Learning Some New Tricks From a Multidrug Transporter

Michael M. Gottesman, M.D.
Chief, Laboratory of Cell Biology
Center for Cancer Research, NCI
National Institutes of Health

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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%

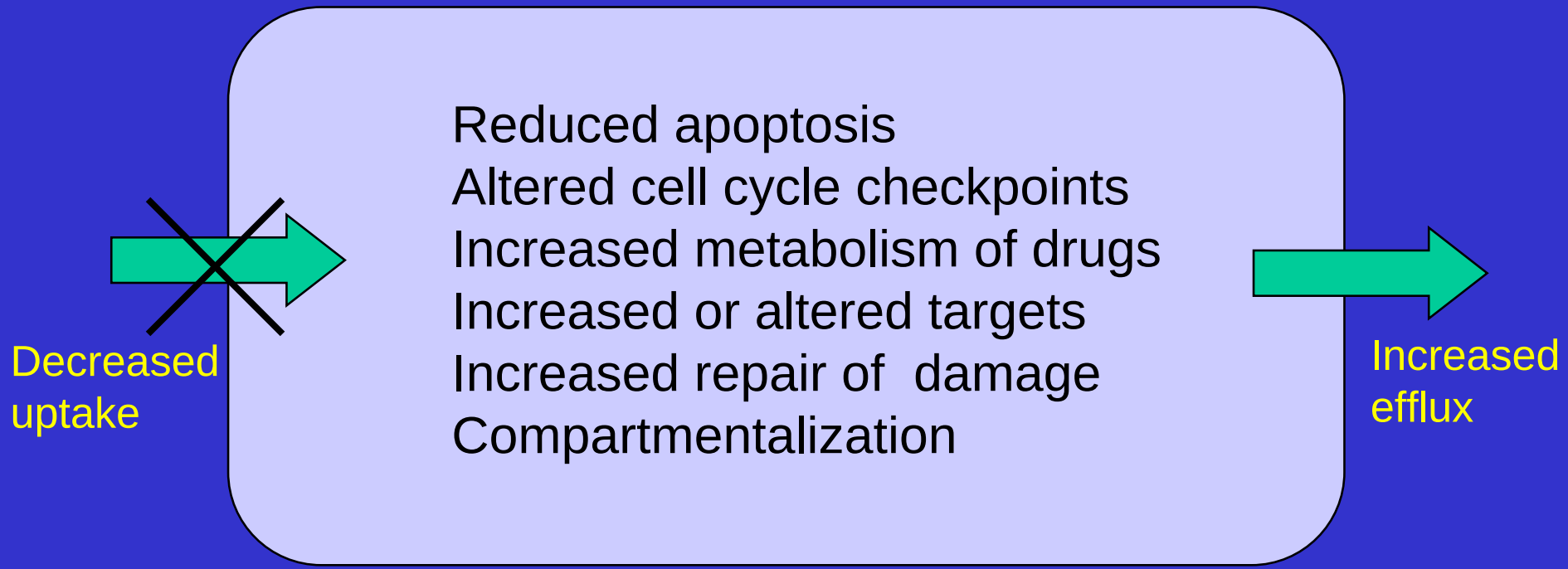
*Virtually all deaths are due to chemotherapy resistance

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

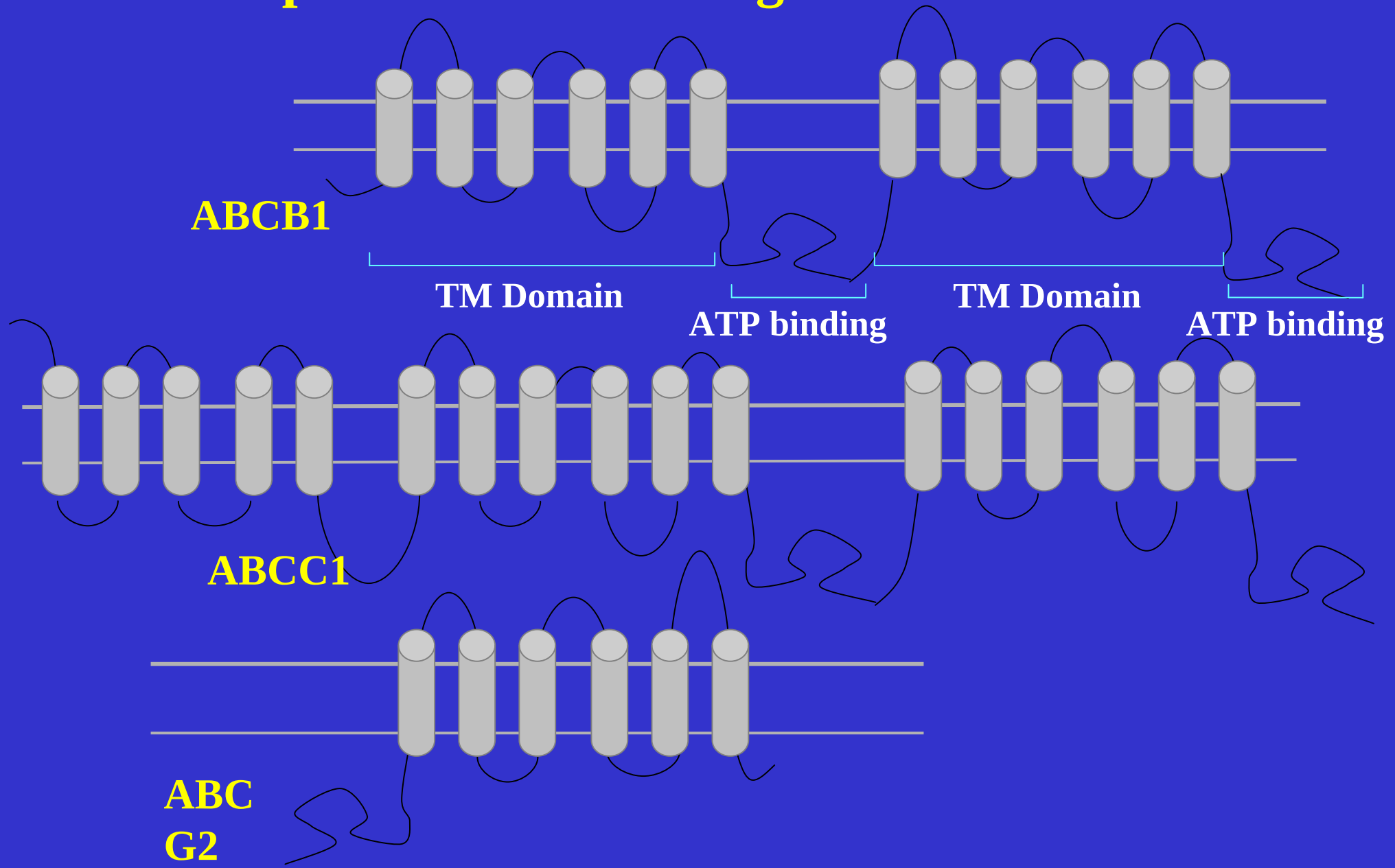
Mechanisms of resistance to anti-cancer drugs



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

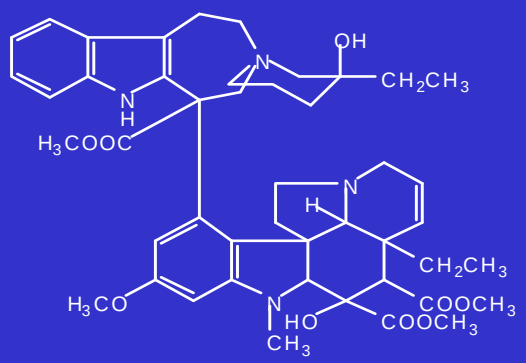
ABC transporters: Domain organization



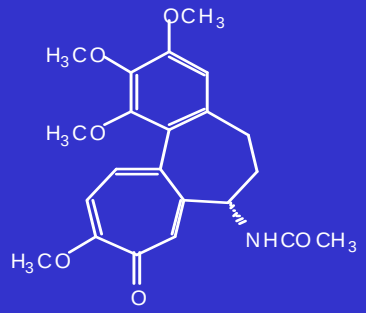
Hypothetical Model of Human P-glycoprotein

POINT MUTATIONS (●),
PHOTOAFFINITY LABELED
REGIONS (|—|), AND
PHOSPHORYLATION SITES (P)

