# Timing and application of transporter studies in drug discovery & development

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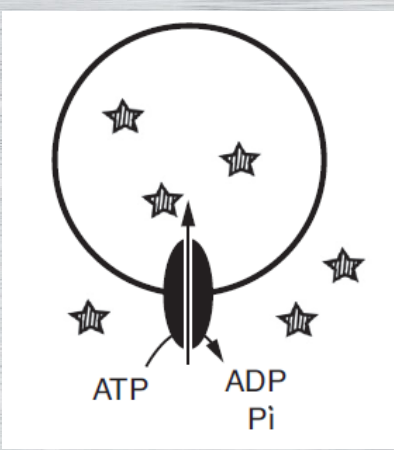
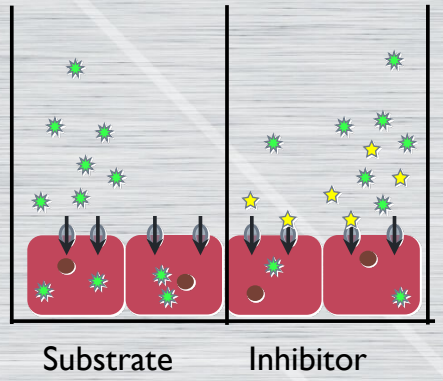
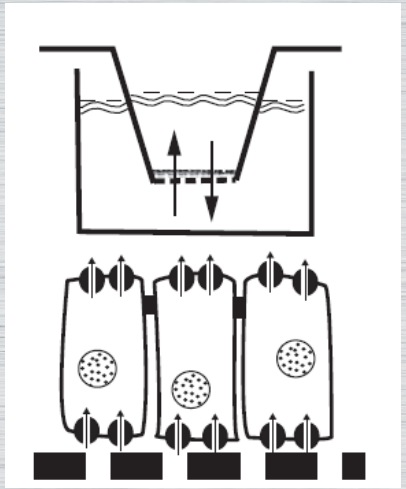
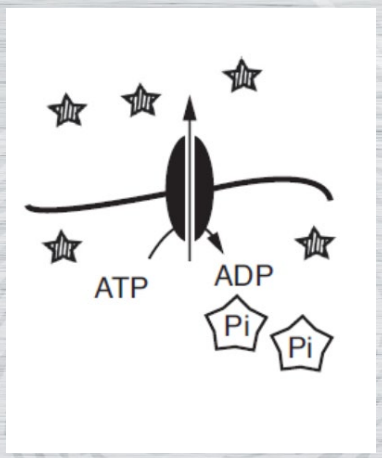
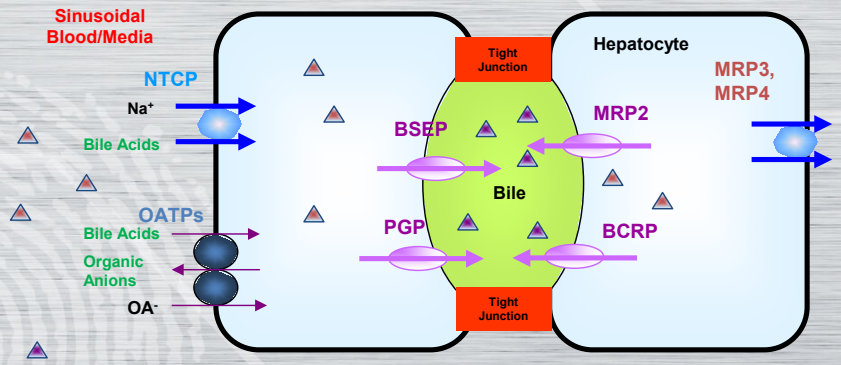
# Multiple methods available for studying transporters

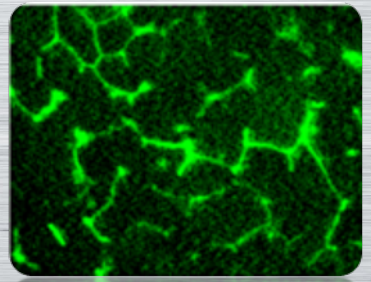




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why

# When should transporters be studied?

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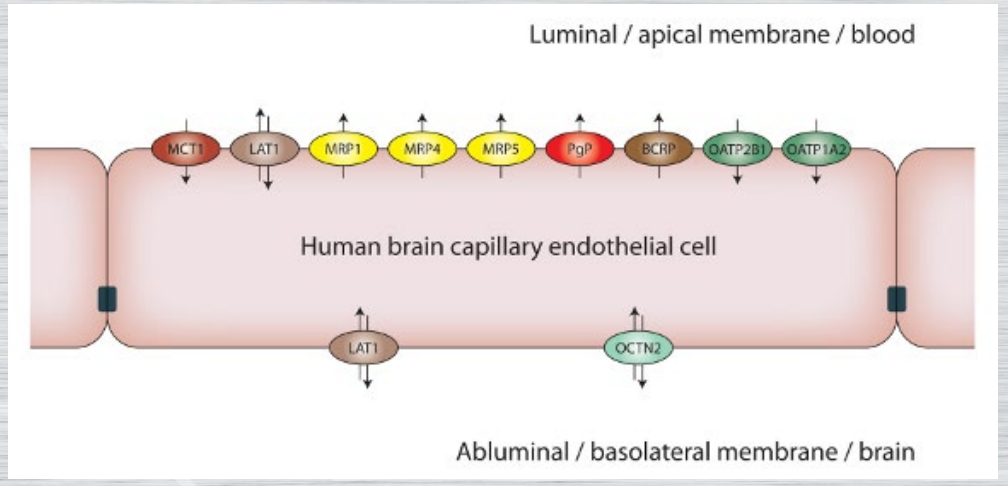
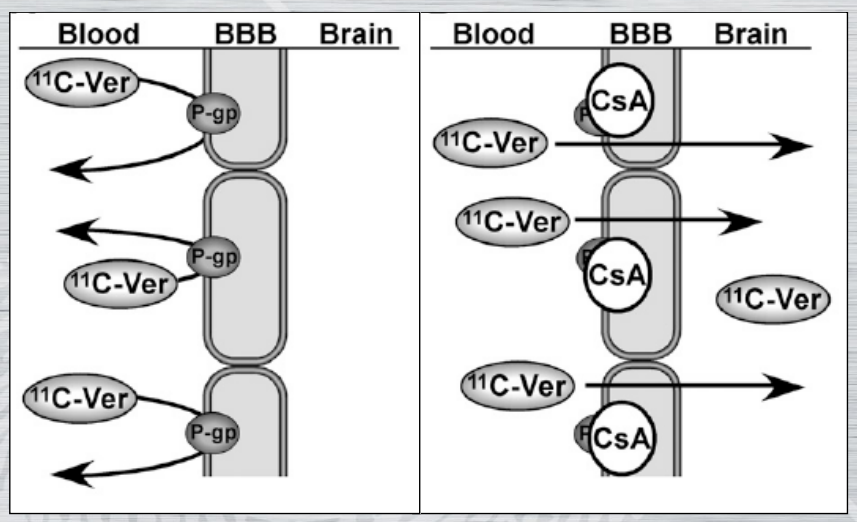
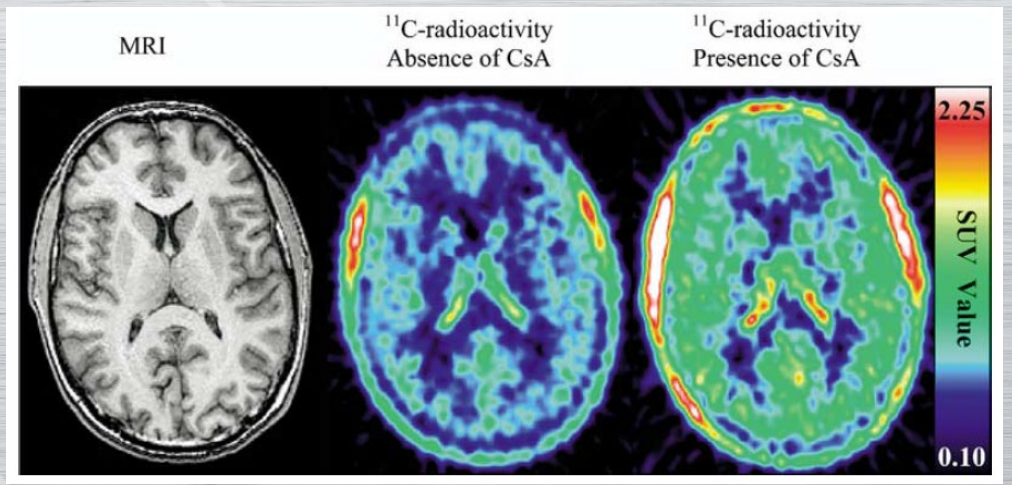
# Transporters can limit tissue distribution

## CNS Drugs

- P-glycoprotein (P-gp/MDR1;ABCB1)
  - Efflux transporter expressed on luminal side of blood-brain barrier
  - Prevents entry to CNS for transported substrates
  - PET imaging of $^{11}$C-labeled verapamil (P-gp substrate) shows marked increase in brain accumulation following co-administration with cyclosporine A (P-gp inhibitor)



