our next lecture is by dr craig thomas

science degree from the university of

indianapolis in 99

and his phd from syracuse university in

000

he then undertook postdoctoral work at

arizona state university

in 00 dr thomas moved to the nih as

the director of chemical biology core at

niddk

currently dr thomas serves as the

chemistry technology section group

leader at ncats

im confident you will enjoy todays

lecture

good morning

afternoon or evening as the case may be

my name is craig thomas im going to be

providing you with some background

and current information on how weve

arrived at

our ability to provide drug combinations

for the treatment of various diseases

why are drug combinations desirable many

reasons

primarily

the combination of drugs can yield

increased efficacy in the disease state

for which theyre trying to treat

uh decreased dosing which oftentimes

will be

desirable for

the elimination or reduction of side

effects

and the ability to overcome resistance

um

currently drug combinations represent

the standards of care for multiple

indications uh as for instance the

treatment of cancer nonshot skins

lymphomas in particular

our chop is the standard of care

this is a combination of five drugs

rituximab

monoclonal antibody targeting cd0

cyclophosphamide which is a dna

crosslinking small molecule doxorubicin

which is a topoisomerase ii inhibitor

vincristine which is tubulin

polymerization inhibitor

and prednisone which is a corticosteroid

for the treatment of hiv

we rely upon

multiple different drug combinations

that are collectively referred to as the

highly active antiretroviral therapies

or heart therapies

one example is a triple which is a

combination of the nucleoside reverse

transcriptase inhibitor

in tricyte to being

another nucleoside reverse transcriptase

inhibitor uh tenofovir

and efeverazine nonnucleoside reverse

transcriptase inhibitor

the treatment of

malaria relies upon what are referred to

as artemisinin combination therapies or

acts

one of the primary acts is cohartine

which is a combination of

artem ether

and lumafantrin

both are widely referred to as

antimalarials as their mechanisms are

not fully understood

how have drug combinations been

discovered in the past um this is a a

really interesting topic and before we

can really understand where we are today

its important to consider how weve

gotten there

i would recommend

reading this cancer research article

which was published in 00 as a good

window into the history of how

chemotherapy has evolved for the

treatment of cancer

further i would recommend the reading of

the pulitzer prizewinning novel the

emperor of all maladies by siddhartha

mukhterji which came out several years

ago

which also gives a terrific retelling

of

how cancer is treated

over the years

um

the development of drug combinations

for the treatment of cancer is is very

storied um

ill start with the

first well

received combination of drugs for the

treatment of pediatric leukemias which

evolved in the 90s

referred to as vamped its actually

developed here at the nih

by

incredibly bold and interrupted

researchers and clinicians

the vamped combination refers to

vincristine again the tubulin

polymerization inhibitor

aminothren which is also known as

methotrexate which is a dihydrofolate

reductase inhibitor

mercaptopurine nucleotide metabolism

inhibit modulator

and prednisone

not only is the discovery of this

combination for the treatment of

leukemia

a rather interesting story but each of

these drugs uh has their own really

remarkable uh history to them vince

christian uh is a drug that was um

discovered and um

championed by eli lilly uh first in hope

that it might be an antidiabetic

drug

methotrexate a

long story history to that drug

developed by

a team led by sydney farber

the

mercaptopurine classes of drugs

developed by several scientists at the

university of wisconsin in particular

they went on to win the nobel prize for

this work

each of these molecules has a very

storied and interesting history and then

of course their combination

as treatments for pediatric leukemia

became some of the first successful

treatments for that disease

the same researchers that championed the

vamped protocol

moved forward with additional protocols

including the mumped and mopped

protocols for pediatric acute leukemia

and hodgkins lymphomas which were

developed and reported in 90s

many of the same drugs were incorporated

but

addition additions including uh mustagen

which are dna alkylating agents were

incorporated into these these treatments

and again these dna alkylating agents uh

derove with a remarkably interesting

history

particularly

the use of mustard gases in world war

one

led to the stockpiling of those

weapons in world war ii

exposure to some of these mustard gases

in ships in the in the port of bari

italy during world war ii uh led

physicians to study the sailors who were

exposed they noticed uh reduction in

bone marrow

cells and in

lymph nodes

which led to the theory that possibly

these would be good therapies for the

treatment of leukemias and lymphomas

and that did turn out to be the case and

and dna crosslinking and alkylating