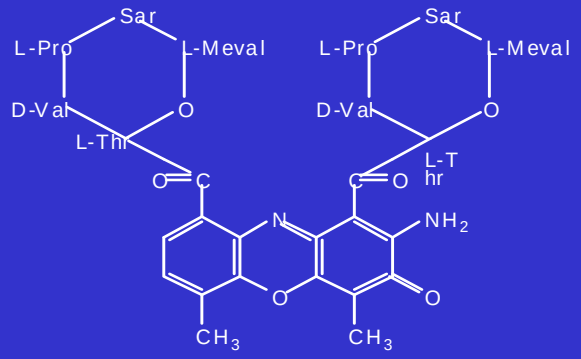
Substrates and Reversing Agents of Pgp



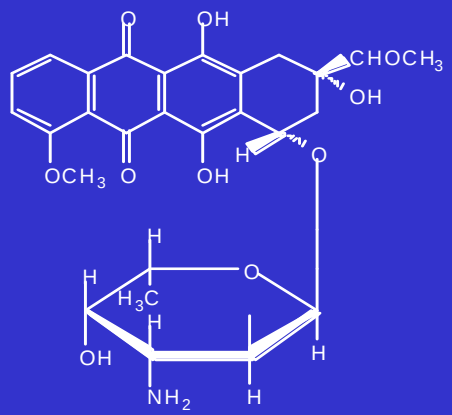
Vinblastine



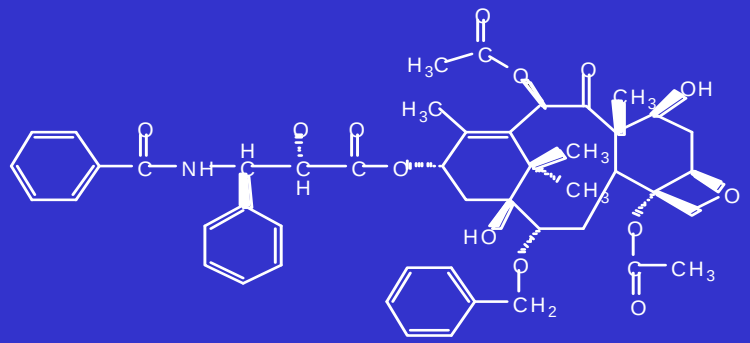
Colchicine



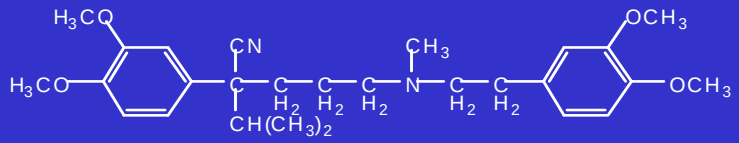
Actinomycin D



Daunorubicin



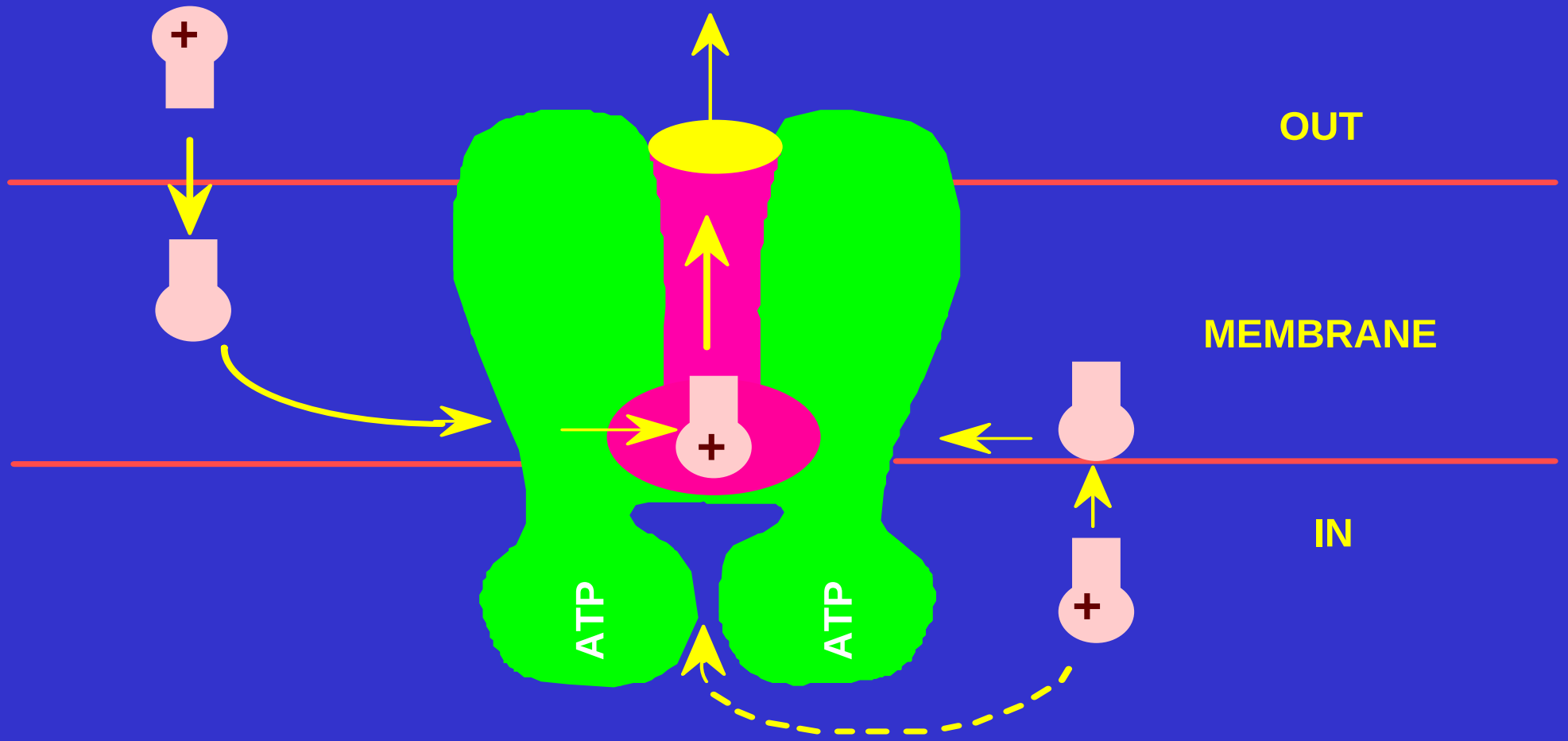
Taxol



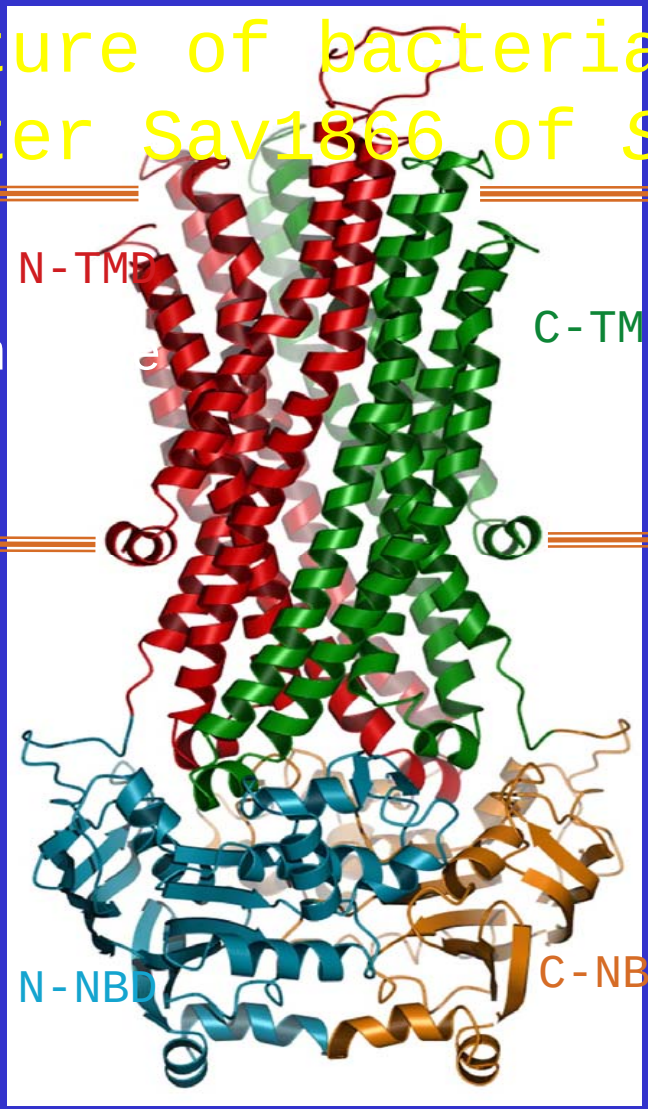
Verapamil

Rapamycin

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*



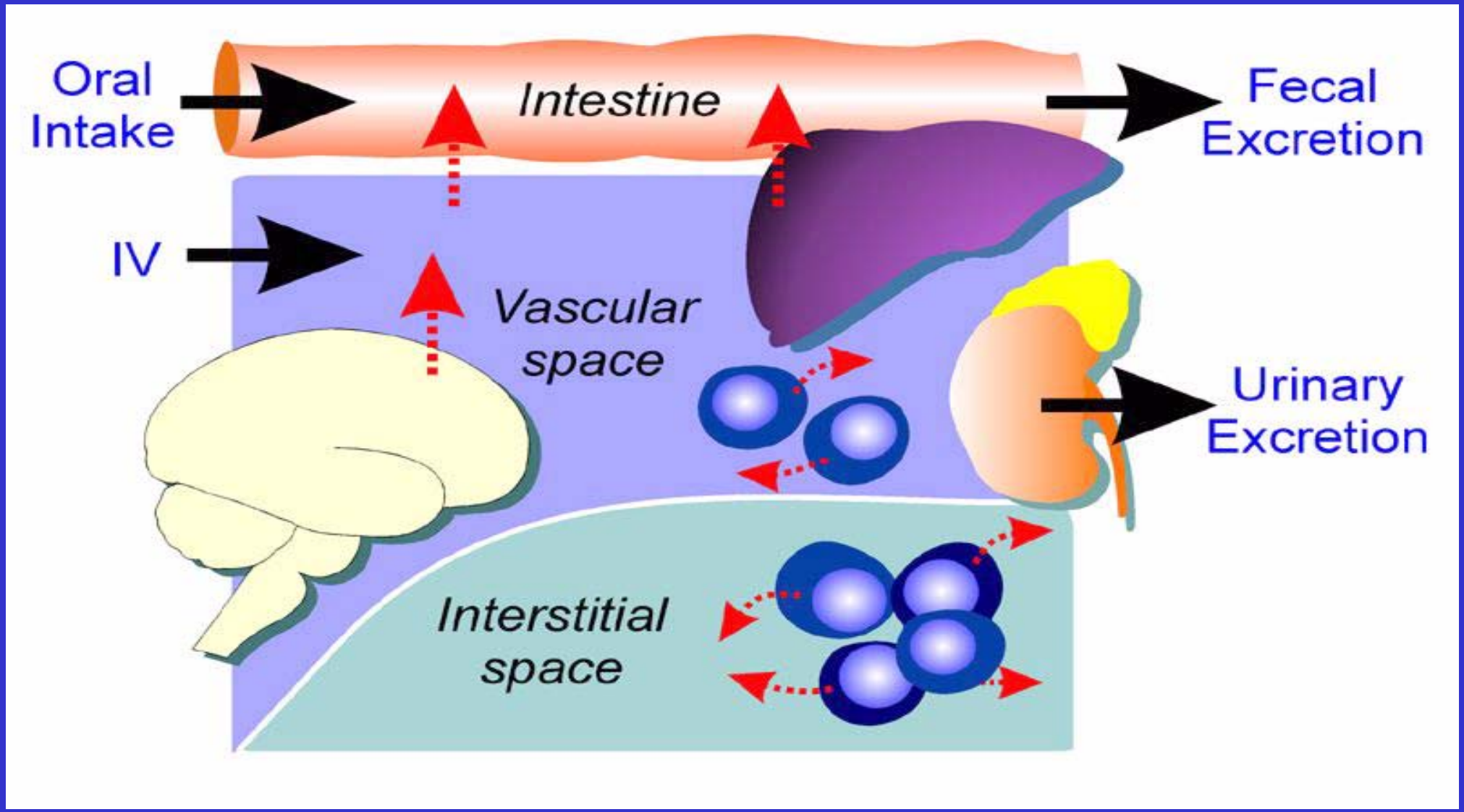
Plasma mem

Cell

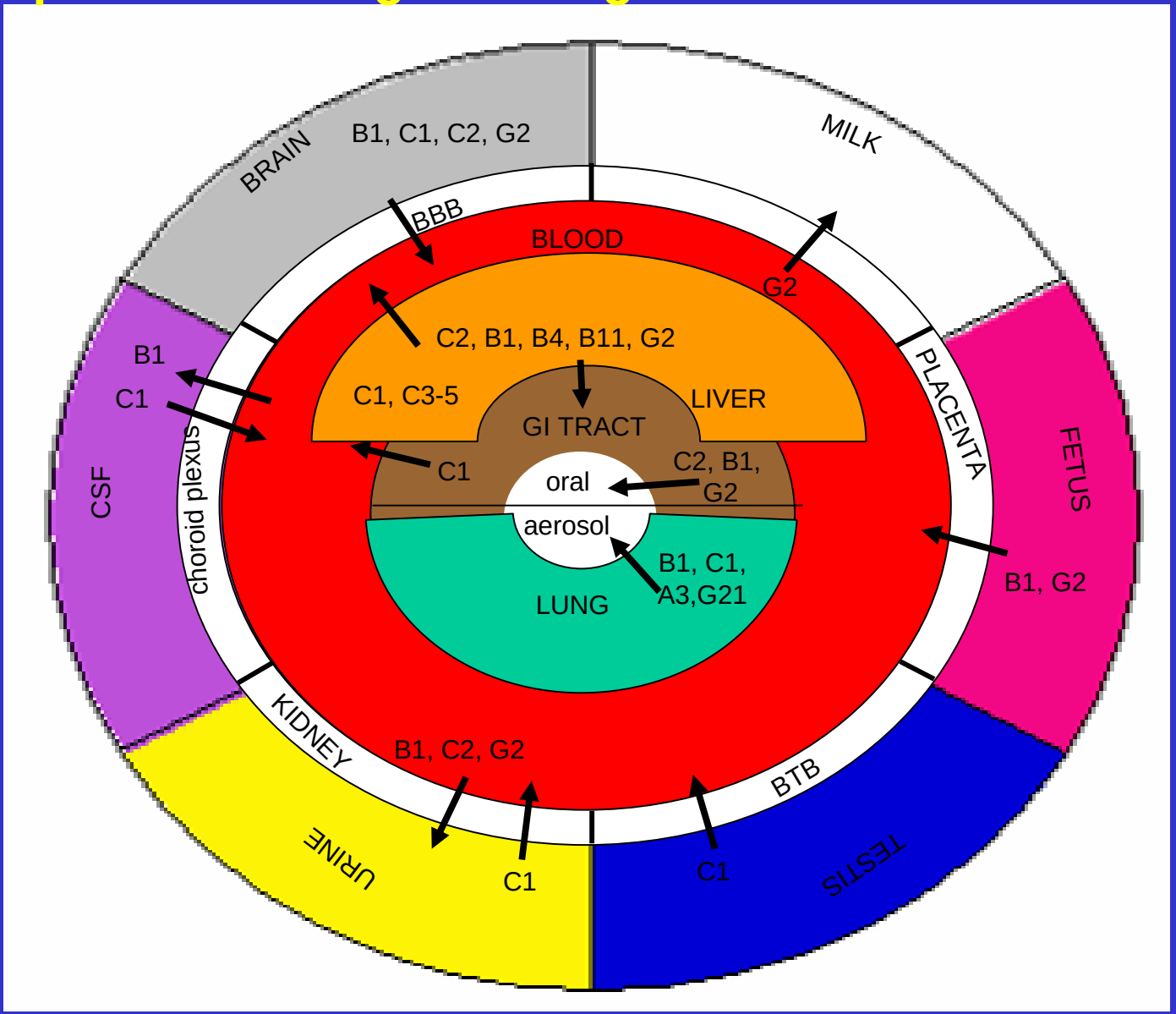
Role of P-glycoprotein in cancer

- 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.