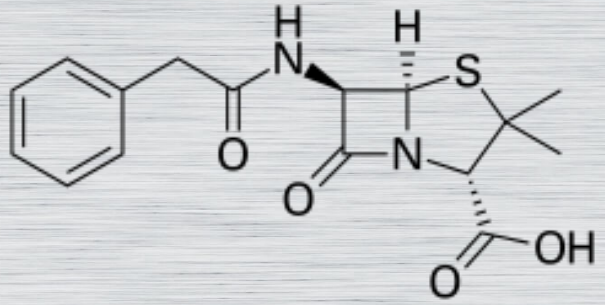



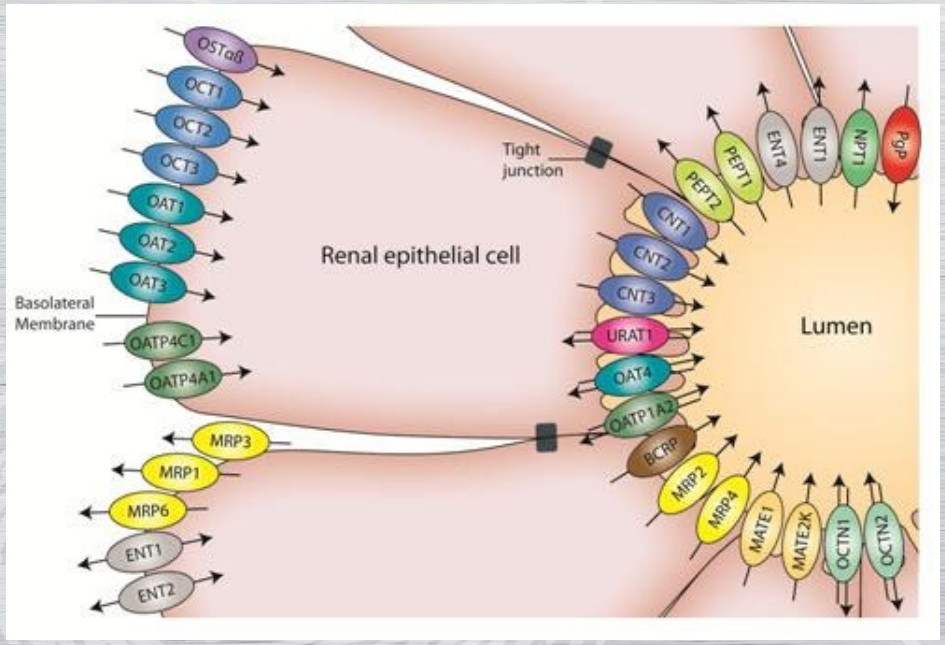

Sasongko *et al. (2005)*, Clin. Pharmacol. Ther., 77: 503-514; Muzi *et al. (2009)*, J. Nucl. Med., 50: 1267-1275

# Transporters can mediate drug clearance

## Benzylpenicillin (Penicillin G)

- Discovered in 1929
- Rapidly cleared via renal elimination (half life ~30 minutes)
- Supply so limited during early use, drug was re-isolated from urine of patients

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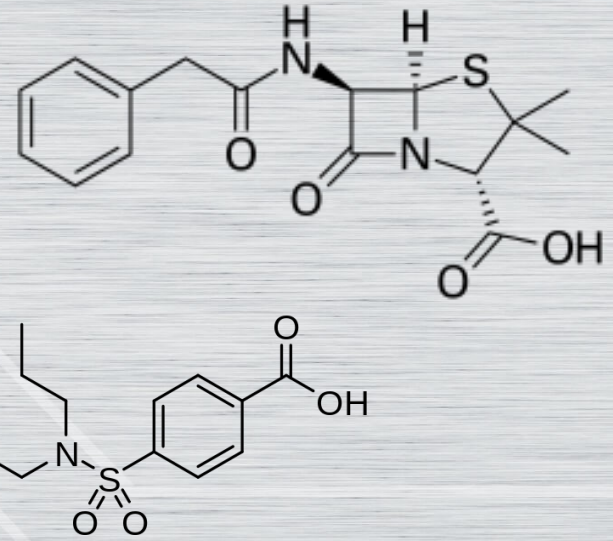
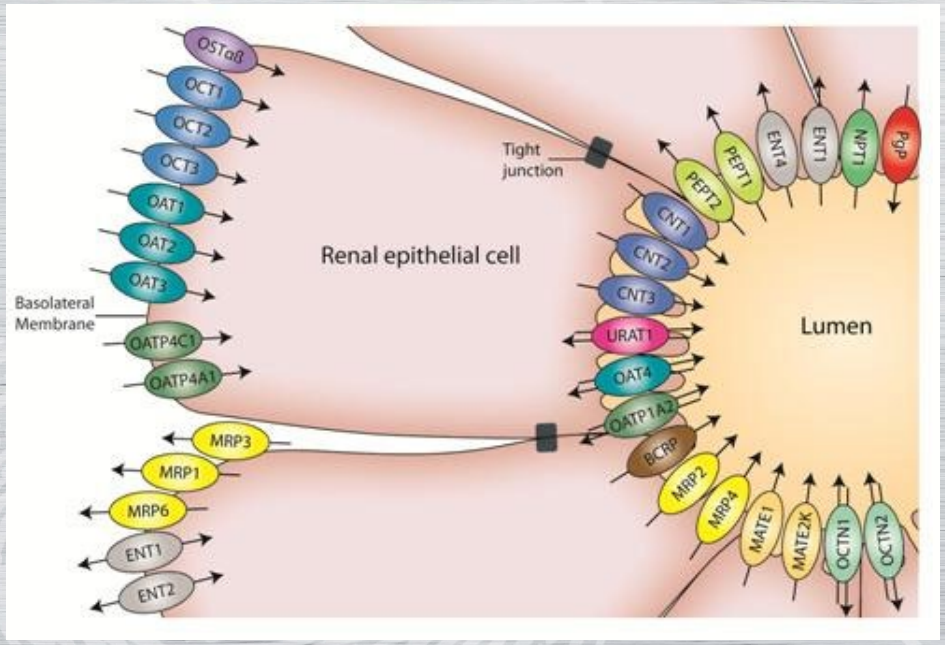
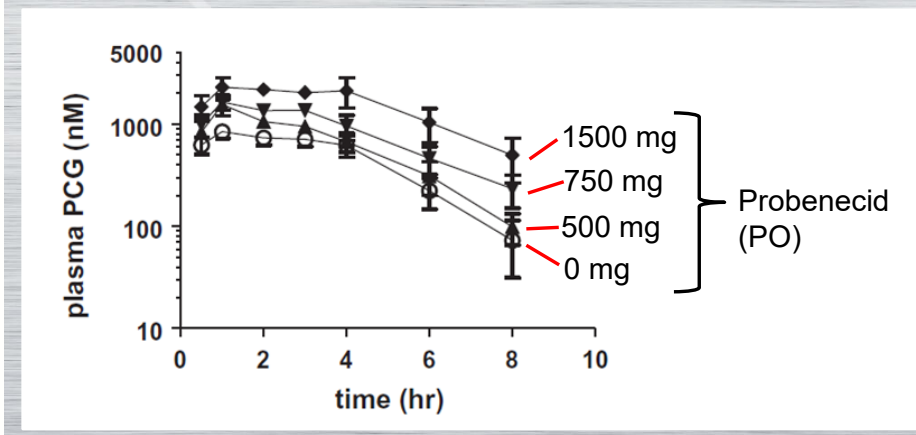
# Transporters can mediate drug clearance

## Benzylpenicillin (Penicillin G) + Probenecid

- 3.3-fold increase in benzylpenicillin (PCG) AUC at highest dose of probenecid
- Renal clearance equal to GFR at highest dose (no active secretion of PCG)



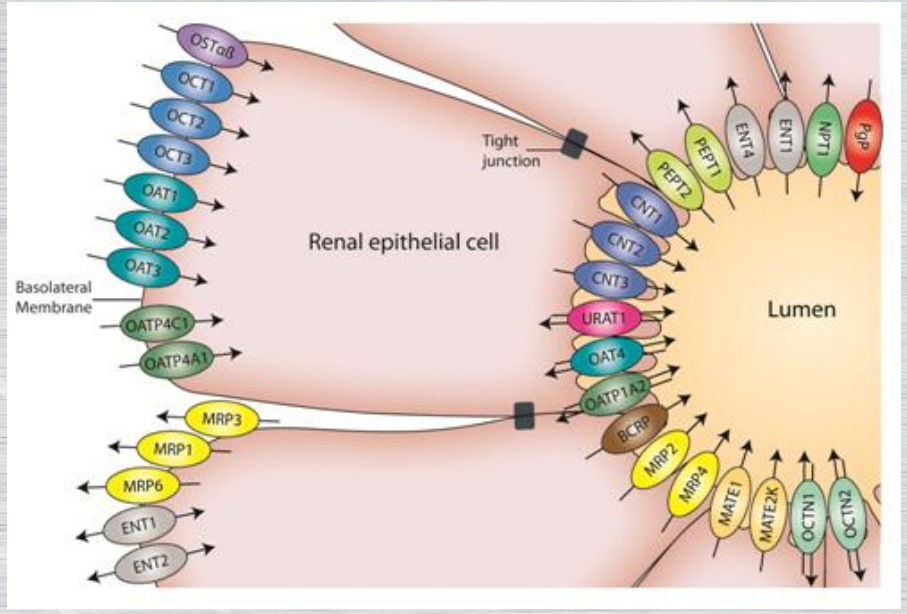




Maeda *et al. (2014)*, Eur. J. Pharm. Sci., 59: 94-103

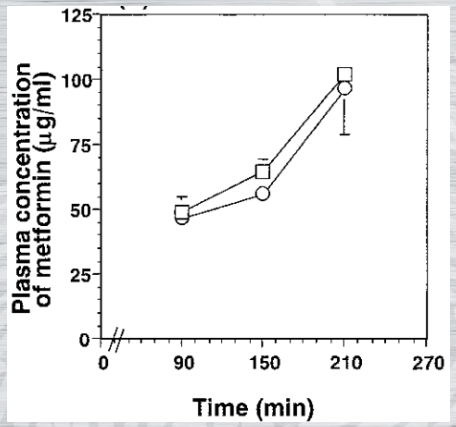
# Transporters can mediate tissue distribution

