we are honored to have dr lansing taylor

dr taylor is currently the director of

the university of pittsburgh drug

discovery institute he began his

academic career at harvard university

and remained at harvard until 9

dr taylor then moved to carnegie mellon

university as professor of biological

science and the director of the center

for fluorescent research

his research interests are linking

largescale cell and tissue profiling

with computational and systems biology

to optimize drug discovery

im sure youre going to enjoy todays

lecture

hello my name is lance taylor and im

pleased today to be speaking on

quantitative systems pharmacology or qsp

in drug discovery

the agenda for my presentation starts

with discussing the present paradigm of

drug discovery and the status of

discovering and developing therapeutics

next ill introduce precision medicine

to make sure we see the connection to

quantitative systems pharmacology this

is important because of a challenge due

to patient heterogeneity

then ill introduce quantitative systems

pharmacology or qsp

a new paradigm for drug discovery and

finally ill introduce a platform for

applying qsp for drug discovery and

development

reductionism has been a major driver in

the recent history of biology and drug

discovery

and this is characterized by identifying

all of the components of a complex

system in this case its a car

and understanding how those individual

components work and then reassembling

the knowledge into the whole system

humans are complex systems and

reductionism is a challenge

there are major organ systems

theres two to 00 cell types

0 to 0 trillion total human cells

to 0 000 protein encoding genes

approximately 00 different

posttranslational modifications

greater than 000 binary interactions

between proteins

over 00 metabolites

and approximately a hundred thousand

nodes in the interactome

as well as many different dna and rna

variants

so that thinking about identifying an

individual molecular target within this

complex array is a major challenge and

has been one of the difficulties in the

present paradigm of drug discovery

we know that humans are heterogeneous

systems and this adds a further

challenge for drug discovery and

precision medicine

were aware of the heterogeneity between

patients in a population

but its also a fact that theres

heterogeneity within a single patient

going from the whole body

to organs and tissues and cells

and the major components that make up

the cells

this is important because these are

potential molecular targets for drugs

and many of these are modified due to

mutations

we also know that these components and

others interact in time and space within

pathways that bring about either normal

cellular functions or abnormalities due

to disease

one of the things that is a core

component of quantitative systems

pharmacology

is building computational networks this

is the quantitative part of the system

this is based on field of mathematics

called graph theory and this enables

investigators to literally

develop a computational model of a

disease process where it can then be

used to predict changes and then they

can be experimentally tested

so the present paradigm of drug

discovery uses

targetcentric discovery

this is also called molecularbased

discovery it starts with

basic science in a therapeutic area

and then the next step is to do target

identification

usually a protein but other

macromolecules are being approached

today

then theres target validation and this

is usually done by a knock down

experiment in a disease model

and then assays are developed in order

to look at this particular

target

under investigation

then theres screening lead generation

lead optimization through medicinal

chemistry

early safety profiling and in vitr in

vitro admin

and then preclinical testing in animals

and then phase one two and three in

clinical trials

in the last few years computational

methods have been applied to the

standard methods including computational

methods for docking

molecules into the target in order to

optimize the medicinal chemistry steps

the advantage of this approach is that

you can investigate a single mechanism

of action on one target

the disadvantage is that target

modulation may not produce sufficient

therapeutic index

and multiple target modulation may be

important

in the last few years a

a separate method called phenotypic

discovery has been applied

and here youre not starting with an

isolated identified molecular target but

you find modulators of disease

phenotypes or functions

in this example we can see a

transcription factor thats found in the

cytoplasm in this figure

its green and upon activation it

translocates into the nucleus where it

can regulate a set of genes

in this assay we want to find molecules

for example that block this

translocation and it may involve

interaction with one or multiple targets

within multiple pathways

so in phenotypic discovery we start

again with the basic science in the

therapeutic area

we identify a phenotype that we want to

manipulate

we validate that phenotype and then do

assay development and screening this

time usually with imagebased screening

called high content screening

then do lead generation early safety

profiling lead optimization early safety

