Learning Some New Tricks From a Multidrug Transporter

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January 17, 2008

Estimated New Cancer Cases & Deaths, 2005

Sites	New Cases	Deaths**	%
All Sites	1,372,910	570,280	42%
Prostate	232,090	30,350	13%
Breast	212,930	40,870	19%
Digestive System	253,500	136,060	54%
Pancreas & Liver	59,730	47,220	79%
Lung and Bronchus	172,570	163,510	95%
Bladder	63,210	13,180	21%
Kidney and Renal Pelvis	36,160	12,660	35%
Genital System, female	79,480	28,910	36%
Lymphoma & Leukemia	98,550	43,180	44%
Brain & Nervous System	18,500	12,760	69%

**Vast majority of deaths due to chemotherapy resistance

CA Cancer J Clin, 2005

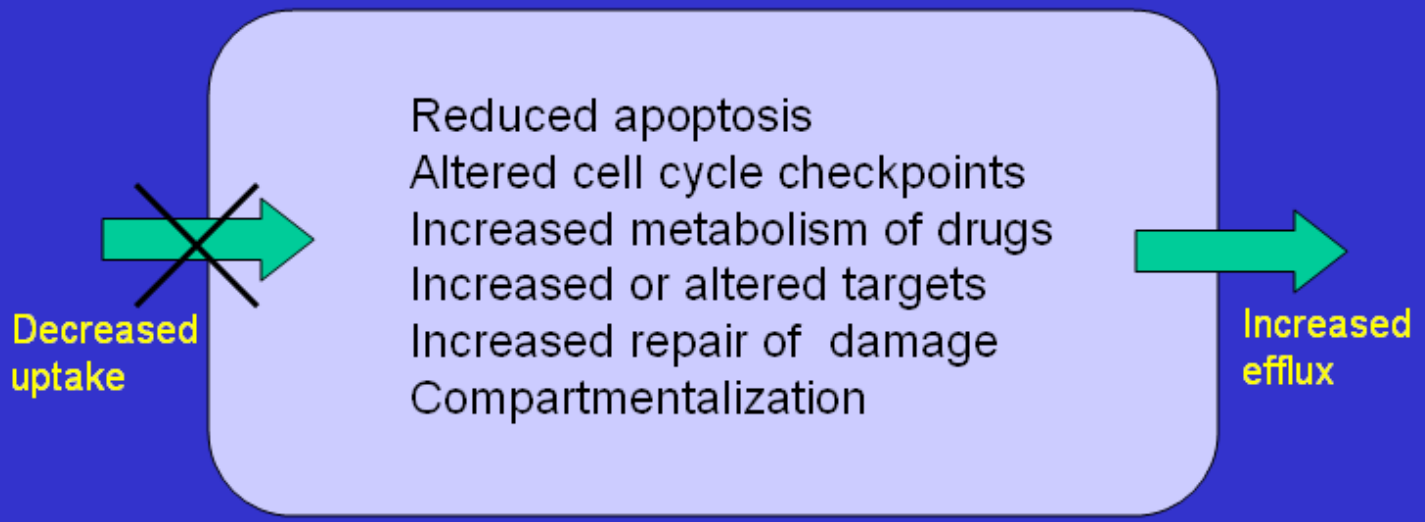
Drug Resistance in Cancer

* 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

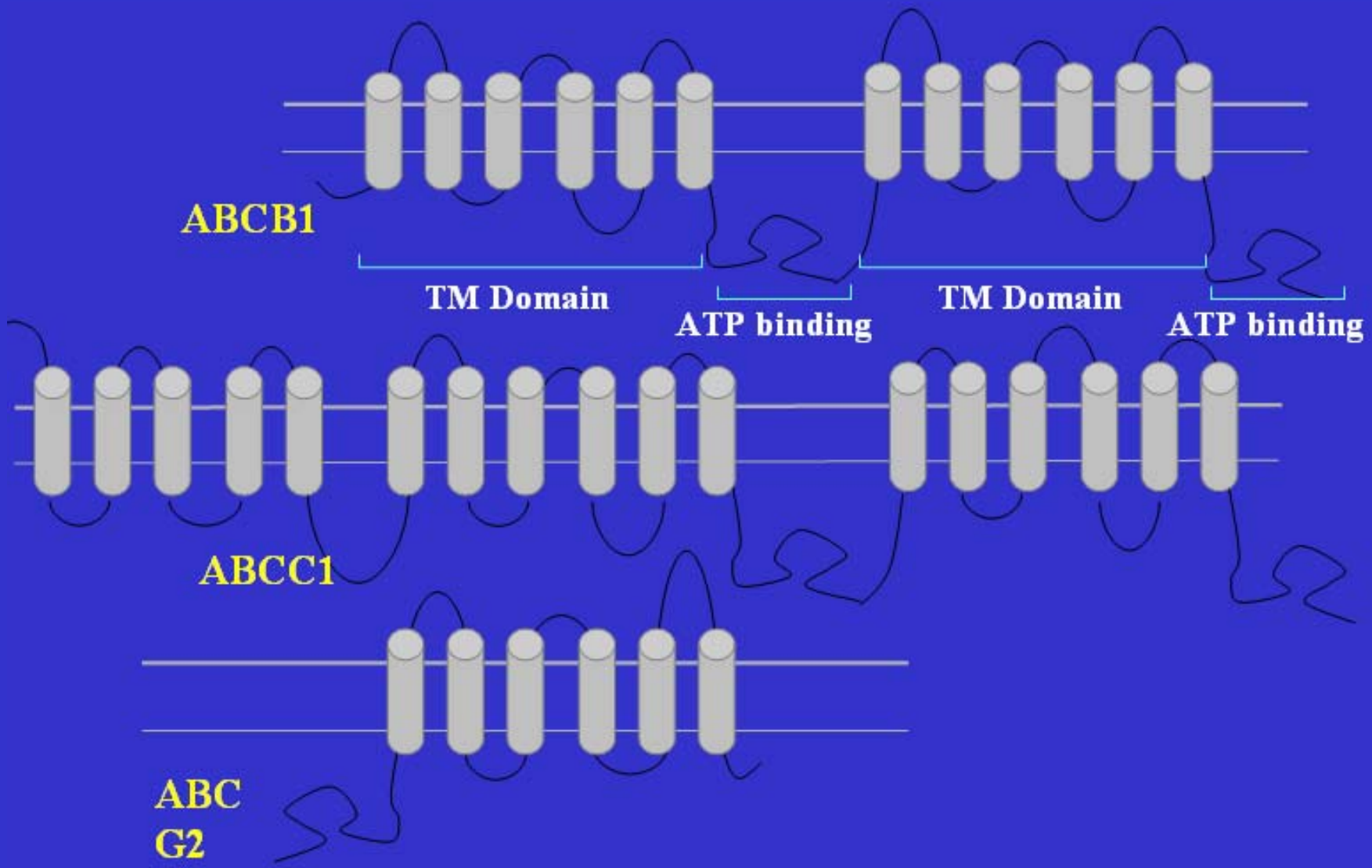
Mechanisms of resistance to anti-cancer drugs



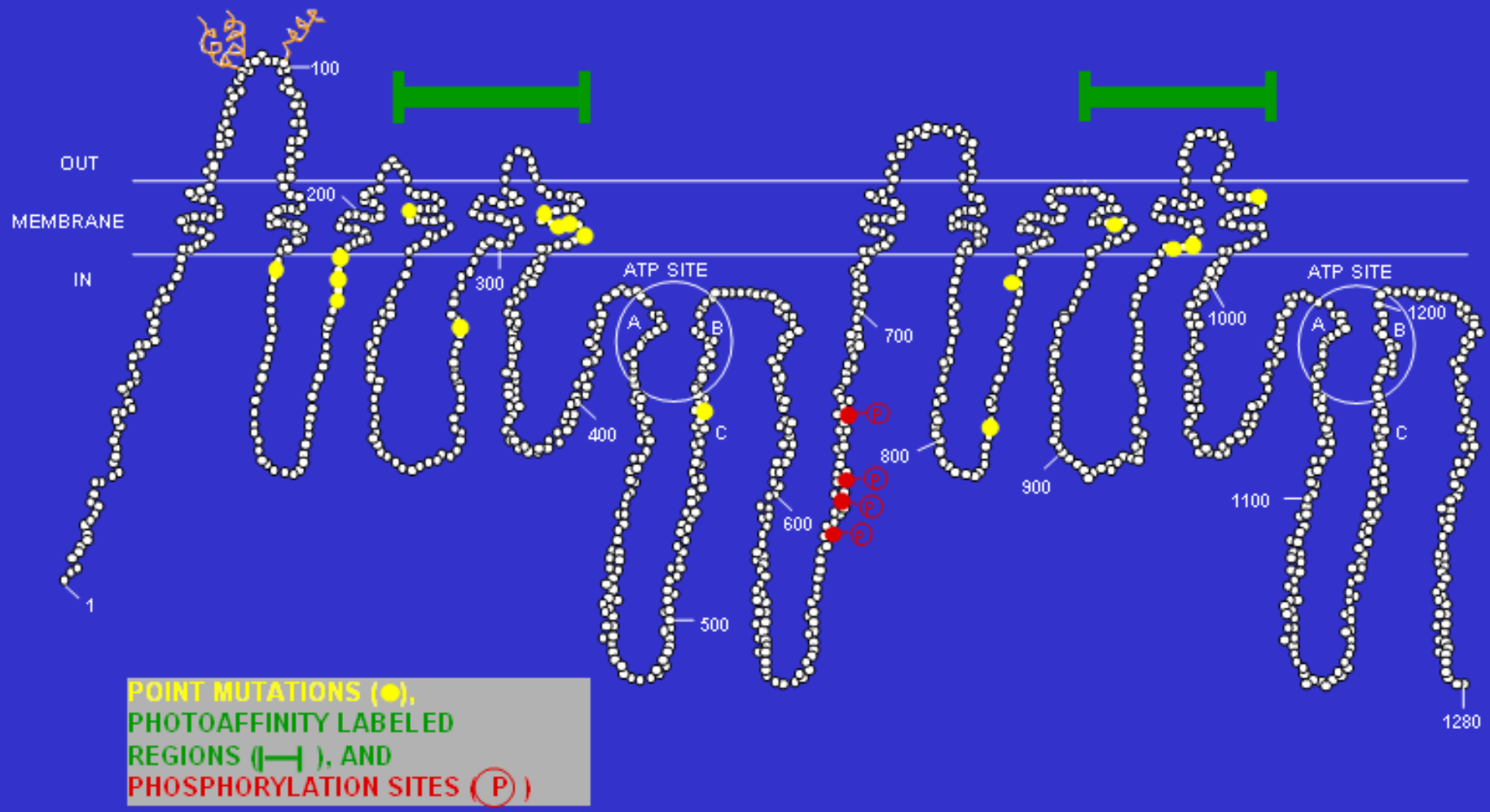
Ultimate Goals

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

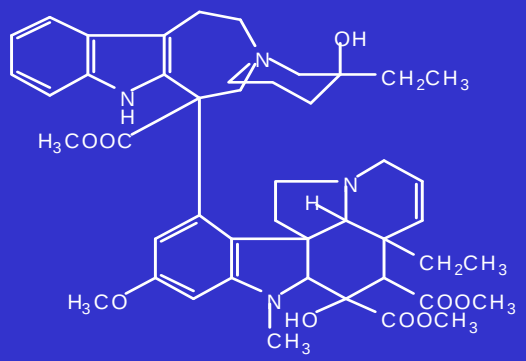
ABC transporters: Domain organization



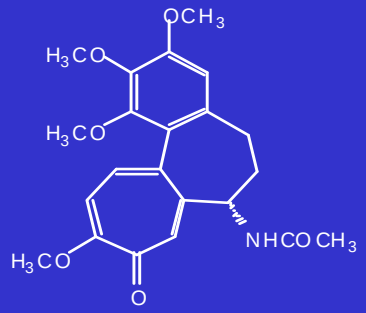
Hypothetical Model of Human P-glycoprotein