## Metformin

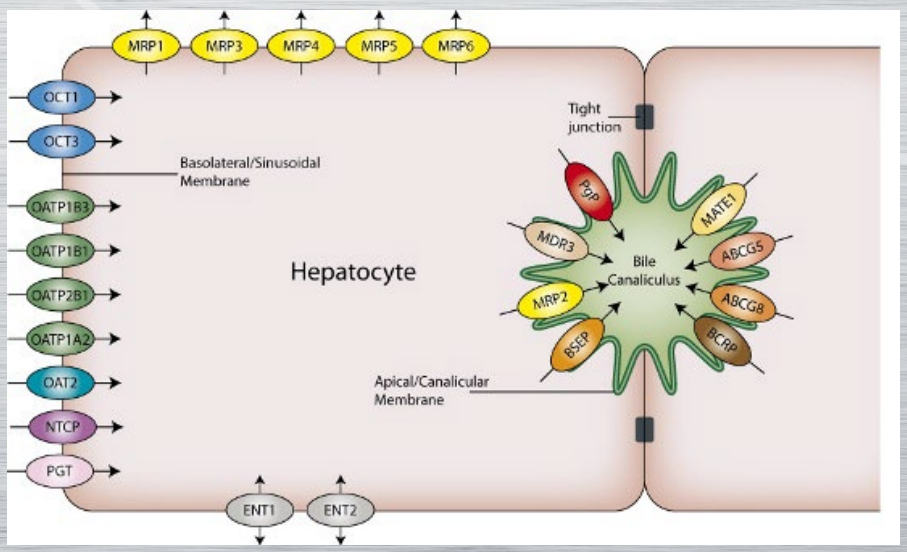
- First line therapy for type 2 diabetes
- Site of action- liver
- Primarily excreted in urine (active secretion by OCTs & MATEs)




| | Liver | |
|---|---|---|
| | $C_{plasma}$ | $C_{liver}$* |
| | $\mu g/ml$ | $\mu g/g$ |
| Wild-type | 102 ± 11 | 417 ± 178 |
| Oct(−/−) | 97.7 ± 15.1 | 49.3 ± 10.4 |




- OCT1(-/-) mice show no change in metformin plasma AUC
- ~90 % reduction in liver distribution

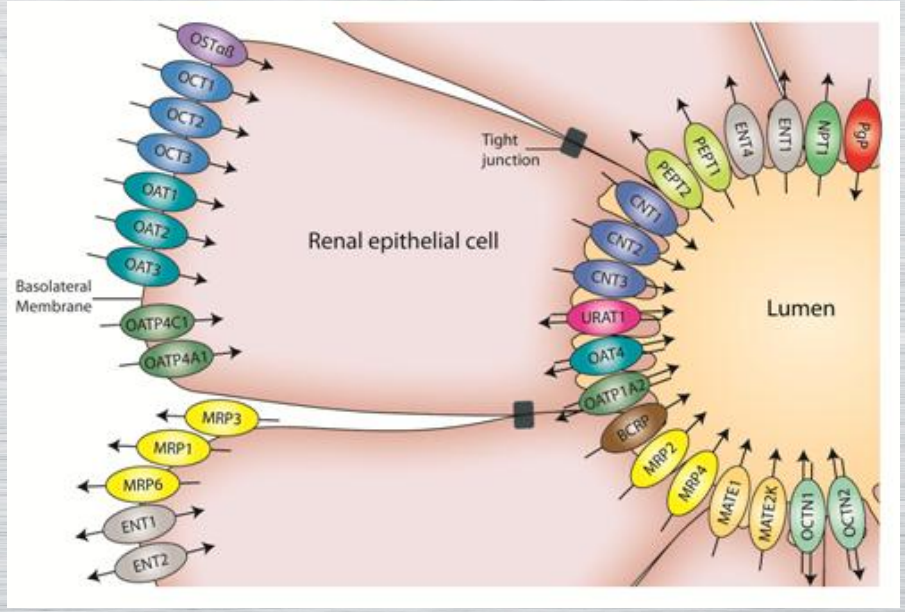



Wang *et al. (2003)*, Mol. Pharmacol., 63(4): 844-848

# Transporters as targets

## Transporters as targets

- Transporters are being harnessed as drug targets
  - Sodium-glucose cotransporter 2 (SGLT2; SLC5A2)
    - Canagliflozin (Invokana)
    - Dapagliflozin (Farxiga)
    - Empagliflozin (Jardinace)
  - Uric acid transporter 1 (URAT1; SLC22A12)
    - Lesinurad (Zurampic)
- Transporters are being utilized to improve absorption/distribution profile
  - Peptide transporter 1 (PEPT1; SLC15A1)
    - Valacyclovir
    - valganciclovir

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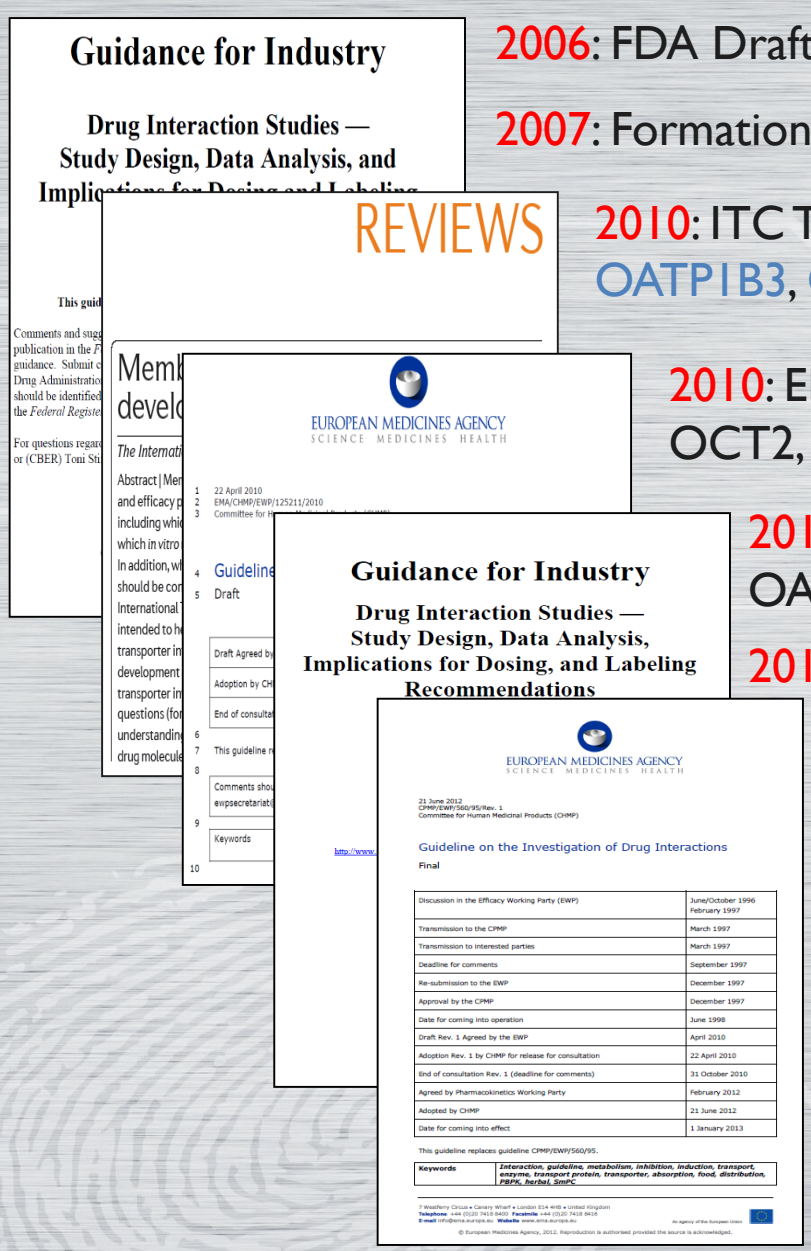
# A changing regulatory environment




2006: FDA Draft Guidance (P-gp)

2007: Formation of International Transporter Consortium (ITC)

2010: ITC Transporter White Paper (P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3)

2010: EMA Draft Guidance (P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, BSEP, OCT1)

2012: Revised FDA Draft Guidance (P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, BSEP, MATEs, MRPs)

2013: Seven ITC Whitepapers Published

2013: Final EMA Guidance (more detailed)

2014: PMDA Guidance published

2017: EMA Guidance Concept Paper Released

2017: Revised FDA Draft Guidance Released

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# EMA Guidance Update

Update to Guideline on the investigation of drug interactions

Concept paper released Q1 2017

Proposed changes include:

- Recommendations for *in vitro* studies on
  - Transport
  - Time-dependent inhibition
  - Induction
- Update of transporter list (for inhibition screening)
- Update on cut-offs for transporter inhibition
- Transport as rate-limiting step
- Clarifications of guideline text
- Discussion of DDI study requirement with contraceptive steroids

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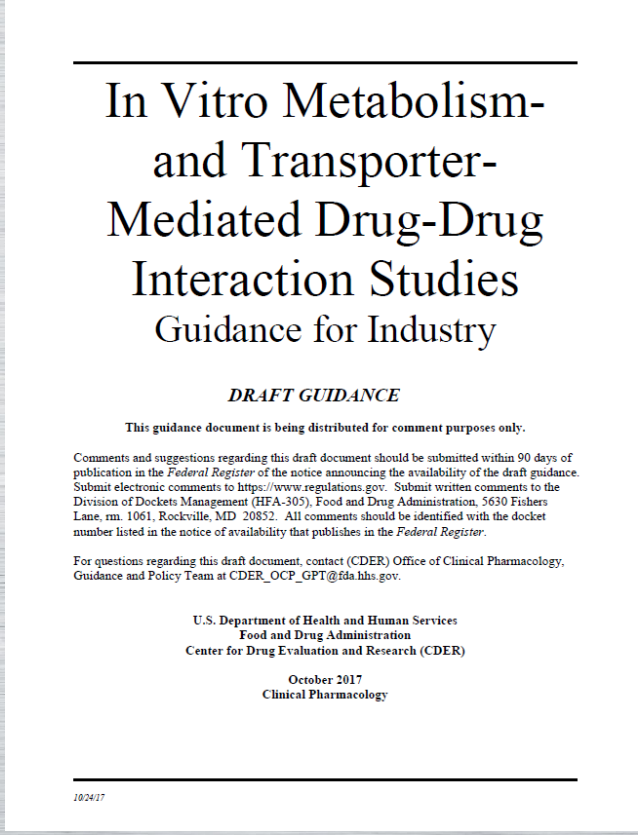
# FDA Draft Guidance Revision

Revision to Guidance for Industry Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

Released October 2017

Changes include:

- Preparation of two separate guidance documents
  - *In vitro*
  - *In vivo*
- Update of transporter list (for inhibition screening)
- Update on cut-offs for transporter inhibition
- Additional recommendations for *in vitro* experiments



In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies

Guidance for Industry

***DRAFT GUIDANCE***

**This guidance document is being distributed for comment purposes only.**

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology, Guidance and Policy Team at CDER_OCP_GPT@fda.hhs.gov.

