today we are fortunate to have dr jim

dr dorsal is currently the deputy

director for clinical and translational

research of the national cancer

institute

and the director of the ncis division

of cancer treatment and diagnosis

from 9 to 00 dr dorsal was the

chairman of the city of hope

comprehensive cancer center department

of medical oncology and therapeutic

research

dr dorsal received his undergraduate

degree magnum laude from harvard

college in 99 and graduated from

harvard med in 9

following the internal medicine

residency at the mass general he

completed fellowship training in medical

oncology at the nci

we are pleased to have him give todays

lecture

my name is dr james dorso im the deputy

director for clinical and translational

research at the national cancer

institute and i have the pleasure of

speaking to you about the subject of the

role of pharmacodynamics and the

development of new drugs

this really is an over all view of my

talk

and really is the most important this

and the next the most important slides

of the presentation

because were going to define

what it is were aiming to understand

along the lines of pharmacodynamic

biomarkers in the development of new

agents so what is pharmacodynamics well

simply put is what the drug does to the

body

as opposed to pharmacokinetics is

actually what the body does to the drug

how it metabolizes the drug how its

excreted etc

and to understand pharmacodynamics one

really needs to understand that what

youre really trying to do

is to develop biomarkers that will give

you additional information about how a

drug might work in a specific situation

so were looking for the molecular

changes that result from drug action or

alterations in the intended target of

the drug socalled proofofmechanism

studies

also if the target is a cancer driver

for example

were interested in developing ways to

demonstrate

how that response to hitting the target

has an impact on disease response

socalled proof of concept

so more definitions

what happens

what is the first thing that youre

trying to measure youre trying to

understand the action that the drug

produces along a specific biochemical

pathway so the primary pharmacodynamic

effect is actually the intended

molecular target and the effect of the

agent on that target socalled target

engagement an example would be

a

receptor for a growth factor like

epidermal growth factor receptor and the

binding of its natural ligand or the

binding of a drug that alters

the effects that the downstream effects

of that particular target whats a

secondary pharmacodynamic effect well

that would be some type of proximal

biochemical change that is a direct

consequence of target modulation so an

example again from the egfr

an egf pathway

might be the phosphorylation of irk

or something downstream of erc

that is a direct response to the initial

phosphorylation of the epidermal growth

factor receptor by

the its natural target

and then

what about what is a tertiary

pharmacodynamic effect well that is a

response that looks at whether its in a

patient or

in a cell line or an animal model what

is the biochemical basis for the

cellular response what is the end

product of that youre looking for in

terms of target engagement so that could

be some form of cell death

some other form of altered structure of

the cell or alteration in the organ

that is

and must go through a variety of

different biochemical steps and before

one produces that desired proof of

concept

target

so the other thing that ill try to make

clear through a series of examples today

is

examples of what a fit for purpose

biomarker means what does it mean to be

fit for purpose well this is really an

essential concept because

it basically means that what youre

trying to measure

and the assays that you develop to

measure those things

must be directly tied to the mechanism

of action of the drug

the measurements need to be robust and

they need to be suitable for clinical

samples because after all we want to

demonstrate in patients

whether or not the drug youre utilizing

and were examining has actually hit its

target and then we want to make sure

that the effect produced by the drug is

not simply due to chance

due to chance changes in variability of

the target expression or effects of on

the target the change must be large

enough so that one can actually

definitively measure an effect of the

interaction lets go a little deeper

into

the concepts of proof of mechanism and

proof of concept

so you have a drug

its administered to an animal to a

person

and one wants to know whether or not the

target is modulated

it can be a very proximal target like a

growth factor receptor as i said it can

be something that is downstream from

that target

related to the effect that youre

overall looking for but it has to be

directly

measurable

and it needs to be measurable in a

tissue that youre interested in im a

medical oncologist so im interested in

measuring the effects for the most part

in tumors

but if the drug is an antihypertensive

drug for example or a drug that affects

the vascular system there must be ways

to measure the effects the direct

effects of modulating the target

one has to be able to measure how much

the target has been inhibited

and how long the inhibition has gone on

which well discuss in greater detail we

need to understand and be able to

correlate the relationship between how

much drug is in the body

namely its pharmacokinetics or its

tablet how often and how long

metabolites are present and the proof of

hitting the target and what are the

relationships

and also

very useful is to understand whether

hitting the particular target

in tissues that are not your initial

goal to evaluate for example the

cardiovascular system when youre

looking at a cancer patient

or the kidney when youre trying to

evaluate

an immune modulation

in rheumatoid arthritis what is the

relationship between hitting the target

and the toxicology and the as well as

the efficacy of the agent and finally

how does hitting the target actually

have an impact on the overall

effectiveness of the agent as well as

its toxicity

well thats the the early

pharmacodynamic response is one related

to demonstrating that the proof of

mechanism exists in a clinical situation

either in an animal or

in in a patient

but ultimately one wants to understand

whether theres a relationship between

hitting the target

and socalled proofofconcept that is

what are the functional consequences of

altering the target inhibiting the

target activating the target does it

affect proliferation does it affect

metabolism of an organ or or of a tumor

does it affect blood flow

and

does it affect some critical endpoint

namely dna damage that is actually

essential to the viability of the tumor

or of other tissues per se

proof of mechanism

resulting in proof of concept

two critical uh concepts that id like

to make sure that you understand

so why worry about this uh major uh

undertaking of trying to understand

whether or not youre actually engaging

the target of interest

and that really is demonstrated in this

pie chart which comes from a paper

that reviews

along for a whole variety of different

types of drugs across many drug classes

not just oncologic drugs but vascular

drugs drugs for

arthritis drugs for pulmonary

diseases and what you see is that over

the last 0 or years

its very clear that we can produce

drugs that have their appropriate

bioavailability and pharmacokinetics

very rarely do drugs fail

because the pharmacokinetics are bad

sometimes unavoidably there are safety

issues and that accounts for less than a

quarter of the reasons that phase two

trials fail

um about 0 of the time it turns out

that drugs

dont really fail but theyre withdrawn

from after or during phase two

evaluations because there are strategic

issues another drug comes on that is

more effective and theyre withdrawn

from the market but most importantly