profiling in vitro add me

and now because we dont yet know the

molecular target or targets we can apply

chemical proteomics in order to identify

the molecular target

then we move on to preclinical testing

and clinical trials one two and three

once again computational methods have

been applied in the last few years

including doing

studies with rna seek where we can infer

pathways of disease progression

that these

functions may involve

the advantage is that the molecular

targets are unknown so there can be a

focus more on function in the pathways

and networks

the disadvantage is that there is a need

to identify molecular candidates

sometime during the process

and these assays you usually have a

lower throughput

by

0 the pharma research and

manufacturing association which is a

trade organization that most of the big

pharmaceutical companies belong to

did an analysis of the state of the

field

first of all the average time to develop

a drug is greater than 0 years

and the percentage percentage of drugs

that actually get out of clinical trials

to make it to becoming a drug is less

than percent

the development costs have increased

dramatically over the years for example

in 90 it was a little over 00 million

a year

whereas in 00 it was already over two

billion dollars a year

the r d spending has also increased

again comparing to 90 it was only

about billion a year

and in 0 it was already over 0

billion a year

and finally theres been an impact of

generics on sales which means revenue to

the industry

in 000 there was less than 0 percent

of the sales of prescriptions

less than 0 percent

by

0 it was almost 90 percent

so looking at the reasons for the drug

failures which has made the cost of the

developing therapeutics so high

we see that over 0

of the reasons

are based on efficacy

and

less than that but an important impact

is that of safety

so this leads us to

the opinion

that we need to explore new paradigms

for drug discovery and development

so the pharmaceutical industry has made

progress over the years a best example

of that is looking at

the effect on attrition of pk

measurements between 99 and 000 using

a combination of computational

approaches and experimental approaches

there was a dramatic decrease in the

impact of pk analyses on nutrition but

theres still a serious attrition based

on efficacy and toxicity

so the major challenge for the

pharmaceutical industry today is the

drug attrition rate in phase clinical

trials

about 0 percent of new drugs that enter

phase ii clinical trials fail

and the major reason is drug efficacy

some of the explanations for those

failures include targeting the wrong

mechanism

targeting the wrong patient population

sub optimal dosing of the drug for the

right target and drug combination

therapies actually being needed for the

disease

the present paradigm of drug discovery

also depends on animal models and

testing

so for animal studies we know the

physiology is very distinct between

animals and man

so for example the concordance of target

organ toxicity between laboratory

animals and man is not very good using

the example of the liver or in this

slide hepatic its only about 0 percent

concordant between human and animal

testing in toxicity

it also has a very low throughput

its very expensive to

use animals

the methods actually date to the 90s

and theres an increasing amount of

societal pressure to minimize the use of

experimental animals in drug discovery

and testing

so there is value in finding

alternatives to animal testing and

creating a new paradigm for drug

discovery

although ive identified multiple things

that are not optimal in the present

paradigm of drug discovery we have

developed some significant drugs

for hiv aids for hypercholesterolemia

chronic myelogenous leukemia autoimmune

diseases and herpositive breast cancer

these are all great drugs the only

challenge has been its been very

inefficient in developing these drugs

so one of the things that i want to put

in perspective when i start talking

about quantitative systems pharmacology

is precision medicine

this is an approach for disease

treatment and prevention that takes into

account individual differences in

lifestyle environment and biology

in an important paper that was published

in 0 by francis collins and the

harold varmus

they defined

precision medicine

as the prevention and treatment

strategies that take individual

variability into account

it is not new blood typing for instance

has been used to guide blood

transfusions for more than a century

but the prospect of applying this

concept broadly has been dramatically

improved by the recent development of

largescale biologic databases such as

the human genome sequence

as well as powerful methods for

characterizing patients such as

proteomics metabolomics genomics

diverse cellular assays and even mobile

health

technology and computational tools for

analyzing large sets of data

this slide shows the flow of information

in precision medicine today starting on

the left with an individual patient you

can collect clinical characteristics

from the electronic health records and

then data based on

genomics transcriptomics and proteomics

that can be added to prior knowledge

such as physiology biochemistry cell and

molecular biology

all of this information being fed into