**U.S. Department of Health and Human Services**
**Food and Drug Administration**
**Center for Drug Evaluation and Research (CDER)**

**October 2017**
**Clinical Pharmacology**

*10/24/17*

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# Types of transporter study

## Inhibition studies

- "Perpetrator" studies
- What is the risk of your NME causing a clinically significant change in disposition of co-administered drug(s)
- Same basic panel required for all NMEs

## Substrate studies

- "Victim" studies
- What is the risk of co-administered drug(s) changing the disposition of your NME
- Transporter panel based upon important clearance pathways of NME

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# Regulatory Requirements

| | | INHIBITION STUDIES | | SUBSTRATE STUDIES | |
|---|---|---|---|---|---|
| | Transporter | EMA | FDA | EMA | FDA |
| EFFLUX | P-gp | yes | yes | consider | yes |
| | BCRP | yes | yes | consider | yes |
| | BSEP | prefer | no | consider | no |
| | MRPs | no | no | consider | no |
| UPTAKE | OAT1 | yes | yes | consider | ≥25 % of elimination is active renal |
| | OAT3 | yes | yes | consider | |
| | OATP1B1 | yes | yes | ≥25 % of elimination hepatic | ≥25 % of elimination hepatic or biliary |
| | OATP1B3 | yes | yes | | |
| | OCT1 | consider | no | consider | no |
| | OCT2 | yes | yes | consider | ≥25 % of elimination is active renal |
| | MATE1 | consider | yes | consider | |
| | MATE2-K | consider | yes | consider | |

# NDA Approvals - Transporter Information

| Period | Percentage of Total NDA Approvals * | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pgp | BCRP | OATPs | OATs | OCTs | MATES | BSEP | MRPs |
| 2006-2011 (n= 183)** | 86 % | 15 % | 18 % | 8 % | 15 % | - | - | - |
| 2012 (n=30) | 73 % | 37 % | 40 % | 23 % | 30 % | 3 % | 3 % | 13 % |
| 2013 (n=21) | 95 % | 38 % | 52 % | 24 % | 33 % | 10 % | 14 % | 14 % |
| 2014 (n=25) | 96 % | 68 % | 72 % | 64 % | 72 % | 16 % | 20 % | 44 % |
| 2015 (n=32) | 84 % | 66 % | 75 % | 59 % | 69 % | 28 % | 34 % | 38 % |
| 2016 (n=14) | 93 % | 93 % | 86 % | 71 % | 79 % | 36 % | 43 % | 43 % |
| 2017 (n=35) | 86 % | 77 % | 77 % | 74 % | 69 % | 20 % | 34 % | 20 % |
| to Q3 2018 (n=36) | 89 % | 89 % | 86 % | 83 % | 83 % | 53 % | 42 % | 22 % |

* Total approvals adjusted for 2013 onwards to exclude “Biologicals” (MABs and Peptide therapeutics), imaging agents and topical products.

** Tweedie et al, CPT, 94(1), 113-25, 2013

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# NDA Approvals - Transporter Information

- Predominantly in vitro data
  - Predominantly inhibition (perpetrator) studies reported
  - All transporter families significantly represented
- Total number of clinical investigations broadly similar
  - 19, 35, 28, 11, 29 and 30 each year for 2013- Q3 2018, respectively
- Range of transporters tested clinically has broadened markedly
  - Consequence of increased range of transporters investigated in vitro
- Occasional post-marketing commitments
- Negative and positive findings referred to in labels
- Trend to combine metabolism and transporter interactions
  - Increasing use of PBPK modelling approaches

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# When should transporters be studied?

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# When should transporters be studied?

- Regulatory considerations
- Patient population
  - Therapeutic indication and likely co-meds
- Physicochemical properties of compound
- Distribution/site of action
- Safety assessment

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# Regulatory considerations - EMA Viewpoint

## Substrate studies

*In vitro* information supporting the prediction of the effects by other medicinal products on the pharmacokinetics of the investigational drug should preferably be available before introducing the investigational product to patients (phase II) and is generally required before starting phase III.

## Inhibition studies

*In vitro* data on the effects of the investigational parent drug on the pharmacokinetics of other drugs should preferably be available before starting phase II studies unless all concomitant drug treatments at risk of being clinically relevantly affected can be avoided in these studies. The *in vitro* information should be available before starting phase III.

- Preferably available before starting phase II, required before phase III

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# Regulatory considerations - FDA Viewpoint

The timing of the in vitro evaluation of each transporter may vary depending on the therapeutic indications of the investigational drug. For example, if the intended population is likely to use statins, the sponsor should examine the potential of the investigational drug to interact with OATP1B1/1B3 before clinical studies in patients. If in vitro experiments indicate a low potential for an interaction between the transporter and investigational drug, subjects taking statins may be included in clinical studies to better represent the intended patient population.

- Timing of in vitro studies depends on therapeutic indication and patient population
- Can be before clinical studies

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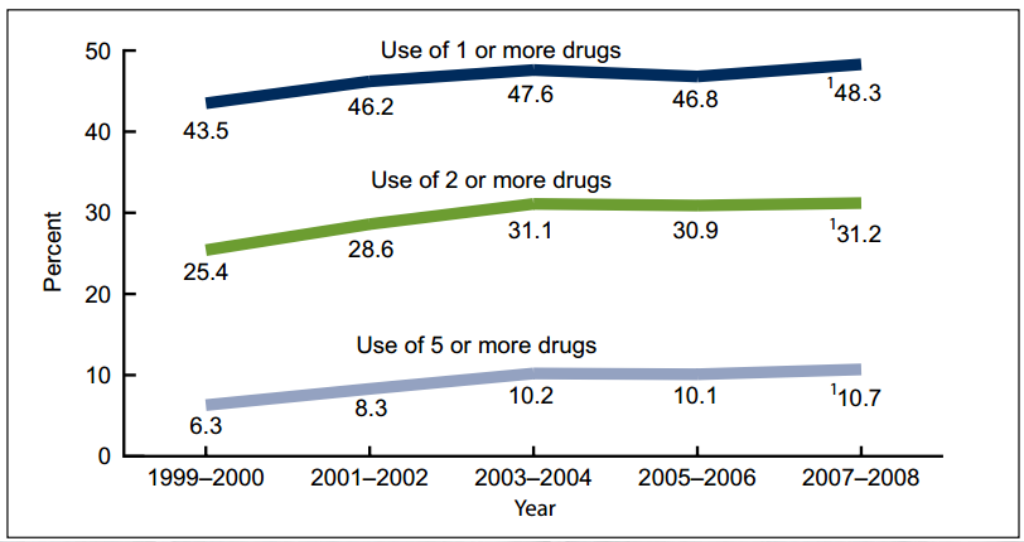
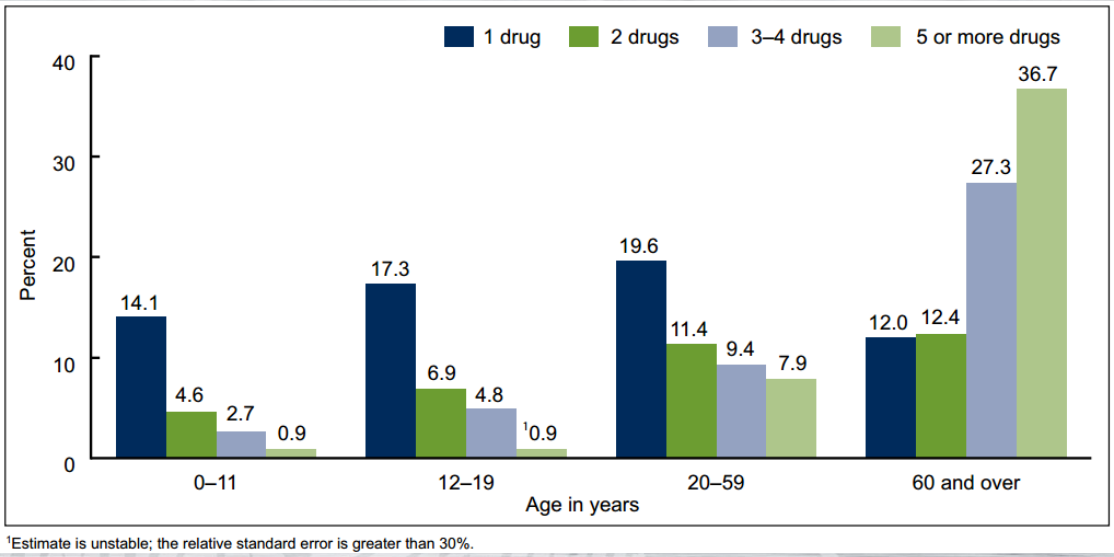
# Patient population - Polypharmacy




1 Estimate is unstable; the relative standard error is greater than 30%.

- Increasing prevalence of polypharmacy
- Increased risk of DDI and ADR
- 'Prescribing cascade'