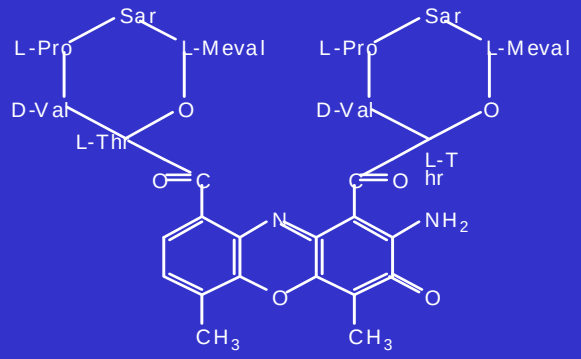
Substrates and Reversing Agents of Pgp



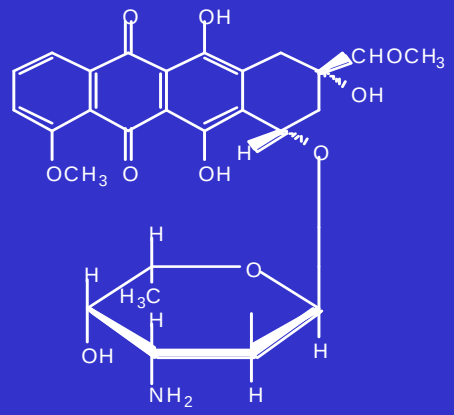
Vinblastine



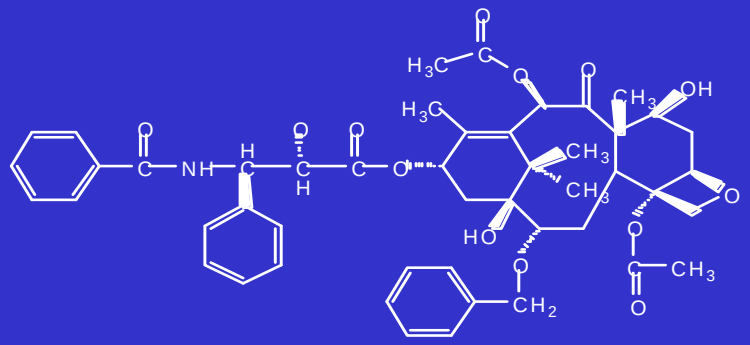
Colchicine



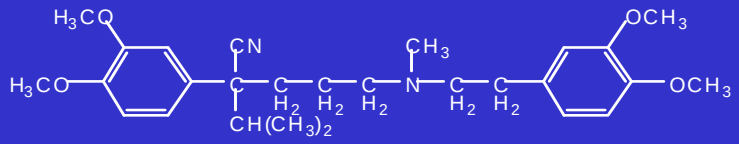
Actinomycin D



Daunorubicin



Taxol



Verapamil

Rapamycin

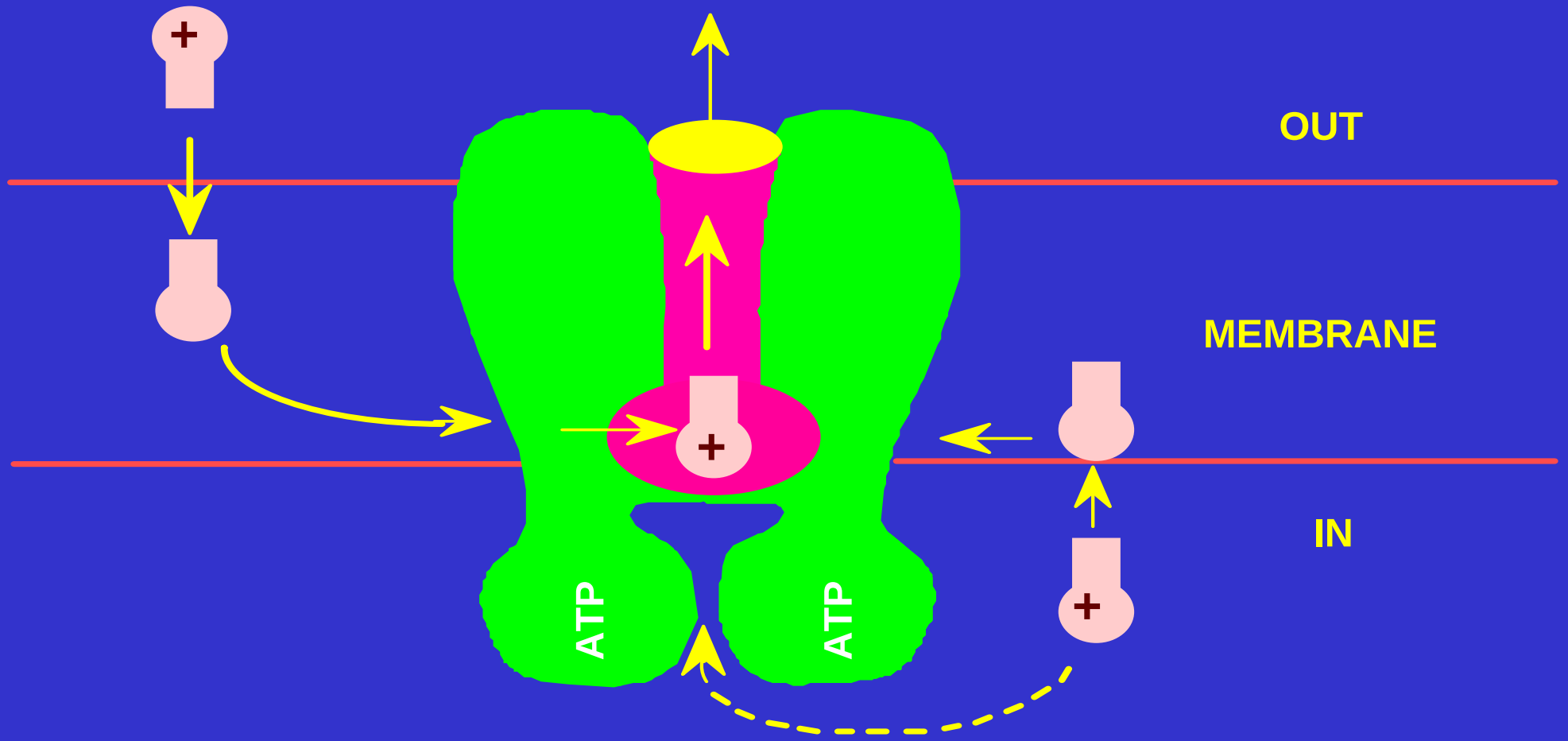
Questions about the mechanism of action of P-glycoprotein

* How does P-glycoprotein recognize so many different substrates?

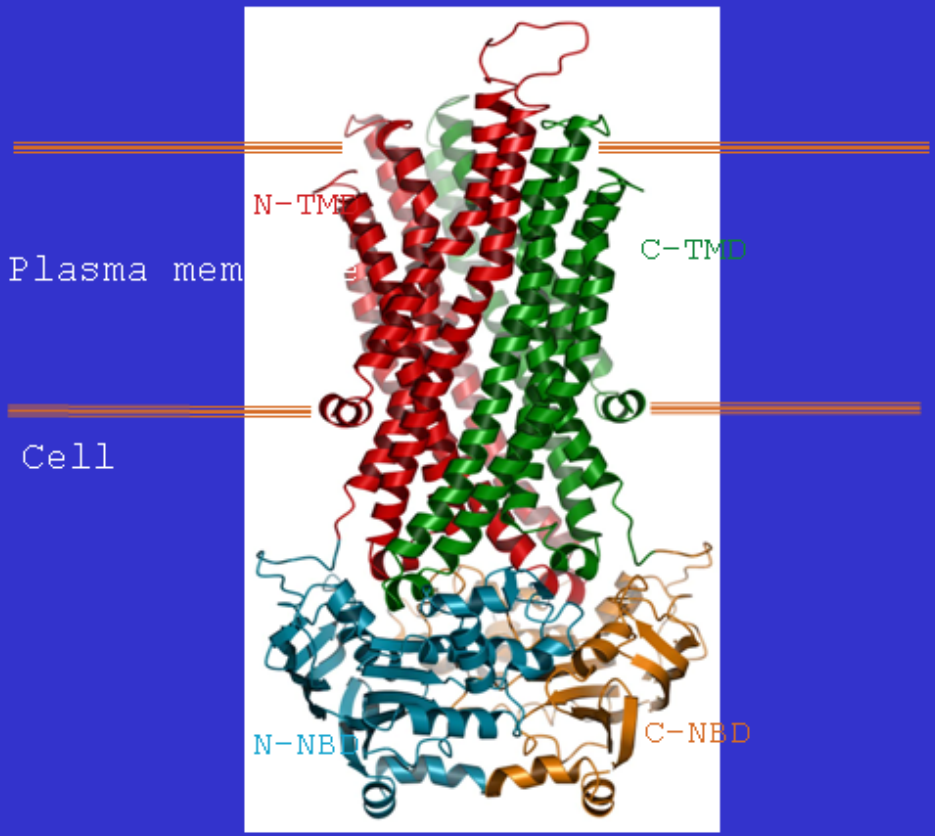
* What do the two ATP binding cassettes do?

* How is substrate binding linked to ATP hydrolysis?

P-glycoprotein removes hydrophobic substrates directly from the plasma membrane



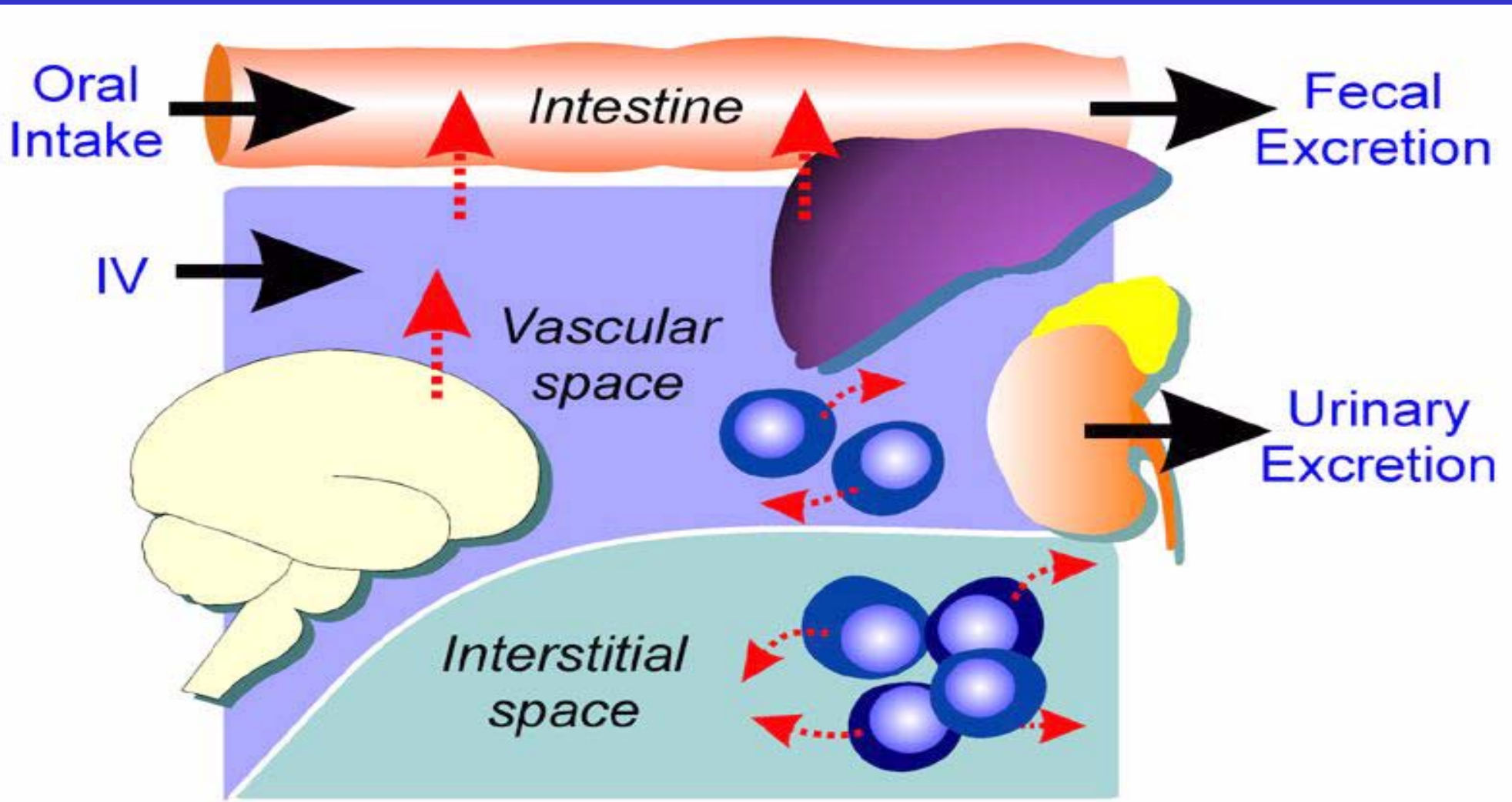
Homology model of human P-glycoprotein based on the structure of bacterial ABC transporter Sav1866 of *S. aureus*



