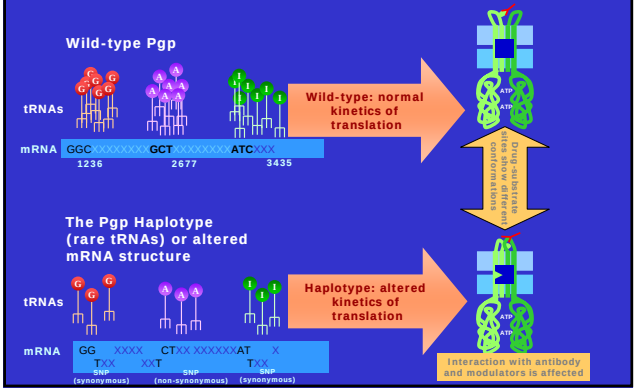
B

| | Wild-type | SNP 3435T | SNP 3435A |
|---|---|---|---|
| Nucleotide | AUC | AUU | AUA |
| Codon usage | 47% | 35% | 18% |

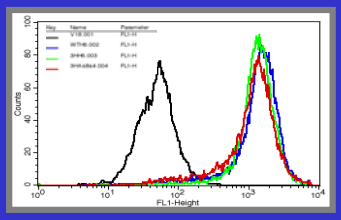
C




## Synonymous SNPs affect P-glycoprotein conformation and function

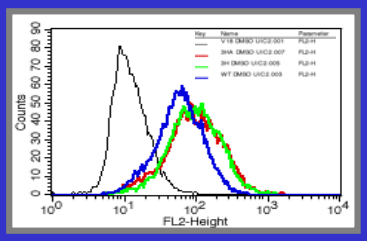


## P-gp expression on cell surface (MRK16) of stably transfected LLC-PK1 cells



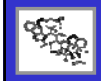

$1^0$ Antibody: MRK16
$2^0$ Antibody: Goat anti-mouse FITC

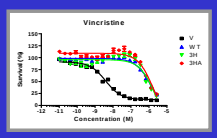
## P-gp expression on cell surface (UIC2) of transfected LLC-PK1 cells

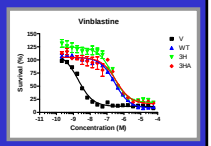



*Conformation-sensitive Monoclonal antibody*

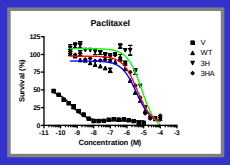
## Vinca-alkaloids

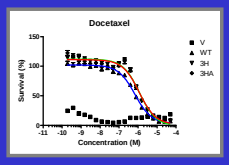




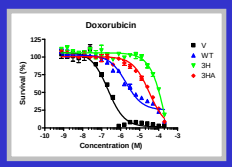



## Taxol

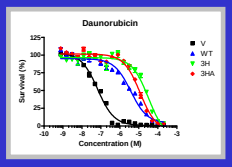



## Anthracyclines




Doxorubicin

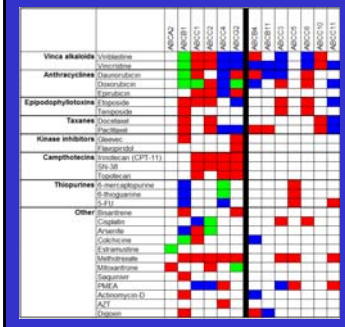



Daunorubicin

## Implications

- Explains conservation of third position for many codons
- Might explain some non-Mendelian inheritance
- Might explain linkage of phenotypes to other synonymous polymorphisms
- For P-gp, the haplotype could have selective advantage and/or affect drug distribution
- For cancers, could affect pattern of MDR and ability to respond to specific inhibitors

## ABC Transporters Confer Resistance to Anti-Cancer Drugs

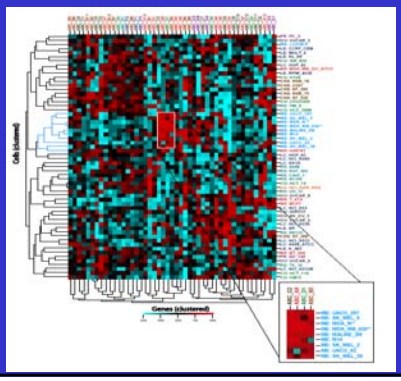



- Confers resistance
- Selected
- Doesn't transport

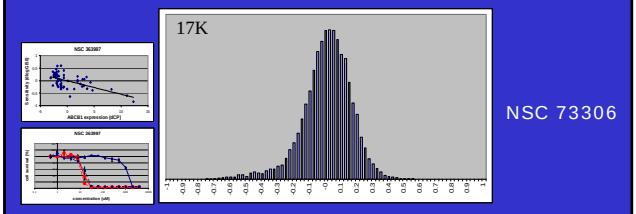
## Can we discover new drugs that interact with ABC transporter genes?

- Use Real Time (RT)-PCR to measure ABC mRNA levels for 48 ABC transporters and 23 solute carrier proteins
- Exploit NCI-60 cell line database, with known resistance to 100,000 different drugs, to correlate patterns of drug-resistance and expression of these transporters

## Expression of ABC-transporters in the NCI60 panel



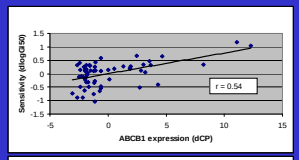
## Search for MDR1-potentiated compounds in DTP's database

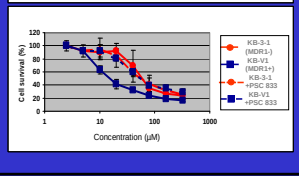


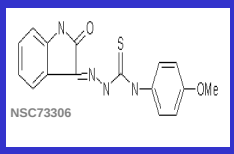

Substrates

MDR1-potentiated compounds?

