we are pleased to have dr joseph ware

dr ware received his phd in

pharmaceutical science from wayne state

university and completed his

postdoctoral fellowship at nhlbi in the

laboratory of molecular medicine and

cellular toxicity

dr ware is the principal scientist in

the department of clinical pharmacology

at genentech please enjoy todays

lecture

hello my name is joseph ware

and i work at gen in tech in the

department of clinical pharmacology

today i will provide an overview of drug

transporters in admin drug action

and in particular im going to stress

more of an industrial perspective

the implications of drug transport in

drug development are multifactorial we

know that drug transporters are an

important determinant of oral drug

absorption

of drug distribution and drug

elimination

moreover it is becoming increasingly

apparent that they are a major

determinant of drug drug

interactions and over the last 0 years

insight has been

found

through the use of pharmacogenetics as

to how important drug transporters are

as dr gottisman mentioned in his lecture

we know that drug transporters are an

else are an important factor in

multidrug resistance

and we also know that theres an

emerging role for drug transport and

toxicology

today ill primarily focus on the role

of drug transporters in ade

as drug drug interactions are a key

issue

to be addressed in the india

in nda of all drugs the fda ema and pmda

have published guidelines on how best to

evaluate drug drug interactions

these guidelines

propose the use of clinical drug drug

interactions where needed to actually

delineate the role of drug transporters

but more importantly the use of in vitro

systems are used to guide and steer

these drug interactions

it is important to recognize that in

order to understand when a drug drug

drug transporter interaction is

important we must understand

when the drug transporter represents the

rate limiting step

and this hypothesis was first presented

by yuichi tsugiyama

more than a decade ago

and i think thats a very very important

consideration for

all drug transport

work

so todays lecture objectives

ill

provide an overview of drug transporters

that are important in drug absorption

and disposition

explain why transporters can be major

determinants of drug drug interactions

ill describe the process of transport

induction

and provide a time course of this

induction well also examine the role of

drug drug

interactions from the point of

inhibition of drug transport and how

that influences pharmacokinetics

and i will provide a few examples of

integrated drug transport strategy

to address drug absorption and drug

distribution limitations

so i work in oncology clinical

pharmacology

and its really important for me

to stress to you this is my perspective

and in how we actually examine drug

transporters

and in particular its the best of times

and the worst of times from the point of

making progress in the fight against

cancer

however only one in 0 of our oncology

molecules survive pass from phase to

registration

moreover even if we work on molecular

targeted agents the therapeutic endo or

therapeutic index or window of a drug is

quite narrow

therefore a personalized approach is

needed to define optimal dose and

schedule to achieve efficacy with an

acceptable safety profile

and finally each patient

with cancer represents a special

population

cancer patients may take up to 0

medications

and also be receiving

complementary alternative medicines

moreover some patients may have had

gastric surgery or develop hepatic

metastases which also lead to altered

drug metabolism or clearance

so when is a drug drug interaction

really clinically significant

and in this case ive adapted used a

slide from

dr xiaomi wang of the fda

just iteratively showing within the

therapeutic window if you have a broad

window a small change in exposure will

not impact the safety

however on the figure on the right with

a narrow therapeutic index or narrow

therapeutic range drug a small change in

exposure greatly impacts your safety

profile

i was lucky to be part of the membrane

transporter white paper published in

00

and this was a key collaboration between

academia fda and industry

which helped to

provide a foundation for future

work and research in the area of drug

transport

the key issues that were addressed in

this white paper were first which

transporter should we study

and how should we study them

and then how do we evaluate whats the

recommended outcome as we proceed from

in vitro experiments to the clinic

so the original international

transporter consortium seven

transporters of interest are shown on

this figure and this figures reproduced

from the nature medicine paper published

in 00 and

pgp which is abcb bcrp which is gene

name abcg oat oat oct

oatpv and oatpb

were the initial transporters that were

proposed to be studied because they

represented

case studies that demonstrated the

impact of these transporters on drug

disposition

from the second itc meeting additional

transporters were added