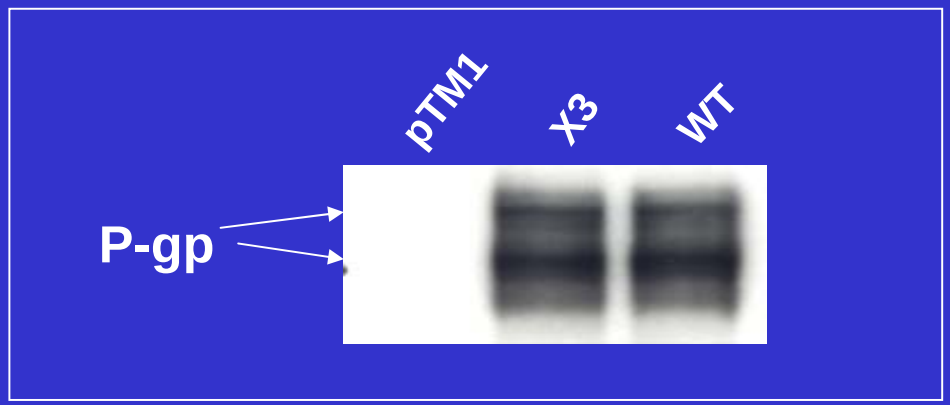
2. 5 common coding (non-synonymous) polymorphisms have no demonstrable effect on drug transport function.

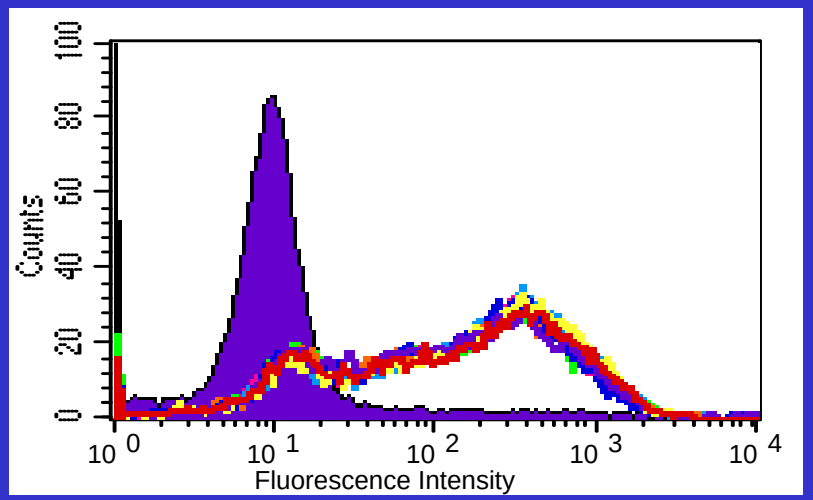
3. The synonymous SNP in exon 26 (C3435T) was the first associated with altered MDR1 function and is often part of a haplotype including another synonymous (C1236T) and a nonsynonymous SNP (G2677T).

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

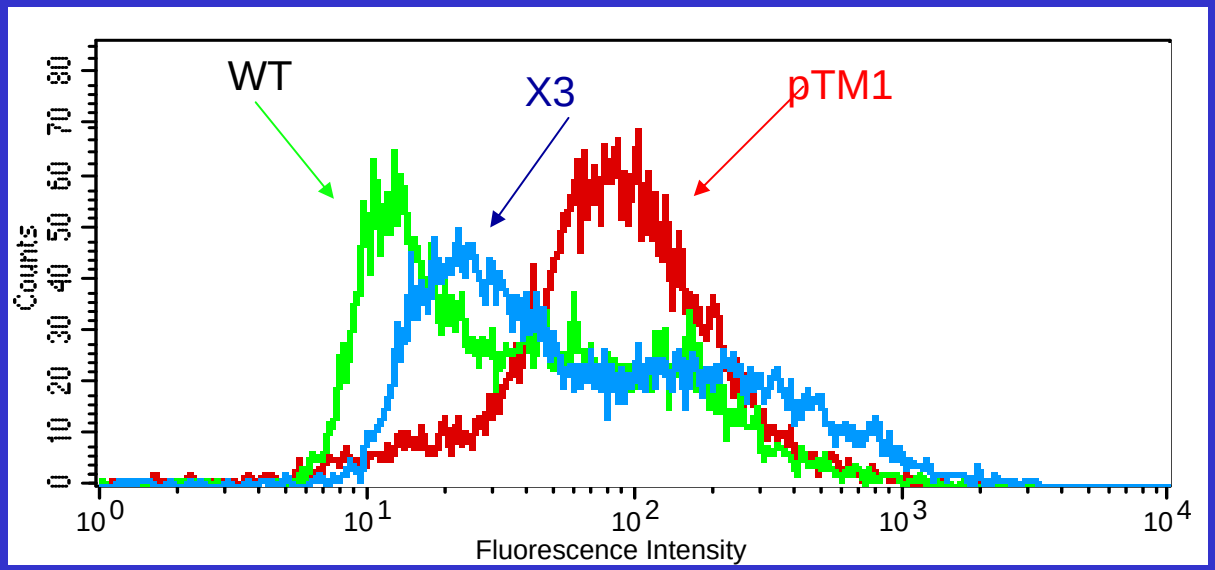
- Altered digoxin and fexofenadine pharmacokinetics
- Altered toxicity in transplant patients from cyclosporine A, tacrilimus
- Altered incidence of Crohn's disease, colon cancer, and Parkinson's disease

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

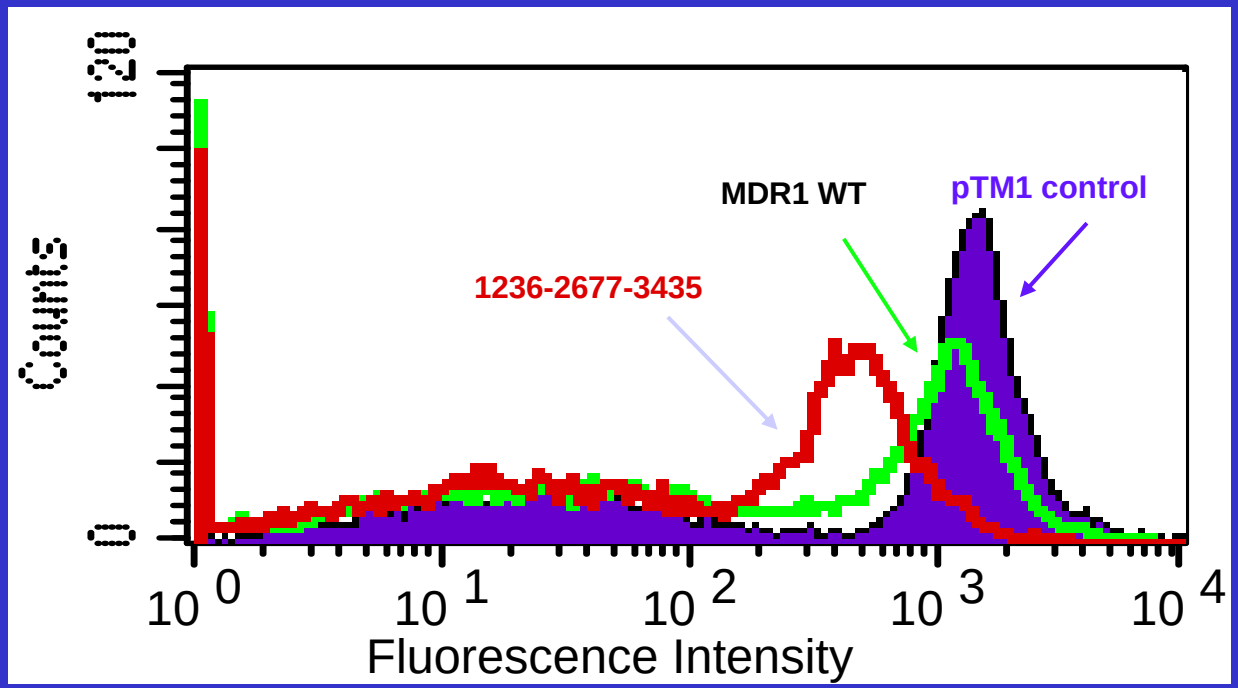




*MDR*1 wild-type and the haplotype (1236-2677-3435) do not exhibit similar Bodipy-verapamil accumulation

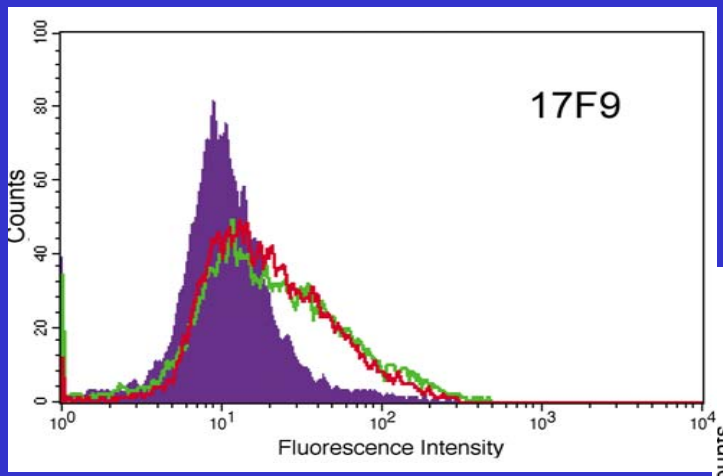


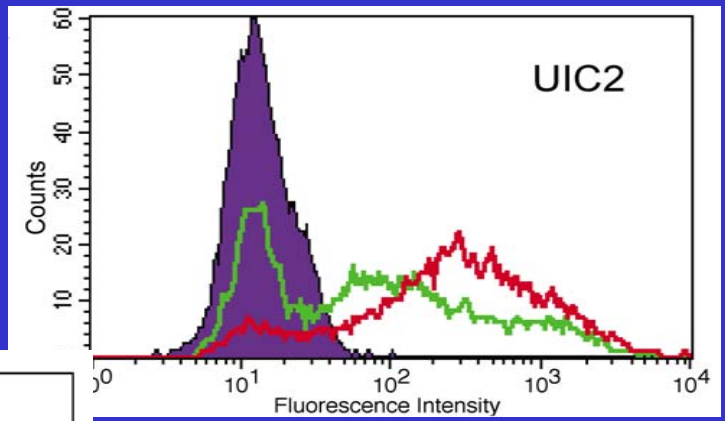
*MDR*1 wild-type and the haplotype exhibit different patterns using rhodamine 123 efflux with cyclosporin A reversing agent