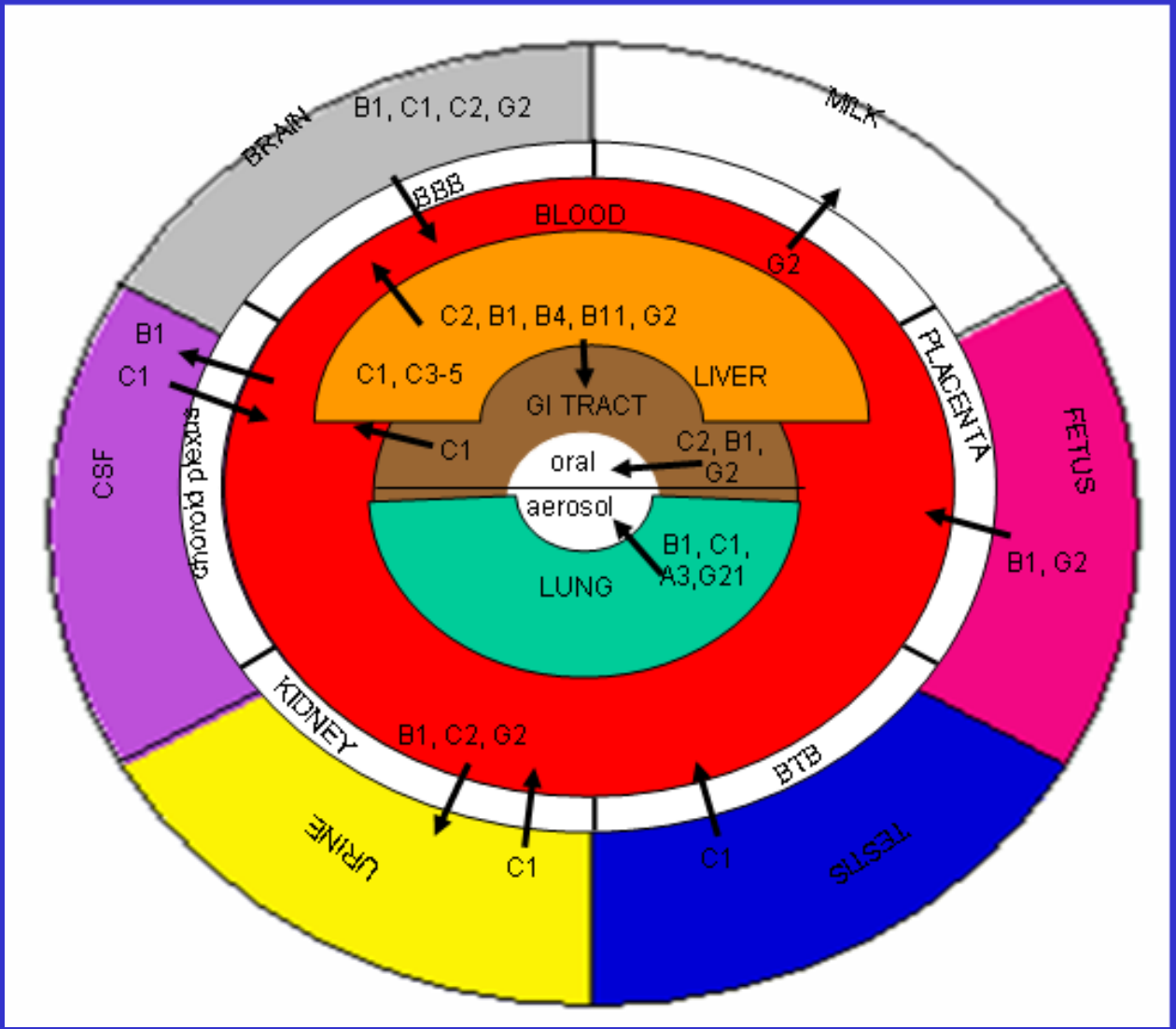
Role of P-glycoprotein in cancer

* 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

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

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

* **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).**

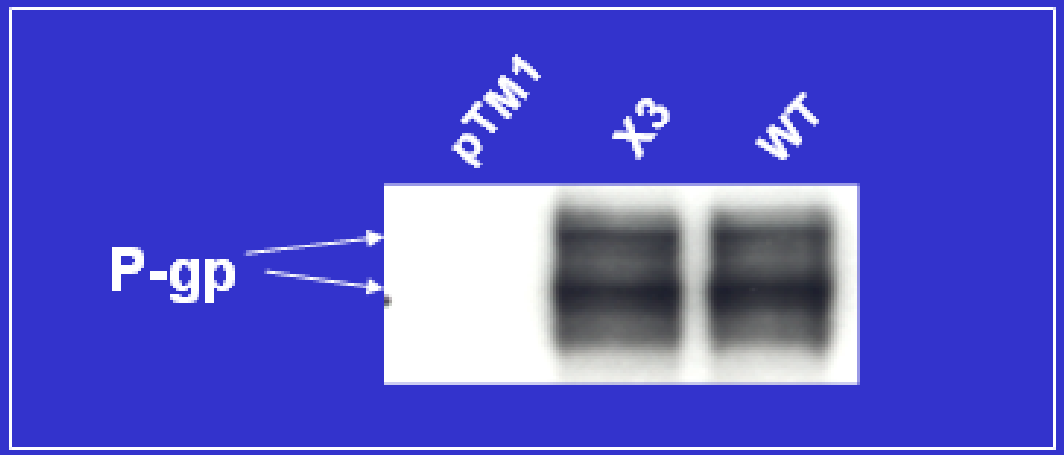
The C1236T, G2677T, C3435T haplotype has been linked to several different phenotypes

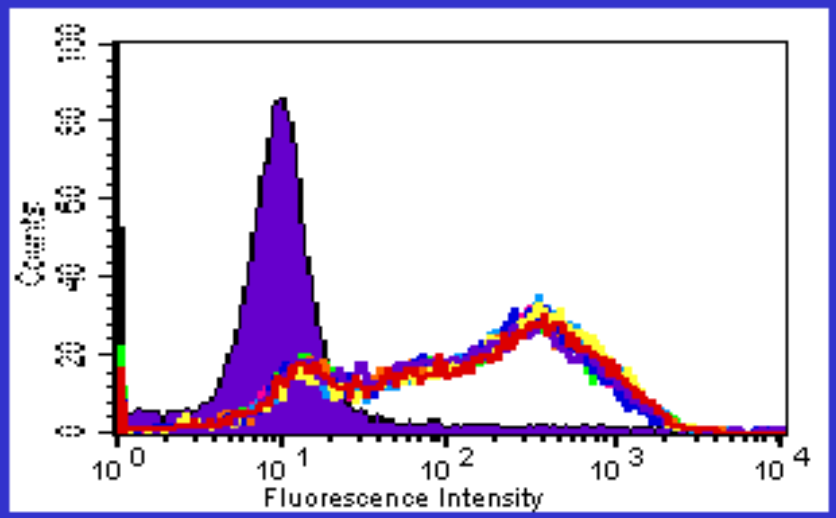
* Altered digoxin and fexofenadine pharmacokinetics

* Altered toxicity in transplant patients from cyclosporine A, tacrilimus

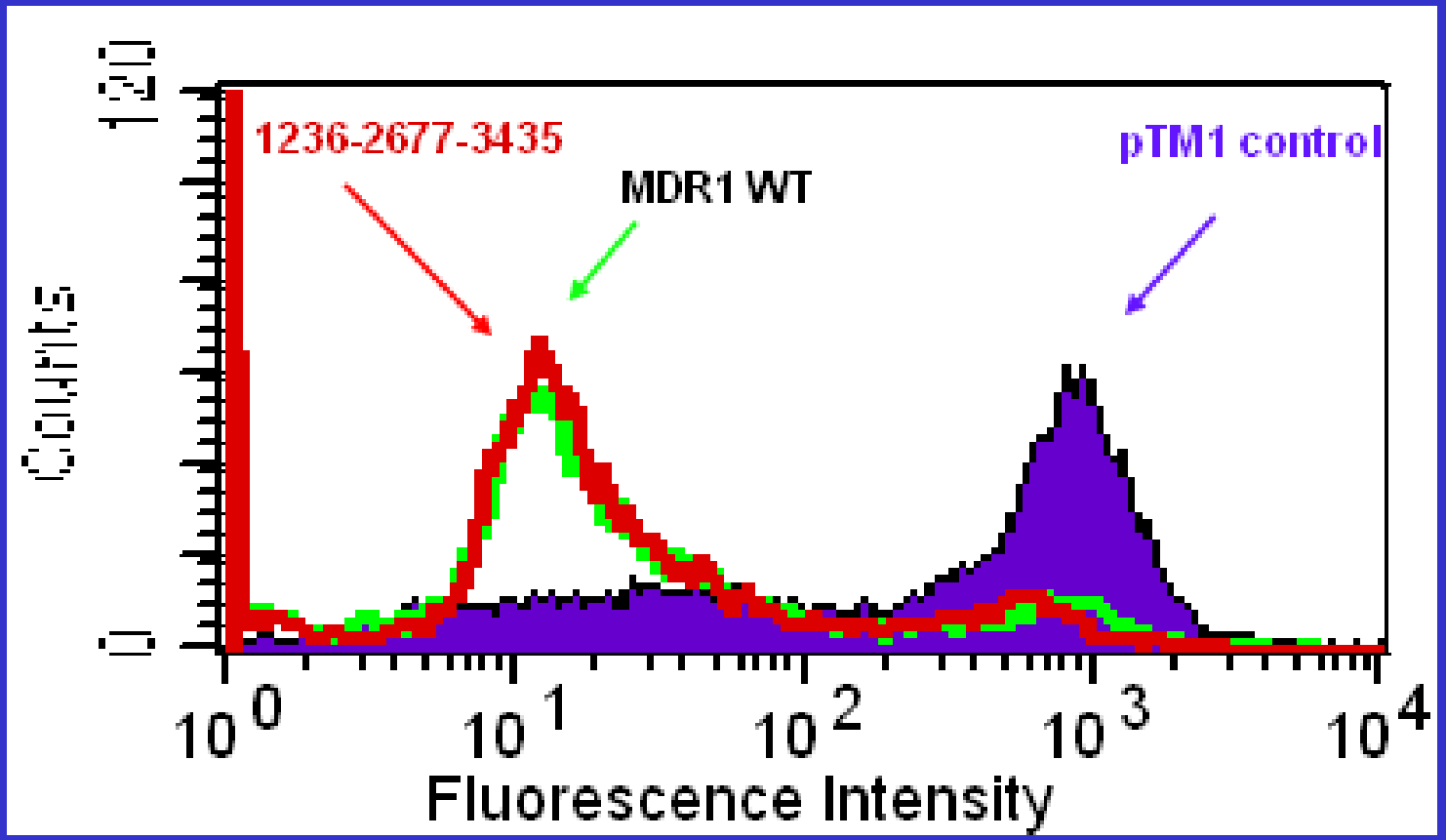
* Altered incidence of Crohn’s disease, colon cancer, and Parkinson’s disease