over half of the time

the reason that drugs fail is they

simply dont work

and so if one gets to the point of

having finished a phase one trial you

know the safe dose and schedule you know

the toxicological profile

it still turns out that half of the time

your drug is not going to demonstrate

efficacy

and all of the efforts related to

understanding molecular pharmacodynamics

are really efforts trying to decrease

that percentage

to increase the

number of times that when you have an

agent and you go to understand how it

might work that you have a better chance

of succeeding

looking at this in another way and again

this is data from pfizer published about

five years ago now

for agents across the spectrum of

molecular entities in which they tried

to understand

when and how they could avoid phase two

failures

so it turns out that about a third of

the time

if you have a way of understanding the

proof of mechanism

then the drug is effective

then youve youve succeeded and its

very highly likely that your phase three

trial will then be successful

its also true that for about uh 0

of these

of these cases

when the

evaluation done in the clinical trial

allowed for proof of mechanism

and then if the drug didnt work even in

those circumstances and there are many

reasons why that should happen

um you know that you can walk away from

the development of that agent because

youve hit your target but some

understanding of the downstream

biochemistry and pharmacology of the

agent was probably faulty and so hitting

the target did not necessarily produce

tumor shrinkage or lower your blood

pressure or any other of the proof of

concept to endpoints that you were

looking for

whats truly amazing and this was these

are data only i say say five years old

is that somewhere between 0 and

percent of these agents that pfizer was

developing

failed in testing

and there was no evidence of

in fact no attempt to understand proof

of mechanism

and so

while the trial was a failure there was

no better understanding of why the drug

failed and so the socalled three

pillars of survival and success for drug

development that devised by pfizer

scientists are one that to be successful

you need to be able to measure the

required exposure at the site of action

you need to know that your drug

is getting to the target

for a sufficiently long period of time

and at sufficient concentration to

actually inhibit the target you need to

know that

there is appropriate binding

and that it may and that the effects on

the receptor for example

may be longlived

and then finally and perhaps most

importantly

one needs to be able to demonstrate that

hitting the target hitting altering the

receptor produces the relevant

downstream effect that youre looking

for so that you will get youll have

proof of concept

lets talk about a variety of drugs im

a medical oncologist and so im most

familiar with oncologic drug classes but

clearly this applies in a variety of

different ways to antihypertensives to

antiinfectives

and its its its in many ways much

easier in some other uh aspects of drug

development um its not hard to

understand what the biomarker is for an

antihypertensive its blood pressure

its control of blood pressure duration

depth etc

and you have you can demonstrate

directly if that is the proof of

mechanism

that that has occurred

on the other hand it is truly remarkable

how many very effective anticancer

agents

have been developed with no biomarker at

all so heres the heres the distinction

we have drugs for example that are

targeted against the her oncogene

trastuzumab is a wellknown antibody

also known as herceptin

we know that that drug only works in

women whose breast cancers express

her at a sufficiently high level or

have amplification of the her gene

so we can find those 0 or percent of

women who have tumors that

demonstrate that biomarker and the

success for that antibody transfusion

map is on the order of two thirds to

percent of patients will have a response

in patients who do not have that

biomarker the odds of success are very

small not nil but somewhere in the range

of

five percent or less

we know very clearly from studies done

about a decade ago that drugs that

target the egfr

receptor

in

nonsmall cell lung cancer if one has a

specific mutation in that receptor one

can

increase the

likelihood of knowing that the

likelihood of response is going to

increase from to 0 percent in

unselected patients to probably over

twothirds maybe threequarters of

patients if that mutational status is is

clear but many of our

standardly used commercially approved

drugs whether theyre cytotoxins that

target dna like alkylating agents or

theyre targeted agents for example the

met inhibitors that

affect the phosphorylation status of the

cmn oncogene

we know that theres efficacy but we

cannot predict the efficacy because we

have no way of knowing and no assays

that we can use to understand how and in

what groups and at what concentrations

the drugs must be administered and under

what schedules that we definitely are

can expect a clinical response

so im going to make the argument and

the argument

throughout this talk

that its really

the ability to understand proof of

mechanism that allows one to do modern

drug development based on

pharmacodynamic biomarkers

you need to have the ability to

understand whether proof of mechanism

can be demonstrated

in

an in vivo situation and in particular

in patients so that the hypotheses that

have led to the development of the drug

and surrounding its mechanism of action

actually can be shown to be true in vivo

many many agents in the history of

oncologic drug development or the last

0 or 0 years have had hypotheses about

specific mechanisms of action once they

have been applied in patients either

that mechanism of action has been shown

to be unequivocally not the case and

other mechanisms have come forward

or

those drugs have often failed in

development because we could not come up

with another hypothesis to really better

test

that drug or members of its class

knowing whether or not the drug in

question modulates the target

also assists in understanding whether or

not one should move from phase one to

phase two or even further

into very large very expensive phase

three trials

this is particularly important when

understanding drug schedules and

the concentrations of the exposures that

occur in patients because there are many

drugs that we have at least the

oncologic field

because of differences in metabolism

and a variety of pharmacogenetic

parameters that exist in mouse models

rat models

even in outbred dog models

demonstrate that an agent that works

fantastically well in a mouse model

because of altered drug

clearance in humans versus mice it

doesnt work at all in patients so

ultimately this an understanding of

whether theres proof of mechanism

thats been demonstrated needs to be

done in patients

the other thing these early proof of

mechanism studies allow one to do is to

find out whether or not there is value

in noninvasively understanding proof of

mechanism

its because it is difficult and well

talk about that

to obtain multiple biopsies in patients

sometimes tumors are in sites that are

not safely

accessible

if there are potential pet scanning

approaches metabolic approaches that can

be assessed by mri for example that

really reflect proof of mechanism it

gives you the value doing these early

studies where one very carefully and

intensively evaluates possible

mechanisms of action

in patients are of great value and also

it can give you some understanding of

whether you should

expect efficacy so if the drug does not

hit its target in a pilot study then the

expectation that there will be an

effective agent moving into phase two

and later studies is lowered

the other whole area of exploration that

has not been done

in a way that uh as carefully as it

might have is trying to understand

molecular effects of a particular agent