these were the multidrug resistance

proteins mrp

mrp mrp

the mates which kathy giacomini will

discuss

and

pista

psap itself is a major determinant of

hepatic injury and maybe something

thats needed to be screened for for

certain drugs that interact with this

hepatic cannonicular membrane

transporter

so ill describe some case studies first

where drug transporters are an important

factor of drug absorption

and ill focus on the expression of drug

transporters in the intestinal

epithelium

so as we know most drugs that are

administered orally must first

undergo disintegration dissolution

and after theyre in solution then they

have to trust several membranes before

they actually reach the target of

desired action

and in this case right here

on the figure shown

to the left is that in the intestine

both pgp or mdr and bcrp

are apical efflux transporters and these

transporters will help

will

keep drugs out of systemic circulation

once the drug enters the enterocyte it

can

pass to the basal lateral or blood side

to be absorbed systemically to the liver

and then from there it enters systemic

circulation

so inhibition both induction and

inhibition of intestinal transporters

can have consequence on the oral

bioavailability of some drugs

this is a partial list of p glycoprotein

substrates

some of these substrates are known drugs

or all of these substrates are known

drugs

and some of these substrates are

administered orally

so suffice it to say independent of

which therapeutic area that you work in

its important to understand

whether or not your molecule has a pgp

liability

now whats interesting and it became

apparent

in the uh

mid000s is that many pgp substrates

are also substrates or inhibitors of

cytochrome p0 sub a

and in this situation you can end up

with a double or nothing impact in terms

of a drug interaction

and this consequence itself is important

when youre running a drug interaction

study whereby you use itraconazole to

inhibit civa you may also be inhibiting

peach glycoprotein

if we think about impacting the

expression of pgp where theres drug

a drug or a xenobiotic that induces the

expression a peak like a protein the

expression of pglycoprotein on the

in the enterocyte

increases this would have a net effect

of decreasing systemic bioavailability

of the drug

this was nicely demonstrated in 999

by a group

where they measured

intestinal expression of pglycoprotein

both before and after rifampicin

administration rifampicin as everyone is

aware is an inducer of cytochrome p0

it also induces pglycoprotein

and as shown by the red color

the

expression of pglycoprotein increases

after the administration of rifampicin

what if you inhibit pglycoprotein

if you inhibit pglycoprotein

and you are administering a substrate

which is a sensitive substrate for pgp

eflux in the gut you can actually

increase systemic auc

and this is nicely shown

by the administration of lopinovir and

retonavir with the victim digoxin

as theres

approximately doubling of exposure to

healthy volunteers who were treated with

digoxin

the digoxin label has many interactions

listed and attributed to p glycoprotein

and i would

turn your attention to this label and

even this is probably somewhat updated

outdated

so its important at least with digoxin

first because of the narrow therapeutic

index

and the significant safety concerns

whereby

the therapeutic window for digoxin is

0 to nanograms per milliliter

and if you exceed nanograms per

milliliter there can be significant av

nodal conduction disturbances

as well as other toxicities

therefore change in exposure might be

clinically significant

this figure shows a number of drugs

that

have been shown to have been studied to

interact with digoxin well digoxin is

not the perfect peak like a protein

substrate it represents a p glycoprotein

substrate a drug with the p glycos

protein substrate a liability and is

therefore of great safety concern when

youre administering

new medications with it

so less than a twofold change in

exposure in either cmax or auc

is significant

utilizing in vitro data we know that

some of the

the predictions are actually correct

however theres still a high rate of

false negatives when comparing in vitro

to in vivo

for digoxin or other enemies that have a

narrow therapeutic index its important

to understand the relationship and the

interaction between a new molecular

entity

and digoxin

and many times

its important to really understand the

limitations of the victim substrate

which in this case is digoxin

for this reason our group started to

understand a little more about rifamixin

and rifamixin itself is a gut targeted

antibiotic

which has a very low

absolute ba which is 0 percent

it was found in the development of this

drug

that when administered with cyclosporine

there was almost an to 00 fold

increase in cmax and auc

we wondered whether or not

pglycoprotein is the only transporter

interacting with this drug

to this end we study

in vitro the transport of rifa mixin in

the presence of a victim or a