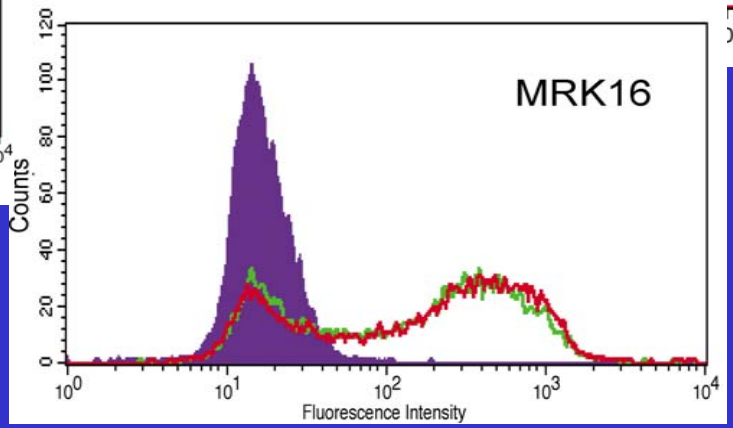


5 mM CsA

*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

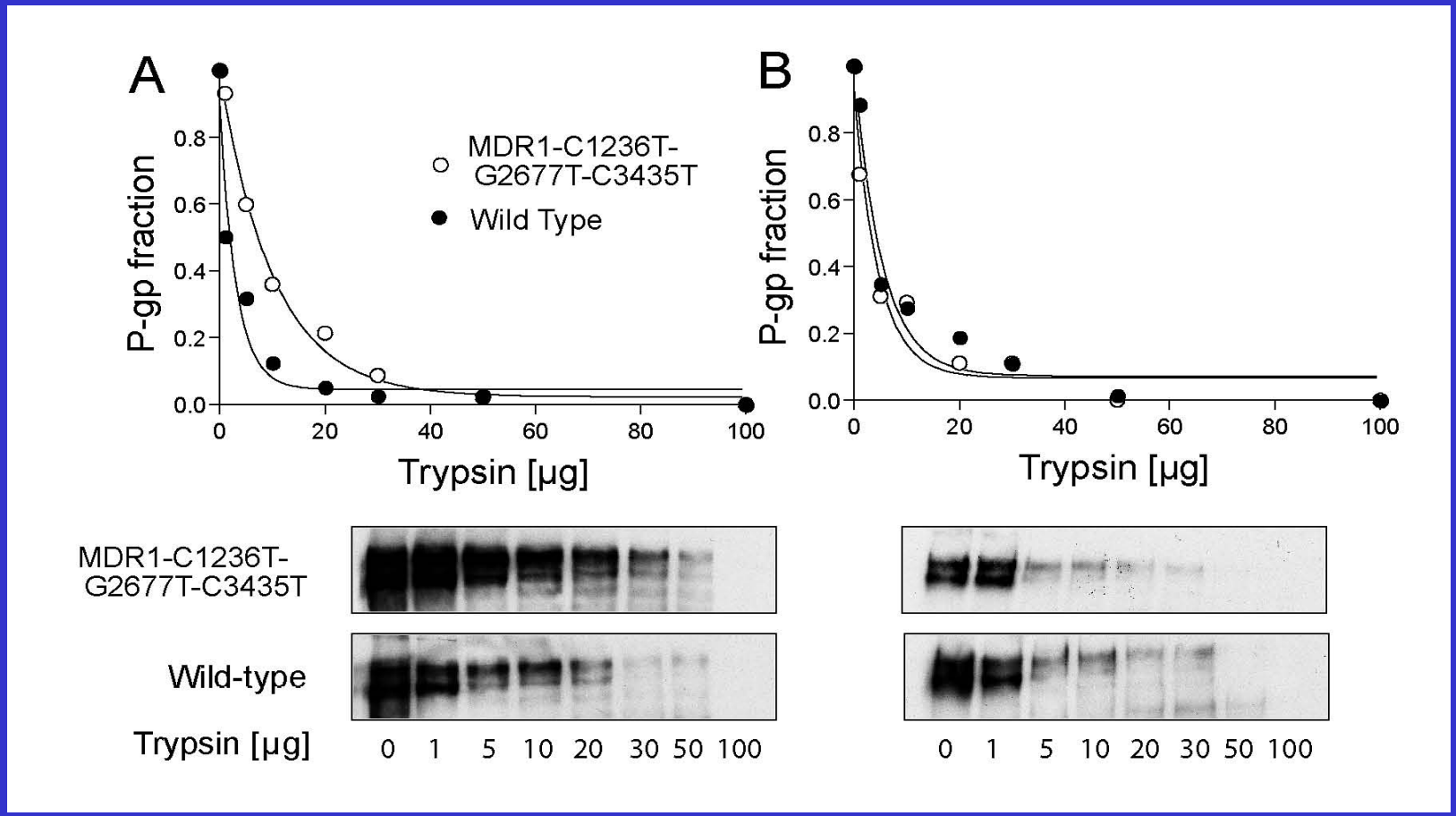






pTM1
WT
1236-2677-3435

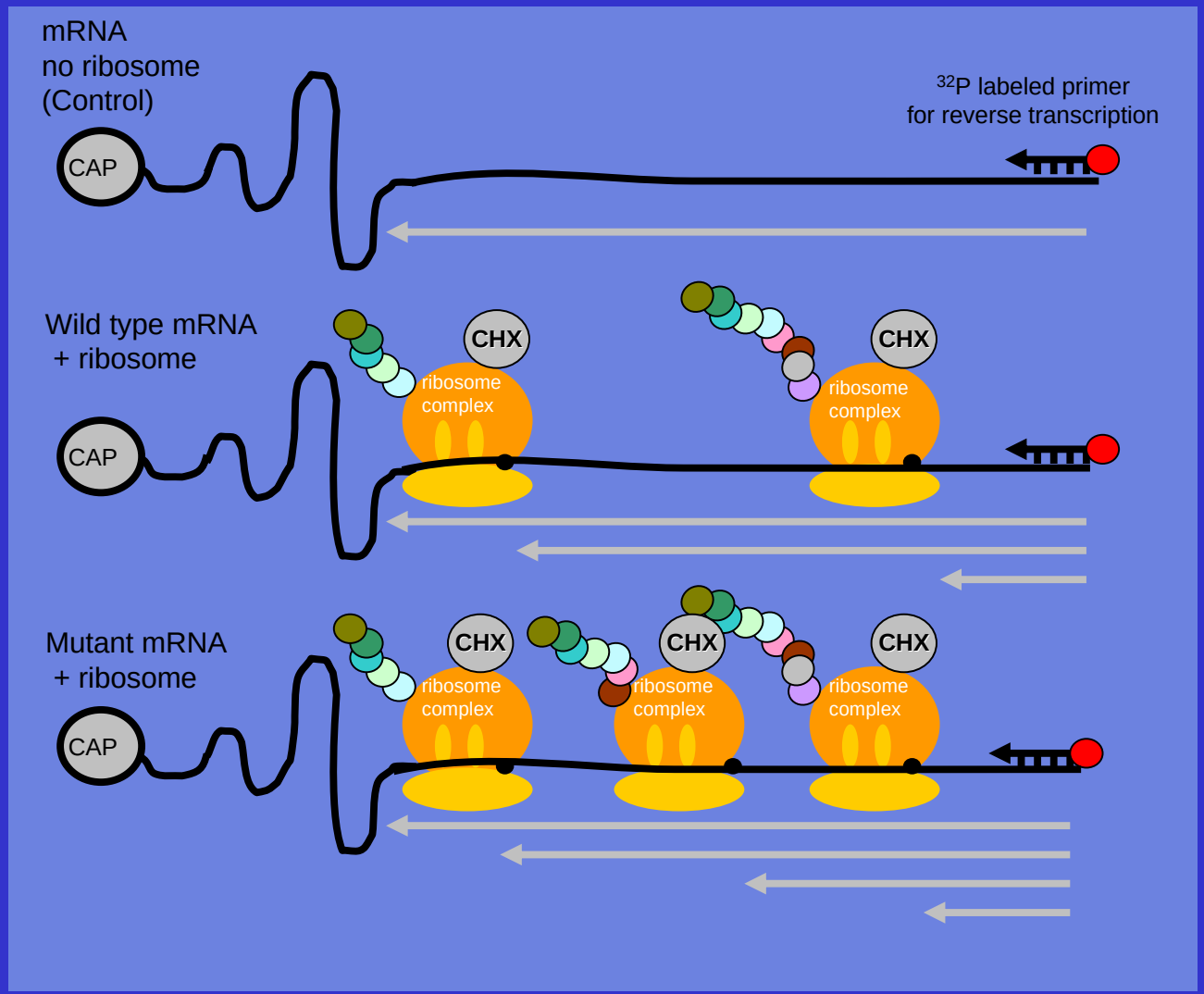
*MDR*1 wild-type and haplotype show different trypsinization patterns confirming altered conformation

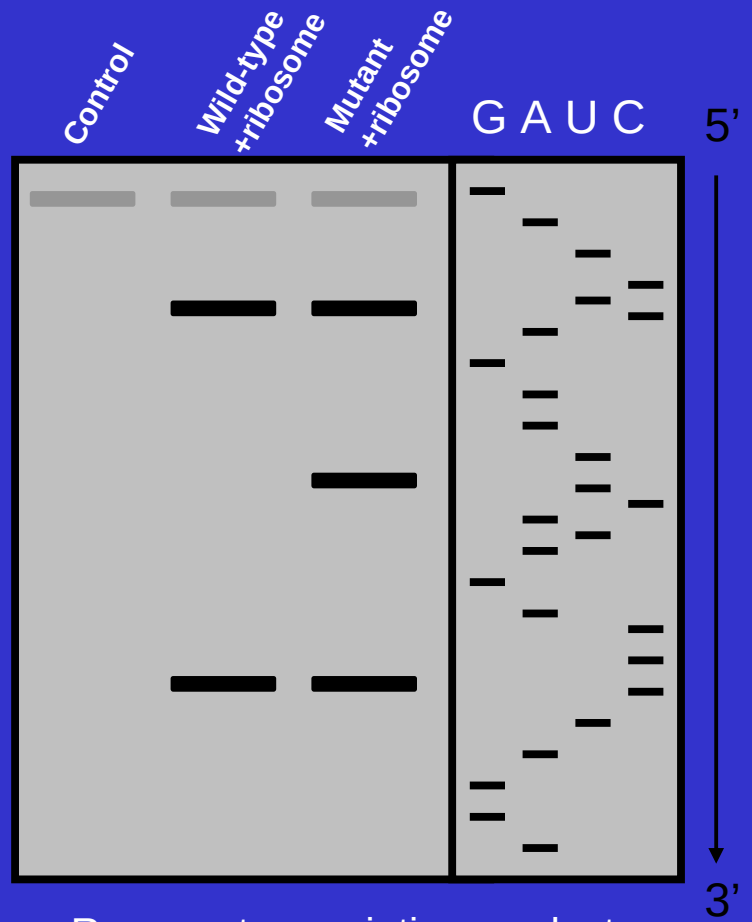


Polymorphic forms of P-gp with alleles that don’t change amino acid sequence change the conformation of P-gp

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

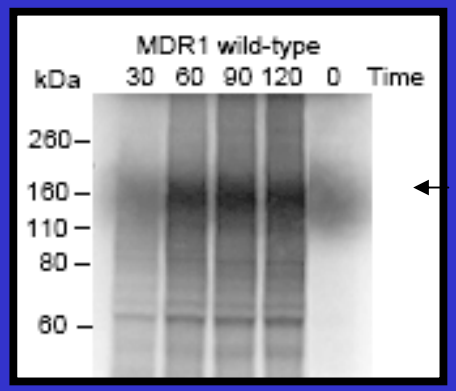




Reverse transcription products were resolved by polyacrylamide gel electrophoresis