pharmacodynamic effects in the in the

understanding of effects of the agents

on nonmalignant tissues so trying to

understand the molecular toxicology of

an agent

this has been done to some degree

eat the first generation egfr inhibitors

all inhibit

the signaling of the egf receptor not

just in tumors but also in normal

tissues and so its very common for

patients treated with those diseases to

get a very particular

maculopapular rash that looks like acne

around hair follicles

and at first this wasnt clear why this

happened and its of course now been

well documented that the

density of egf

or set the egf receptor around the base

of the hair follicle is very high and so

this is really demonstrative proof of

mechanism binding to the target in a non

malignant tissue it also helps you

understand

the

potential for toxicity at that site

lastly i think its really very

important when considering

this first step

in doing

the development of a new drug to

understand that

demonstrating proof of mechanism in an

early study

is not necessarily predictive of

clinical benefit

only large later stage studies

can demonstrate that a particular

biomarker which has been

demonstrated to be fit for purpose in a

mechanism study is actually going to be

predictive of activity in the clinic

so for the rest of my talk im going to

try to hit by example

several of these if not all of these

topics were going to talk about the

development of the right assay for the

target in question

i think its critical to understand

whether the target is suitable

for the purpose envisaged

were going to talk

a fair amount about assay validation and

clinical readiness before you start your

trial

demonstrate demonstrating tissue

acquisition and handling characteristics

for each particular assay is also

essential because its often these

somewhat boring

repetitive kinds of tasks that

are hard to be

from a scientific perspective be

enthusiastic about are the are the major

reasons why the development of

particular drug doesnt work because we

didnt know how to handle the specimens

of

of interest

were going to talk about and show

demonstrations of

how you approach proof of mechanism

studies

and what that what you can learn when

the proof of mechanism is not

demonstrated

were going to talk a little bit about

combinations and understand the

relationship between dose and schedule

and target inhibition

finally im going to close with

an evaluation of how were trying to

develop a multiplex approach to pde

biomarker assay development so we can

understand at this in simultaneously how

a whole variety of different

markers change in the same geometric

space in d and finally im going to

suggest to you how drug development at

least in cancer has changed very

dramatically over the last few decades

and suggests what the state of the art

is now

so the first thing id like to make

clear is that

i doubt very many of

you who are watching this presentation

actually know what some of these

various

instruments actually are

because some of them are really very old

but the point is that in when youre

trying to understand a proof of

mechanism youve got to match the

technology to the

your needs

at hand

and so

youve got to first be able to measure

the amount of drug

in the blood and in the tissue and then

youve got to measure various parameters

specific parameters about the target

so

in the mid to late 90s were talking

over 0 years ago

one could measure

concentrations of any cancer drugs very

early drugs in the range of 00

micromolar

00 micromolar rarely if ever achieved

in patients through the use of a very

simple spectrophotometric approach

that then morphed to various

chromatographic approaches the second of

these images

shows a paper chromatogram probably this

is so old that not even

grade school or junior high school

biochemistry biology classes do this i

doubt theres anybody that knows what

drug we used to measure using thin layer

silica gel chromatography but this was

the standard way to measure the

pharmacokinetics of the anthracycline

antibiotics oxorubicin for example

in the 0s through the early 0s

finally in the early 90s various types

of instruments that could measure

the

levels of drugs

in blood or in tissue into the nanomolar

range namely high performance liquid

chromatograms became available and now

if youre talking about the last 0 or

years no selfrespecting

pharmacokinetic laboratory would not

have the latest

liquid chromatograph mass spectrometer

that measures picomolar levels of drugs

that which can be measured both in blood

and in tissue

for almost any agent that we choose to

measure

and so id like to make the point that

that is the evolution of

pharmacogenetic evaluation and the same

thing has happened in terms of

developing assays for early stage proof

of mechanism studies

prior to about years ago one measured

drug growth in animals

with a caliper

one had the ability to measure the

amount of blood

amount of drug in the blood

with an hplc

but the notion that we would have

genomic biomarkers we would be able to

apply those to tumor

using next generation sequencing that we

would be able to have

very sophisticated mass spectrometry

that could demonstrate proteomic

changes including phosphor proteomic

changes really were only in the at the

very earliest stage of having the kinds

of very very sensitive ways to measure

whether or not the drug of interest has

actually hit its target and

the blooming of this

of the variety of assays that are

available makes it essential that as

youre considering

doing a new drug study that you have a

very good understanding of both the

specificity and the limits of detection

of the molecules that youre attempting

to evaluate in your trials so it takes

some time to understand what is the

right assay and what is the right tool

that you need to employ to measure what

you want to measure

the next issue is as i described before

is the issue of having a fit for purpose

biomarker so this may sound

difficult to understand its actually

quite simple

depending on the type of study thats

what youre doing

youve got to be able to demonstrate a

statistically significant response

in the biomarker of interest

so

as some of you know about 0 years ago

the us food and drug administration put

out an exploratory drug development

guidance for socalled phase zero trials

these are pilot studies in which one has

a very very sensitive assay and a drug

that produces the changes in that

particular molecular characteristic at

very low dose at nontoxic doses and if

that is your goal to be able to

demonstrate a proof of mechanism

with a perhaps a single only a small

number of doses of a very potent drug

then its essential that

the baseline variability

for the analyte of interest

be small

so that when nontoxic doses of a

particular drug are administered one has

the opportunity when its even possible

to demonstrate a significant

impact on the analyte on the other hand

if you are doing a study that involves

escalating the dose of a drug

into the toxic range

which is still unfortunately

often the case and sometimes its

necessary for phase one and two

oncologic studies then the amount of

variability can be greater but the

necessity

is that you go to a much higher level of

drug

potentially even a toxic level of drug

so that the impact on the analyte is

sufficiently great so the differences

can be demonstrated and one can be sure

that the target has been engaged

id like to next turn to

something that

has for many years at least in the

oncologic field is still an issue of of

time and money and expertise and hinders

and continues to hinder oncologic drug

development and that is

the development of sufficiently robust

assays for the estimation of the effect

of a drug on a particular biomarker

this is not a simple undertaking

it is an expensive undertaking because

for the most part not always but for the

most part the essays that are