perpetrator pgp inhibitor

and what we found was

the ba transport of this molecule was

significant in cells that were

transfected with pgp or mdr

and that this ratio of efflux could be

brought down by administration or adding

an inhibitor of p glycoprotein

so in other words

we could demonstrate the presence of

eflux as a liability

we then studied rifamixin in

pgp and vcrp knockout mice

and what we found was

there was a significant increase in

exposure

in mice where pgp had been removed

what we found though however was that

for at least vcrp

and pgp knockout mice

the the addition of an additional

knockout did not impact the auc

wild type ratio

and this wild type ratio was greater

than 0 to one for both

pgp and pgv and bcrp knockout mice

i want to discuss now bcrp as it relates

to drug absorption

bcrp or abcg was first identified

as a pluripotent marker for stem cells

and at the same time investigators at

the nih were investigating its role

in mitosanthrone resistance

and this transporter is an abc

transporter

of the seventh family

and its a

known by many names but well call it

bcrp

the substrates that are known to

interact with abcg or bcrp

are the topoisomerase inhibitors

also many drugs in nononcology interact

with this

including

po byte a estrogen sulfate

odipiperazoson there are also many

inhibitors of which molecular targeted

agents have been shown including

many drugs that are commonly known so

its theoretically possible that if

youre administering a targeted

molecular targeted inhibitor in

combination with the chemotherapy there

could be bcrp interactions

originally i started studying bcrp our

lab started studying bcrp

when this work was published by the

netherlands cancer institute

and what they found was when topo is

topatekin was administered

in mice it had very poor oral absorption

and after the administration

of what was known to be a pan pgp vcrp

inhibitor the area under the curve for

this molecule increased about eight fold

and then they studied the presence of

of

drug transport in knockout mice

so they used a knockout mouse construct

in the presence of a pgp and vcrp

inhibitor

and thats shown in figures

top right bottom left

that there was an increase in exposure

and what they demonstrated was

this was actually related to pgp and

vcrp but they defined it to be a bcrp

only interaction

and

this experiment was then conducted

in healthy or in cancer patients where

they administered topetekin

in the presence of gf09

and they could increase the exposure of

this chemo

approximately eightfold

so why study this

in the olden literature there was even

examples of high variability so

variability after the administration of

an orally administered drug is a major

problem

in drug discovery and development

and even with old drugs such as cell

phosalazine

theres

a wide range of pk exposure

and this was a published paper from

the developers of cell phosalazine in

the late 90s

sulfasalazine itself though is not

metabolized by cytochromes p0 the

rate limiting step of metabolism is an

azo reduction that occurs by bacteria

in the distal

small intestine in the proximal large

intestine

after the drug is cleaved theres a

nonabsorbable fragment which is five

amino salicylic acid which is thought to

benefit patients with crohns disease

and ibd

and then sulfa pyridine

we hypothesize that interindividual

differences in intestinal expression of

abc g or bcrp could contribute to the

variability absorbed with sulfasalazine

and also this pk variability would

translate to pharmacologic response

variability

to understand how bcrp could contribute

to sulfasalasine absorption we use the

bcrp knockout mouse

and whats shown in the top figure is

the oral administration of sulfasalazine

to both wildtype blue circles and knock

out red triangle mice

we found over a hundred fold increase in

the ac of sulfus alazine

iv administration of sulfusalazine

demonstrated that there is a small

difference

but not anything as significant as what

was shown

now to answer the question whether or

not bcrp only or pgpm vcrp interacts

with sulfasalazine clearance we

administered selfestablishing to knock

out mice and we found only a twofold

increase in aec

and no difference in iv clearance

therefore selfestablishing we believe

was a sensitive substrate

for bcrp

we studied selfestablishing pk in north

american healthy volunteers and at the

time there was

we were the first to report

pharmacogenetic differences in abc g

expression

and an impact on a model substrate

sulfasalazine pk and healthy japanese

volunteers also demonstrated

pharmacogenetic variability

and in this situation there were low and

high differences in sulfasalazine with

the intermediate zygotes also falling

into place

so what about drugs that could interact

or

xenobiotics that could interact we found

that in collaboration with sunit shukla

and

the nih we found that cucumberin could

interact with bcrp cucumber in itself

is a drug that the nci has studied in

over 9 clinical trials its

antiinflammatory antioxidant