building a network on that patient and

the disease

in terms of network theory all of the

components that make up the network are

referred to as nodes

and all the connections between these

components are referred to as edges

and again the mathematical field that

drives the development of an analysis of

these networks is called graph theory

so the idea is to create a large number

of these networks

and this library of networks of human

diseases that are patientspecific can

then be used in making predictions about

therapeutic strategies for the patients

so at the present time precision

medicine is driven to find the right

existing therapeutic

getting it to the right patient at the

right dose and at the right time

so now i will introduce quantitative

systems pharmacology

which is a new paradigm for drug

discovery and development and this

really had its birth

in two workshops held at the nih one in

00 and in late 00

and it included representatives from

academia industry and government that

reviewed the state of the art in two

very quantitative fields that were then

distinct systems biology and

pharmacology

to determine if a combination of these

two disciplines might transform drug

discovery development and clinical uses

of therapeutics

and a white paper was published which is

listed at the bottom of this slide

indicating that there was a need for new

approaches to drug discovery and

development through the introduction of

concepts technologies and researchers

from the fields of computational biology

systems biology and biological

engineering to pharmacology

this new field of qsp has roots in

classical pharmacology and physiology

but adds a molecular and systems level

approach that allows the investigation

of the responses to drug treatments in

the context of complex signaling

transcriptional and metabolic networks

and the patient variability

theres a very good definition of qsp in

this white paper and its defined as an

approach to translational medicine that

combines computational and experimental

methods

to elucidate validate and apply new

pharmacological concepts to the

development and use of small molecule in

biologic drugs

further qsp will provide an integrated

systemslevel approach to determining

mechanisms of action of new and existing

drugs in preclinical and animal models

and in patients

qsp will also create the knowledge

needed to change complex cellular

networks in a specified way with mono or

combination therapies

alter the pathophysiology of disease so

as to maximize therapeutic benefit and

minimize toxicity and implement a

precision medicine approach to improving

the health of individual patients

this figure describes the

difference between how the

pharmaceutical industry was beginning to

investigate qsp around 00 and academia

on the right side

is what the pharmaceutical industry was

doing back in 00 and continues that

today

and that is focusing on systems

pharmacology particularly pk and pd

analyses

especially in the phase one two and

three and now four phases of clinical

trials

in contrast in academia they focused on

systems biology chemical biology and

genetically engineered mouse models

building computational network models

doing target identification

screening using chemical biology and in

the

in the middle of those was chemistry and

animal studies being applied by both

however it was clear there was a

disconnect between these two directions

and it had to be brought together and

qsp promises to make this a functional

continuum

theres also the complexity in

thinking about systems biology and

systems pharmacology separately

systems biology kind of represents a

horizontal integration of understanding

how drugs interact with targets

target in cells cellular networks and

multicellular networks

this is a focus of systems biology that

has been present

the vertical integration comes from

systems pharmacology where people

started studying the interaction of

drugs with purified components cells

organs animals patients and populations

with the goal of all of the technical

applications to get to the point of

defining the systematic holistic

understanding of drug action

this is an integrated view of

quantitative systems pharmacology

one of the things that became apparent

was that we needed a new type of

investigator in order to join the drug

discovery

team

so

when we incorporate computational

modeling and simulation into the

pharmaceutical r d

function with experimentation

we have to start by gathering all of

that data the big data with the

analytics

build the computational model you can

then make

predictions

from that model and then do the

hypothesis testing through

experimentation

this leads us to the point which is the

key in qsp

in that its an integrated and iterative

computational and experimental approach

with the standard drug discovery

scientists we now have to add

computational scientists

modeling engineers

data

programmers and computational

biologists

in building these

simulation and computational models

we start with a model scope where we

have to identify physiological pathways

disease processes

organ systems

pharmacology pharmacokinetics

that are to be included in the model

then develop detailed physiological maps

representing the model variables and