agents remain standard of care even

today and part of many drug combinations

this is a quote directly from the cancer

research article based upon these

efforts to develop new combinations of

drugs

in the united states by 9 the

national mortality from childhood

leukemia and hodgkins disease had both

fallen by percent as these new

therapies were adopted broadly

um so how are modern drug combinations

being discovered uh

the aforementioned combinations uh

the mom and the mop

these were clinical trial and error

these were physicians uh adding drugs

in patients in the hopes of better

outcomes

today the iterative exploration

of drugs is different

we could pursue the

iterative

exploration of drug combinations in

humans in the 90s 90s and 0s

because there werent that many drugs

and of course thats changed

the good news of that change is that we

have many more therapies for the

treatment of human disease specifically

cancer

tamoxifen for breast cancer in the 90s

all trans retinoic acid for the acute

promyelomyelosectic leukemia

in the 990s herceptin for her positive

breast cancer in the 000s

imatinib for cml uh

gefetniv or lotinib for egfr mutant

nonsmall cell lung cancer protuzumib

emerged during that time period for the

treatment of multiple myeloma

seraphinib for renal cancers

sunitinib for

gastrointer

intestinal stromal tumors

and just to name a few and of course in

the current decade

over 00 new drug approvals for oncology

indications have been made

in addition to

these more recent approvals the older

drugs but still useful chemotherapies

over 000 new drugs are currently

undergoing clinical investigation in

humans for the treatment of cancers

now

this is great news this means that there

are more opportunities more options for

patients and of course many of these are

targeted therapies

for specific cancers with

a genomic

cause

but it does create a difficulty as we

try and combine these new therapies with

one another for the most effective

treatment of those cancers

lets consider the testing of only 0

drugs

for possible combination studies

the way we do this math is 0 times 9

divided by two that results in three

thousand our thirty one thousand one

hundred and twenty five twoway

combinations if we were to consider the

iterative uh evaluation of threeway

combination that number explodes

uh up to over million different

possible combinations of those drugs

obviously thats an untenable

consideration for the treatment of these

therapies in humans

so

modern drug combinations can be

discovered in two different ways one

rational

combinations of agents based upon

a mechanistic uh guide

lets consider for the

moment uh the story of the b raf

inhibitors and melanoma

as melanoma was better understood from a

molecular basis it became clear

that mutations within the gene and

resulting protein b raf were one of the

causal elements of

that particular disease

as a result many organizations

designed and developed braf inhibitors

when those molecules were applied in

patients with advanced

melanoma

staggeringly good responses were

discovered

however

these responses were shortlived with

aggressive disease relapsing often

within six months

sometimes sooner

a remarkable thing happened a scientist

quickly

pounced on that discovery

and

revealed the mechanistic cause for those

relapsed and aggressive cells within

patients who were treated with braf

inhibitors

this led to the discovery that alternate

activation of the sig braf signaling

pathway which

includes the

mech

kinases

were leading to these

relapsed events

so the rational combination of b rap

inhibitors with mech inhibitors

became one of the

obvious things to try clinically

with

remarkably good results

some of the kaplanmeier results are

shown here

much better clinical outcomes when that

those drugs were applied

in combination

of course not all

diseases cancer in particular

have good

obvious mechanistic reasons for

combining specific drugs with specific

mechanisms

happily though advances in robotics

compound management informatics have

enabled the high throughput evaluation

in in vitro models of disease

to survey tens of thousands

of drug combinations for synergy

additivity or antagonism in only a

matter of days

good references for how those methods

evolved are shown here at the bottom of

the slide

so before we get into some of the

methods that

are used to

do those

types of studies lets uh lets cover a

couple up front questions uh foremost

what is

synergy what is additivity what is

antagonism these are actually not the

most straightforward

questions to answer

we can consider this from a number of

different experimental inputs uh for

instance from a single dose of each drug

lets consider a scenario where drug a

at a single dose is combined with drug b

at a single dose where both of those

drugs alone create a

percent response in a specific assay of

around percent

and the combination creates a specific

response of around 0 percent

an alternate scenario drug a a specific

concentration plus drug b at a specific

concentration actually creates a weaker

response

an alternate situation where drug a plus

drug b at those doses creates a