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

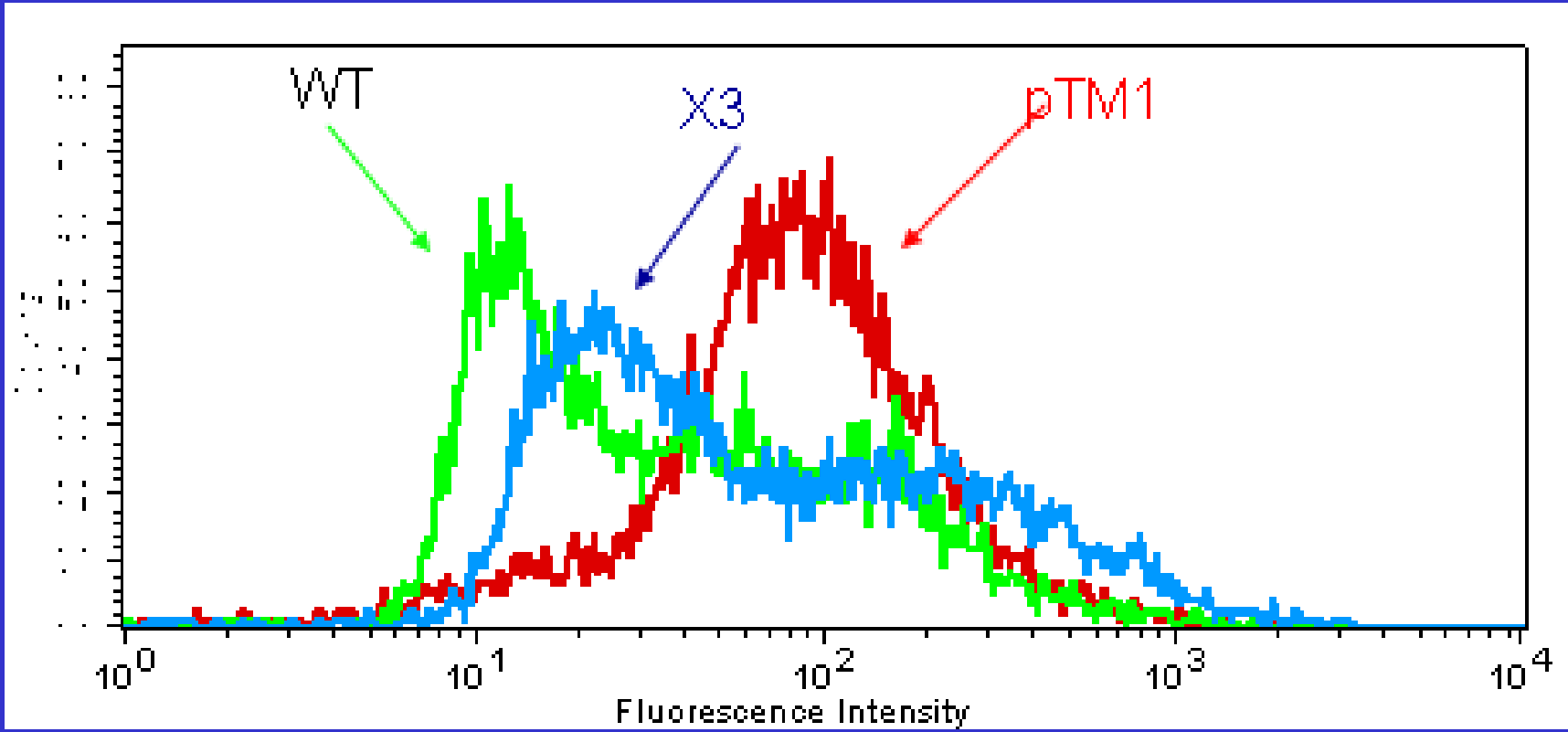




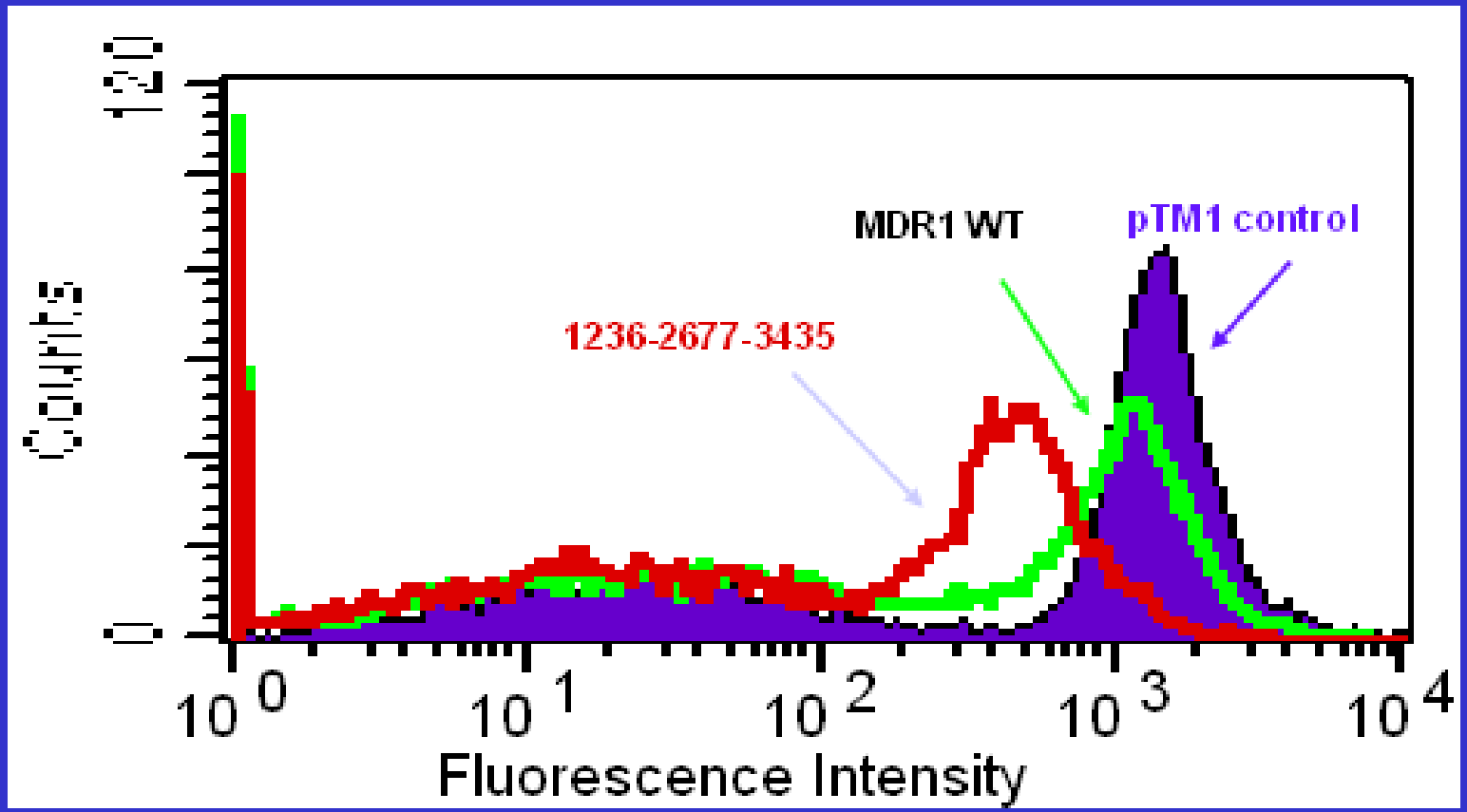
*MDR*1 wild-type and the haplotype (1236-2677-3435) exhibit similar rhodamine 123 efflux



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

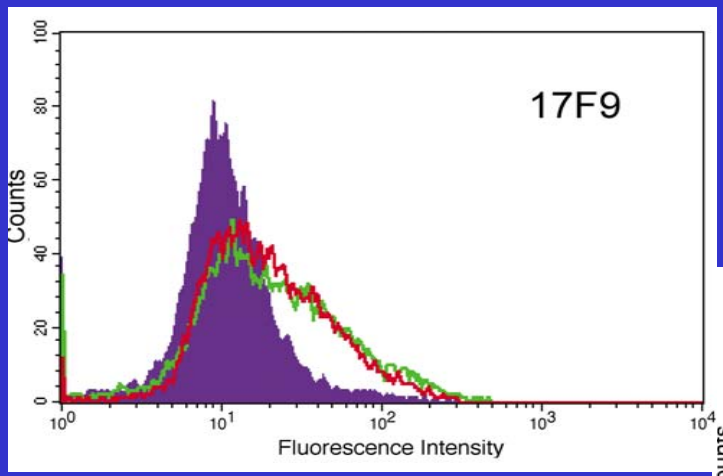


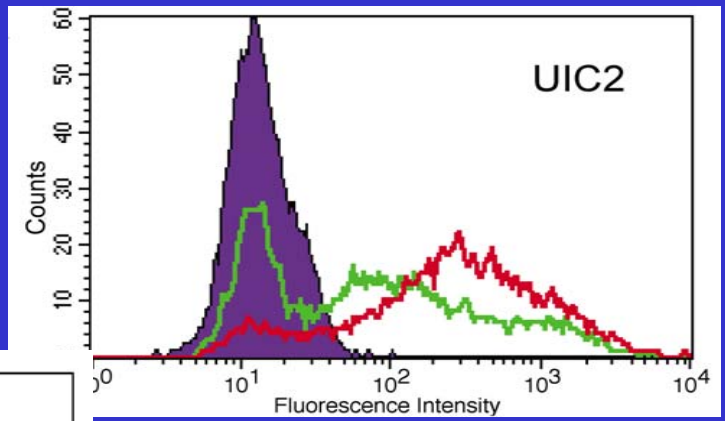
*MDR*1 wild-type and the haplotype exhibit different patterns using rhodamine 123 efflux with cyclosporin A reversing agent