and its being investigated

and marketed many places

for its chemo preventive

antiinflammatory activity

in collaboration with trash ambikar and

sunit shukla

we found that cucumberin increases the

bioavailability of sulfasalazine in the

mouse

and that cucumber in itself was

selective inhibitor of bcrp

whereby there was no impact of curcumin

on the efflux of pgp

dr sukiyamas lab or kushiharasan

in

japan studied

the impact of curcumin and there was

several interesting facts

first there was a dose dependence when

sulfasalazine was administered as a

victim and curcumin was administered as

a perpetrator so a lowdose

sulfasalazine displayed about a twofold

increase in ac

where

high dose sulfusalizine therapeutic dose

which is grams

there was an increase of approximately

volt ac

most recently from ucsf and other

investigators have demonstrated

that uric acid is a substrate of vcrp

and uric acid itself and its

administered drug allopurinol both can

be impacted by pharmacogenetic

differences in avcg

so im going to speak now about

intrinsic and extrinsic factors

impacting drug absorption we know that

intrinsic are host dependent which

include disease age gender

physiochemical and cmc properties are

also important in the absorption of

small molecule drugs

extrinsic factors related to drug drug

interactions ive provided you some

examples im just going to digress here

shortly where im going to go into ph

dependent absorption issues these are

not transporter mediated but they impact

the ability of a drug to interact with

drug transporters its also very

important to recognize that food effect

is an important determinant of pk

variability

we found or reported that many of the

molecular targeted agents display ph

dependent solubility and in this format

many of the commonly administered

targeted molecular targeted agents such

as desatinib or lotinib gemfetinim

all have ph solubility

issues and in this situation because the

molecules are weak bases

an increase in ph from your normal

gastric ph of two to five

could

decrease the solubility sometimes over

ten thousand fold

we also found that many cancer patients

take a large number of acid reducing

agents and in this situation we found

that patients with glioblastoma

and gastrointestinal stromal cancers

actually between 0 and 0 percent of

those patients take ppis

we ran a healthy volunteer study in

collaboration with dr les bennett and

his graduate student at the time mark

yago and we reported that to satniv in

the presence of rebeprazole which is a

model ppi

inhibitor decreased the exposure of

the satinib over eightfold

we came up with this reactivification

hypothesis whereby we could administer a

small amount of acid to subjects to

overcome the pharmacologic

hypochlorhydria

and in this situation we could increase

the dasatinib exposure in the presence

of rebeprazole within 0 percent of the

baseline values

im going to just slowly go through the

role of drug transporters and drug

distribution

the role of pgp in the bloodbrain

barrier in the placenta was first

characterized in mice

alfred schenkel during his postdoc at

the netherlands cancer institute knocked

out mdra and b and found that the

offspring of these mice were viable

fertile and without an observable

phenotype however the mice developed a

mite infection and were treated with

ivermectin

after the mice were treated with

ivermectin

this dose response and survival curve

dramatically shows that the mice that

did not have mdr were greatly impacted

by the administration of ivermectin

and the mice with an intact mdr at the

bloodbrain barrier there they were

relatively resistant to uh that this

toxicity

investigators at merck sharp and dome at

the time were investigating ivermectin

in cd mice or cf mice and were found

to actually have clap pallet

the mdr in this situation was actually

demonstrated to have an impact at the

blood placental barrier

theres not a human equivalent of this

knockout

certainly dogs

have a for base pair deletion and

collies in particular are known not to

have functional pgp at the blood brain

barrier therefore if you have a collie

or

dogs in the shelti family its important

that their dose of hydrobactin be

greatly reduced

how this relates to drug discovery and

development

is in oncology

we believe that glioblastoma is highly

unmet

in medical need and patients with

glioblastoma theres over 000 new

diagnosis per year and once a patients

diagnosed with glioblastoma their median

survival is less than a year and a half

one of the best types of cancers

characterized at a molecular and genetic

level

however due to the heterogeneity of

genetic and morphological differences

diffuse infiltrate of disease all are

challenges that are encountered there

are relatively limited treatment options

including surgery radiation temozolomide

there are a number of clinical trials

that are unprocessed

but however this is an area where there

are multiple targeted agents that have

failed

and part of the reason that they have

failed

is because the molecular targeted agents