Toeprint analysis of *MDR1* mRNA around the 3435 SNP

A

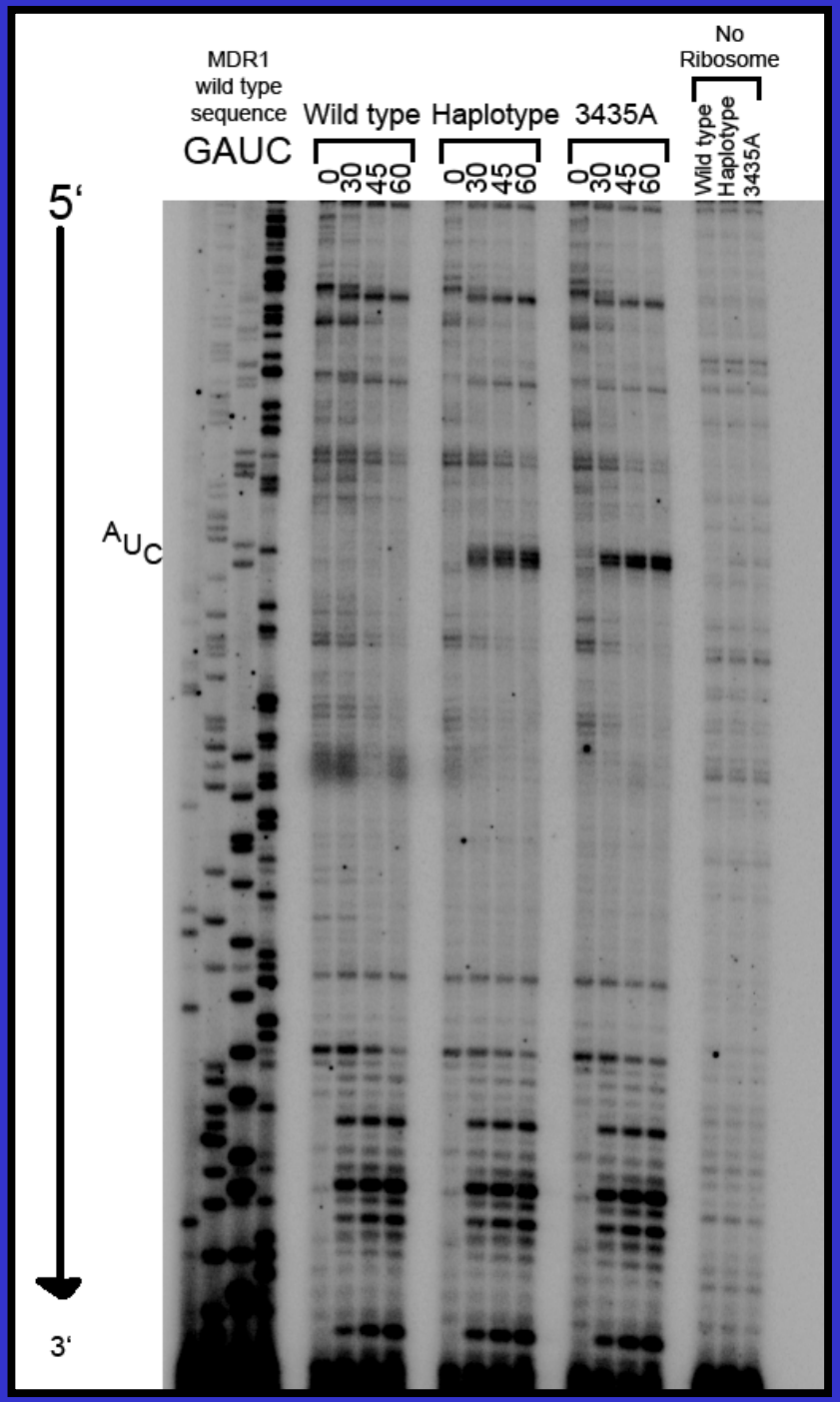


In vitro translated MDR1

B

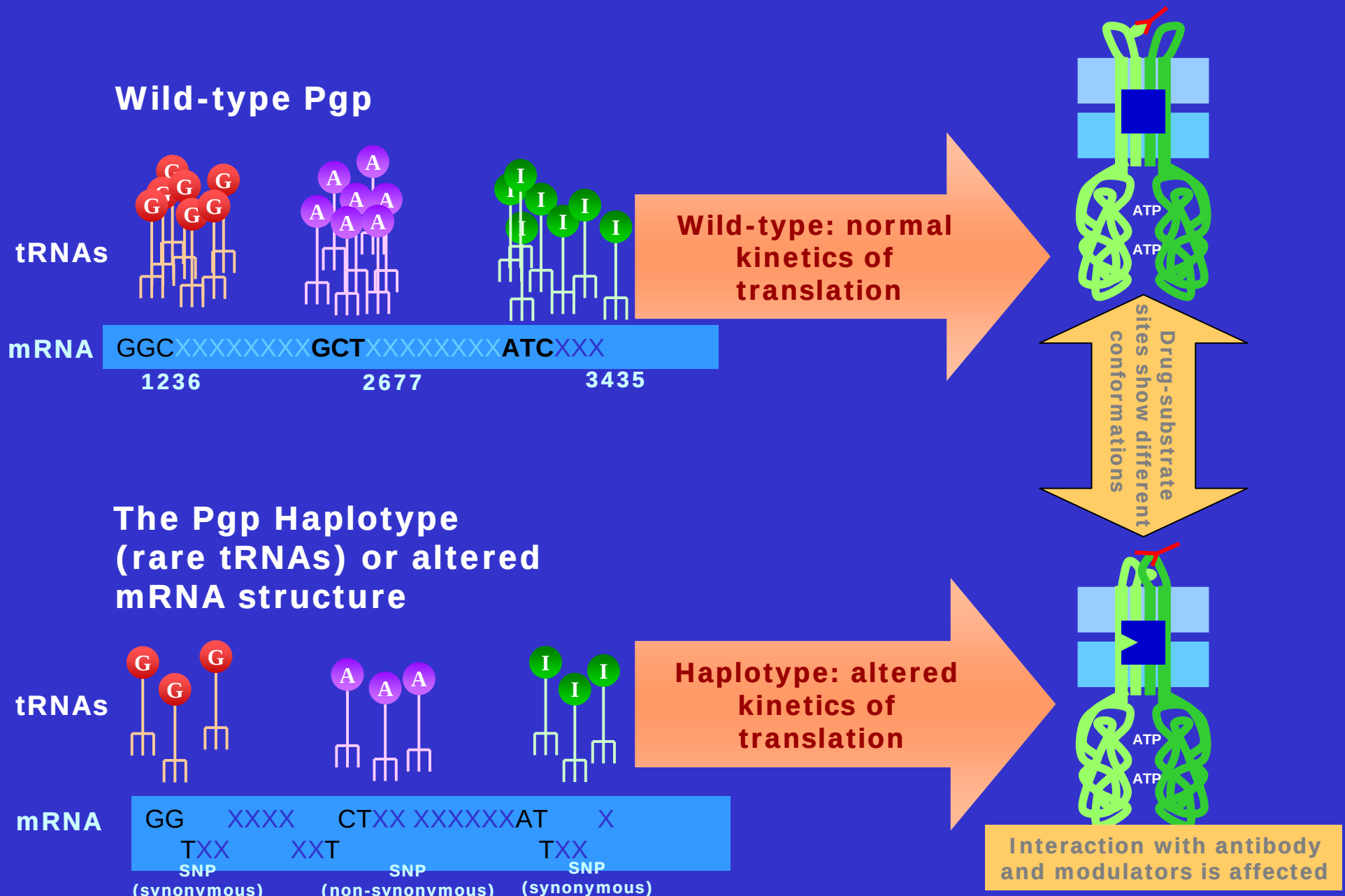
	Wild-type	SNP 3435T	SNP 3435A
Nucleotide	AUC	AUU	AUA
Codon usage	47%	35%	18%

C

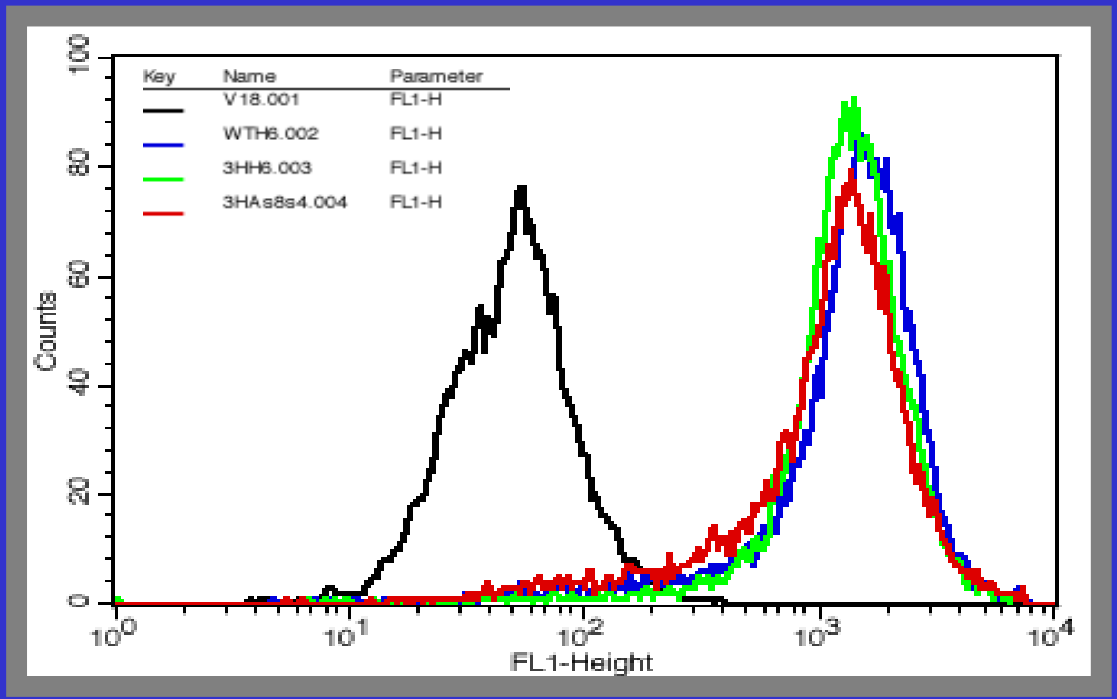


Toeprint signal

Synonymous SNPs affect P-glycoprotein conformation and function



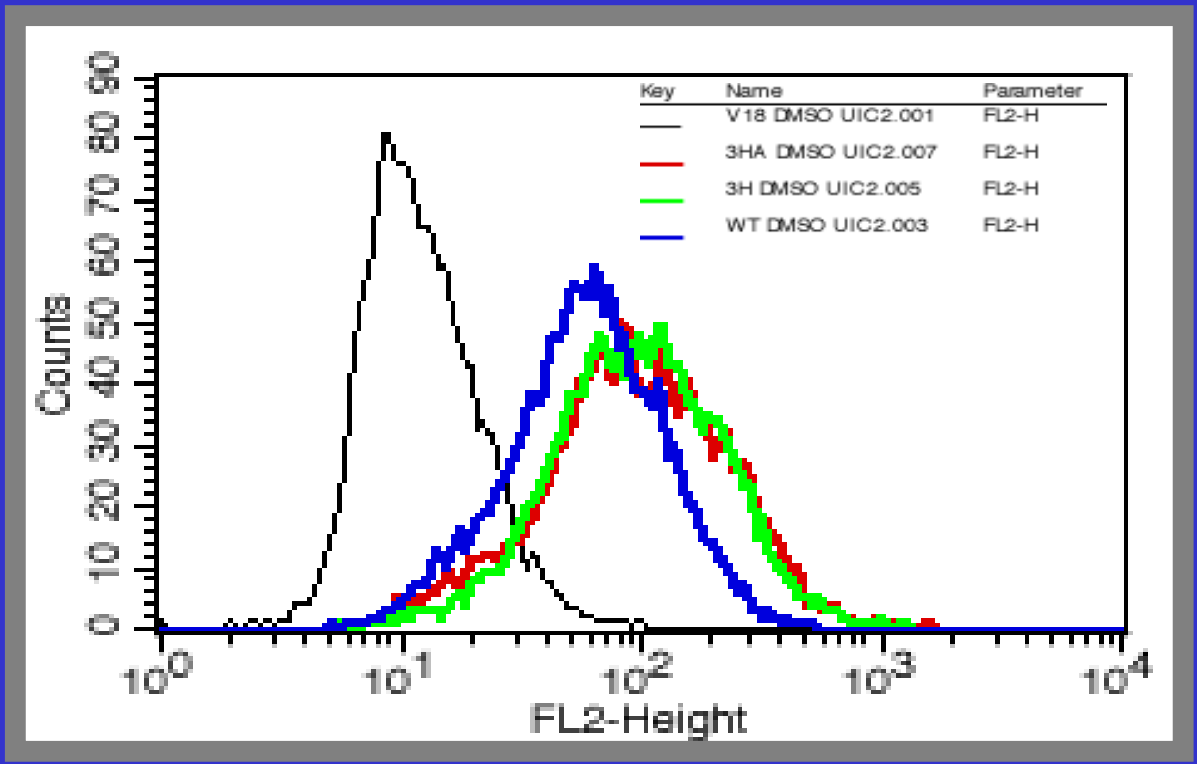
P-gp expression on cell surface (MRK16) of stably transfected LLC-PK1 cells



1^0 Antibody: MRK16

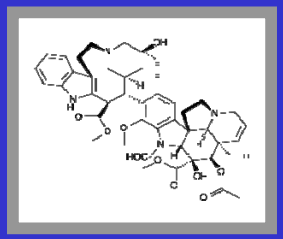
2^0 Antibody: Goat anti-mouse FITC

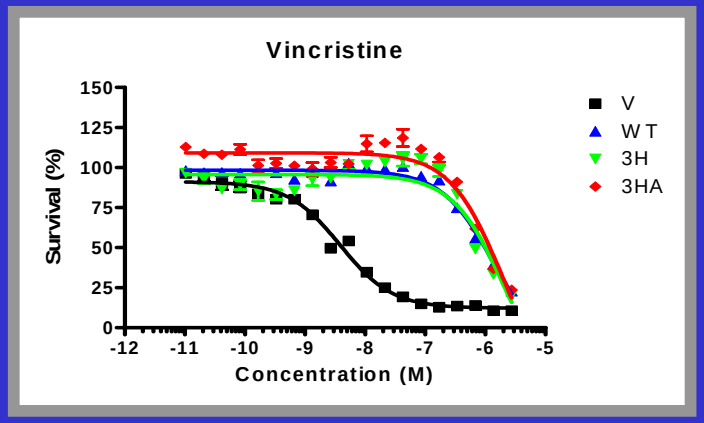
P-gp expression on cell surface (UIC2) of transfected LLC-PK1 cells

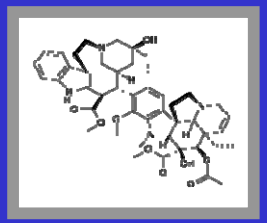


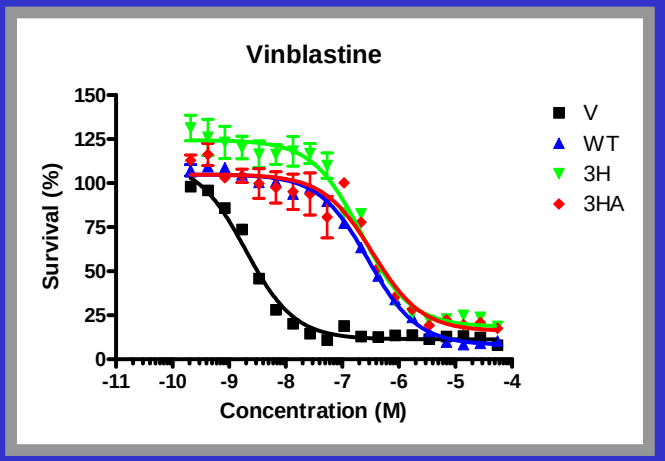
Conformation-sensitive Monoclonal antibody