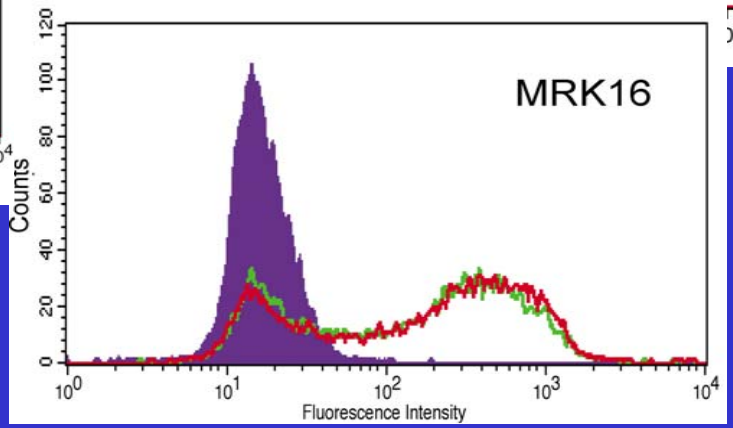


5 mM CsA

*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

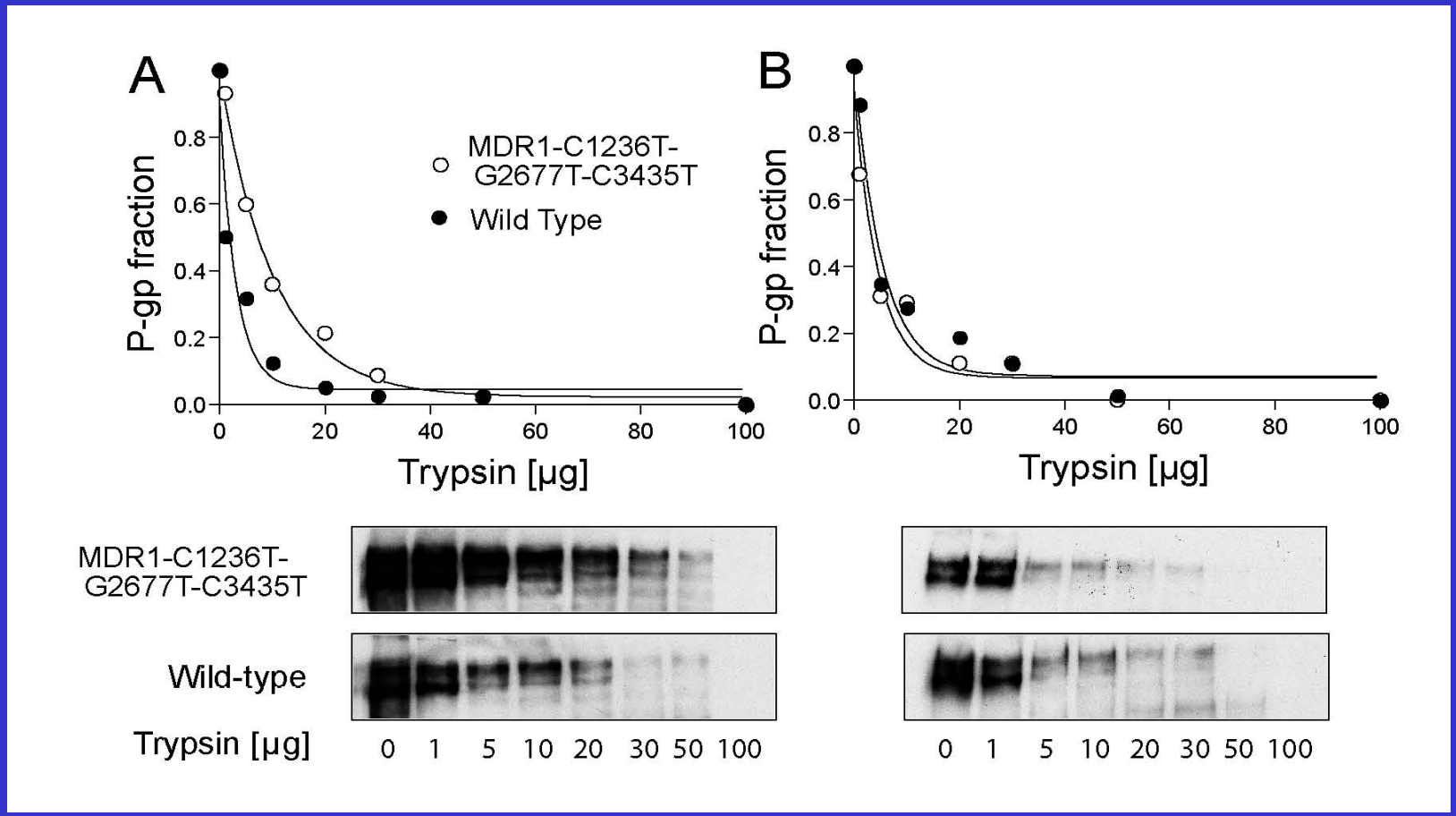




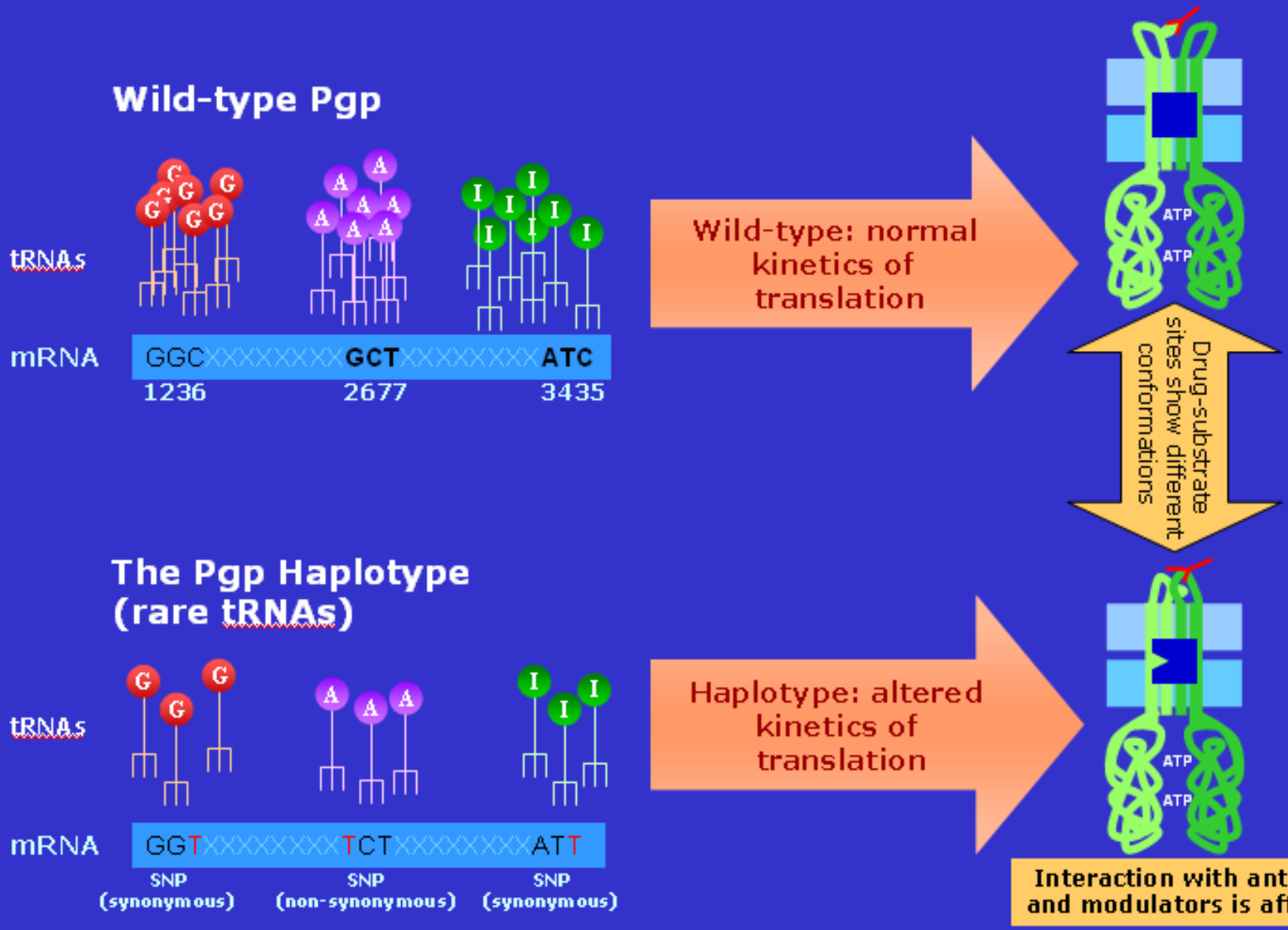


pTM1
WT
1236-2677-3435

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



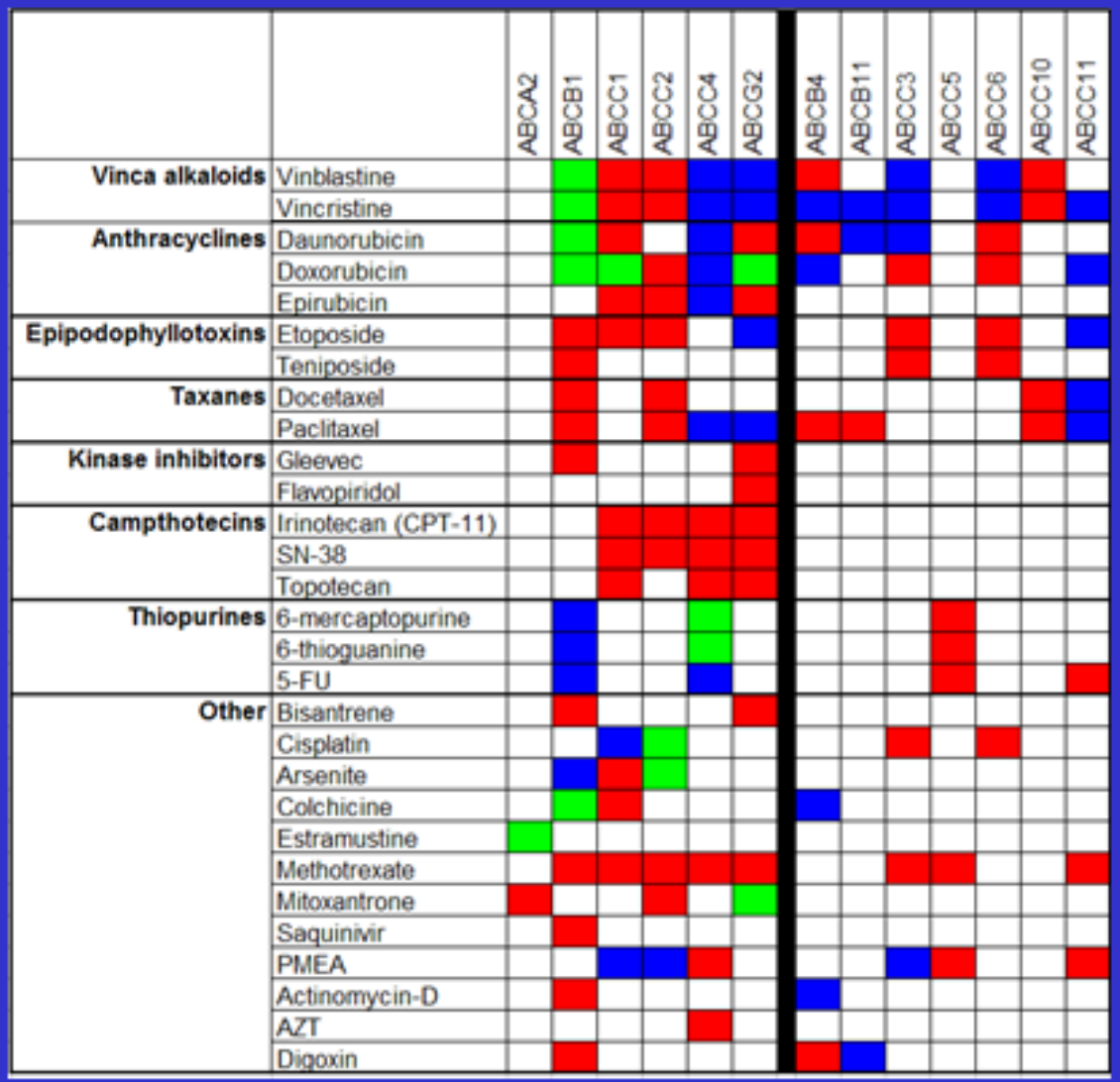
Synonymous SNPs affect P-glycoprotein conformation and function