## The cytotoxicity of NSC 73306 is increased in KB-V1 cells



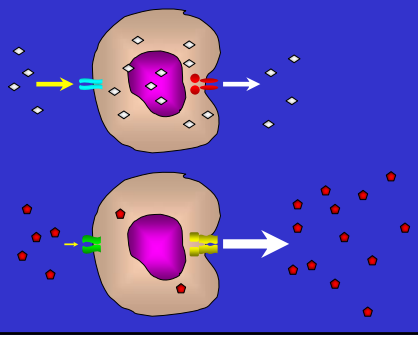




## Potential Clinical Utility of Discovery of Compounds that Specifically Kill MDR1-Expressing Cells

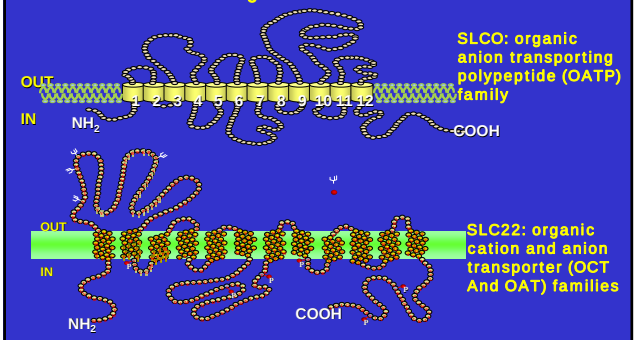
Can be used in combination with standard chemotherapy to eliminate MDR1-expressing cell populations

Preclinical development of thiosemicarbazones and search for additional compounds with similar properties is underway

## Balance of uptake and efflux determines drug accumulation in cancer cells



## Solute Carriers are plausible uptake transporters for anti-cancer drugs




## Summary of SLCO and SLC22 Transporters

Most of the SLCO and SLC22 family members we tested are expressed at some level in cancer cell lines.

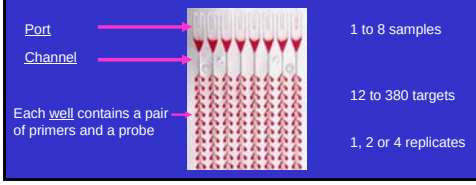
By correlating the expression profiles with the growth inhibitory profiles, expression of 3 of the SLCO and SLC22 family members were found to correlate with sensitivity to specific drugs.

Expression of SLC22A4 in KB cells confers sensitivity to mitoxantrone, doxorubicin, carboplatin and cisplatin.

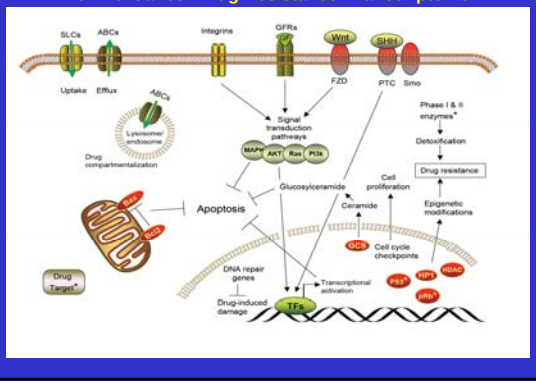
## Microfluidic technology using TaqMan Low Density Array (TLDA)-based detection to study the mechanisms of multidrug resistance in clinical samples

**Goal: To correlate expression of drug-resistance genes with response to chemotherapy in clinical samples**

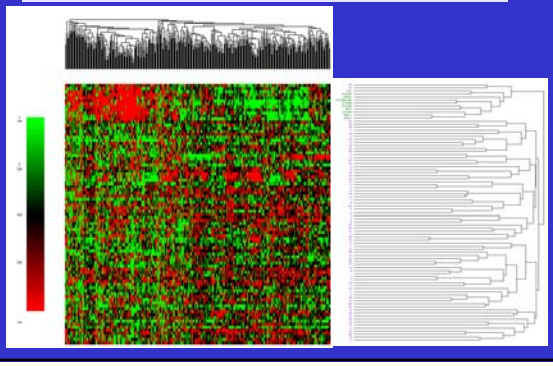
**Method:** a customized TLDA with probes for 380 drug-resistance genes enabling high-throughput quantitative real-time PCR based on TaqMan chemistry




## The Anti-Cancer Drug Resistance Transcriptome




No ovarian cell line has a drug-resistance gene expression profile similar to any clinical sample



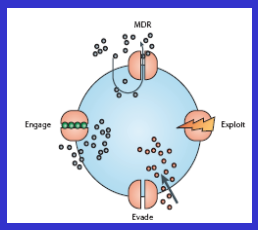
## Strategies for dealing with MDR1-mediated multidrug resistance

**Development of specific inhibitors of P-gp**

Poor performance in clinical trials for a number of reasons:

-Poor trial design, e.g., cancers don't express MDR1

-Side effects due to inhibition of endogenous functions




Drug structural variation
Dose escalation

Can we use MDR1 Expression as an Achilles heel to kill MDR cells?

Imaging of P-gp in vivo in cancers can enable all of these strategies

## Acknowledgements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- Ira Pastan
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