their interactions

the next step is model development

and in fact there are models floating

around that have been under development

so you can collect prior models

nonclinical and clinical data that will

be used to develop mathematical

relationships in the model

apply mathematical functions to describe

the rates of processes in the model

and the volumes of all the physiological

compartments

in the next step which is the model

qualification you collect the relevant

clinical data in patient populations so

it would be used to calibrate and

qualify the model

and then calibrate the model at all

relevant scales of time and physiology

in these qsp inspired computational

models

theres multiple steps

one to analyze the networks to identify

optimal points of intervention

remembering that were not starting with

a isolated molecular target but were

identifying processes defined by

networks

we then use models to improve selection

of primary and backup targets

we model outcomes and variants

predict on target and off target safety

model absorption distribution metabolism

excretion and target engagement

sustain target validation throughout

drug development

and model therapeutic repurposing

this requires the integration of diverse

data sources into the computational

models

bringing about the need for the new

areas of socalled big data and

analytics for patients

we bring together data

thats mechanistic from in vitro studies

target characteristics drug properties

in vivo data human physiology genetic

information as well as human pathology

based on prior clinical data

this is all fed into the construction of

the network

the mathematical equations there are

represent the fact that ordinary

differential equations are usually used

to develop these mathematical models

once the model is in place and validated

it can then be used to make

translational predictions and ill give

an example in a few slides

so computational modeling

has evolved in terms of different levels

of mechanistic detail

starting with the drug focus early

models were focused on pk and pd

and they described

the gathered data but did not

attempt to make quantitative insights

into underlying mechanisms because there

wasnt enough detail it just explained

the data at hand

you can increase the mechanistic detail

and this has now been done using

pharmacologybased pk

models

and this

facilitates the use of additional

mechanistic data to make predictions and

now there are even commercial packages

that perform this

computational work

finally with the disease focus this

requires a large amount of mechanistic

detail

so the mechanistic data for predictions

of efficacy or changes in safety signals

are a focus

so

in getting started with computational

modeling theres already a large number

of computational models available in the

public domain

this is one site that is worth

investigating its the bio models

database and its managed by the

european molecular biology

laboratory in the link is shown in the

slide

so

multiscale networks are needed to

understand and to predict drug action

and this starts all the way back with

computational modeling of drug receptor

interactions or drug target interactions

the socalled atomic or molecular

interactions scale

that is fed into cellular and tissue

level networks

and those that are used to help build

organ level networks and physiology

with the ultimate goals we want to do

whole body outcome predictions looking

at things like what affects blood

pressure control

heart attack arrhythmia issues

so in order to understand the mechanisms

of drug action and predict efficacy we

have to integrate these network models

from the molecular level all the way

through to the human

so ill give an example of how one of

these

computational models is used

and this is on asthma which is a chronic

inflammatory disease involving many

immunological and stromal pathways

this table lists the things that were

taken into consideration and quantified

within the model

that included innate immune cells

adaptive immune cells including thy two

lymphocytes

and thats something ill focus on in

the example i give

airway resident cells soluble mediators

clinical measurements including

the forced expiratory volume in one

second

thats called

fev

interventions used to develop the model

these are known drugs with known targets

that can be used to manipulate the

network

and then patient types

including patient subtypes which ill

use in the example i give of thy two

high

patients patients that have a large

number of

type lymphocytes

so in a in this figure we see the

computational network thats been

developed

it includes the airway stroma and

functions cytokines and chemokines

adaptive immune immune immunity

innate immunity as well as the

therapeutic interventions

and ill just focus on the top graph in

b

where

we had a patient sample

or cohort that had high thigh two

as patients

and it was demonstrated that a single

agent

that was an antiil

showed in the solid blue line an

increase in the fev capability of the

patient

it was then predicted from the

computational model

that using a bispecific

molecule that included

antiil

plus a proprietary

target would increase the fe fev and

in fact experimentally shown here with

the blue dotted line the experiment