response which is significantly uh

enhanced

its tempting to call these uh uh

individually additivity

antagonism or synergy

um and its theres theres nothing

wrong with really doing that although

the science has emerged in ways that

allow us to with less

ambiguity

make those make those labels

we can also examine

molecules

drugs in dose response um so in blue we

see a curve a dose response curve for

drug a uh in yellow a dose response uh

curve for drug b

um the dotted red line would could be a

the the

uh

theoretical additive curve if those

molecules were combined

whereas the combination sometimes could

create a

stronger increase in the potency curve

or the dose response curve

or a stronger

percent response which we refer to as

efficacy in many situations

an alternate way and probably the most

uh

useful way to

consider drug combinations whether

theyre synergistic additive or

antagonistic

are from dose response matrixes these

are oftentimes referred to as

checkerboard plots

we call them matrix plots in our lab

and what youre seeing here is an

increase concentration of drug x along

the x axis and increasing concentration

along drug y

uh along the y axis

and as these drugs

are combined you get a more rich

survey of the responses

as they uh

combine

so

this kind of work has been done for a

long time early antibacterial research

really spearheaded this technology

enterprising scientists many that came

out of the schreiber lab at harvard

university actually started a company

which

spearheaded many of the high throughput

methods for creating these types of

plots

this company did a lot of

insightful and

innovative things and and one of the

things that we should all be happy they

did they published their methods labs

like mine have copied those methods

significantly

and much of that work is detailed in

this nature by our technology paper from

009

so

multiple methods do exist for the actual

labeling of specific outcomes as

synergistic additive or antagonistic

many of these are very old models

derived from

models that were

put in place to consider

the actions of multiple agents on

enzyme processes

the bliss model from the 90s

is an independence model assumes that

the drugs affects

a process or system by the independent

action

uh and unrelated actions of the two

drugs involved

the low model from the 90s is an

additivity model it assumes the drug

the drugs in in in question are

affecting a process or system by similar

or identical actions

the gatta model sometimes referred as

the highest single agent uh model is a

noninteraction model and assumes that a

drugs effect on a processor system can

mask the individual actions

of the second drug

um

i do want to point out on this slide and

youll see these references that are

listed at the bottom of the slide below

that

this these these are reviewed well uh in

the published literature many of the the

the publications i list are are

tremendous resources for more

information

about these models

um

the ciao talalay combination index

theorem

worked on by this team of scientists in

the 90s and 0s as a unified theory of

drug combinations which integrates dose

effect curves regardless of

whether its first or higher order

dynamics and regardless of the mechanism

of action of the drugs

the actual calculation

that calculations that are used to label

a drug combination as synergistic

additive or antagonistic

are

best reviewed

by reviewing the the publications that

ive ive shown at the bottom of the

slide

i wont go through the math for several

reasons one i dont purport to be an

expert

and two it deserves more time than we

have to give to the subject today

the bliss model can be solved in this

way

solving for a beta

variable

where

when the beta variable is greater than

one the drug combination is considered

at those doses to be antagonistic when

its equal to one additive and when its

less than one synergistic

in a similar way this equation can be

solved for a gamma

metric which again greater than one

equals antagonism less than one synergy

um

the chao talalay approach actually

solves for a combination index value

very similarly

when the ci value is less than one the

drugs at that concentration are

considered synergistic when its greater

than one the drugs at that concentration

are considered antagonistic

the child cali approach can also

yield several outputs visual outputs of

the data because this is being done in

dose response you can see a broader

swath of whether or not these drugs are

combining

in a synergistic or antagonistic fashion

at multiple dose overlaps

fraction affected plots can be generated

when the combination

is done in a constant ratio

normalized isobolograms can be used for

nonconstant combination ratios

additional ways to label a drug

combination as either synergistic

additive or antagonistic

can be surface response modeling as

shown here and a good example is in this

paper in

00

um

additional approaches when the system

gets more complex when multiple drugs

can be

put into a combination

these get more

complicated

researchers at uh the aforementioned

company started by a a number of