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

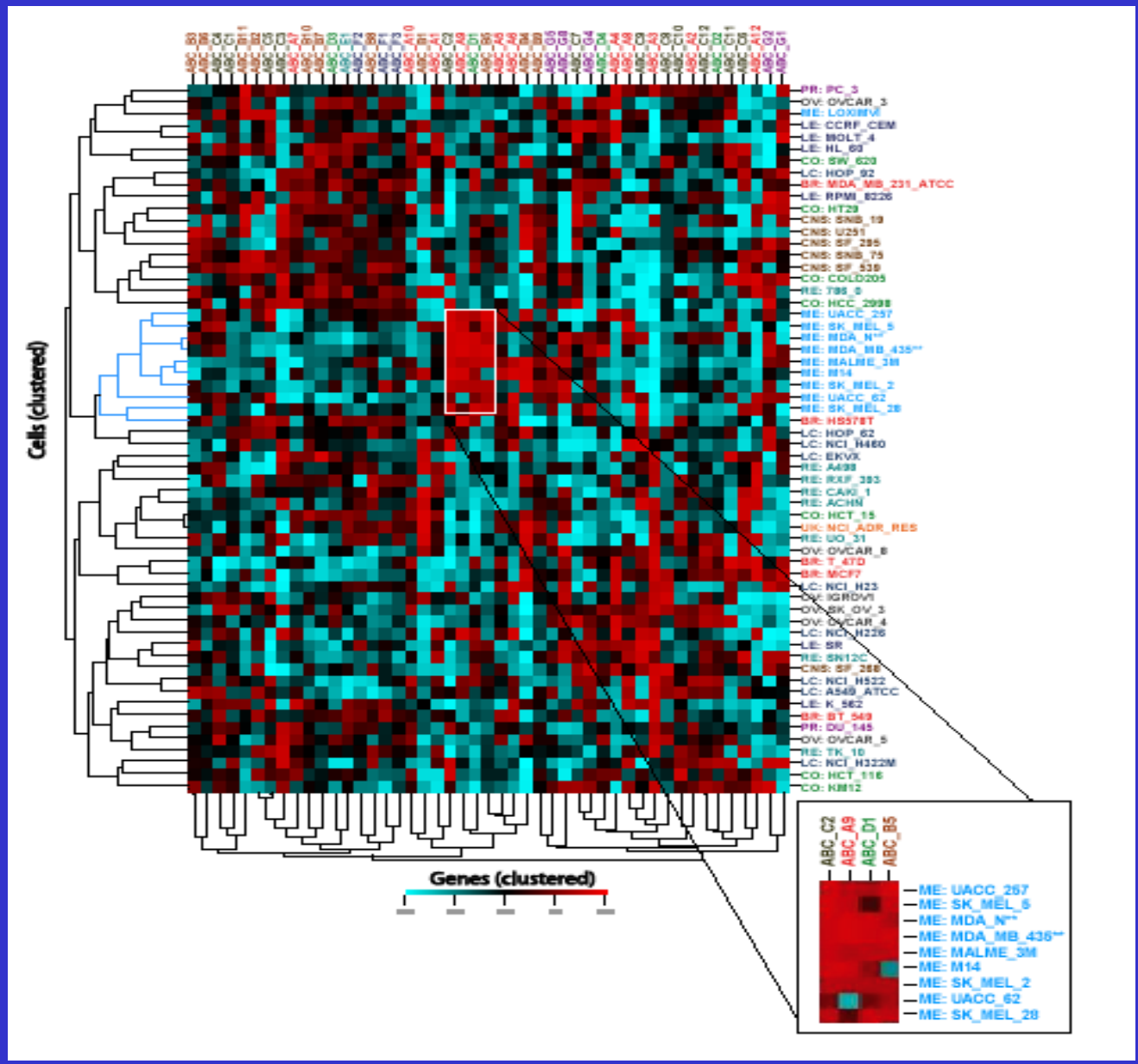
Selected

Doesn't transport

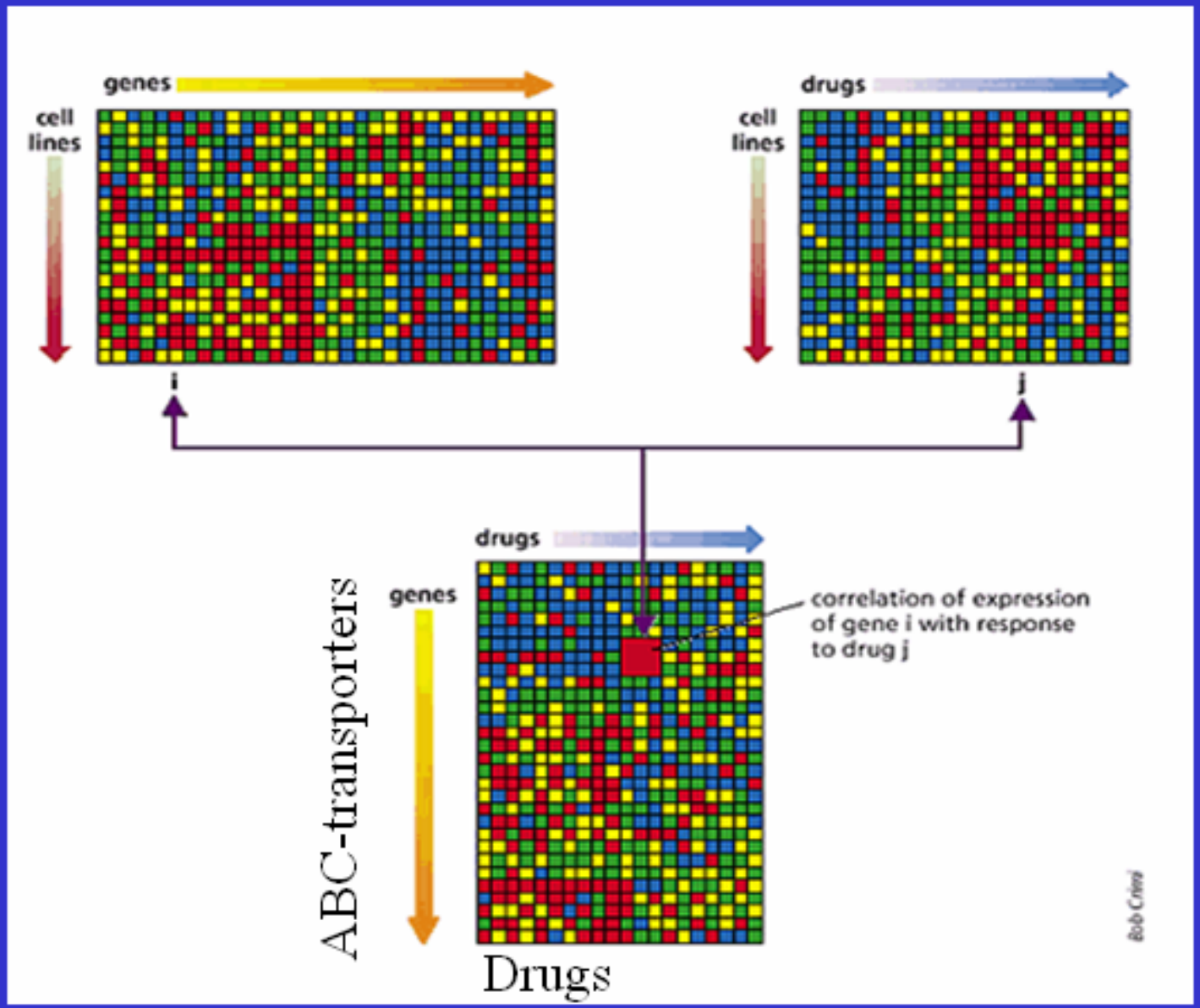
Can we discover new ABC transporter genes responsible for drug resistance?

* 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



Correlation of Matrices

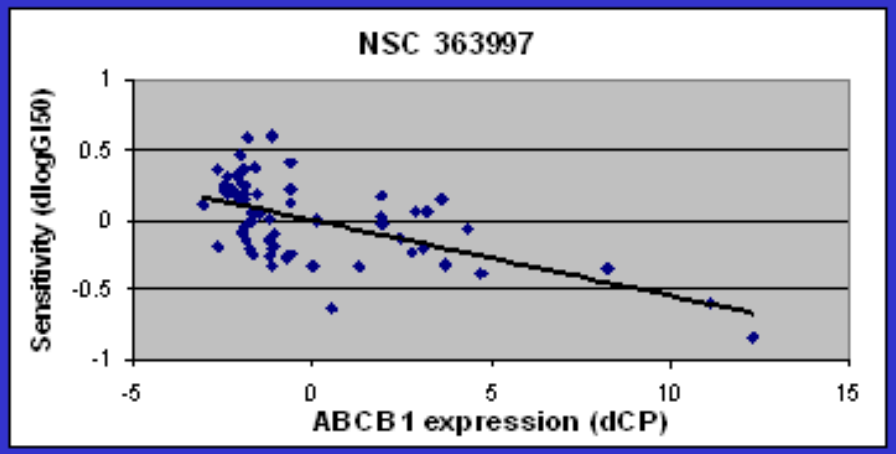


Pinkel D, *Nature Genetics* **24**, 208-9 (2000)

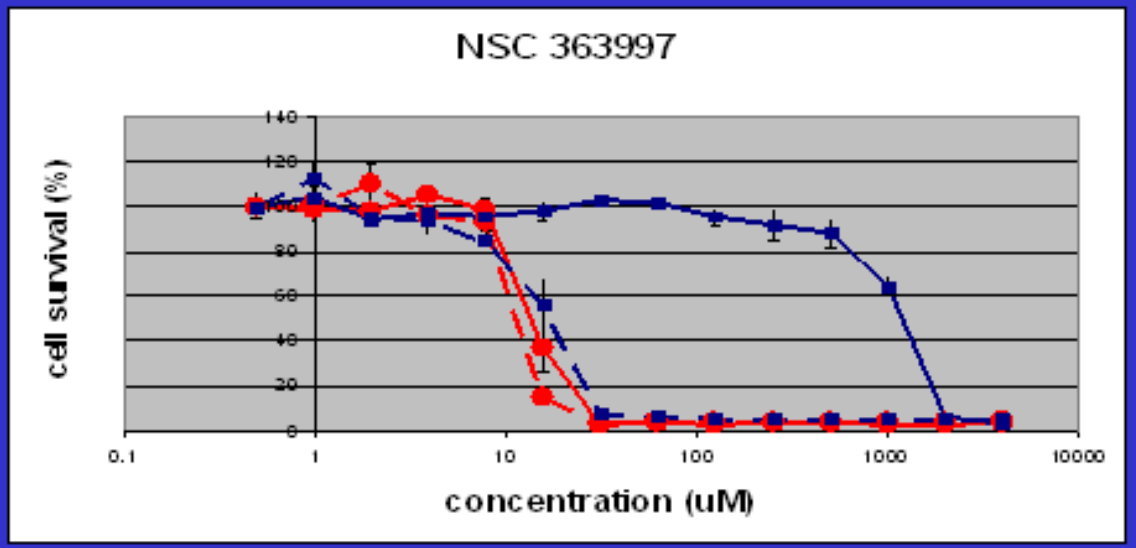
Conditions which must be met to correlate expression with resistance

* mRNA levels are quantitative and reflect levels of functional protein in cells
* Drug resistance data are accurate and quantitative
* Resistance is determined by levels of transporters, i.e., they are limiting

Inversely correlated compounds are ABCB1 substrates



KB-V1: MDR derivative of KB-3-1 overexpressing P-gp

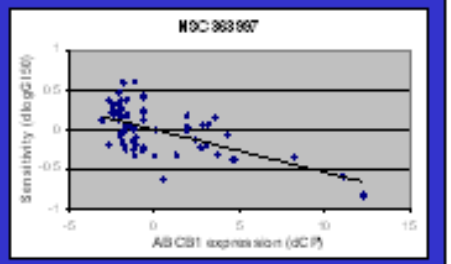


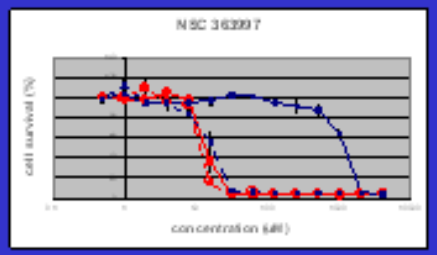
Inversely correlated compounds indicate potential substrates for 28 ABC transporters