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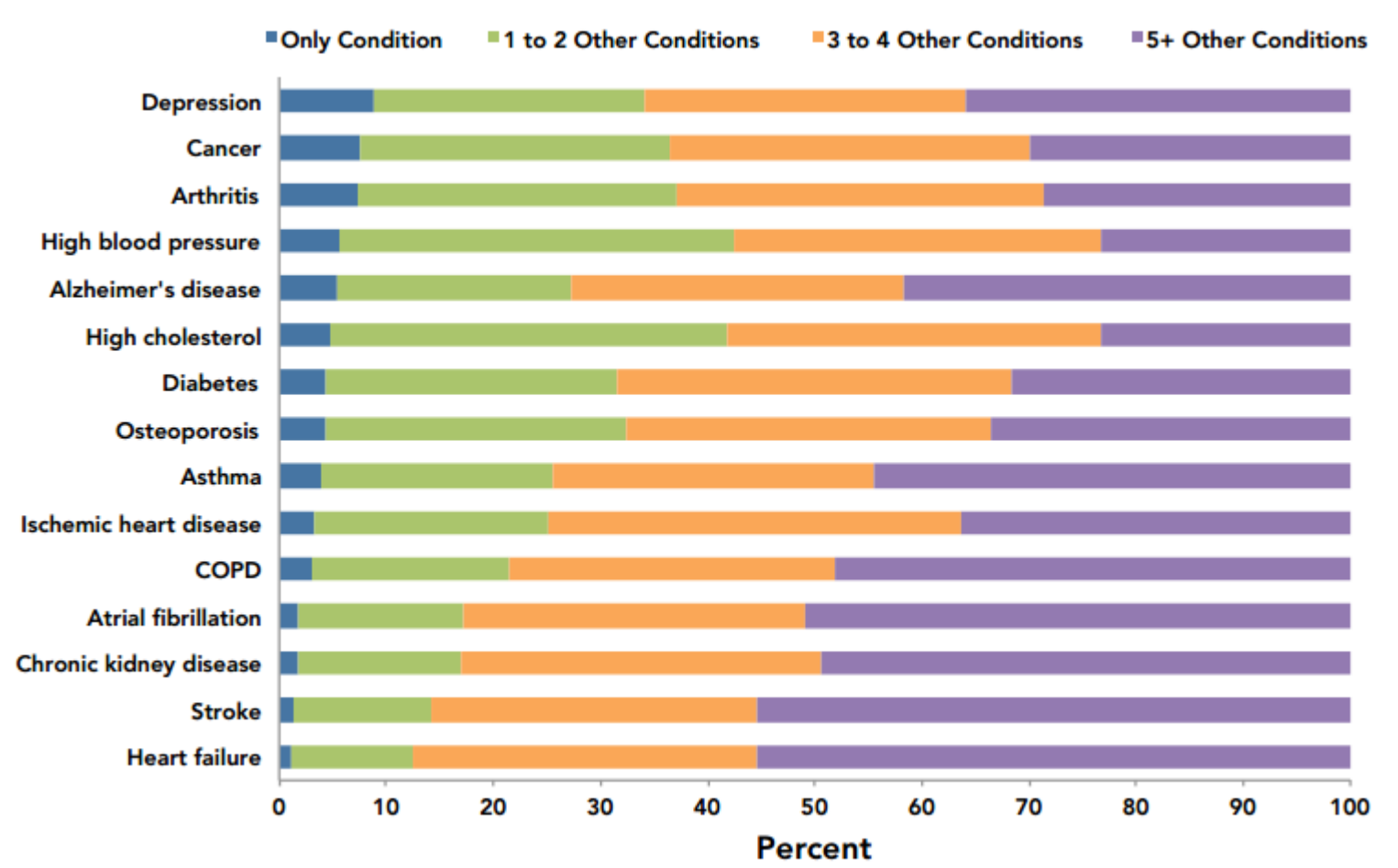
Gu *et al.* National Center for Health Statistics Data Brief No. 42, September 2010. Centers for Disease Control and Prevention

# Patient population – Co-morbidities

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"Co-morbidity among Chronic Conditions for Medicare FFS Beneficiaries: 2010", from Centers for Medicare & Medicaid Services Chartbook, 2012 Edition; US Department of Health and Human Services

# Patient population – Co-morbidities

FIVE MOST PREVALENT DYADS

| Dyads | Prevalence (%) |
|---|---|
| High cholesterol and High blood pressure | 52.9 |
| High cholesterol and Ischemic heart disease | 36.2 |
| High cholesterol and Diabetes | 32.3 |
| High cholesterol and Arthritis | 31.1 |
| Ischemic heart disease and High blood pressure | 29.6 |

FIVE MOST PREVALENT TRIADS

| Triads | Prevalence (%) |
|---|---|
| High cholesterol and High blood pressure and Ischemic heart disease | 33.7 |
| High cholesterol and High blood pressure and Diabetes | 29.9 |
| High cholesterol and High blood pressure and Arthritis | 25.7 |
| High cholesterol and Diabetes and Ischemic heart disease | 21.5 |
| High cholesterol and Ischemic heart disease and Arthritis | 19.3 |

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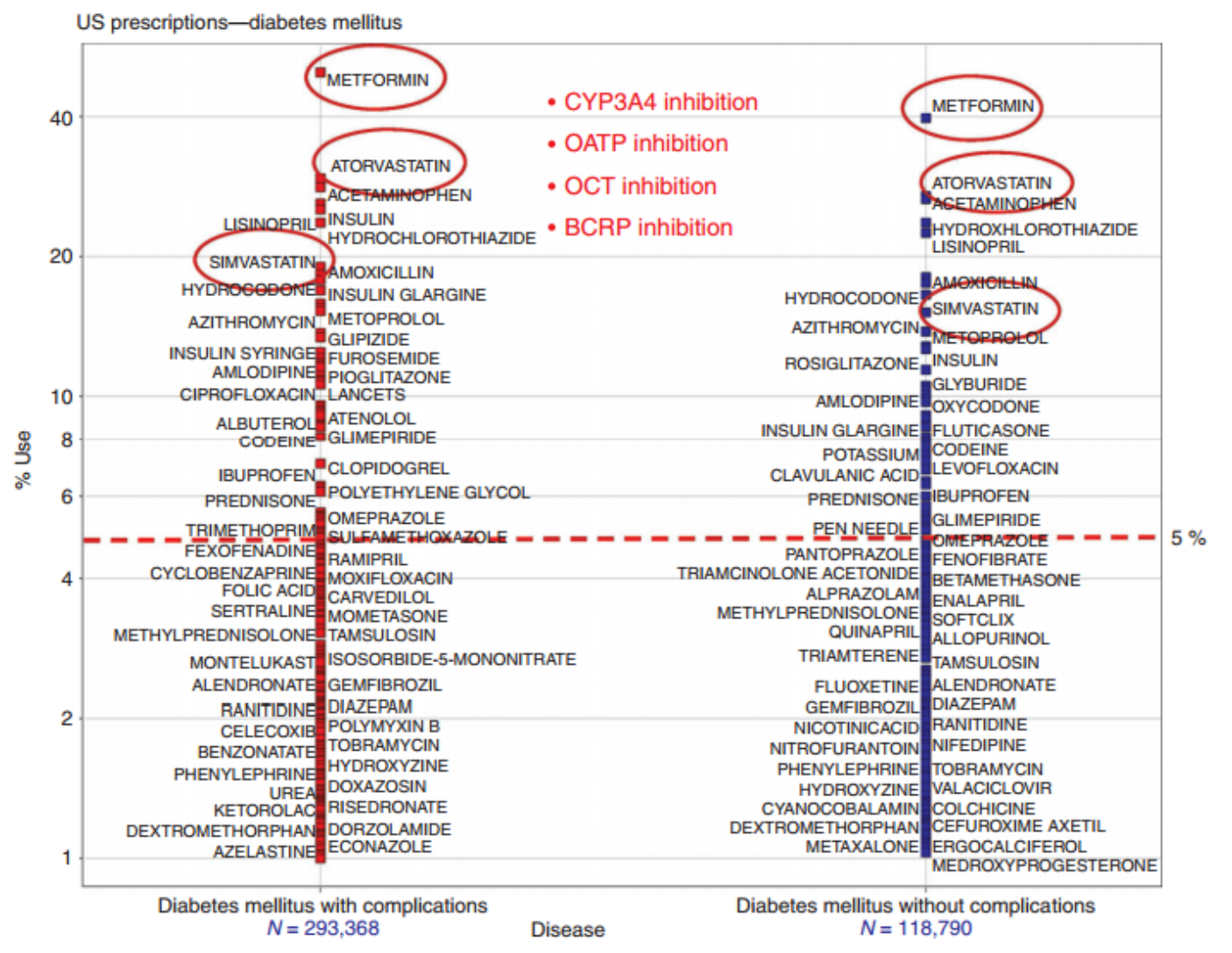
"Co-morbidity among Chronic Conditions for Medicare FFS Beneficiaries: 2010", from Centers for Medicare & Medicaid Services Chartbook, 2012 Edition; US Department of Health and Human Services

# Patient population – Co-meds

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Tweedie *et al.*, CPT, 94(1), 113-25, 2013

# Victim Drugs - Therapeutic Index

| Victim DDI for NTI drugs | Pgp | BCRP | OATP | OAT | OCT |
|---|---|---|---|---|---|
| Severe clinical risk (toxicity severe in nature, options for management include contraindication, dose adjustment, directed monitoring) | Digoxin | Topotecan | Repaglinide | Methotrexate | Dofetilide<br>Pilsicainide<br>Procainamide |
| Moderate clinical risk (potential consequences of toxicity are less severe in nature and/or risk is moniterable before serious sequelae occur) | | Atorvastatin<br>Fluvastatin<br>Rosuvastatin<br>Simvastatin | Atorvastatin<br>Pitavastatin<br>Pravastatin<br>Rosuvastatin<br>Simvastatin<br>Glyburide | | Metformin |

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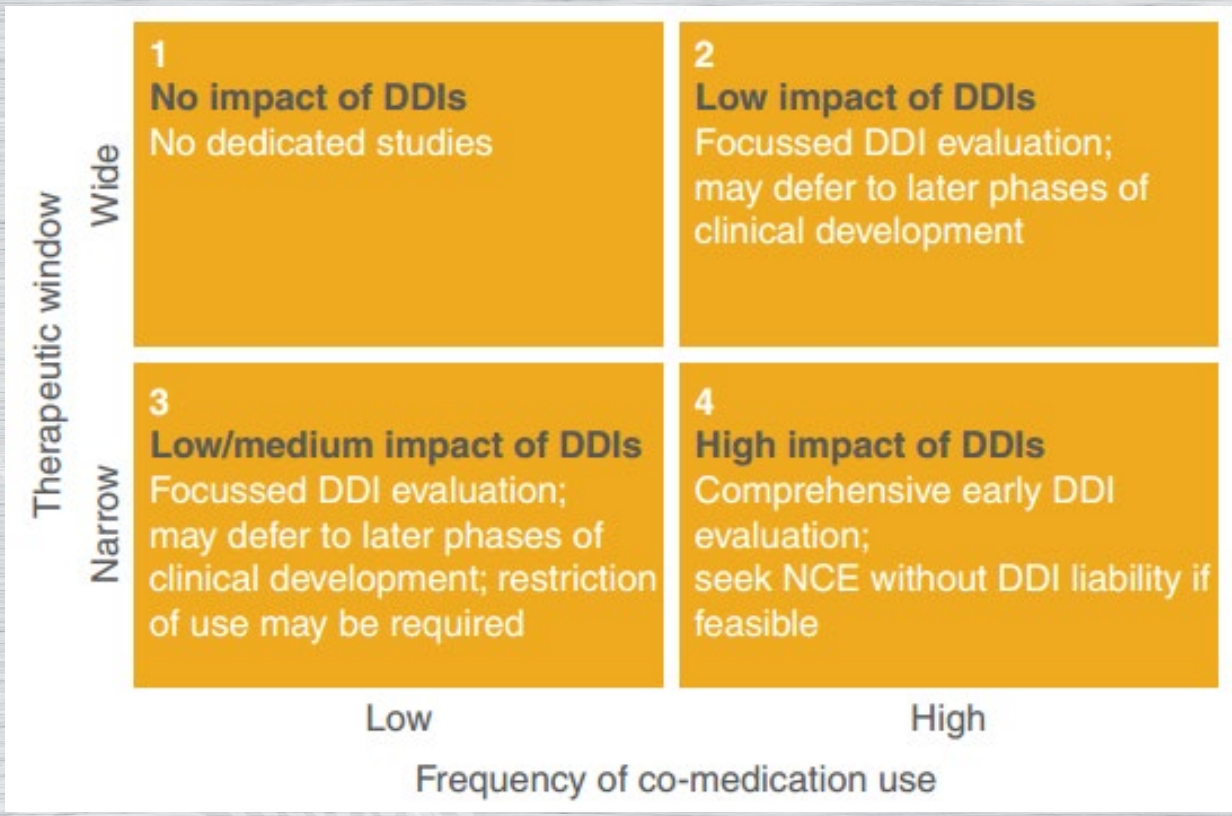
Bloomer *et al.* (2013), Expert Opin Drug Metab Toxicol, 9:737-751