Vinca-alkaloids

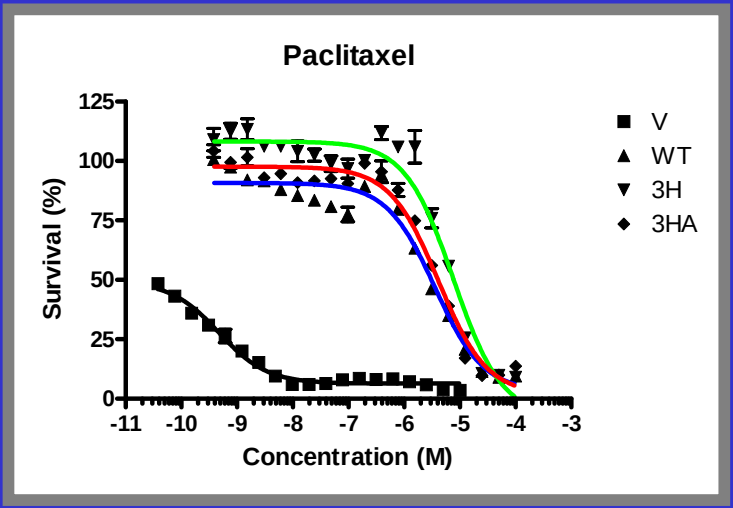


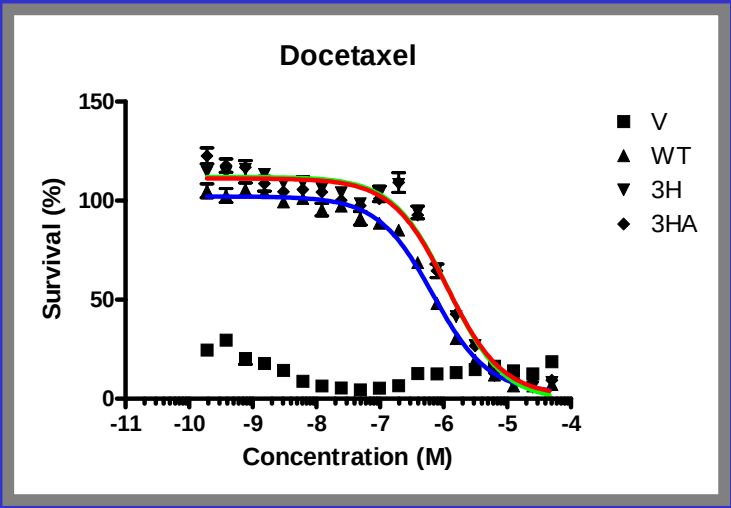




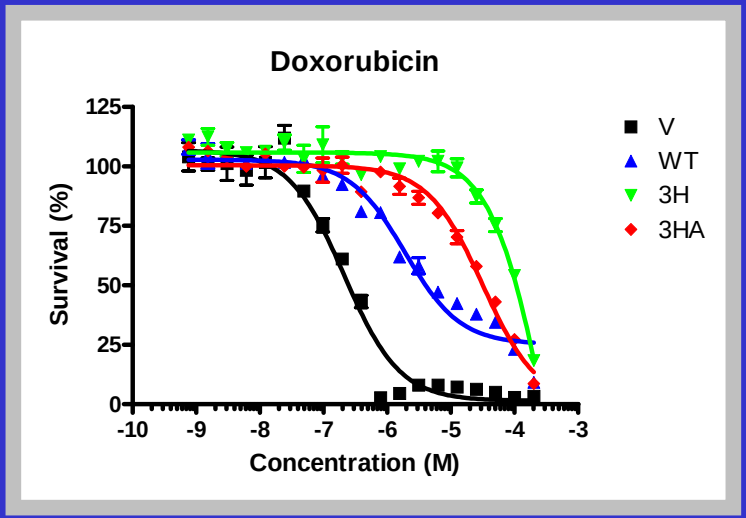


Taxol

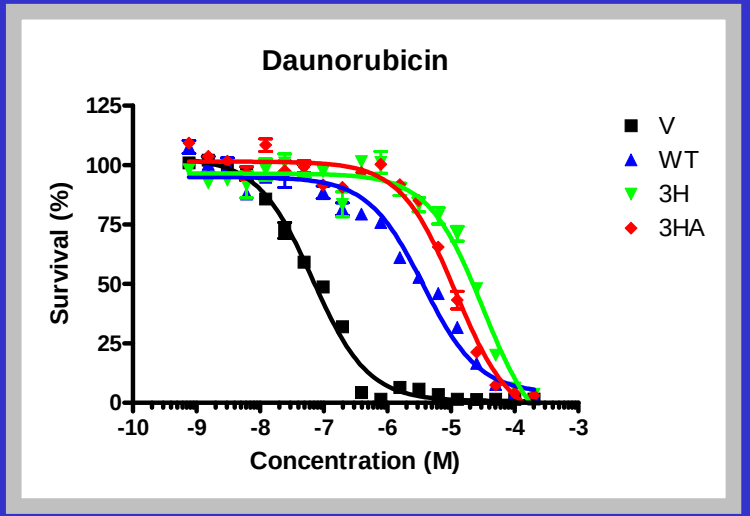

Paclitaxel
Survival (%)
125
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
Concentration (M)
V
WT
3H
3HA


Docetaxel
Survival (%)
150
100
50
0
-11
-10
-9
-8
-7
-6
-5
-4
Concentration (M)
V
WT
3H
3HA

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

		ABCA2	ABCB1	ABCC1	ABCC2	ABCC4	ABCG2	ABCB4	ABCB11	ABCC3	ABCC5	ABCC6	ABCC10	ABCC11
Vinca alkaloids	Vinblastine													
	Vincristine													
Anthracyclines	Daunorubicin													
	Doxorubicin													
	Epirubicin													
Epipodophyllotoxins	Etoposide													
	Teniposide													
Taxanes	Docetaxel													
	Paclitaxel													
Kinase inhibitors	Gleevec													
	Flavopiridol													
Campthotecins	Irinotecan (CPT-11)													
	SN-38													
	Topotecan													
Thiopurines	6-mercaptopurine													
	6-thioguanine													
	5-FU													
Other	Bisantrene													
	Cisplatin													
	Arsenite													
	Colchicine													
	Estramustine													
	Methotrexate													
	Mitoxantrone													
	Saquinivir													
	PMEA													
	Actinomycin-D													
	AZT													
	Digoxin													

Confers resistance

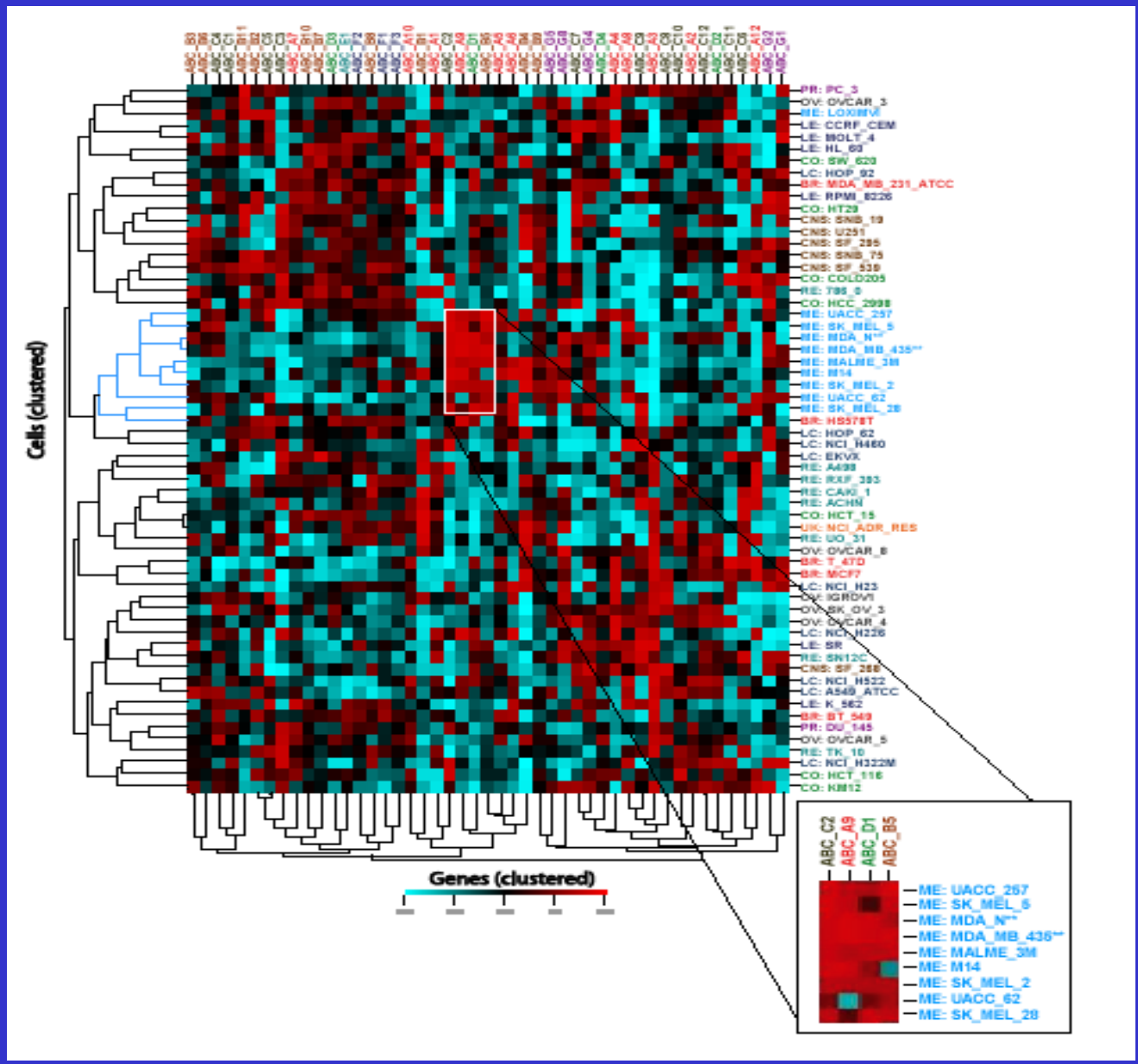
Selected

Doesn't transport

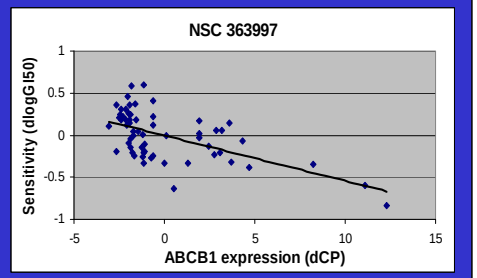
Can we discover new drugs that interact with ABC transporter genes?

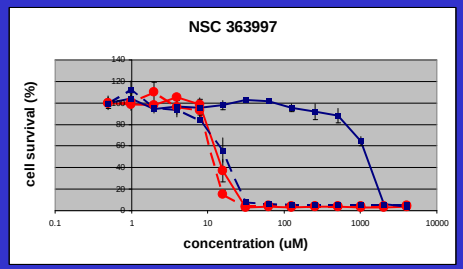
- Use Real Time (RT)-PCR to measure ABC mRNA levels for 48 ABC transporters and 23 solute carrier proteins
- Exploit NCI-60 cell line database, with known resistance to 100,000 different drugs, to correlate patterns of drug-resistance and expression of these transporters

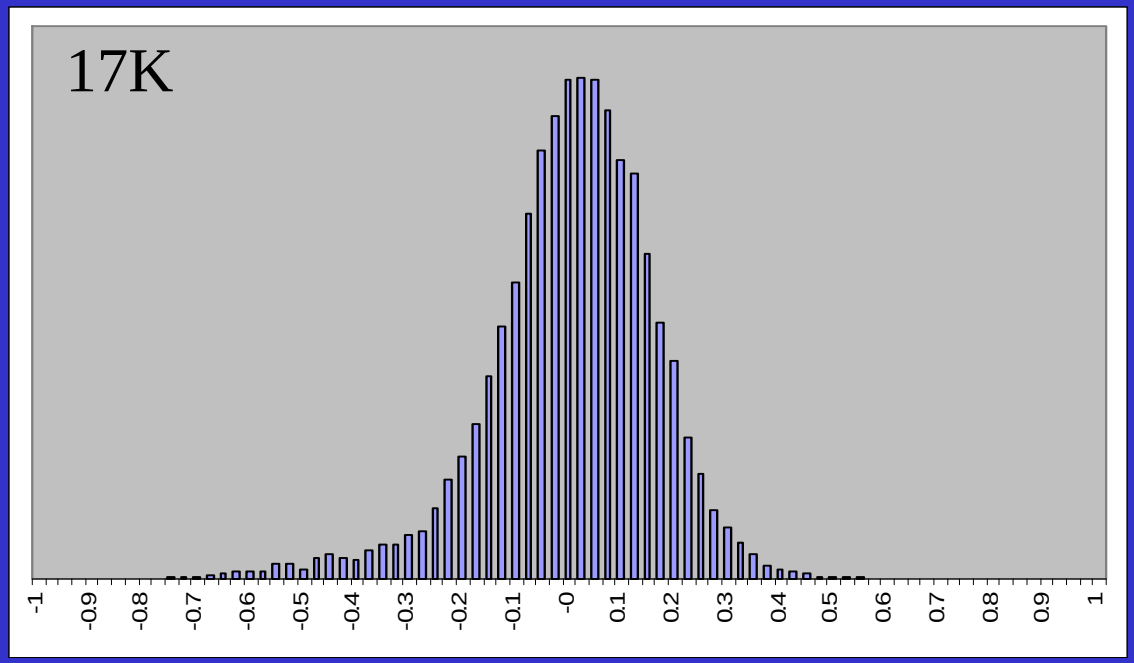
Expression of ABC-transporters in the NCI60 panel