clearly appropriate for a laboratory

phenomenon thats being investigated are

not sufficiently sensitive for the kinds

of changes that are likely to occur in

patients

and so as i said the assay form format

and the platinum form that are chosen

and the appropriate instrumentation are

essential

but

one has to do something more than just

giving the drug

doing a biopsy and then measuring

something because one has to have the

appropriate

controls to understand

that is your analyte actually changing

and is it changing in the tissue of

interest

can you develop

your own standards

that have been quality controlled

and can be spiked into a tissue sample

to demonstrate that what youre actually

trying to measure is stable in the

tissue of interest

one needs to know what is the

appropriate concentration relation dose

response relationship for the agent on

the target market modulation in cultured

cells and in in vivo models

whats the assay sensitivity

if there are going to be multiple

determinations on the same sample

can you freeze and fall and freeze and

fall the sample that youve obtained

how do what is the level of variability

thats acceptable in the measurements

and i think perhaps the most important

and the thing that is least often done

to what degree do you pay attention to

these validation issues

do you have master lots of the reagents

do you understand that when you buy an

animal an antibody or for that matter

various kinds of animals for your animal

models that there can be genetic drift

there can be changes in the antibody

even though the

when you go to the website for that

manufacturer

they tell you that youre getting the

same uh

antibody

targeting the same

particular receptor or protein

but do you know that it has changed what

happens when they run out of a

particular lot

do you have the wherewithal to to

actually qc multiple lots of a

particular antibody so that you can

guarantee that when you do the essay

over the course of a year two years or

three years that your measurements will

be stable

is the assay

is it linear

and

can you do the essay in different places

this is this again may sound

very trivial but

ive had the experience

of having

two laboratories performing precisely

the same

with with a standard operating procedure

and sop in place in both laboratories

using the identical instrument in both

places

and then having great concordance

between those laboratories and have all

of that fall apart over the course of a

week or two why because at one of the

sites

the company came in doing its routine

maintenance for the

instrument installed an upgraded

software package which completely

changed the

linear dose response curves the standard

curves

we actually had to get the company to

come back and uninstall the new software

reinstall the old software so that we

can have values that were maintained and

were concordant from laboratory to

laboratory many things thats

under standard

basic

preclinical laboratory situations would

not be something that you would be

concerned with necessarily

let me give you an example about

how important analyte handling tissue

handling is

this is

obviously a nude mouse

that has

on its flank a human a

melanoma xenograft

and what youre seeing

are examples of three different ways to

do a small gauge needle biopsy of

that tumor in the upper left

is using a standard biopsy needle

one basically does exactly what an

interventional radiologist might do when

going after a liver metastasis

at the bottom right you see what would

happen if a surgeon excised a nodule in

the liver and then that were used to

measure a particular biomarker and then

the lower left and the upper right

you see an gauge needle biopsy

instrument that

actually when the trocar is inserted

into the

tumor

is attached to a

dry a co source which freezes the tumor

in sight in situ before the tumor is cut

and then

disengaged and then processed

so we have standard excisional biopsy

standard needle biopsy

and a special biopsy needle which

freezes the tissue in place before the

blood supply is cut off and then cuts

the tumor

so what happens heres a western blot

for

the phosphoakt in those various samples

and if i hadnt just gone through the

previous slide showing you how the

different tissues were handled but

rather had told you that we were

evaluating a new akt inhibitor you might

have imagined that the third fourth

fifth and sixth lanes

were really potent

akt inhibitors

but in fact these animals got no drug

whatsoever

the

these animals had a standard fine needle

aspiration and with a cutting biopsy

they had some phospho akt that was left

here we see that the time it takes to

resect the tumor and to put it into

liquid nitrogen

namely

a minute two minutes

produce a substantial decrease

in the phosphoa akt signal

this is something that

most individuals are not aware of

it can dramatically affect the results

of your correlative studies and

basically is as ill show in a few

slides

is not uncommon across a variety of

different phosphate proteins but its

also not universal

so basically depending on what the

target might be one has to perform these

kinds of experiments for each individual

target to understand and provide

the

the techniques necessary

to employ

that need to be employed to preserve the

analyte if at all possible

so weve talked about

the

need for

kind of doing a

if you will a clinical dry run on a

preclinical sample so that the clinical

procedures for sample acquisition and

handling are well characterized and

heres a mouse getting an gauge

needle biopsy here you see this very

small piece of tumor that to the h e

stain

of this tumor is shown

on the right and we what we routinely do

and what

laboratories that are actively engaged

in developing pharmaco

kinetic assays keeping pharmacodynamic

assays do is to utilize these models to

understand what the storage requirements

and transferability of the tumors might

be

what the time frames are

can we in the handling

how long can we freeze these samples

before

the signal deteriorates is there

variation and how much variation

goes on from a tumor on the right side

of the animal versus the left side of

the animal from one biopsy site to

another biopsy site

we can

basically do a clinical readiness

assessment

to understand the minimum dose required

to

engage the target

and we can understand

from preclinical experiments in

living models whether its a mouse a rat

or dog

whether or not surrogate tissues are

useful

and then last but absolutely not least

one can mimic

in a dry run the actual clinical

process by which these specimens will be

obtained this is particularly useful and

important

if you are working in an institution

where for example not all the buildings

the hospital your laboratory are

connected

this is something we get a lot of

practice doing

at the nih because the clinical center

hospital where the interventional

radiologists perform these procedures is

actually a substantial walk

to our laboratory on a different part of

the campus

one needs to demonstrate unequivocally

that in the time required

to carry the samples even if they have

been

suitably obtained

in that time that it takes to move the

sample from one place to the laboratory

where thats going to be obsessed there

arent very substantial differences in

the levels of your biomarker and only

then after youve done all of that can

you start

to

understand whether you can demonstrate

proof of mechanism in patients

so let me talk a little bit about

heterogeneity and the types of assays

that you can perform in the different

kinds of tissues that you have available

because this is again an issue that has

gotten

a scant appreciation in the literature

and it really is of great great

importance

we have done over the last year and a

half two years a very detailed analysis

of results of our biopsies obtained from

patients that are treated in the phase