# Victim Drugs - Therapeutic Index

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Bloomer *et al.* (2013), Expert Opin Drug Metab Toxicol, 9:737-751

# Physicochemical Properties – BCS and BDDCS

## Biopharmaceutics Classification System (BCS)

| | High Solubility | Low Solubility |
|---|---|---|
| High Permeability | **Class 1**<br>High Solubility<br>High Permeability<br>Rapid Dissolution | **Class 2**<br>Low Solubility<br>High Permeability |
| Low Permeability | **Class 3**<br>High Solubility<br>Low Permeability | **Class 4**<br>Low Solubility<br>Low Permeability |

## Biopharmaceutical Drug Disposition Classification System (BDDCS)

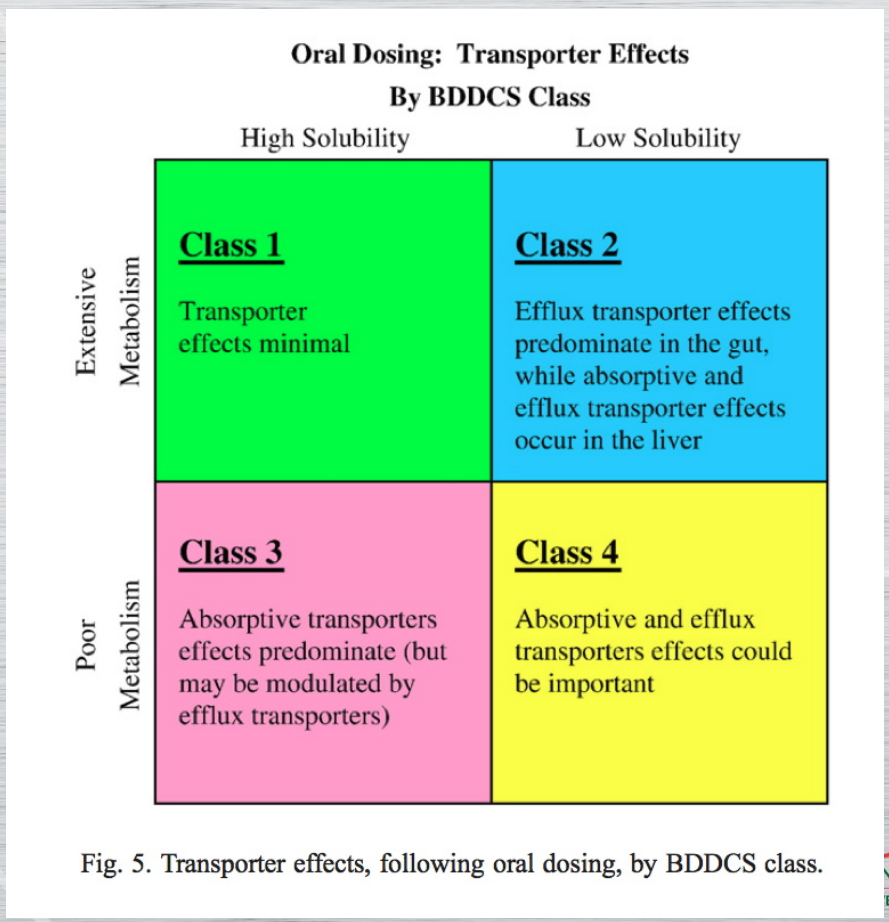
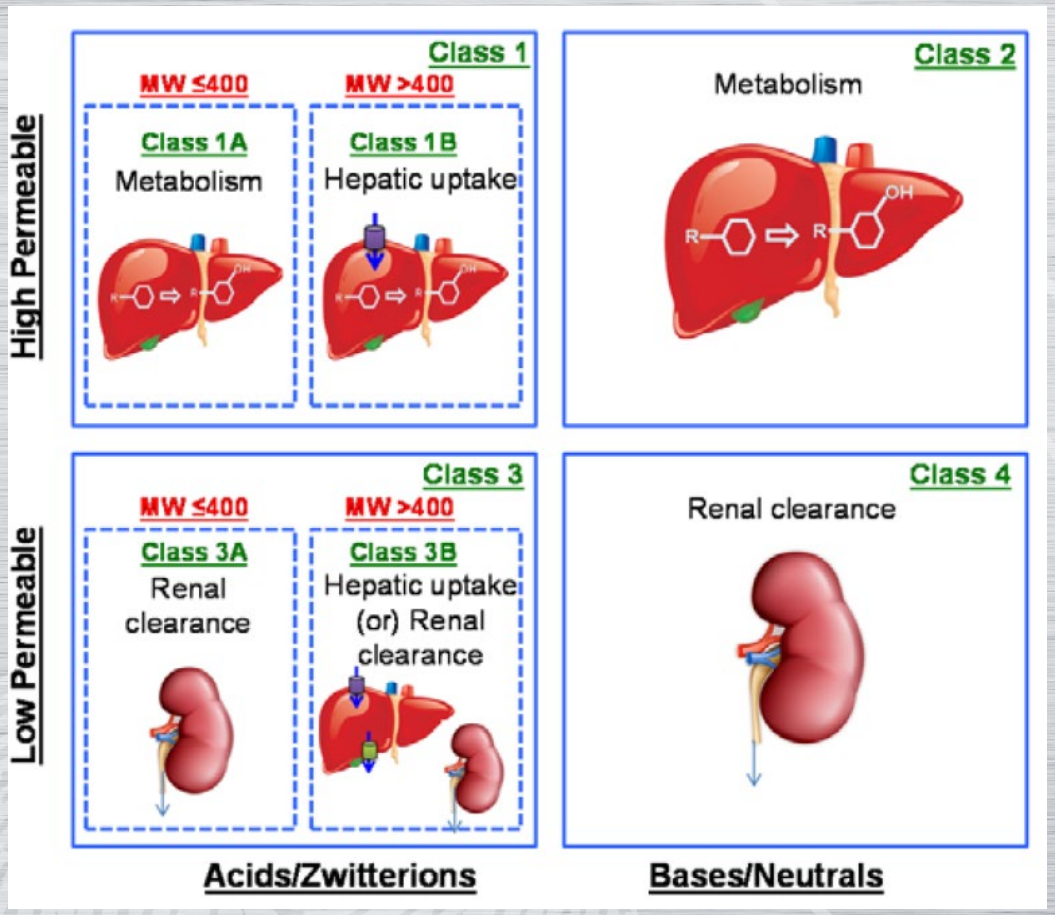


Fig. 5. Transporter effects, following oral dosing, by BDDCS class.

Amidon *et al.* (1995) Pharm. Res.; Wu, and Benet (2005) Pharm. Res.

# Physicochemical Properties – ECCS

## Extended Clearance Classification System (ECCS)




- Prediction of clearance rate-determining step using physicochemical properties (permeability, molecular weight, ionization)

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Varma *et al.* (2015) Pharm. Res.; Varma *et al.* (2017) Clin. Pharm. Ther.

# Safety considerations - cholestasis

- Ranking cpds based on BSEP $IC_{50}$, 25 µM cutoff
- 79 % of cpds with BSEP $IC_{50}$ < 25 µM associated with DILI
- $C_{ss}$ / BSEP $IC_{50}$ > 0.1 gave 95 % correlation with DILI incidence
- Recommendations:
  - BSEP-VT screen for potent inhibitors
  - Confirmatory transporter assays (MRP2, MRP3 and MRP4) may be helpful

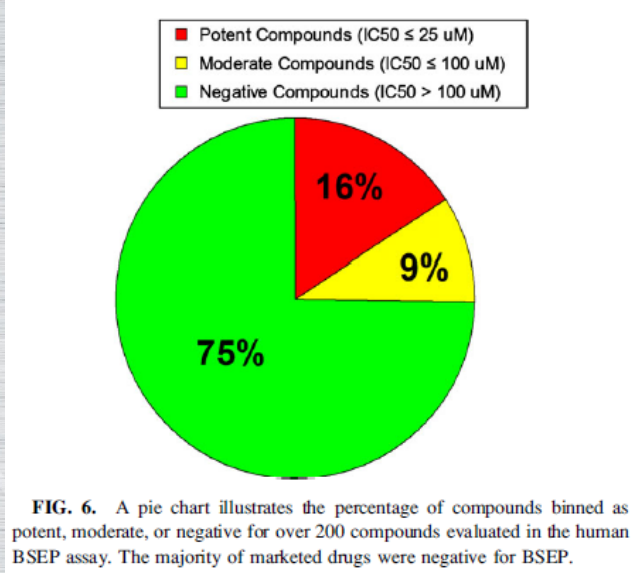


**FIG. 6.** A pie chart illustrates the percentage of compounds binned as potent, moderate, or negative for over 200 compounds evaluated in the human BSEP assay. The majority of marketed drugs were negative for BSEP.