matched the prediction

so this is a key value of incorporating

simulations and quantitative models in

drug discovery

very early on is that you can avoid

making a large number of experiments

make predictions and test those

predictions

in addition mechanistic the mechanistic

basis of the model allowed investigation

of which pathways drove the predicted

response and it also predicted changes

in the circulating eosinophils which is

actually a biomarker for asthma

so we

decided to define

qsp in a more functional way

and so our definition of qsp which we

published on a year or so ago

was determining the mechanisms of

disease progression and mechanisms of

action of drugs on multiscale systems

through iterative and integrated

computational and experimental methods

to optimize the development of

therapeutic strategies

this is a simple summary of

the approach

which at the same time advances

precision medicine

through quantitative systems

pharmacology

so presently precision medicine is

practiced today as i mentioned before

you start with patients patient data

you get patient samples to perform the

various omics analyses like genomics

then use patient data analytics to

extract information from that data

and patient big data management and then

youre in a position to predict make

predictions from patient data using

computational and systems biology this

is the point where i mentioned before

you get the right drug to the right

patient

you can continue around this circle

in order to practice and by the way at

this point youre only able to get

existing drugs to the right patient at

the right time at the right dose if you

want to develop new therapeutics you

continue around this circle

implement therapeutic area basic science

develop experimental models of disease

and in our perspective they need to be

humanbased experimental models

build computational models of the

disease

and then apply the usual drug discovery

sciences

in implementing this you get both

precision medicine and

precision drug discovery

so i mentioned before that humans are

heterogeneous systems and its a further

challenge for drug discovery and

precision medicine

ive introduced the concept of the

networks computational models to make

predictions and then test those

predictions

but we need a better experimental

platform as well

remembering qsp is both computational

and experimental

so one of the things that is under

active development in many laboratories

is building human tissue based

experimental models

either d models and standard

microplates or more recently and

potentially more powerfully in

microfluidic devices

the real impact of this technology for

building better human experimental

models will be brought around

by the use of induced pluripotent stem

cells from patients themselves so you

can have the proper genetic background

in developing experimental models as

well as the proper disease

background

weve actually developed a platform to

functionally

do a continuum from precision medicine

to drug discovery

again were focused on patients and

patient samples and data

its also possible to have validated

target knowledge from the literature

an example of that that ill use is that

we know in metastatic breast cancer

there are mutations in the ligand

binding domain of the estrogen receptors

this is well established and we want to

incorporate that into our knowledge and

models

but we also do an unbiased study

where we can infer pathways of disease

progression and we do this in metastatic

breast cancer by performing rnaseq on

both primary tumors and the metastatic

tumors and then using computational

methods to infer the pathways of disease

progression from primary to metastatic

state

once you have these inferred pathways of

disease progression

this is now essentially a list of

molecular targets within each one of

those pathways

and we can use

tools such as machine learning and

databases of drugs and targets like drug

bank and stitch

and actually make predictions about what

existing drugs could interact with the

targets

in your listed pathways

this is actually an important step

for a repurposing drugs as well as

making

tools

to probe the experimental systems

so in the next step we make phenotypic

models of disease and safety again we

focus on humanbased models particularly

using microfluidic systems

and like any other

manipulation approach

theres tools such as rnais crispr and

mutant cdna libraries and annotated

focused compound libraries that can be

used to manipulate the experimental

models

profiling is done by high content

screening and a key element of this

approach is the fact you get

heterogeneity analysis at the same time

because by imaging these devices youre

measuring cell by cell

so that any heterogeneity is quantified

coming out of those screens

are potentially drug hits from profiling

and those can be

the drugs that were predicted in the

machine learning stage or from the focus

compound libraries and of course

medicinal chemistry can be applied right

away to optimize

these molecules

again we dont know at this point what

the molecular target or targets are

so at this point we implement chemical

proteomics and rna eye knockdown studies

in order to identify the molecular

targets

that are involved in the phenotypic