one clinic at the national cancer

institute at the nih clinical center and

these were procedures were all performed

by expert

in interventional radiologists but what

im going to show you is

how important it is

for those individuals to understand

as well as the pathologists that one is

working with that trying to obtain

tissue for pharmacodynamic

estimation is very different than trying

to obtain

the a biopsy to demonstrate that a tumor

has metastasized for which one might

need a very small number of cells

we are used to in medical oncology and

other forms of oncologic practice

needing to obtain only the tissue

necessary to make a pathologic diagnosis

that mindset

is really diametrically opposed to

obtaining as much tissue as possible to

understand whether or not

you have affected a particular molecular

target in the patient so let me just run

through these are these are actually

samples obtained from patients in the

clinic that i attend

and on the far right

is a piece of tissue from an gauge

needle biopsy

that shows beautiful normal liver not a

single cancer cell um next to it on on

the right

it shows a very hypocellular

tissue

from that actually has a few

tumor cells might be enough for a

pathologic diagnosis of recurrence

nowhere near enough to do a

pharmacodynamic essay

in the middle you can see outlined in

green outlined by a radiologist i see a

bad pathologist

areas of

nests of pathologic

tumor

of in this case it was a colon cancer

that had metastasized to the liver

no question theres enough tissue for

pathologic

confirmation of metastasis but nowhere

near enough tissue to analyze

for a particular molecular marker

the

samples on the left and then and one in

from the left

demonstrate on the far left a tumor that

is about 90 90 percent

tumor

and

that makes our life much easier

whereas

in the sample that is one in from the

left you show marked in green multiple

areas sufficient areas of tumor that are

amenable to certain kinds of assays

which ill talk about

but insufficient for other kinds of

assays and what am i talking about well

if youve developed a way to measure

proof of mechanism

by developing an elisa assay for example

youre going to grind up this entire

piece of tissue

and then

try to measure the analyte of interest

in this case it might be

dephosphorylation of the metal protein

measurement of

dephosphorylation of akt and so on

in the case

of a tumor that is has

more than 0 percent is more than 0

percent homogeneously tumor

one can get an estimate of the

particular target effect

that is likely to be uh quite

representative of the tissue as a whole

on the other hand if theres percent

to a tumor

less than 0 somewhere between and

tumor its very unlikely that an assay

that requires homogenization of the

tissue will actually give you a

representative sampling of tumor versus

normal cells that is useful for the

estimation of the impact of your drug on

the on the tumor and hence what we have

done and which ill explain in a minute

is to try to evaluate and demonstrate

assays that can utilize

intact tissue from

fixed tumor specimens

utilizing quantitative immunofluorescent

assays that can estimate from small but

definitive amounts of tumor whether the

target has been

occupied or altered

in such a way

that you have confidence that in the

tumor of interest

the target has been altered

so heres a a rough estimate of

a series of about

biopsies from our clinic over the

last couple years

and what you see is that

starting out a very substantial

percentage of our tumor tissues

roughly twothirds had insufficient

amounts of tissue for any kind of assay

so putting through a patient through the

kinds of procedures invasive procedures

that are really important for

understanding mechanisms of action of

these drugs when twothirds of the time

one doesnt have sufficient tumor in the

pre or posttreatment biopsy to actually

make a measurement

really is boarding on bordering on

something that might be conceived to be

unethical and so weve spent a

considerable amount of time trying to

understand how can we make the tissues

that we utilize more how can we improve

the analyzability of those tissues so

one thing that weve done

is to be able

to utilize a

a larger number of biopsy passes

whenever the tissue is biopsied in the

first place not one or two but we

routinely get three or four different

passes both pretreatment and during or

after treatment so that their odds go up

in terms of finding analyzable tumor

weve also

basically said that if a pathologist can

give us

a site of tumor that is sufficiently

representative we really only need one

such site

as shown in the image in the upper left

to be able to do for quantitative

immunized chemistry an analysis that is

likely to be legitimate when you do that

the likelihood that youll get be

successful is somewhere between two and

three and three out of four

the other way that we have

substantially increased

our ability to get these tissues and

analyze them is actually to meet with

our team of interventional radiologists

on a regular basis

show them the

scans of the patients that we hope to

biopsy and really assist them

in trying to go after the lesions that

are less likely to have necrotic cores

and more likely to have a sufficiently

high amount of viable tumor to enhance

the ability to analyze those tissues but

as you see in the upper

panel

that

most of the time when the tissue is

insufficient its because the the tumor

content is just too low either theres

no tumor possible

that can possibly be detected or it is

too low to do the assay of interest and

so we are working diligently to try to

find better ways to develop needles that

can be placed in tumors

with an enhanced understanding and

confidence that the material that we

will withdraw from the patient

is actually

something that is recognizable as

malignant and representative

for that tissue of interest

so let me give you an example of an

assay that we recently developed to get

a sense of how one goes about doing that

so

phosphomat is an important to oncogene

it is important on its own for the for

driving the

oncologic and the oncogenic growth of

hereditary hereditary papillary renal

cancers but also appears to

be over expressed in many patients with

nonsmall cell lung cancer as one

mechanism of resistance to epidermal

growth factor receptor inhibitors and so

we were very interested in trying to

much more specifically understand

whether or not the one could improve

upon the immunohistochemical assays that

are available that had been and were

available

for

phosphomat

and also to look at

uh different sites different

phosphotyrosines that to the drugs that

are currently available and being

evaluated actually target and so we

developed a sandwich

immunoassay

and what you can see in vitro is that

in the absence of any treatment for the

gtl which is a gastric

adenocarcinoma cell line that

constitutively over expresses

phosphomet because of gene amplification

its easy to demonstrate the presence of

the phosphomet on the plasma membrane

in the ht9

colon cancer cell line there is

a very modest a real but modest amount

of metaexpression and the a9

human lung cancer line theres basically

no

phosphomet expression

we can treat these cell lines with a

drug

that is commercially available its

called crizotinib and this demonstrates

very clearly that one can inhibit the

expression of phosphomet one can use

another drug another phosphotyrosine

agent that does not target met targets

vascular endothelial growth factor and

other receptor tyrosine kinase and show

that it has absolutely no effect on the

expression of phosphomat

we can then show very nicely

using a xenograph model we can model the

doses

that are required to get both inhibition

of growth so as we get and we have a

nice dose response curve here for

cresottonym showing that as we increase

the dose of cresottonym the growth of