students emerging from the schreiber lab

and later at novartis published this

paper in 00 which gives you some inst

which if you review gives you some

insight into how complex this situation

can get

um

a couple notes on

how to go about describing

drug combinations

its important and

and advisable to be careful with the

labels that are used synergy can often

be uh thought of as enhancement or

potentiation

we oftentimes adopt a label of beyond

additivity

as as synergy the labeling of synergy

can actually be quite complex in certain

situations

uh furthermore

all of the computational methods which i

just quickly reviewed

its advisable to be cautious with using

any of them i quote george boxman saying

that all models are wrong but some are

useful

cell signaling networks

drug mechanism pharmacology in general

the cellular response to the actions of

two or more drugs are very complex

as a result uh

each of the aforementioned models and

the scientists the really brilliant

scientists who put together these

different models

took that into account

um

but each of the models thus incorporates

specific biases and weigh data

differently

so its important to consider

the methods by which

a label is ascribed to a specific drug

combination be it synergy or

antagonistic

with a degree of caution

building upon the thoughts of others and

standing on the shoulders of giants i

will state that

like others its important not to let

this complexity keep you from performing

an experiment or conducting an analysis

but its also important not to take the

outcomes

of those experiments or analyses as

the

gospel

okay so

this is data generated in our lab at

ncats

and and i use it to describe how we go

about ascribing

labels

such as synergy

so this is a a drug combination of a

drug called neraprib and a porn ad and

ill describe how we arrived at this

particular

drug combination

later in the lecture on the left you see

a heat map a matrix heat map or a

checkerboard plot

that describes uh

the percent response

of these drugs in this particular assay

on the right you see the delta bliss

values for each of the discrete outcomes

which represent

the combination of those drugs at

specific concentrations

so where you see a strong delta bliss

value

those are the concentrations where

synergy exists when these drugs are

combined

thats how we describe

the combination outcomes from our lab

that they are synergistic or

antagonistic at specific concentrations

we typically do not say that a drug

combination is synergistic overall

they are synergistic at specific

concentrations

um the reason i think this is very

important and weve recognized it as of

others that uh drug drugs in combination

uh the effects can change at different

concentrations uh heres another example

from a different study

where drug a and im to leave these

vague drug a is combining with drug b

at some of the concentrations where they

are combining

those two drugs are combining in a

synergistic fashion

as detailed by the delta bliss outcomes

on the right

at other concentration overlaps they are

actually antagonistic as detailed by the

deltablis values

that ive highlighted

so lets move on to the experimental

methods um

this is an oversimplification but to

produce the type of data that are going

will allow uh you to understand whether

two drugs are combining in a synergistic

additive or antagonistic fashion you

need four basic needs

one you need a library of agents to

screen drugs

compounds natural product extracts

you need a compound plating method you

have to be able to put the drugs in the

wells where the tests are taking place

at the right concentration

different methods for this pipettes pen

tools acoustic dispensing technology

the third

essential need is an assay

oftentimes cell toxicity assays or

bacterial toxicity acids are conducted

to generate the data that is then

analyzed

and number four a data processing method

a method that will actually bring the

data out of

those assays

and allow you to perform one of the

aforementioned

modeling

of that data

so this is an example library this is

the library that we use we refer to this

library as the my library or the

mechanism interrogation plate all of the

drugs within this library have a known

mechanism

this particular library that we use in

our lab is a collection of around 00

small molecules

around 900 approved drugs 00 molecules

in phase one two or three investigations

and a thousand molecules that we refer

to as preclinical molecules or probe

molecules

uh this like collection of

drugs represents both diverse and

redundant mechanism of action

um the plating method that we take

advantage of

utilizes acoustic dispensing

acoustic droplet droplet ejection

technology

um has emerged over the last decade as a

very useful way to put drugs into wells

this utilizes a pulse of ultrasonic

energy to move low volumes of fluids

typically nanoliters or picoliters and

this can be done using dmso as a carrier

solvent for your drug but also water

and it does so without any physical

contact this is very useful to us as

physical contact sometimes will

alter the concentration of both of the

drugs youre trying to add to the same