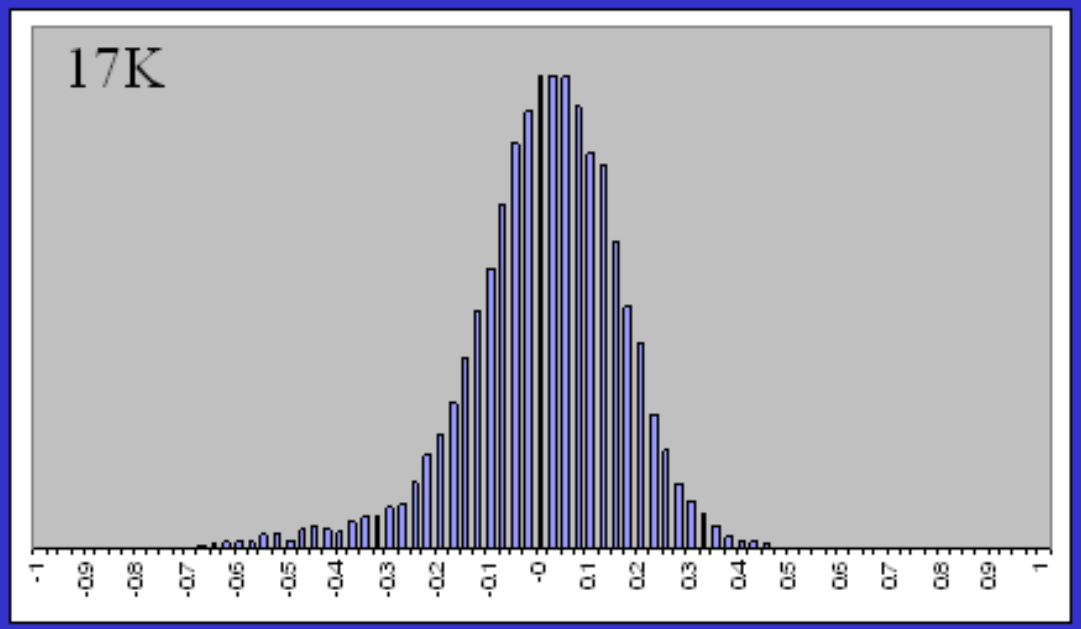
Analysis of a dataset containing 1430 compounds at 99.99% bootstrap confidence interval (equivalent to a significance level of $p<0.0001$)

Substrate assignments confirmed for 4 transporters in transfected cell lines: ABCB1, ABCC2, ABCC4, and ABCC11

Search for MDR1-potentiated compounds in DTP's database





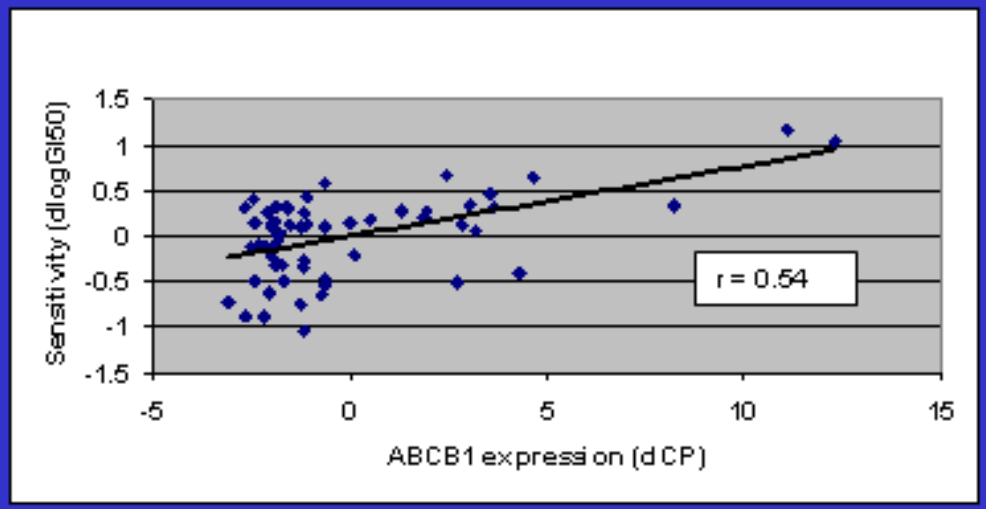


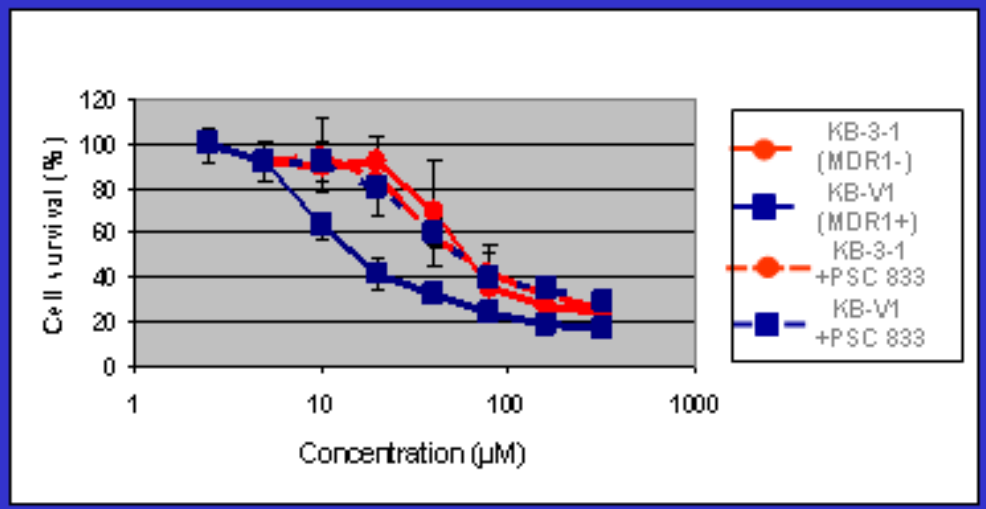
NSC 73306

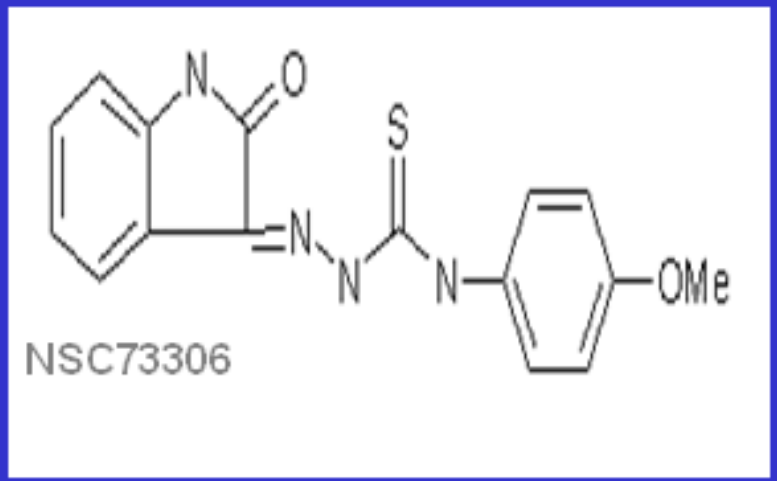
Substrates

MDR1-potentiated compounds?

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





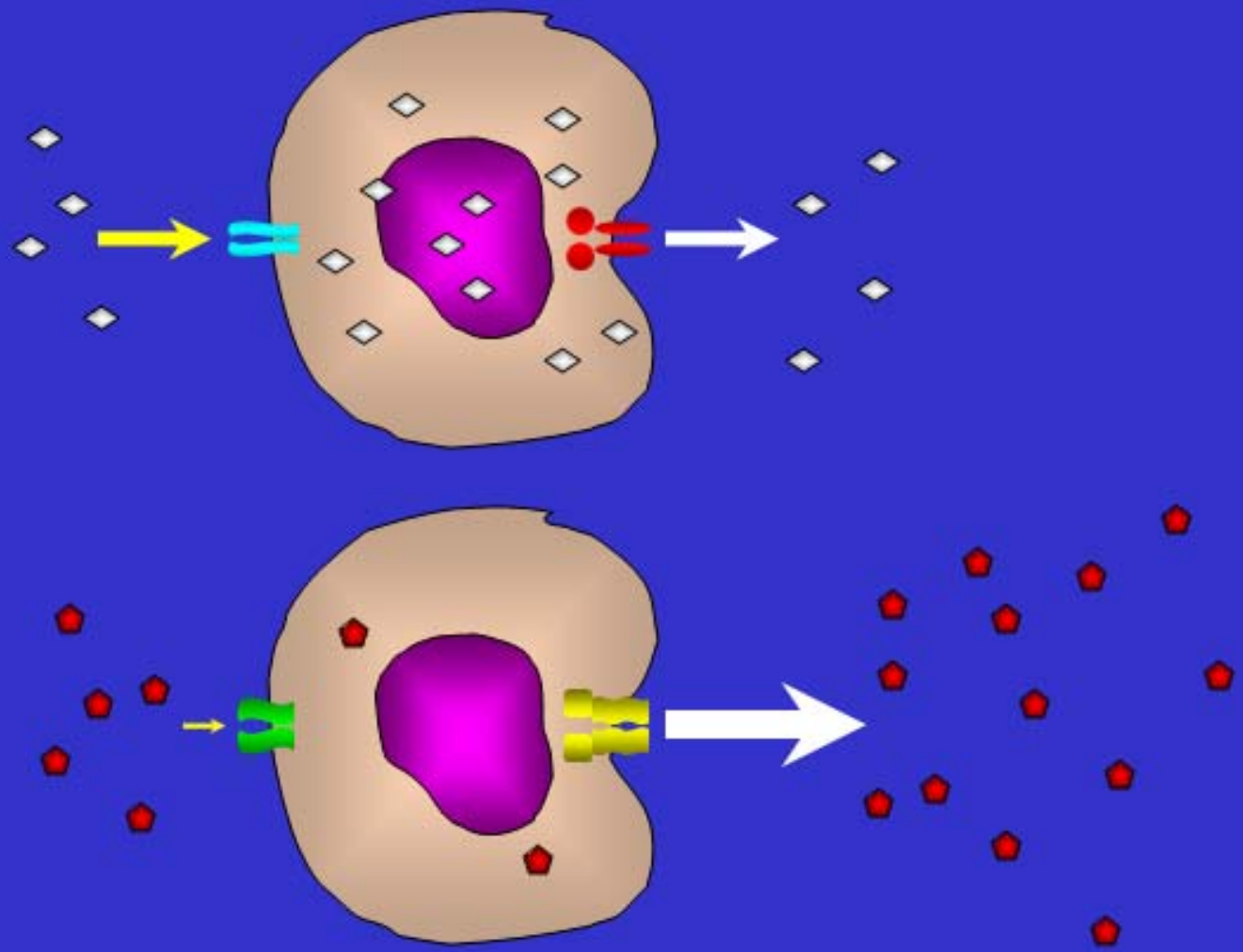


Potential Clinical Utility of Discovery of Compounds that Specifically Kill MDR1-Expressing Cells

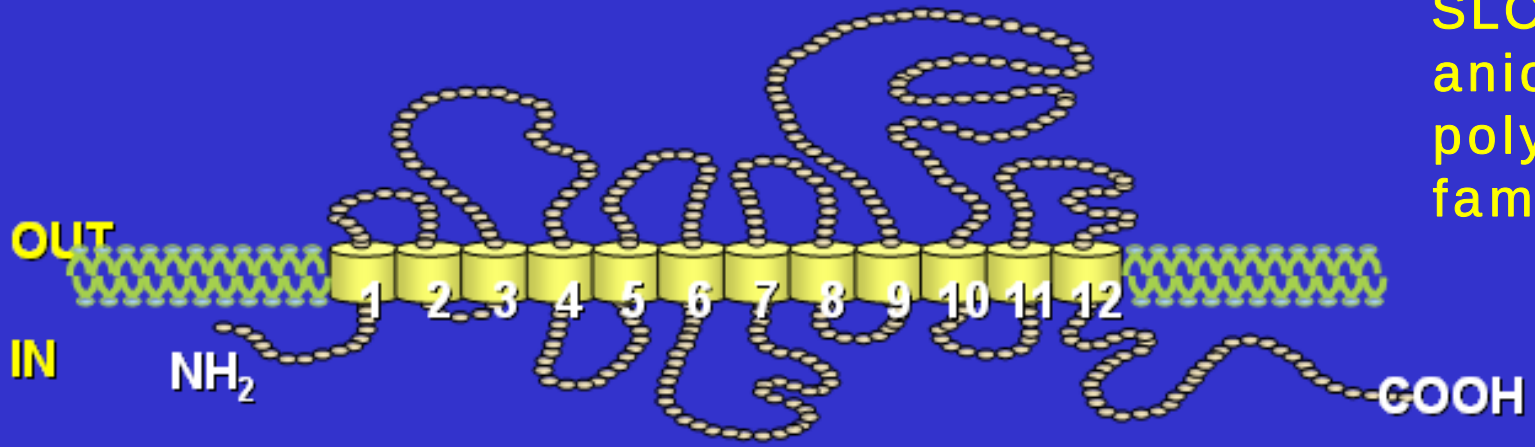
Can be used in combination with standard chemotherapy to eliminate MDR1-expressing cell populations