the tumor progressively declines and

when it actually gets some shrinkage of

the tumor one can at the same time

measure the expression of total met and

more importantly the phosphorylated

tyrosines and demonstrate that in a

dosedependent fashion correlating very

well

with the growth in vivo there is

inhibition of phosphomath so this sounds

great we use this assay to understand

theres a dose response curve

and then next we did what i had

suggested earlier and what youll

remember from the picture for fossil akt

might be very important in terms of

understanding whether the analyte has to

be stabilized can it be stabilized

from in the in vivo situation so heres

an experiment with another gastric

carcinoma that overexpresses phosphomat

the animals were not treated with a drug

even though uh first glance you might

think that this is a a very nice dose

response curve except when you look at

the xaxis the xaxis is in minutes

not

necessarily related to drug doses

and what this demonstrates is that

whether its at degrees or at room

temperature

when you do a tumor biopsy

of this xenograft

that in a in a minute

almost 0 percent of the phosphomat is

gone uh if even when the samples

are done at room temp held at room

temperature if theyre held at

degrees someone can envision

this actually going on in the operating

room

one sees that you have less than a

minute to get the sample before there is

substantial degradation of the

phosphorylation of this protein and so

it would be impossible to demonstrate a

drug effect

unless one

goes to quite extraordinary lengths to

obtain the tissues in such a way

that there is not

a decrease in the target simply related

to the phosphatases that are circulating

within the organ

so we did we went to those extraordinary

lengths and

heres an example of biopsies obtained

from

a single patient with hereditary

papillary renal carcinoma

that has a germline mutation of met

it shows very clearly that theres over

expression of met

in this case full length met

but it also shows

something that had really never been

demonstrated

previously in vivo and that is

using extraordinary links

and attempting to freeze these samples

uh almost immediately after the biopsy

needle

is retracted uh and removed uh from the

upside

one finds out that on the order of 0 to

percent maximum of the met that is

present in the tumor is actually the

activating species

and someone has to be very careful if

youre trying to target and prove proof

of mechanism in a patient

one would be looking at inhibiting these

levels which are on the low side

not these levels uh to demon and

therefore the handling of the tumor

would be essential um and and and

even that

might or might not be possible to

demonstrate that this uh tissue and in

this tumor one can demonstrate to that

the target has been modulated

so now weve talked extensively about

tumor module and modulating the approach

to handling the tissue what about

choosing a dose for pharmacodynamic

study

and its not just dose but its schedule

because

in the and when one does in vitro cell

line experiments

whether we want exposes for an hour or

two hours or hours when it has

complete control over the area under the

curve for the exposure of the tumor cell

to the particular drug of interest

in vivo its of course much more

difficult

lets assume that you know for a

particular drug

what the

lowest biologically effective dose the

socalled biologic bed biologic

effective dose might be

because you have done experiments to

demonstrate that a dose of that drug

causes a dramatic decrease

in the target expression

of

of the af with the agent in question

well its not good enough to demonstrate

that the target is inhibited

eve because in almost every case

one can assert and show that you have to

decrease

the target expression

to a particular level in some cases its

90 in some cases its less than that

but the issue is how long do you have to

inhibit the target

to actually get a proof of concept does

the tumor shrink

and so unfortunately

mostly because the assays are so

have not generally been developed there

are usually very few such experiments

like this done in animal models to

understand what the not just the dose

but the scheduling for that particular

agent that would optimize efficacy by

maximizing the control of target

function

so lets look at this again lets say

that

you know the biologically effective dose

you give one dose every hours

you get an effect but by the time youre

ready for the next dose how the

biological

effect has returned to normal

in that case you would be very lucky to

just stabilize the growth of the growth

of the tumor

in most cases that would lead to some

expansion of the tumor volume

if you are fortunate enough to be able

to understand in vivo

the schedule whether its twice a day

three times a day

perhaps the pharmacogenetics are such

the drug has a long halflife to

understand what the best schedule is you

can then target that schedule so that in

this case one is giving the biologically

effective dose twice a day

measuring and sampling after each dose

and demonstrating that with each

successive dose

one as the

target of interest starts to recover you

are inhibiting the target again and

every time the target of interest starts

to recover its being inhibited so there

youre keeping the function below a

particular level that can be discerned

and demonstrated in vivo will actually

lead to a shrinkage or some other

biological effect

if you give

less than the appropriate dose

and you give it even on a twice

a day schedule the tumor is going to

grow because youre never going to

produce a situation where the tumor

target that controls the growth of the

tumor is actually reached

and is likely to produce

some kind of therapeutic effect so now

id like to share with you a recently

completed clinical trial of ours that

demonstrates that

this theoretical

construct that i just described

actually occurs in human beings

um so this is a phase one trial of the

combination of a phosphoakt inhibitor

and an inhibitor of the map kinase

pathway specifically a mech

inhibitor and in this case we know

that

in when you add these two drugs together

in cell lines one gets synergistic

tumor cell kill

but in animals

uh its clear that

even complete inhibition of akt

will often lead to a feedback up

regulation of ras or upstream

targets that will overcome the

resistance to

produced

by the

uh overcome the therapeutic effect of

inhibiting the phosphorylation of the

drug the very same thing as if it is

true for a form of mech inhibitor

from astrazeneca six two four four and

so

the notion that you could get

synergistic killing by inhibiting two

parallel pathways

um

at least in

animal studies

um

was

found

to be

difficult to reproduce because of this

simultaneous upregulation of resistance

mechanisms that target upstream

targets that will alleviate the

inhibition produced by the drug

and so at vera at minimum

for this combination to be useful one

has to demonstrate that one can achieve

a concentration of both of these drugs

when theyre given together that leads

to a level of inhibition that has been

shown for each single agent to be

therapeutically useful and so we

performed a study

in which

the the merck drug 0 was given on a

weekly basis

the

inhibit the az inhibitor the mech

inhibitor

was was given

and

on the first cycle we just measured uh

the effect

of

the mech inhibitor and then subsequently

added the azd to find out whether there

was a and basically whether both drugs

given together could hit their

individual targets and reach the goal of

shrinking tumors because of this

combined approach

we developed assays for phosphorylation

of phosphoa akt and phosphorylation of

erc as a surrogate marker for mech

inhibition

well what did we find

here are

0 patients first 0 patients that had

pre and post

biopsies of their treatment with these

drugs

and what do you see