well

and

we

utilize this acoustic technology to put

the drugs in the wells as ive described

earlier

drug a along the

xaxis in increasing concentration drug

b and the yaxis and increasing

concentration

so the bottom

row is drug a alone the far right column

is drug b alone and then as they mix

together we see

the effects of these drugs when combined

an example of the assay format many of

the studies that we do are

interrogations of cancer cell models of

disease

so cellular cytotoxicity is a very

useful assay to conduct for those

studies

multiple cellbased assays

formats exist for the interrogation of a

cytotoxicity of a drug or drug

combinations in cells within assay

plates

the cell titer glow

assay is the one that we utilize uh

more than any other

and this is an assay that takes

advantage of

the conversion of luciferin to

oxyluciferin which actually produces

light as well

utilizing luciferase the enzyme

luciferase

this is reliant upon atp that enzymatic

step wont occur without the

contribution of atp

living cells produce that atp at the end

of the assay

if all of the cells in that particular

well are gone the atp is quickly

degraded and that enzymatic event cant

occur and you wind up with a loss of

signal

which represents cell death

um a data processing method um a

really incredible scientist at ncats led

by raj guha created a webbased

method for the output of this data

this is a screenshot of that webbased

interface with the data

on the far right you actually can see

the dose response matrix

a physical representation of those drugs

in combination and the delta plus

bliss

plots of that same data

other columns that we utilize within

this data processing interface

show the cell line that was being

screened in this case tc is a ewing

sarcoma cell line uh the drugs a and b

and then a number of different columns

that represent different

methods for

interpreting whether or not that

combination as an aggregate is

synergistic or antagonistic or additive

so

to go through a project example um ive

already provided some of the data from

the work weve done within you in

sarcoma which is a collaborative work

that weve done with lee hellman whos

now at the university of southern

california

and experts here at nci including

christine heske whos a clinician who

studies ewing sarcoma

human sarcoma is a bone soft tissue

cancer prevalent in teenagers and young

adults

of the cases involve a translocation

between chromosome and resulting

in an aberrant protein thats oftentimes

referred to as the ews fly protein

treatments the standards of care for

this drug include surgery and radiation

and sometimes chemotherapy chemotherapy

aggressive

chemotherapy has resulted in increased

survival rates

but most of those therapies are rather

toxic to the individual and result in

late stage effects for those patients

who receive that therapy

so ewing sarcoma remains a very much an

unmet medical

need

the way we often times integrate these

kind of projects into our lab at ncats

is we bring in the cell models of that

disease in this case we worked with four

different cell models that represent

ewing sarcoma

we screen these

these cells versus

our my collection which at the time was

around 000 approved and investigational

drugs

from this work which i show a a

plot on the right which kind of

represents what this data looks like

from this work we found around 00 9

agents which possessed activity in all

four of the cell models of that

particular viewing sarcoma

mechanistic insight

[Music]

the redundancy of the actions of those

drugs

uh the clinical status of each of those

drugs we were interested in

the approved or or late stage

drugs

um known toxicity limitations were all

utilized to

cull that 9

agents down to a reasonable number of

drugs for

combination efforts

now

obviously were not the only people

doing these kind of studies

this work published out of the business

lab

at harvard

was published in the journal nature in

0

which really was a broad survey of drug

sensitivities across many different

cancer cell lines a number of different

lessons from this study but one of the

more important ones was the realization

that ewing sarcoma cell lines

cell models of ewing sarcoma responded

very strongly to the class of drugs

referred to as parp inhibitors

this is a figure four from that paper

which really illustrates the finding

in our own work we noticed a similar

outcome this is the activity of the

proper inhibitor naraparib versus all

four of the ewin star ewings sarcoma

lines that we studied as part of our

effort

so parp inhibitors were one of the drug

classes we were interested in and

incorporated into the combination

studies we performed

so we screened several cell lines in the

end

in multiple by or 0 by 0 matrix

screens what that means is a combination

of

a six by six combination is actually a

combination of five different

concentrations of each drug

uh including a dmso

control

a ten by ten matrix is a

screening of 9 concentrations of each of

the drugs

and a dmso control in the x

survey we usually use a rather

wide

dispersion of doses within the 0 by 0

setting we

[Music]