are not unable to cross the bloodbrain

barrier

i add this slide as a review

just to state that theres three ways

that a drug can cross the bloodbrain

barrier

first it can permeate the cell through

trans cellular transport there can be

specific uptake transporters

in particular from the slc family

or small molecule drugs can traverse

between the tight junctions but these

molecules have to be extremely small

specifically molecular weight less than

0 to 0

we investigated and developed one

specific molecule

for the treatment or the investigation

of the treatment of glioblastoma

this molecule gdc0

was found to have similar pharmacologic

potency of gdc09

however this molecule had greatly

enhanced bloodbrain plasma

increases on the order of a hundredfold

over pictilosim

and the free fraction that actually

crossed the bloodline barrier was

greater than seven percent

this was this investigation was led by

laurent salvati with shireen shaldi

latham

and in particular

mirroring models of glioblastoma were

found

in

and developed and gdc0

was administrated orally at

milligrams per kilo

and what you see on the left are the

lesions of the tumor itself

and then on the right is actually for

each figure is how much the drug

actually crosses and these figures are

maldi imaging of the cells where

gdc0 is present

gdc09

shown on the righthand figure

is known to not cross the bloodbrain

barrier so even pharmacologically it

hits the right target was very poor

activity and was never studied in the

clinic

therefore

through the use of chemistry and

selective reduction of

pgp interactions there was over a

hundred fold increase in the brain to

plasma ratio for this molecule

what was interesting was that because

many glioblastoma patients actually need

to have surgery

the measurement of

gdc00 was determined in one clinical

cancer patient

and in this situation

the brain to tumor plasma ratio was

greater than

and in the brain to tissue ratio itself

was

so what we learned about this molecule

and this specific patient was

that we could trust our nonclinical

approach

to actually getting drugs into the brain

more importantly into the tumor

im going to speak briefly about solute

carrier superfamily now these

transporters are primarily involved with

uptake

of substrates in organs of elimination

such as the kidney and the liver

this sub this uh the slc sub family is a

super family so theres over 00

approximately 00 genes in this family

of transporters so its much greater

than the abc transport family

and

all of these molecules are named through

slc

slco nomenclature

i wanted to just review simply impact of

slcs and

renal clearance

and its important to recognize that

renal clearance is a summation of gfr

plus secretion minus reabsorption

and in situations where renal clearance

equals gfr we can only say the net

effect of secretion and reabsorption are

equal

if renal clearance is much less than gfr

we would say theres net reabsorption

and if renal clearance is much greater

than gfr we would say we have net

secretion

and the transporters that are shown in

the single isolated proximal tubule of

the transporters ill show you our oet

oat oct

and on the basolateral side of the

transport

they are also abc and other slc

transporters

so renally mediated drug drug

interactions

are some of the earliest examples

of drug drug interactions that were

discovered to be mediated by active

transport during world war ii penicillin

was in short supply so the quest to

understand an inhibitor of drug

transport of penicillin was undertaken

it took almost eight years to identify

that probenocid could inhibit the active

tubular secretion of penicillin

drugs that have specific labeling

precautions related to

drug drug interactions are those with

the narrow therapeutic index such as

dofadelide

and dopalide is tycosin which is known

as an antiarrhythmic but at higher doses

it has very

simple very pronounced cardiac toxicity

sedovavir it was found that in the

presence of an oat inhibitor you could

prevent

the the development of nephrotoxicity

these examples are are clinical examples

of drugs that have high renal clearance

and in the situation of mirapex tycosyn

and metformin the renal clearance ranges

from anywhere from

uh four to fivefold gfr

in in the situation where you administer

a prototypical inhibitor of organic

cation transport which is semedidine or

probenocid which inhibits organic anion

transport

you can end up with either an effect on

the oet or oct transporter

dovetalite again

because of its narrow therapeutic index

and range its important to avoid

cementidine

and also

ketoconazole is known to interact with

dofetalide

metformin which kathy giacomini will

discuss more

is a molecule thats eliminated entirely

unchanged in the urine with the gfr

being much much greater or with renal

clearance being much much greater than

gfr the most common adverse effects with

metformin are gi toxicity such as severe

diarrhea

lactic acidosis which is particularly

rare is still a very severe side effect