well unfortunately what you see is that

in no case

for the

evaluation of the expression of

phosphoerc do we come close to

inhibiting the 0 inhibition target

in two patients we inhibited phosphoakt

by greater than 0

but not the other target

so that we had a very good idea from the

pharmacodynamics

that it would be unlikely to demonstrate

therapeutic activity and certainly no

synergy because we we didnt even hit

one of the targets much less both

and this occurred at a dose level of

both agents

was actually found to be

beyond their maximum tolerated doses

giving these two drugs together produced

synergistic toxicity rather than

synergistic target inhibition

and the trial was

discontinued

with no patient demonstrating

a clear clinical benefit

but because we had the assays we could

we knew exactly why the dosing both in

terms of the schedule and more

importantly the dose delivered was

unlikely to be useful

lets talk about another drug class

where

the idea is that and this may sound

awfully simplistic um and perhaps

something that you would not necessarily

believe

that our colleagues in la big pharma

would do but this is absolutely the case

the the and what im what im about to

show you so were going to show you data

about three different

um inhibitors of polyadeburgus

polymerase uh the abbott drug philip rib

the astrazeneca drug elaborate which is

fda approved now for the treatment of

serous ovarian cancer and in particular

for women

that have mutations in the brca genes

and also a drug called bsi 0

that was developed by a small

pharmaceutical company and then was

purchased

for 00 million dollars by sanofi

this was the first drug in this class to

get to the clinic

the randomized phase ii trial of this

agent in combination with

of two different chemotherapeutic agents

where the rationale for giving it was

that inhibiting polyadep ribosomation

with thought was thought to be a way to

enhance synthetic lethality that is to

to

depress

basic system repair and therefore

enhance the cytotoxic effects of those

drugs on dna

and that it would be likely that the

combination might be a little bit more

toxic but would certainly be more

effective

there was a randomized phase two trial

with about 0 patients in each arm

demonstrating clear superiority for the

combination that required that added

inibrip to cisplatin and gem cytopine

about the time

not too long after that study was

published we performed the following

experiments

in a cell line this is a breast cancer

cell line human breast cancer cell line

that carries brca mutations we could

show very

very good inhibition of the target

and that correlated well

with the

efficacy of

these two

parp inhibitors

but

in our initial experiments demonstrated

for the parent drug or its two major

metabolites we could develop demonstrate

no inhibition of the target at all in

cells none at all

and using

an assay that ill talk about in a

minute that we had developed that was

very sensitive

you might say how could this possibly be

didnt the company that developed this

have an assay that they could use

certainly for in vivo in vitro

experiments if not in vivo or in

patients to demonstrate that the target

was engaged because the target is an

enzyme and so this is actually easier

its not

a phosphorylation site its an enzyme

that should be easy to measure

in small amounts of tissue

and the bottom line which i will show

you in the next slide is that number one

the

agents that are known and clearly were

demonstrated to be

inhibitors of polyadep ribosome

polymerase worked very well just as well

in vivo

as in vitro but to the bsi 0 compound

had no effect

at any concentration in any dose

up to its maximally tolerated dose in an

animal on the target polyadeb ribose

polymerase

what happened

next is not

so

unremarkable about a month after these

data were

presented

the company

in this case now sanofi

dropped development on this compound

because they had invested hundreds of

million dollars of dollars in a

subsequent phase three randomized trial

that showed absolutely no advantage to

adding this compound to the

chemotherapeutic agents that had been

shown to be advantageous in a small

phase two randomized phase two trial

when they tried to recapitulate that

results using a much larger more

appropriate number of patients those

results could not be recapitulated and

almost certainly the reason is because

the agent did not hit its target

and so an enormous amount of money was

spent not only to acquire this drug but

also to perform phase three trials and

it failed miserably in the clinic

because one did not have definitive

evidence of in vivo target inhibition

here are data

actually a phase zero trial of the

volipurit the

abv company abv drug showing very nice

inhibition with it at four hours after a

single administration of milligrams

of this drug

and then this these graphs show two

other things number one here are three

patients treated at the highest dose we

used and two of the three had very

strong greater than 9 percent

inhibition

of

polyadp ribose polymerase and one

patient

whose pharmacokinetics demonstrated

clearly that the drug was present in the

bloodstream

had a very inadequate only about 0

percent inhibition of the target

and we did extensively studied this

patient extensively and still could not

figure out why it was

that the drug was ineffective

we were also in other patients these are

three more patients

we could show that

if you administered a single dose and

did a biopsy within two to four hours

one could produce a very substantial

inhibition

if you administered the drug and then

biopsied at six hours there was

inhibition but less

and if you administered a single dose

and then waited hours

there was

a minimal amount of target inhibition

showing very clearly that for this drug

to be effective and if one believed that

the target had to be inhibited for a

prolonged period of time one had to

administer the drug at least on a twice

a day basis

let me now turn to the issue of the

detection of heterogeneous effects

and weve also implied and and

uh discussed the issue of an assay that

needs to be done with the entire piece

of tissue that you obtain namely an

immunoassay

and how do we get around the fact that

we simply dont have the tissue often to

be able to do those assays or we can do

the assays but the amount of tissue

available is much less than one would

have expected

we do that by developing

socalled multiplex immunofluorescence

assays we take tissue that is

fixed in formlin and then

cut from multiple slides

and then develop antibodies they can

react with the targets of interest

develop

um

tumors

that and and known calibrators that have

low medium and high levels of the

analyte and then develop ways to put

tumors of interest

on the same slides with our analyte and

then process them for immunofluorescence

that can be quantitated

by a computer program that can

can measure either the total amount of a

particular color in a cell the amount in

the nucleus

or the amount of foci that are present

so this is an example

done in cell lines but i will show you

the same thing can be done

in tissues where we have treated

a

human breast cancer cell line

shown in brightfield here

with

the active metabolite of a topoisomerase

one inhibitor

tobotican

and what you see is even in tissue

culture

you can measure

different components

of the dna repair pathway namely

gammahax which is critical for

doublestrand brake repair

phosphombs which is an earlier which

is an earlier part of the pathway for

base excision repair and ercc one a

different component of the dna damage

repair pathway and in adjacent cells

there are different levels of expression

and this is in tissue culture where

there cannot be any specific

pharmacokinetic kinetic effect the drug

is just poured into the tissue culture

dish and yet you get activation of these

different signals in adjacent cells