make the dilution factors between doses

much tighter

in hopes that

we see a

a broader survey a more more detailed

survey of how those drugs combined

all told in this series of experiments

we screened over 00 agents

which were examined in over 000 total

drug combinations

the plot here just shows one of those

experiments where 9 drugs were

screened or 9 discrete combinations

were assessed

and you can see the the the rankings of

the most synergistic drugs based upon

the highest single agent

metric

including a combination of the drug

navidiclax

and the drug

azd0 which was the fourth ranked

drug surveyed in that particular

experiment

the fourth highest excess hsa value

the highest ranked combination of a parp

inhibitor neraparib was number which

was a combination of naraparib with

deporned

the

drugs which demonstrated strong synergy

in those pilot studies were then

advanced into the aforementioned 0 by

0

combination

studies

one of the outcomes we were particularly

interested in is shown here and ive

already shown you this this particular

heat map the combination of the wraparib

and depornade

both the percent response com on the

left and the

and the delta bliss plot on the right

the

mechanisms uh the parp inhibitor and

the parp inhibitor neraparib and the

porn add which is inhibitor of an enzyme

called namt uh was of particular

interest to us now one of the things

that we often try to do is assure

ourselves that drugs which are

displaying synergy like this

combination of naraparib and depornade

are based upon their mechanism of action

one of the quickest ways to define that

is to show that all parp inhibitors

[Music]