manipulation

once youve identified those molecular

targets you can revert to traditional

targetcentric drug discovery and do the

medicinal chemistry based on

targetcentric work

we also build mammalian models of

disease and safety as part of the

multiscale needs

and from published literature as well as

the assay data generated here we can

construct the computational models

so once a computational model is

constructed its not going to be

complete with one round so this is an

iterative process we can go back around

this

make predictions from the computational

model test those predictions by using

rna knockdowns for example

rerun the assays and then update the

model and you can do that very early on

in the drug discovery process before you

get to expensive steps

because this is a systems biology

approach essentially you expect disease

specific emergent properties

like optimal therapeutic strategies pd

biomarkers of drugs and prognostic and

predictive biomarkers and of course you

want to get to the point of helping to

design a clinical trial

and then simulate that clinical trial

so this slide kind of is a simple

summary of the first part of

that

qsp

approach

and thats the computational and

database selection of existing drugs to

probe the modulation of the phenotype so

on the left we can see patient rnaseq

data from a normal versus a disease

state or the example i gave a primary

tumor versus a metastatic tumor

this identifies

genes that are upregulated or down

regulated

from those genes we can infer the

pathways of disease progression that

they take part in

from that you can

determine molecular targets from those

pathways

and then you can use the database and

machine learning

to computationally predict drugs that

would interact with those targets

and then those predicted drugs can be

experimentally tested in a humanbased

phenotypic model of the disease

ill take a short attack here to add an

important new omic in our perspective

and that is really

characterizing and quantifying the tumor

microenvironment

based on computational pathology which

we think is an important new omic to add

to genomics proteomics etc

on the right you can see a classic

diagram of a tumor

its very heterogeneous theres cancer

cells at different stages of genomic

evolution

theres normal epithelial cells

theres a

migratory immune cells that have been

filtrated into the tumor

so its a complex tumor micro

environment and its been demonstrated

in recent years that depending on the

makeup and the spatial relationships

within the tumor of these different

types of cells

the tumors can respond differentially

to different therapeutic agents

so we want to characterize the tumor

microenvironment determine the cellular

content the state of activation of cells

like immune cells

and then define quantitatively the

spatial relationships between the cells

then we can infer mechanistic basis of

disease progression using network

systems biology but based on the cells

and their spatial relationships within

the tumor

its possible to create prognostic and

diagnostic tests

to then recapitulate the tumor

microenvironment in a human experimental

model

and then iterate computational

experimental models of disease to

further

develop a therapeutic strategy

so

the key is quantifying spatial

interactions

and the measurement of multiple

biomarkers and tissue sections

coupled with machine learning tools to

characterize the spatial interactions

and infer signaling networks responsible

for tumor progression is a major area of

development today

so if we start with a primary tumor

we can take tissue sections and now

label it with multiple

fluorescently based antibodies for key

tumor and nontumor cells

multiplexed fluorescence is anything

from one to

seven different biomarkers in a single

section

and then hyperplexed fluorescence

involves cyclical

labeling imaging quenching of

fluorescence and rounds of labeling to

look at up to 0 or 0 different

biomarkers in the same section

the spatial analysis

is developed first by learning patterns

of each individual cell in the field of

view

what biomarkers it is showing a positive

response for

and then each cell is identified and

then around it spatially defining whos

near it

this can be quantified by a machine

learning approach called pointwise

mutual information so you can literally

define the tumor microenvironment based

on spatial relationships between

different types of cells

when you know the different types of

cells and how close they are to one

another you can infer the signaling

networks that exist between those cells

and this

we believe will lead to better diagnoses

prognoses

and

therapeutic strategies

so moving to the experimental models of

disease one of the major advantage

advances made over the last five or six

years is the development of human

microphysiology systems or mps

as experimental models in drug discovery

and development this is a program put

together

as a collaboration between the nih

particularly ncats darpa the fda and the

epa

the goal was constructing microfluidic

d human organs on chips

linking these organs together in a

platform to provide physiologically

correct human model systems

and in particular

incorporating induced pluripotent stem

cell derived cells in order to test