one can look at different ways to

measure different things including ki

pro for proliferation

one can look at dna breaks not as if an

effect on dna damage but as part of the

apoptotic cascade and show that one can

get

a timedependent change in the same

tumor in vivo so this is a xenograft

treated with an agent that

alters apoptosis and one sees first

within a few hours this is two hours

activation of the castbased cascade

within four hours theres evidence of

apotic related dna damage and then

only at a later time point one sees that

that you begin to get inhibition of

proliferation

well what about in a clinical trial

so this is a clinical trial in which the

compound that inhibits polyadp ribose

polymerase

abt or veliprib which i discussed

previously was combined with another

topoisomerase one inhibitor a

combination that is shown to be

synergistic both in culture and in

animals previously

and here we have

the same three biomarkers ercc one gamma

hax and phosphombs but this time this

is a clinical gauge needle biopsy

sample

in the patient

treated with this combination

the biopsy was taken somewhere between

four and six hours after treatment and

in a quite remarkable

demonstration of heterogeneity you see

that there are doublestranded the cells

that are undergoing

doublestrand breaks repair

there are cells in which theres

activation of fossil mbs

but in an adjacent cell theres no

activation of phosphombs theres no

activation of dna double strand repair

and theres

minimal if any activation or change in

ercc one so we see that cells over the

over a very small

radius

in that geometry these cells are almost

certainly in different phases of the

cell cycle and so they may or may not be

amenable to drugs that attack

dna in one fashion or another and were

now able to measure

upwards of five or six different

parameters in different ways that cells

repair

the damage caused by

different kinds of targeted agents or

cytotoxic agents to understand even in

tumors that have relational biopsies

have relatively small amounts of tissue

uh what whether the targets have been

engaged and whether theres a downstream

effect of that particular drug on those

targets

so now id like to begin to to conclude

some of the points that ive made so why

should we try to do these proof of

mechanism studies why does

pharmacodynamics matter in the

development of new drugs

whether it is a cancerrelated target

its an immune modulated target

or it is a target that is appropriate

for a drug that affects the kidney or

the vascular system i think the most one

of the most important features is that

if you is the notion that if you cannot

demonstrate proof of mechanism early on

you can in most cases avoid the

enormously costly uh effort involved in

performing um an ineffective phase three

investigation

that may cost anywhere from to 00

billion

one can also in a relatively short

period of time

test a variety of different analogs to

understand if one is better than another

in patients as opposed to what works

best

in animals

one could also then be in a position to

see whether as i showed

whether the predictions of targeted drug

combination synergy based on mechanism

action

actually occur and whether that can

occur in doses and at schedules that are

consistent with an appropriate toxologic

profile

one can look at both putative mechanisms

of action and downstream effects to

understand

how these drugs

might work in patients

one can understand the robustness and

potential predictive value at least get

an initial handle on that

for early in the drug development

process and really one provides the

basis for molecular characterization

studies in

as one goes forward into early phase two

investigations so that one can then

definitively correlate efficacy with

proof of mechanism

ive also i think made it clear that

unfortunately at the present time

doing this kind of clinical trial

is is not easy because to really

understand what youre doing you have to

develop fda quality assays

both for target engagement and

downstream effects and toxicity and for

toxicity markers

those assays need to be developed in

advance of the first in human trial

they need one need they involve not only

developing an analytical procedure

for analytical validation and standard

operating procedures but also to

understand all the various ways that uh

one needs to be

facile with respect to definitively

knowing how to and how to handle the

specimens

and

to do so

in a way that is appropriate to either

the outpatient clinic or the inpatient

service that are the arena of operations

i think its also clear that

it takes a lot of time and effort to

produce the kind of

robust

sets of sample data

and

analytes used for controls

as well as having a supply of the

necessary components of the assay so

that you can analyze

all of the tissues obtained for your

test

under what would be the same

the same

set of standard operating procedures and

finally

it is no small task to understand how to

disseminate the technology so that

extramural sites and a variety of

different labs can

perform these tests

at a high with a high level of

confidence that there would be

concordance from laboratory to

laboratory if you do that

and you invest in these supporting

assays i would propose that certainly

for the development of oncologic drugs

and probably for other classes of drugs

one can develop in a small number of

patients

data that demonstrates whether your

assays are qualified in humans

during these pilot studies qualified in

a way that you can then go on to phase

two investigations which give you an

estimate of the accuracy of the drug

effect on the tumor

and get an early read on the efficacy of

the compounds in the context of whether

or not the mechanism of action has been

been demonstrated and in that case the

size of the trials needed for definitive

studies whether theyre randomized phase

twos or phase three investigations is

undoubtedly smaller

than what would be required for studies

in which one has no handle

on whether or not youre hitting your

target

so lastly id just like to compare

the paradigms for developing oncologic

drugs

several decades ago for what the state

of the art is now

when the time that i was starting out in

this in this process

one basically took compounds that had no

no clear demonstration in vivo of

mechanism of action

and used them to try to estimate

response rates and side effects in

nonrandomized studies

one did this in specific tumor types

using tumor histology as a surrogate for

target

we now know very clearly that there are

some targets that are present across

histologies

it makes no sense to only use a

particular agent

in one disease or another when it might

be perfectly suitable

for a variety of different

tissues and so tissue agnostic clinical

trials are clearly very appropriate and

we almost always used drugs that had a

relatively nonspecific mechanism action

and now

for a wide variety of types of patients

we have agents that are less toxic

where previous treatments are unlikely

to affect

the mechanism of action of the new agent

and whether and when we can

use specific ways to demonstrate whether

or not the molecules of interest produce

specific effects

on the targets that have been previously

defined so that we can then

use the minimum amount of data and the

minimum number of patients to understand

whether the drug

development paradigm should be exercised

to any one specific

study or any one particular type of

disease

in other words to define very early

whether or not a drug is worth taking to

large expensive randomized trials

so thank you very much for your

attention and id like to thank all of

my colleagues at the nci

both the colleagues intramurally and all

of the investigators that with whom we

actually have a variety of different

interactions at academic sites across

the country whove made these studies

possible thank you very much