when combined with all namt inhibitors

universally

display a degree of synergy

luckily there are several clinically

relevant parp inhibitors including

naraparib olap rib and bellapurib

and several named inhibitors including

depornade

a molecule referred to as gmx

and a newly emerged knapped inhibitor

from gin in tech called gne

gratifyingly when all of these drugs

were combined with one another they all

displayed a very similar synergistic

outcome

uh as the original

combination of naraparib and depornade

so

moving forward and this is an example of

something that i recommend for all uh

studies that explore drug synergies or

antagonisms

before one invests too much into

advanced studies in an in vivo setting

or even translational studies into

humans

its recommended that

the mechanism by which the synergy is

affected is explored

the best way to do that is to consider

the mechanism of each drug

on its own

namt

is the

rate determining step of the twostep

process which governs the salvage

pathway for nad biosynthesis

that salvage pathway shown here

nad being a ubiquitous biomolecule all

cells require

and governs governs multiple enzymatic

processes governs much of the

metabolic state of a cell

um parp

is part of the dna repair pathway

when dna is damaged parp is recruited to

that site of damage where it creates

something called the par complex

to create that par complex

parp relies upon two metabolites two

biochemicals nad and atp

once the par complex is created

other dna repair elements are recruited

to that site where

the lesion is repaired and dna fidelity

is restored

based upon these two mechanisms we

surmised that

parps ability to kill

or

significantly

cause issues for a cancer cell

parp inhibition when

when parp is inhibited to kill cells is

reliant upon the presence of nad as a

biochemical

if we are reducing the available pool of

nad by inhibiting the salvage pathway

with a named inhibitor

we would exacerbate the effect

of the parp inhibitor

and of course this is uh an easy thing

to test all we have to do is exogenously

put nad back into the system

um when we do that we see that weve

abrogated the

single agent effect of the namp

inhibitor entirely

and and

removed all of the synergistic

nature

of

the combination of the named inhibitor

with the parp inhibitor

mechanistic

studies

going beyond

that type of

unevaluation can include things like

genomics evaluations of

of these cells when theyre treated with

one

of the drugs or the combination of those

drugs

um

so transcriptomics rnaseq data

metabolomics

proteomics can be captured to give a

better sense of

of how the cell is responding to each of

those drugs individually

or the combination of those drugs

i wont go into all of that data

with one exception one of the places

where we saw a synergistic outcome was

from the proteomics examination of these

drug combinations

two key parts of the cell stress

pathways the p map kinase

and the sap junk kinase were noted to be

synergistically induced the

phosphorylated versions of those enzymes

uh synergistically induced when uh the

amp and parp inhibitors were combined

um

and that gave us a better insight into

how these these drugs are acting

in a synergistic fashion

um following

mechanistic explorations into these drug

combinations its imperative uh before

considering a translation of that

discovery into human clinical trials

that these outcomes are shown to be

effective in

established animal models of the disease

two good

xenograph models of human sarcoma exist

and when we applied naraparib and the

genentech inhibitor which is uh

gne in this particular example

the combination of these drugs did have

a synergistic

effect on the outcome or at least a a

beyond additive effect on the outcome

uh in both terms of tumor volume

reduction and

survival of the the mice

so thats a good example of uh of some

of the of how we pursue these types of

projects

um

these kinds of studies are great uh uh

theres theres two different i always

view these kind of studies as two

different uh real key ways that we

pursue drug combination studies in our

lab one is from a systems biology

perspective this is a great systems

biology experiment

the the ability to see synergy when you

when you inhibit two enzymes which might

have been

previously thought to be unrelated or

pathways that

were previously not known to intersect

and then of course the translational

benefit to these studies the actual

vetting of drugs which could be

considered for the treatment of the

human disease

from the systems perspective

the concept is that synthetic lethality

is is a is is more of a genomic based

term at least historically has been

where we consider that a normal cell

when you

knock out a specific gene using

rnai or crispr based technologies

doesnt have any effect on a healthy

normal cell

nor when you knock out a second gene

again no no real effect

but in a cancer cell where a specific

gene in this in this illustration gene b

has been mutated in some way shape or

form

uh the subsequent knockout of gene a

becomes

whats referred to as synthetic lethal

for that those transformed cells

the concepts in synthetic lethality in

terms of drug combinations

is a little bit more

muddy in terms of how we examine or

consider or label two drug combinations

to be synthetically lethal

when combined

going back to the aforementioned wrap

rib and depornade example

the studies that we typically pursue

select for enrich for drugs which are

already active on their own depornat

here you can see is active at a low

animal or concentration so a

response

uh when the drug was dosed in this

particular assay at nanomolar thats

a very strong response by itself before

its ever ever combined with the namton

or the parp inhibitor

um likewise the parp inhibitor uh its

activity right around the four or five

micromolar

ic0 value type of representation of its

activity

um again for parp inhibitors thats

pretty active for an in vitro

cytotoxicity based assay

so these drugs are already active very

active on their own

where we see synergy typically is that

the interface of the active inactive

range and that synergy is actually

fairly localized to a subset of

concentrations

which is why we refer to synergy at a

specific concentration overlap of two

drugs

when we consider the concept of

synthetic lethality were looking for

broad synergy

broad cell death that occurs when drug a

and drug b are essentially inactive on

their own

more recently weve begun to survey

drugs which are less active or wholly

inactive by themselves

heres an example of a matrix plot

generated between a drug a and a drug b

in a specific assay where drug b really

had almost no activity at the

concentrations that we surveyed

drug as activity

really plateaus right around the 0 mark

however when you see the combination

theres broad synergy across many

concentrations for both drug a and drug