Search for MDR1-potentiated compounds in DTP's database





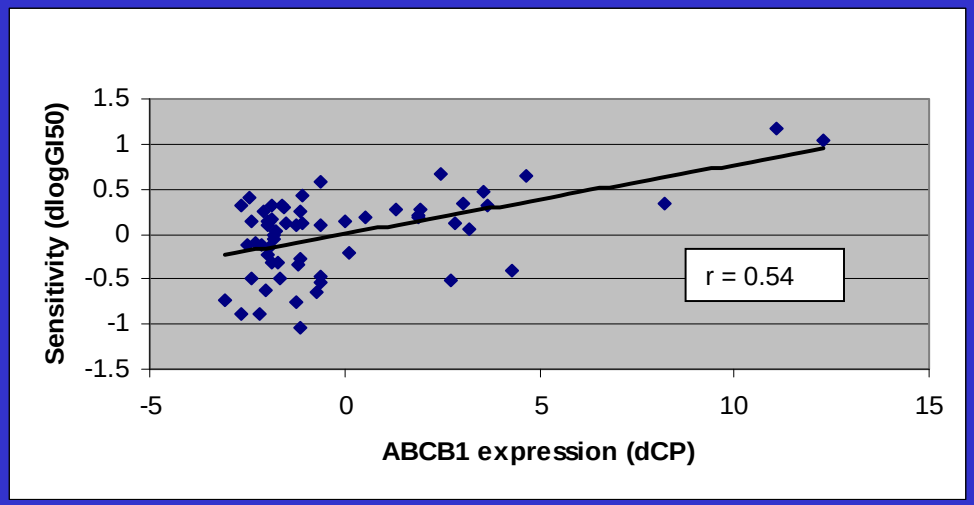


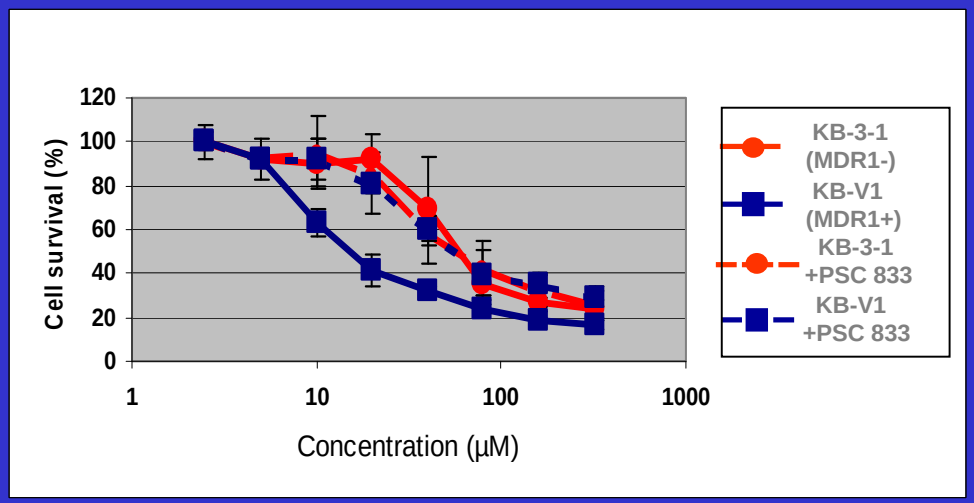
NSC 73306

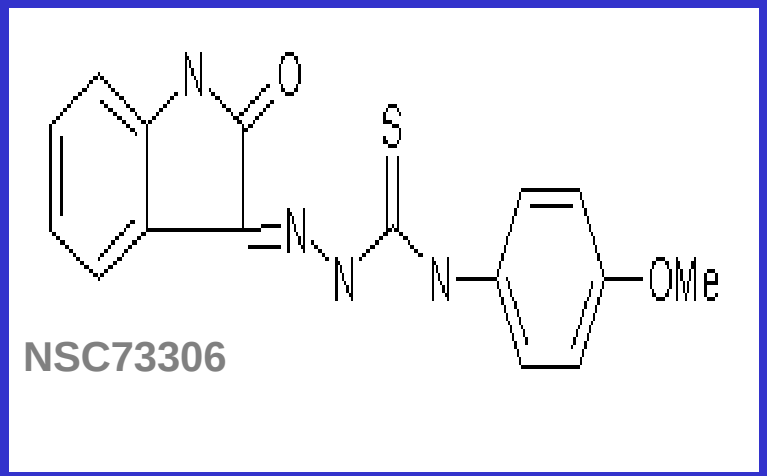
Substrates

MDR1-potentiated compounds?

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





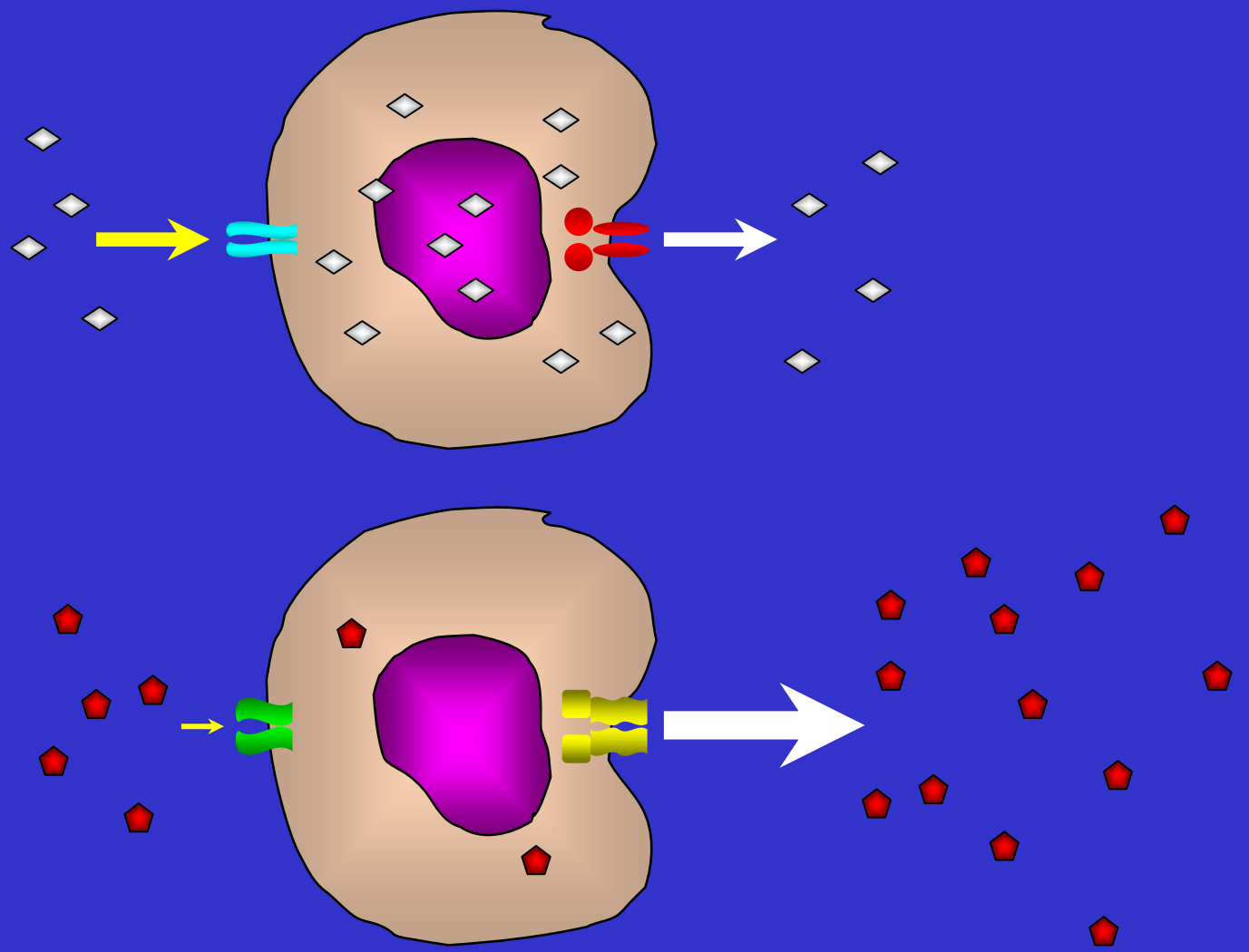


Potential Clinical Utility of Discovery of Compounds that Specifically Kill MDR1-Expressing Cells

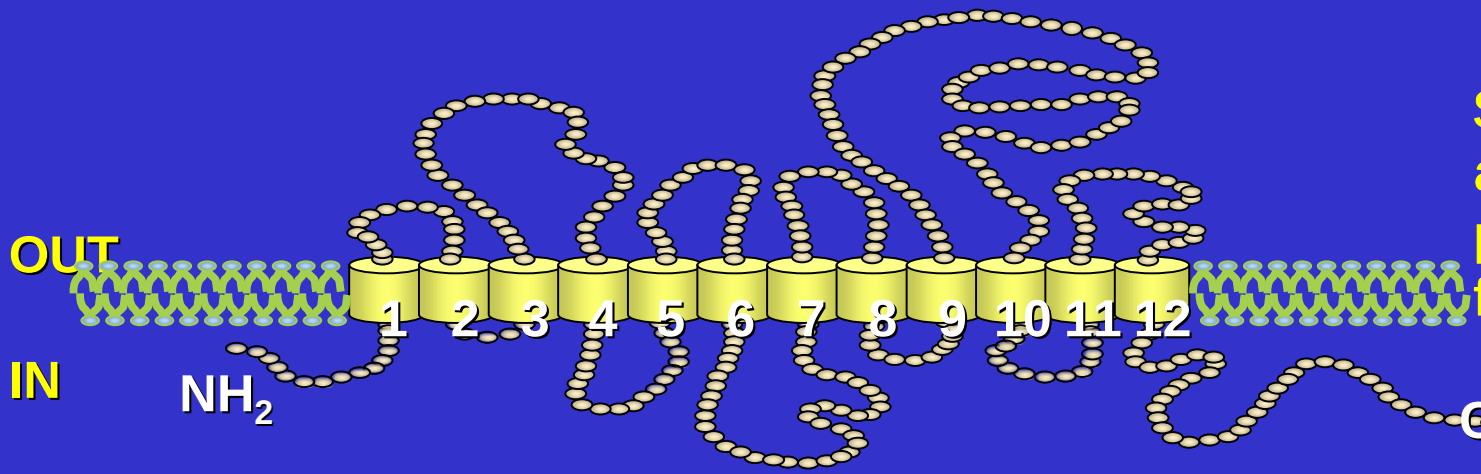
Can be used in combination with standard chemotherapy to eliminate MDR1-expressing cell populations