drugs with distinct

genomic backgrounds and against disease

models

for

precision or personalized medicine

so this slide shows kind of a platform

that has been developed to make a d

microfluidic

liver

for experimentally modeling liver

diseases

phenotypic screening and for early

safety testing

in the upper left

we have

the description of the present

generation model it involves cell

four key cell types from the liver

its layered to the point where there is

a separation between

a

the hepatic chamber and a vascular

chamber so you can move things through

the system just like it occurs within

the liver

can also take a subset of the liver

cells

and label them with fluorescently

labeled biosensors so you can measure

things in real time such as ros

production and apoptosis

since these microfluidic devices are

under continuous flow like blood flow

within an organ

you can take media efflux and sample it

for secreted proteins

measuring oxygen and ph and metabolic

readouts using mass spec

with the biosensors you use high content

imaging and various of the sentinel

cells can be analyzed in time again for

a variety of physiological readouts all

of this data is then captured in what is

called the microphysiology systems

database

where you can also

import data from other databases because

the ultimate goal here is to not only

acquire

analyze

the data from these devices but also do

the computational modeling

in building a liver experimental model

for drug discovery

the focus has been on the liverocenus

its a basic unit of the liver

the blood flow into the one end of the

liver cenus is very high in oxygen

thats called zone one and by the time

the

the media or the blood flows through the

liver theres a consumption of oxygen

principally by the hepatocytes so by the

time the blood flow gets to the central

lane a vein

the oxygen tension is very low

so there are four major cell types the

hepatocytes the endothelial cells the

kupfer cells and the stellate cells that

make up the liver oceanus and the goal

is to recapitulate the content as well

as the d structure and function

so this slide shows the 0day day

function of the initial liver

microphysiology system

and its characterized by

lasting at least a month as evidenced by

the lack of ldh release once its

stabilized

it also demonstrated high levels of

albumin and urea synthesis

more physiological than in static

cultures

we were able to demonstrate that the

sips activities and the phase ii

conjugations were maintained during that

month

and you can induce fibrosis within the

liver with methotrexate treatment

because the stellite cells were

activated they expressed smooth muscle

actin and they also produced

collagen could also demonstrate immune

mediated hepatotoxicity

by combining lps with a drug like

trovofloxacin that induced apoptosis

so the second generation

model was called the lamps a liverocenus

microphysiology system

its physiology was improved by the

addition as you see here in purple of a

thin layer

of liver extracellular matrix put down

between the hepatocytes and the

endothelial cells representing the space

of dc within the liver acenus

this model

maintained its threedimensional

structure and all of the activities

described in the first generation

but we also

recapitulated zone one and zone three in

separate devices to explore

the the biology

of of the microenvironment

so

we created zone and zone models by

modulating the oxygen tension within the

devices

this slide shows

biological data demonstrating that the

microenvironment of zone and zone

with known biological

activity differences

were recapitulated

zone data is shown in red zone in

blue

and as expected in

zone

albumin urea

secretion was greater than in zone

oxidative phosphorylation no surprise

because of the high oxygen tension was

higher in zone one and glucose levels

were higher in zone one

also as expected alpha one antitrypsin

secretion was higher in zone three than

in zone

sipe

expression was higher in zone

as was the ability to induce steatosis

higher in zone than in one and

acetaminophen toxicity was higher in

zone three and zone one so now we have a

model that biologically is representing

what is going on within the liver so now

we can do more experimental

manipulations and create disease states

within these microenvironments

so one of the diseases that weve been

exploring is metastatic breast cancer

and creating a metastatic niche within

these liver devices

and

the communication between the cancer

cells and the endogenous cells within

the liver are actually very important

and this is evidenced by the fact that

since we are interested in the ligand

binding domain

mutations

we have a wild type

mcf cell

and these mcf cells are labeled with a

fluorescent protein so we can measure

them within

the device you can see on the upper

right

the image data sets of red cells and

were quantifying

growth by the measurement of the number

of fluorescent cells

but we also use crispr

in order to put in mutations to the

major mutations

within the

estrogen receptor

one called

ys and the other called dg

and if you look at the bottom left when

we did traditional d

experimentation with these mcf cells we

found that the gray line which

represented the dg