**TABLE 2**
**Number of Compounds With Evidence of Liver Injury/Total Number of Compounds Fitting Column and Row Criteria (%)**

| Transporter Assay | $C_{ss}/IC_{50}$ Ratio < 0.01 | $C_{ss}/IC_{50}$ Ratio < 0.1 | $C_{ss}/IC_{50}$ Ratio ≥ 0.1 |
|---|---|---|---|
| BSEP | 18/44 (41%) | 34/70 (49%) | 36/38 (95%) |
| MRP2 | 6/9 (67%) | 9/13 (69%) | 1/1 (100%) |
| MRP3 | 7/11 (64%) | 17/23 (74%) | 5/6 (83%) |
| MRP4 | 10/23 (53%) | 26/39 (67%) | 14/17 (82%) |

*Notes*. The closer exposure values in humans approach *in vitro* potency values in the transporter assays, the stronger the association with liver injury. Conversely, as the exposure values fall further below the *in vitro* potency values, the weaker the association with liver injury.

Morgan, Toxicol Sci 2010 and 2013

# Safety considerations - cholestasis

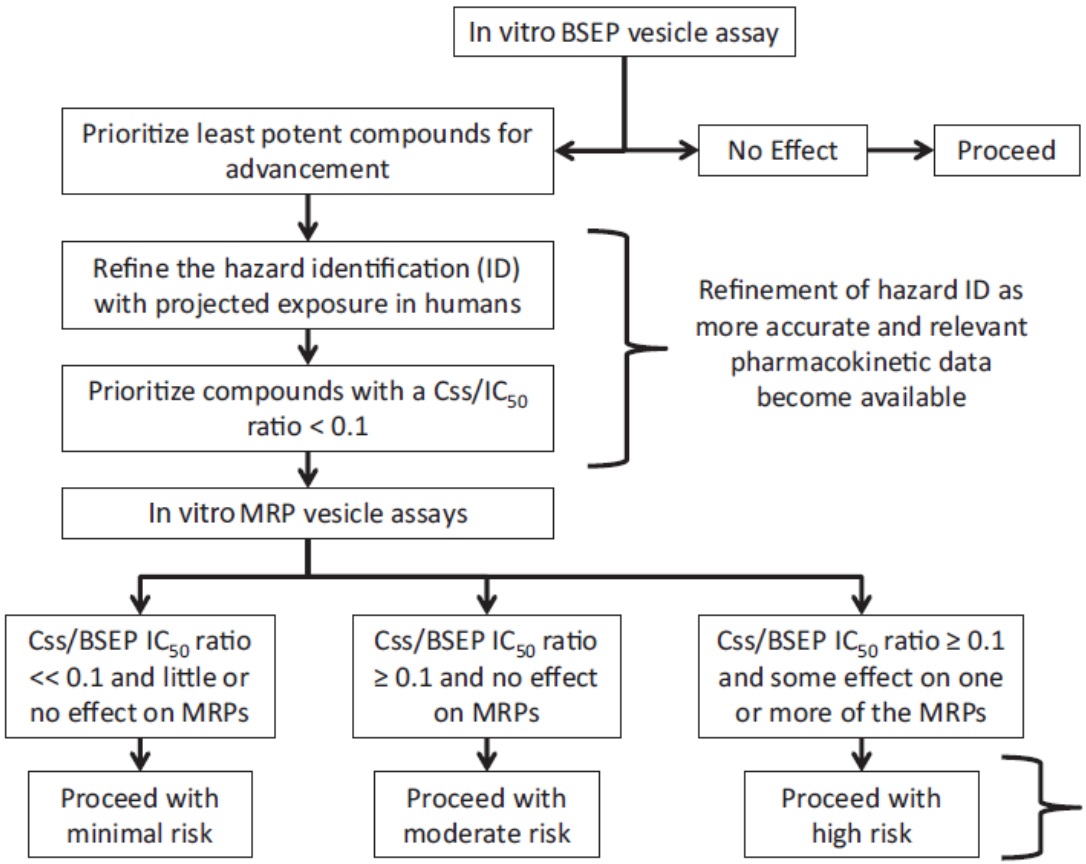


**FIG. 7.** Flow scheme for deploying a transporter panel during early therapeutic compound development. Abbreviations: BSEP, bile salt export pump; DI
Drug-induced liver injury; MRP, multidrug resistance-associated protein.

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Morgan, Toxicol Sci 2013

# Safety considerations - cholestasis

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

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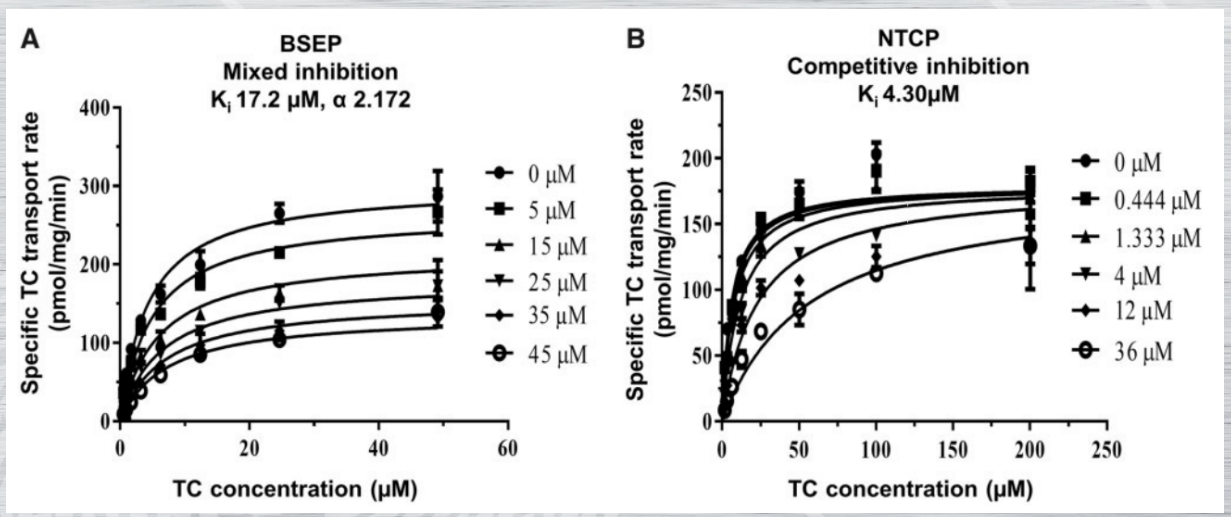
Advance Access Publication Date: October 5, 2018
Research Article

## Quantitative Systems Toxicology Analysis of *In Vitro* Mechanistic Assays Reveals Importance of Bile Acid Accumulation and Mitochondrial Dysfunction in TAK-875-Induced Liver Injury

Diane M. Longo,*,[1] Jeffrey L. Woodhead,* Paul Walker,[†] Krisztina Herédi-Szabó,[‡] Károly Mogyorósi,[‡] Francis S. Wolenski,[§] Yvonne P. Dragan,[§] Merrie Mosedale,[¶,‖] Scott Q. Siler,* Paul B. Watkins,*,[¶,‖] and Brett A. Howell*

- TAK-875 withdrawn in Ph.III due to liver toxicity
- Used *in vitro* mechanistic data together with PBPK modeling (DILIsym)
- Mode of transporter inhibition found to be significant for predicting tox




Longo, Toxicol Sci 2019

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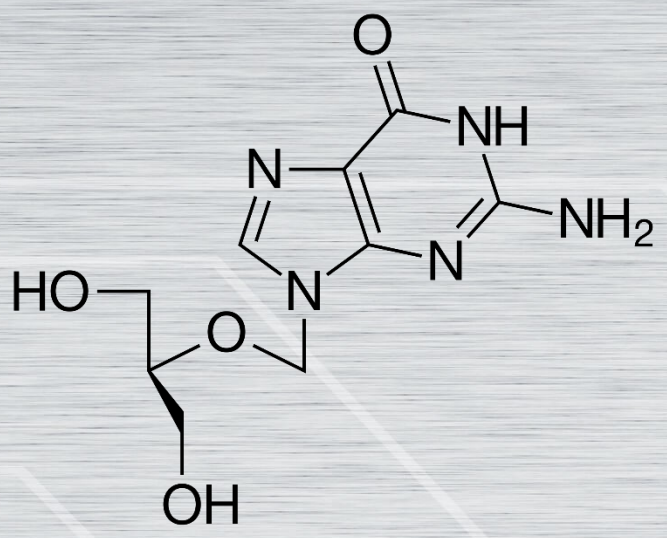
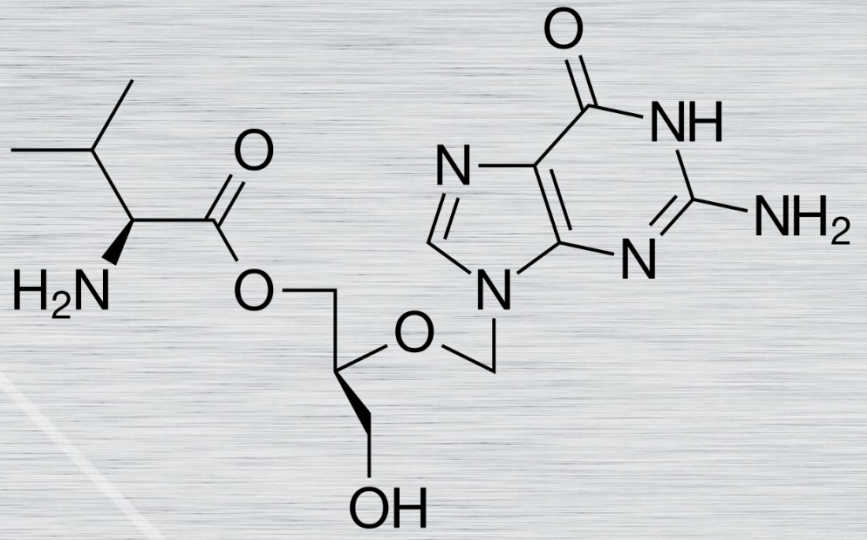
# Distribution – Prodrugs