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

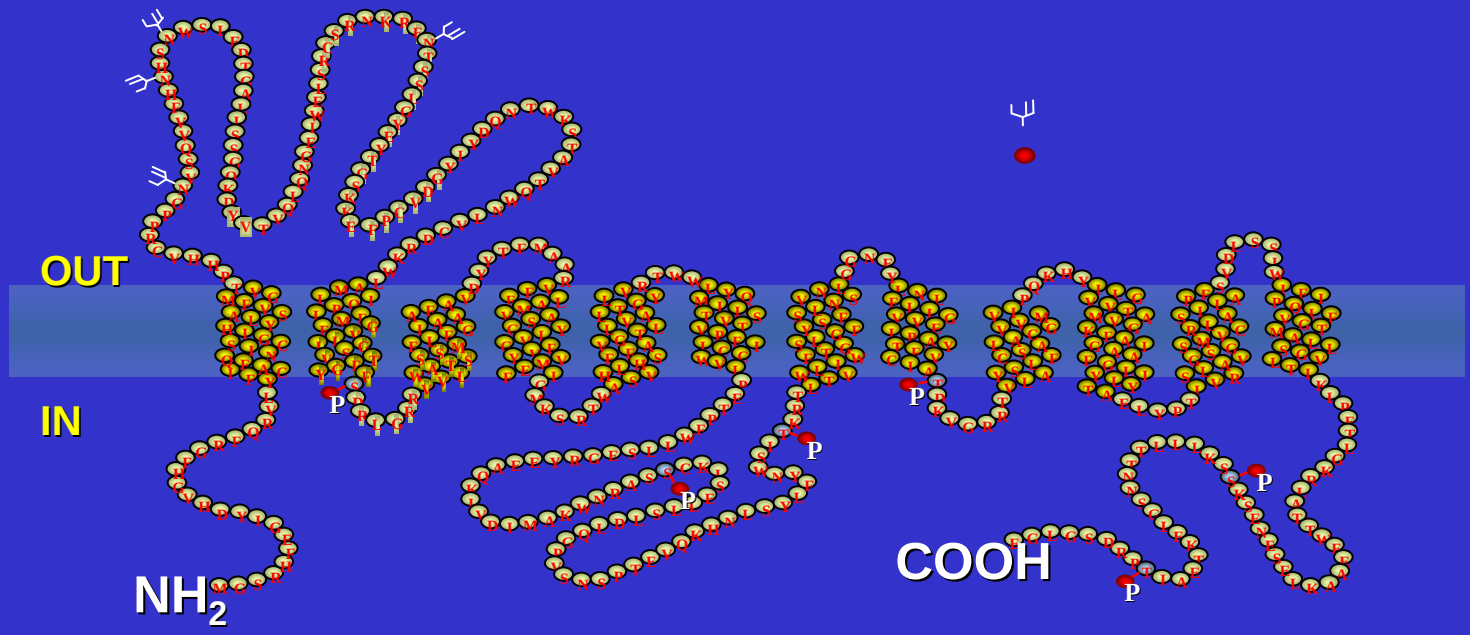
Balance of uptake and efflux determines drug accumulation in cancer cells



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

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

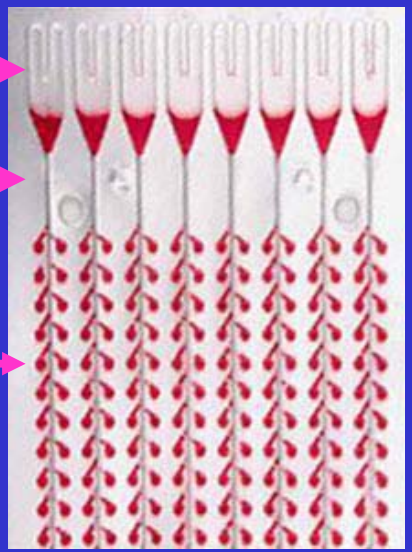
Goal: To correlate expression of drug-resistance genes with response to chemotherapy in clinical samples

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

Port

Channel

Each well contains a pair of primers and a probe

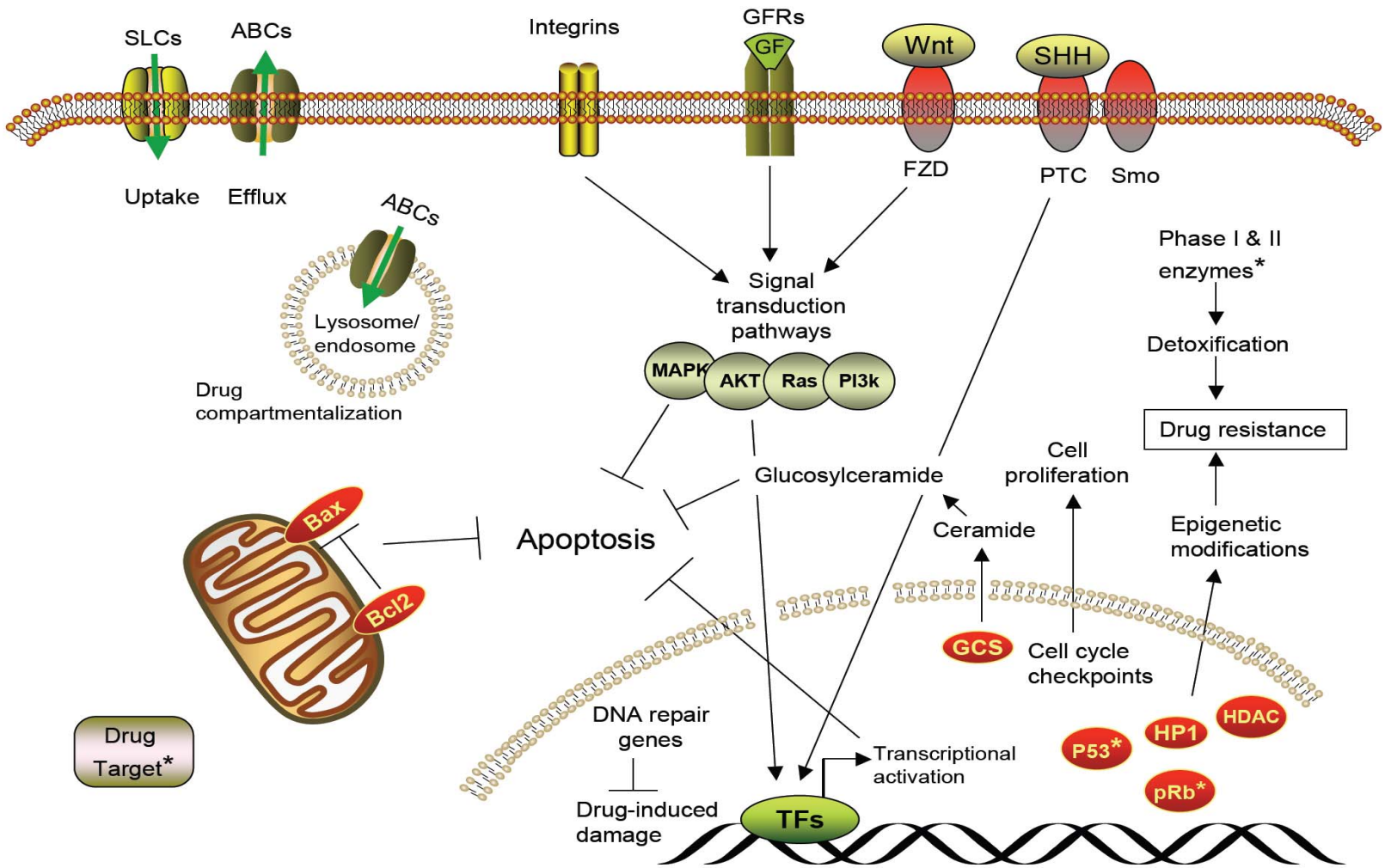


1 to 8 samples

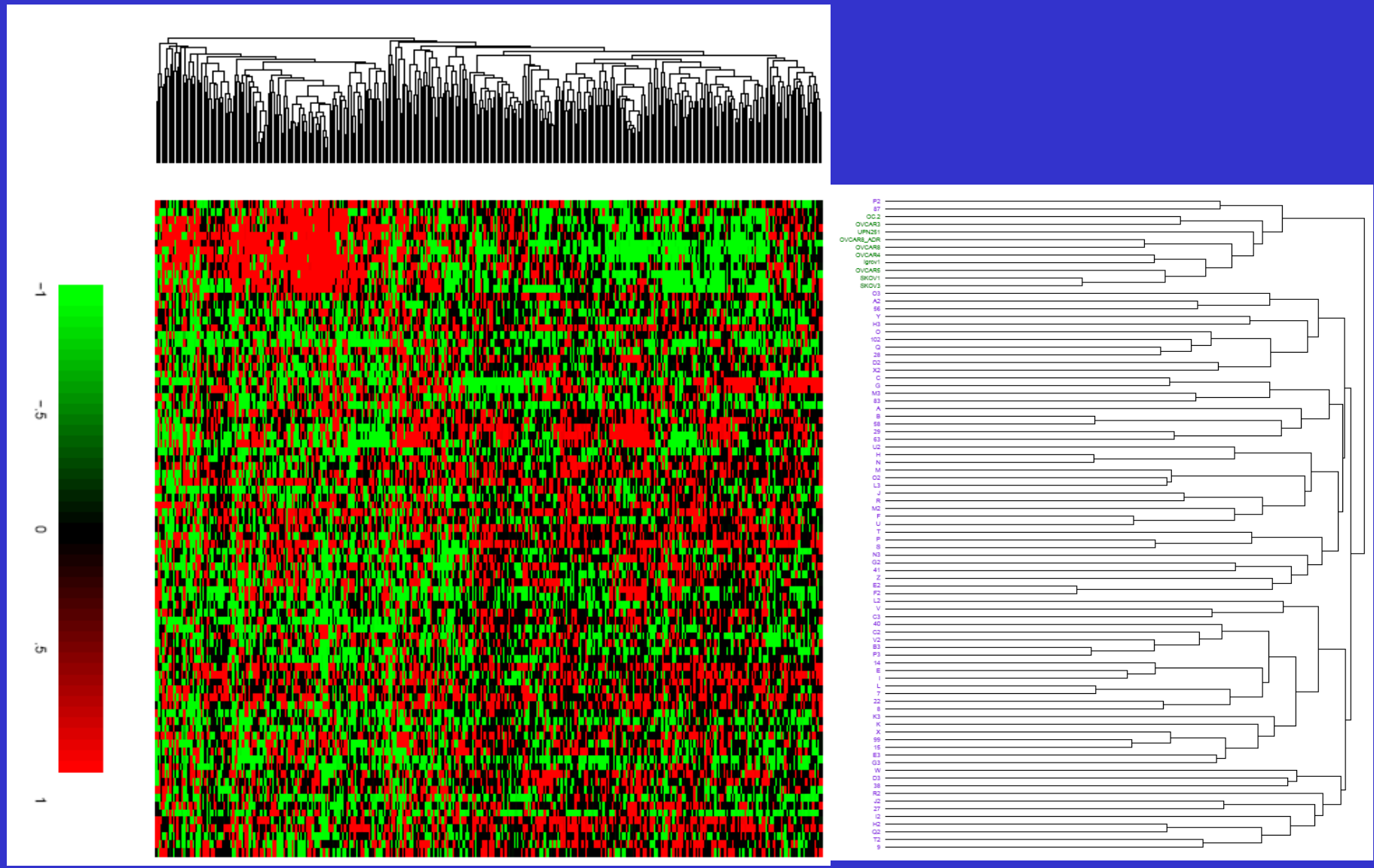
12 to 380 targets

1, 2 or 4 replicates

The Anti-Cancer Drug Resistance Transcriptome



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



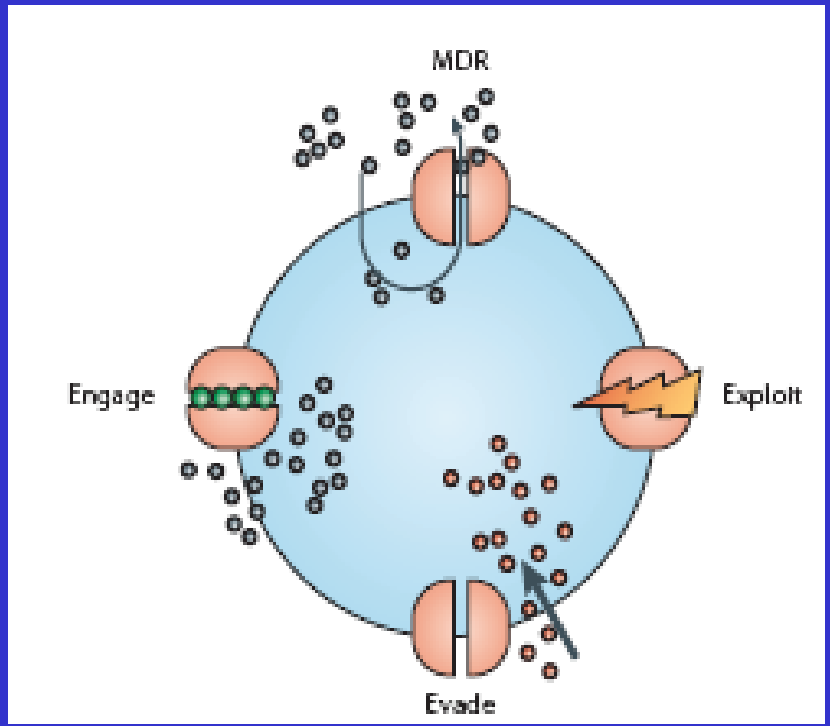
Strategies for dealing with MDR1-mediated multidrug resistance

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

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