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

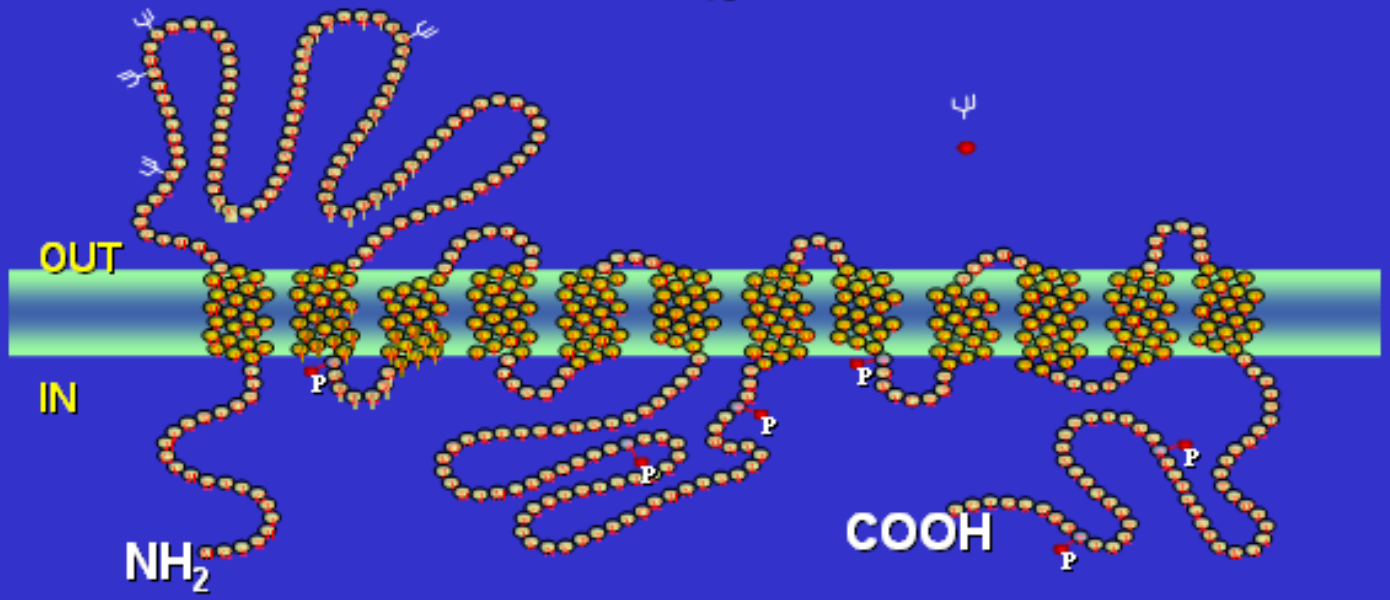
Balance of uptake and efflux determines drug accumulation in cancer cells



Solute Carriers are plausible uptake transporters for anti-cancer drugs

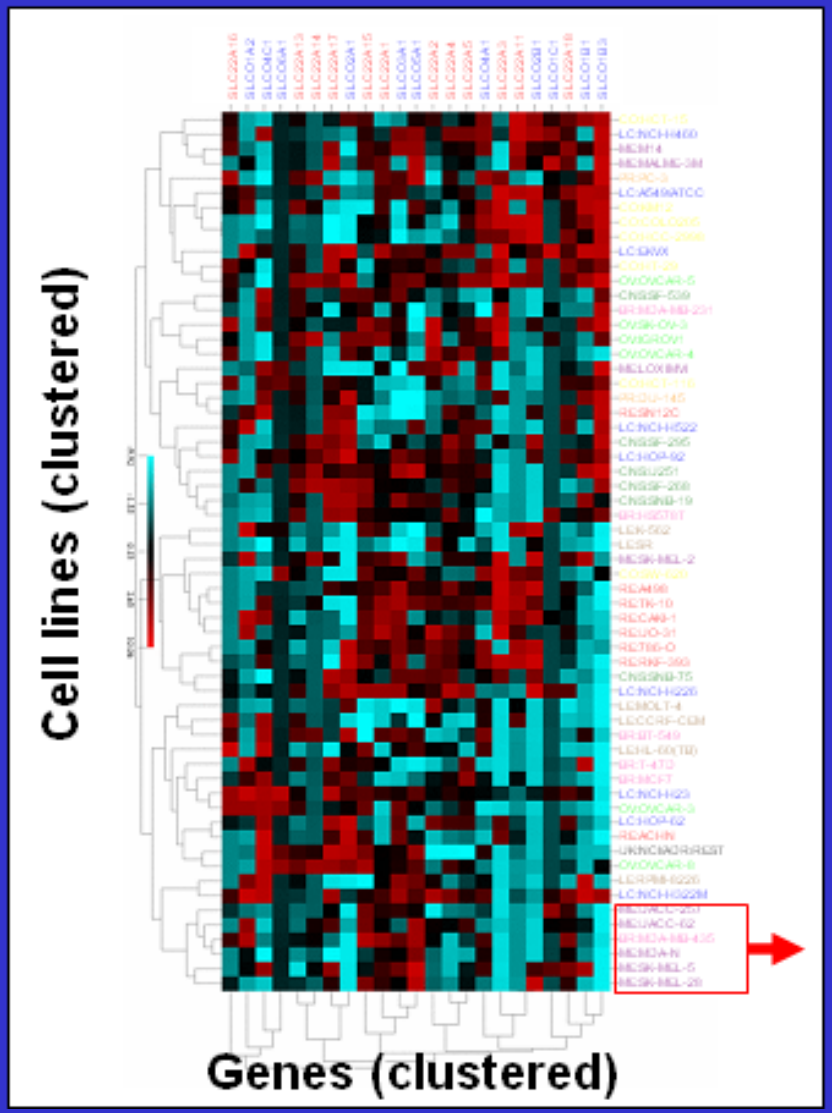


SLCO: organic anion transporting polypeptide (OATP) family



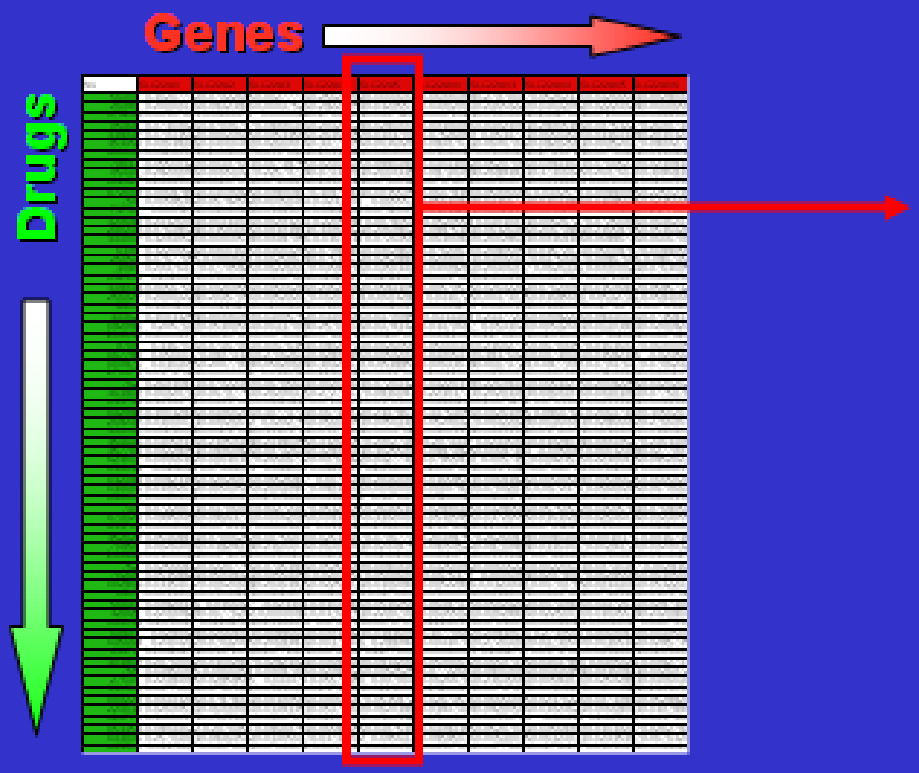
SLC22: organic cation and anion transporter (OCT And OAT) families

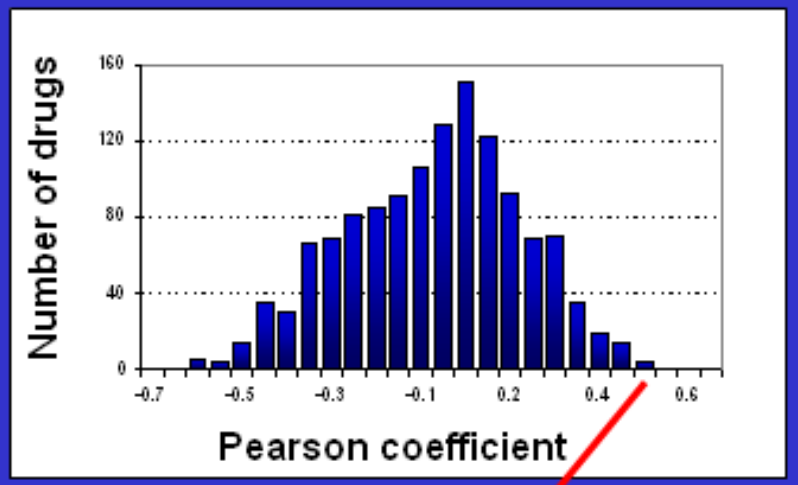
Gene expression of SLCO and SLC22 family members in the NCI-60 cancer cell lines

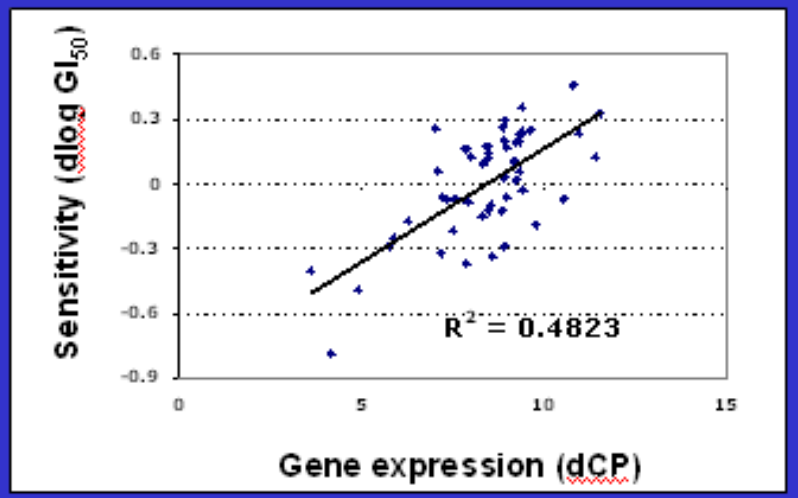


CIMminer tool
(http://discover.nci.nih.gov/)

Identify potential SLC substrates







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.

Acknowledgements

* Gergely Szakacs
* Jean-Philippe Annereau
* Kevin Chen
* Joe Ludwig
* Jill Paterson
* Mitsunori Okabe
* Matthew Hall
* Chava Kimchi-Sarfaty
* Jung-Mi Oh
* Suresh Ambudkar
  - Zuben Sauna
  - InWha Kim
  - Anna Calcagno
* Ira Pastan
* John Weinstein
* Carol Cardarelli
* Takaaki Abe
* Joe Covey
* Henry Fales