Ganciclovir
~ 9% bioavailability




Valganciclovir
~ 60% bioavailability

- Addition of valine moiety makes Valganciclovir a substrate of the PEPT1 uptake transporter in the gut, increasing bioavailability following oral administration

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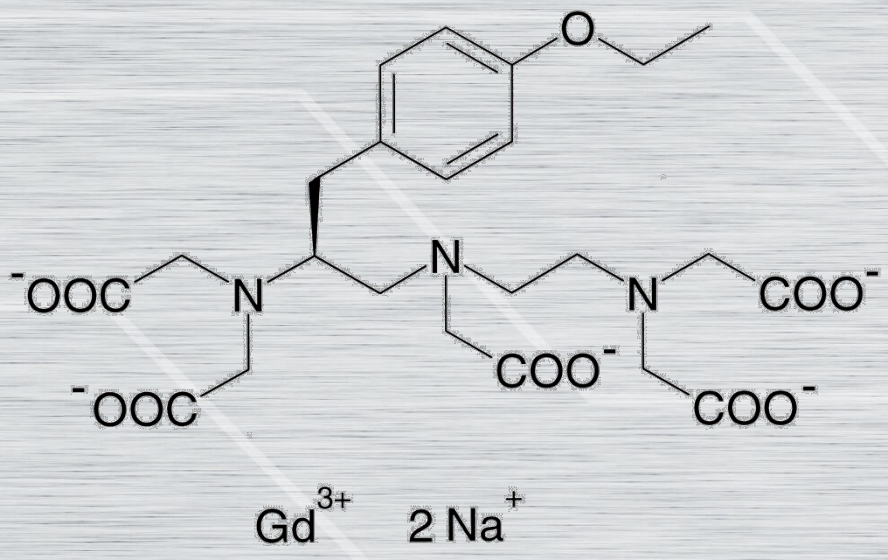
Anderson, Clin Ther 1995; Abete, Antimicrob Agents Chemother 2004

# Distribution – transporter targeting




**Gadoxetate (Eovist)**

- MRI contrast agent
- Used for imaging hepatocellular carcinoma and liver metastases
- Substrate for OATP1B1 and OATP1B3

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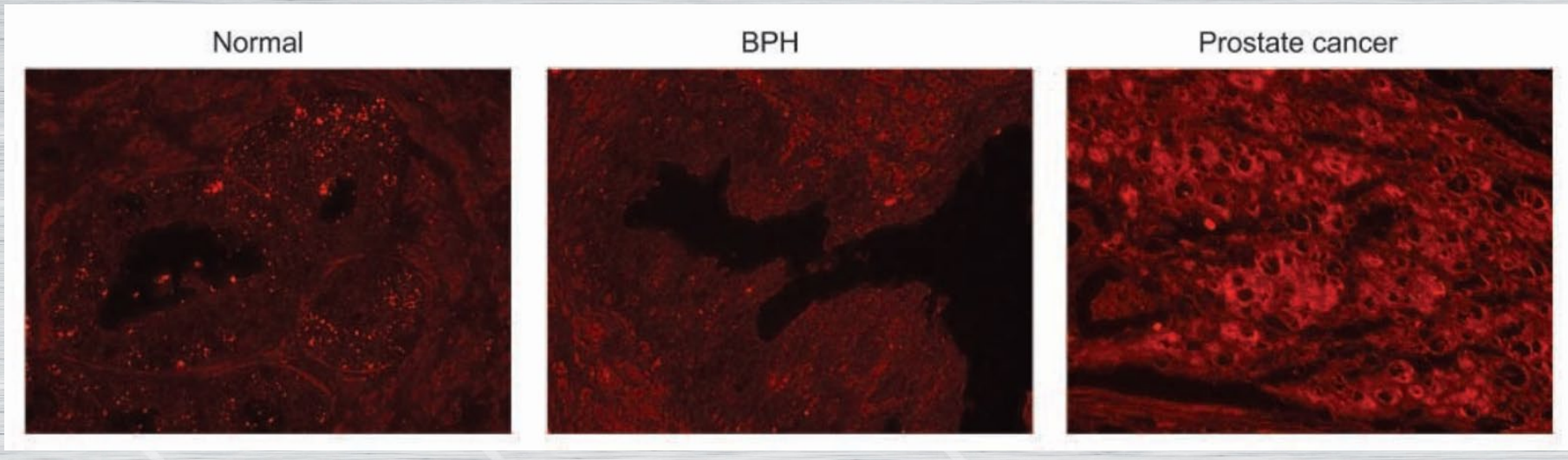
# Distribution – transporter targeting




- OATP1B3 expression is normally liver specific
- OATP1B3 expression is upregulated in prostate cancer

Hamada *et al.* (2008) Clin. Cancer Res.

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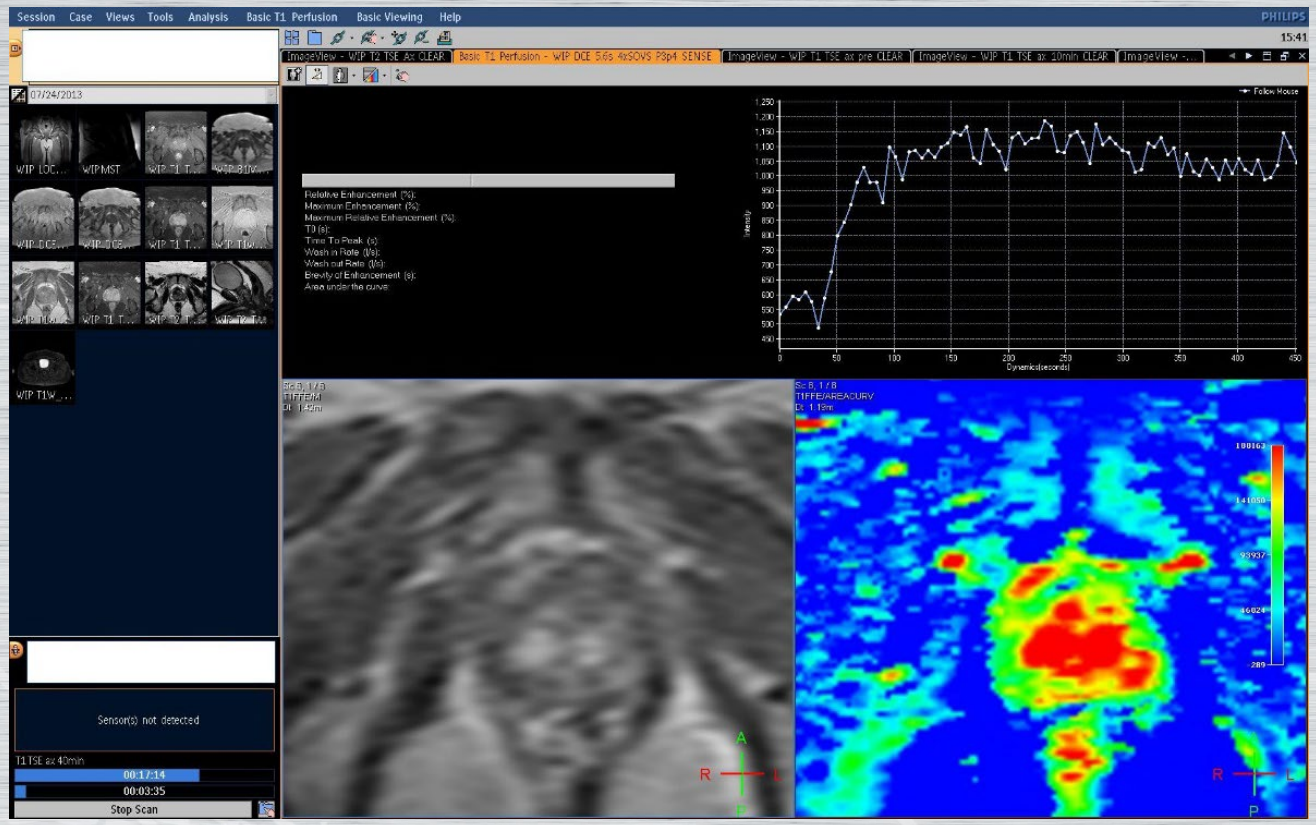
# Distribution – transporter targeting




- Gadoxetate (MRI contrast agent and OATP substrate)
- Accumulation of gadoxetate in prostate cancer

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Slide modified from William Figg, Pharm.D. (NIH)

# Development Stage - Preclinical

- Screening for (hepato)tox
  - BSEP, (& MRP2-4, NTCP)
  - Incorporating *in vitro* data into PBPK models
- Transporter as a target
  - Substrate/inhibition assessment – ie. SGLT2 (eg. Canagliflozin/Invokana) or URAT1 (eg. Lesinurad/Zurampic)
- Route of administration
  - Oral - MDR1 & BCRP substrate assessment
- Target biology (tissue/site of action)
  - Central nervous system? MDR1 & BCRP
  - Organ targeting (ie. liver via OATPs)
- Patient population/co-meds
  - Statins? OATPs
  - Metformin? OCTs & MATEs

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# Development Stage - Preclinical

- Physicochemical properties
  - BDDCS
  - ECCS
- Patient population/co-meds
  - Statins? OATPs
  - Metformin? OCTs & MATEs
- Narrow therapeutic index
  - ie. Oncology drugs
  - Substrate assessment to determine extent of DDI liability as victim

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# Development Stage – Phases I & II

Phase I

- Will have animal PK data (indication of extent of metabolism, renal/hepatic elimination)
- Little/no human PK/PD data
- Adding to transporter data information on substrate studies (test compound as victim of DDI)

Phase II

- Will have human PK data (indication of extent of metabolism, renal/hepatic elimination)
- Additional transporter data required - Substrate studies (test compound as victim of DDI)
- Complete information on transporter DDI typically required for End-of-Ph.II meeting

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# Summary

- Early transporter screening utilizing vesicle/cell-based assays useful to address specific questions
  - Tissue targeting
  - Mechanism of action
  - BSEP (& MRP) inhibition and hepatotoxicity

- Prioritization of inhibition studies for patient population with high rates of co-morbidities/co-medications, or likely transporter-interacting co-meds

- Prioritization of substrate studies for patient population with high rates of co-morbidities/co-medications, narrow therapeutic index

- Some transporter inhibition data may be required pre-IND

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