mutation it had a growth advantage over

the other mutant as well as wild type

however when we put those same cells in

the presence of the liver micro

environment particularly within the

lamps models

we found theres a switch

and that in fact the mutation

ys actually gained the growth

advantage

so now were in a position to explore

the effects of different drug treatments

within a micro environment which is more

or less recapitulating the micro

environment within

within a liver in a metastatic disease

and demonstrates that you really dont

want to be using d models because they

dont reflect the physiological

activity

in vivo

so then

obviously since this is iterative

computational and experimental work

we were interested in a particular set

of pathways and so we wanted to untangle

the

the

insulinlike growth factor one from

insulin signaling in breast cancer

and this

slide just shows the continuum of

generating data in this case the data

was a reverse phase protein array

and then building computational models

first a statistical inference model

which could then be

tested hypothesis generated and tested

and then building mechanistic

predictions and testing those so its a

whole

sequence of things that are ongoing work

but in

looking at

the computational model and how we could

use it

we know that the igf and the insulin

receptors share 0 of their sequence and

that factors bind to each others

receptors

and that hybrid receptors can form

and signaling pathways share downstream

components

so a key question is how do we

specifically target only one of the two

highly overlapping pathways

the answer is building a computational

model and then testing it

so in

testing the model we started with the

rppa data

which had phosphoproteins in the

array

different cancer cell lines

looked at six different time points of

stimulation from five minutes to

hours using stimulation of either igf

or insulin stimulation

so from that data

there was an influence

graph that developed of the major

components

and there are some were involved in that

thats the purple errors arrows igf

only interactions and some were insulin

only interactions

but one of the key things were able to

do is based on this

network

is to make a prediction

and the first prediction was that if we

knock down acc

the map

k activation was predicted to be higher

with igf stimulation

than with insulin stimulation and

looking at the

the western blots at the bottom of that

you can see the igf had a larger

effect than the insulin as

was predicted

similarly it was predicted

that the ecad knock down

the effect on akt activation was

predicted to be higher again with igf

stimulation than with insulin and the

data

supported that

so heres an example where you can build

a network make predictions and then this

can lead you to understanding how to

intervene in an optimal way rather than

just random

testing of compound libraries

so

in the future in recapitulating the

complete liver microenvironment

there is now a new

microphysiology

system

called the vlamps of the vascularized

liver cenous microphysiology system

which has vascular flow like the liver

sinusoid

you can

do things like add immune cells into

that vascular flow and test for

immune infiltration into the disease

model

you can actually also study

extravasation and start by putting the

cancer cells that are labeled into the

vascular flow see them bind to the

primary uh uh

the liver

sinusoidal uh endothelial cells and then

loc translocate into the hepatic

compartment

you can create a continuous

liver oxygen zonation which is

physiological and the system can be

maintained for a month or more so were

now in a position

to have a model system where we can

recreate the disease and use it to test

therapeutics based on predictions made

from the computational approaches

i mentioned before the importance of

induced pluripotent stem cells

the real value going forward is the

ability to collect patient skin cells

to then generate induced pluripotent

stem cells from those skin cells and

then guide their development and

maturation along different paths to

particular cells including cells of the

liver like hepatocytes

then those ips derived cells can then be

put into these microphysiology systems

so you can literally have you on a chip

and get the testing that is optimal for

your genetic and environmental

background for your disease

so this will permit personalized drug

testing

and the ability to investigate in detail

disease mechanisms in cohorts of

patients of similar backgrounds

so the future of qsp

is the ability to implement the full

platform for diseases starting with the

patients patient samples and data

to develop multiscale experimental and

computational models of the diseases for

drug discovery along with pkpd for drug

development

and to develop more patientderived ipsc

based microphysiology

experimental models to account for

heterogeneous genomic and disease

backgrounds for drug discovery and

development

with that id just like to acknowledge

various people that influenced me in

thinking about qsp

thank you for listening to this lecture

i hope this

has given you information about qsp that

you can utilize

in your work and studies

if you have any questions you can

contact the program administrators thank

you