moreover recent evidence suggests

that theres an anticancer effect or

benefit with metformin

finishing up with hepatic uptake in

eflux i wish to discuss the role of

oatpv and v in the uptake of some

drugs but suffice it to say hepatic

permeability is an important factor

interaction with basolateral slc

transporters is an important factor to

determine whether or not your drug will

have rapid clearance

also uptake metabolism efflux energy is

something thats known to occur

the hepatic transporters themselves

are very important for many of the

statins that are administered

and they serve to actually target the

liver and in this situation its

actually a good thing

that the target for the statins and also

the transporters work in harmony

to increase the

efficacy of these drugs

rezuvastatin itself is one of the newer

hmgcoa reductase inhibitors and early

in the development of this molecule it

was found that patients of asian

heritage

had twofold higher increase in exposure

of rosuvastatin that was not related to

the metabolism therefore its important

for resume statin is with other statins

that patients be started at the lowest

dose possible

the role of slcovo

in this mutation actually was

specifically known to increase

the exposure to patients who are on

resume statin

moreover rosuvastatin is also a

substrate of bcrp

so bcrp is expressed in the gut so

patients mutant in the qk

would also have increased exposure to

rezubastatin

this figure is from the new england

journal of medicine and it just

demonstrates that patients who are

treated with a statin who are mutant in

oatpv

have an increased prevalence of myopathy

after treatment with highdose

symphostatin

if you review the labels of hmdcoa

reductase inhibitors

its nicely highlighted

that oatpb

is the

important transporter for

the movement of these drugs into the

liver and that if you inhibit these

transporters with cyclosporine

this can be a significant impact this

can have a significant impact on the

exposure to patients

both in the liver but also in the

systemic circulation which then you end

up with the more with more of a risk for

skeletal muscle injury

over the last few years actually in the

last two years the discovery of

endogenous biomarkers have been proposed

first it was discovered in patients with

rotor syndrome that they had

over

they had high levels of copropriation

and this could be a biomarker for

oatpvv

and in the study

where rifampicin was studied on

resuvastatin

theres a significant increase on the

left

and if you look at coproportion

the two actually parallel each other

from baseline to inhibited situation

so

one idea is could we replace clinical

drug drug interactions through the

knowledge of endogenous drug

transporters

substrates

however this needs further validation

especially in mild to moderate

situations where your inhibitor is

not a significant or potent inhibitor of

oatpb or v

we had such molecule with gdc00

where we studied the impact of this

molecule on pravastatin and we found a

very modest or slight interaction

with pravastatin pk

and when we studied the impact on

coporforfern

the

increase was about fold

so we believe that in essence that

coporporin or cp cp could be an

important biomarker

for

understanding the role of small molecule

drug interactions with hepatic uptake

transporters

other key endogenous transport

biomarkers which could serve as victim

substrates include

creatinine which is always measured in

can in cancer and also internal medicine

patients

uh oatp oat one substrate taurine

and then also six beta hydroxy cortisol

so theres a large

in itc effort to characterize

endogenous transport

biomarkers with the hope that drug drug

interaction studies could be understood

quite early in the clinic when a

molecule is first studied in human or

first inpatient

so summary and conclusions

we believe that many drug transporters

are important for moving drugs in

xenobiotics into or out of cells in the

body

transport or mediated drug drug

interactions

caused by induction or inhibition have

the potential to influence pk and pd

transport or mediated drug drug

interactions are routinely taken into

account in drug development therefore

its important to have robust in vitro

assays to steer the clinical

pharmacology plan

the pharmacogenetics of drug transporter

function is important consideration for

many oatpb and bcrp substrates

and an integrated approach which

consists of in vitro vivo pvpk and

clinical ddis

are really needed to define the impact

of drug transport on pk pd and ddi risk

much of this work has all been supported

by collaborators both within the itc

ic and the original itc

and

my department at genon tech id like to

thank specifically kenta yoshida

and sharmila rajan for the slides carrie

morrissey also for her work on her

projects along with lauren silvati

thank you for listening to this lecture

and if you have any further questions

please reach out to the program

coordinator at the nih principles of

clinical pharmacology thank you

you