b

i i think

i think if i were to call this this

combination synthetically lethal i think

there would be plenty of reason to push

back on that

but this gets closer to a synthetic

lethal event

when combining two drugs which are

either fully inactive or largely

inactive at the constant at a broad

swath of concentrations

additional considerations from the

systems perspective

drug polypharmacology

may very well complicate the analysis of

two different drugs when combined

consider

some data that weve generated

for the combination of this molecule

called dinocycline

this is a molecule thats

reached phase three evaluation in a

handful of

of oncology indications

its mechanism is reported to be

an inhibition of the cyclindependent

kinase isoforms and 9 these are

isoforms of the cyclindependent kinase

which are

essential for the

dna transcription process by

rna paul ii

we were excited to see uh

combinations of dino cyclip

with multiple drugs including what im

referring to as drug a

strong synergy for this particular

combination at specific

concentrations and you might consider

that this is potentiation of the

activity of dino

cyclin

by drug a since drug a has has little to

no activity on its own

now

this was a

easy thing to elaborate upon there are

many inhibitors of the cyclindependent

kinases including specific inhibitors of

the two seven and nine isoforms

including this molecule

sns0

which has been in phase one clinical

evaluations in humans

however the

combination of drug a

with sns0

did not result in a similar level of

potentiation or synergy

as wed seen with dynacyclip

we were interested to note

a year maybe two ago

the report

that this drug dinosaur is also a

effective inhibitor

of several epigenetic factors including

bromodomain isoforms two and four

the bet bromo domains have become

emerging drug targets

in recent years

and

there are good established inhibitors of

brd and brd

including this rather remarkable

molecule called jq

now when we went back into this specific

assay and asked does drug a

synergize with bromodomain inhibitors

we did see a broader element of synergy

akin to what we saw for dinocycline

this led us to

theorize that the

mechanistic rationale for the

combination of drug a

with dinocycline was more based upon its

ability to target

uh bet bromodomains than its activity as

a cyclindependent kinase inhibitor

so

uh additional considerations this time

from the translational perspective

if youre interested in considering

drugs that from an in vitro screen

showed synergy if youre interested in

translating those to a potential human

clinical evaluation

its paramount that you consider

the drug pharmacokinetics

here are

these are generic

illustrations of drug

exposure concentrations

over time from a human clinical

evaluation of those drugs

so

for foremost you have to ask yourself

are the drugs acting

at concentrations which are achievable

in the human host so the more potent the

better is a general rule

so you can see it marked as x

this is the theoretical

minimal concentration that is needed for

drug

a

and then on the right plot

another theoretical concentration

minimum that needs to be achieved by

drug b

if youve achieved exposures over what

you believe

those drugs are required to work at

then its important to consider the

pharmacokinetics of drug a and drug b to

make sure that theres a window of

overlap

if you assume that the synergistic

output of those drugs

is reliant upon them being present at

the same time

and of course what ive done in this

particular genetic generic

example is shown a window of activity

for drug a which occurs between hours

and 9

after dosing

and a window of activity for drug b

which occurs

between hours 0 and after dosing

based upon those

pharmacokinetic outcomes

it might not be advisable to combine

drug a and drug b

in a human

condition in a human host because their

activity

overlaps would not occur

thats not to say that drug

combinations

have to be present at the same time

a paper that was reported in

the journal cell in 0 does a very

good job at showing that sequential

applications of drugs may yield better

outcomes this is work

out of the yaffi lab at mit a really

remarkable

lab um this these are these are plots

out of the paper that show

how

an exploration of the sequential

application of drugs um resulted in a

combination of doxorubicin and

erlotinib where

preapplication of the drug erlotinib

followed by

application of doxorubicin resulted in

a much stronger

increase in the percent of apoptotic

cells

than the combination of that those drugs

contaminatedly

this was

later shown in the paper to be true in

in vivo experiments as well

additional considerations from the

translational perspective

if youre considering moving

specific drug combinations into human

host its essential that you consider

the clinically defined toxicities for

both drugs these are just uh just made

up examples where drug a and drug b

have an overlapping

toxicity liability

in terms of thrombocytocytopenia

it would be

not advisable to combine two drugs with

a similar

welldefined clinical toxicity

um

the clinical

toxicities uh its important to go

beyond

those those more

blunt assessments of a drugs

toxic toxicities

digging in into

key

preclinical toxicity

data is also important

as a for instance the

transporter bisup

has emerged as a a

preclinically validated

transporter to evaluate to make sure

that your drug or the drugs dont have

activities as a b step inhibitor

of course a single drug by itself

inhibiting b cep at a at a low

percentage or 0 percent might not be

cause for

that molecule not entering into human

clinical trials but if two drugs uh with

low b sub activity are combined the

combined actions of those drugs might

take it over

uh what would be a reasonable amount of

bcep inhibition that would not uh

be advisable

so evaluation of preclinical toxicology

packages is also something that needs to

be done before

two drugs are considered for human

clinical combination

so finally id like to introduce

the fact that with these emerging

technologies the ability to survey tens

of thousands of drugs

its well worth the effort of the

scientific community to uh

go back and challenge existing dogma

id like to to highlight a

a story from our own

work our own labs work in combination

evaluations of the drug ibrutinib this

is work we did in collaboration with the

stout lab at nci

and later

windham wilson whos a clinician here at

nci

a brute nib is an emerging uh or an

emerged therapeutic uh for bcell uh

driven lymphomas uh approved currently

uh already in cll and mcl i believe

um

it was also active

clinical activity established for

diffuse large bcell lymphoma

we surveyed the combination landscape of

this molecule in um

in vitro in the in vitro setting

in diffuse large b cell lymphoma cell

line models

we saw strong synergies between

ibrutinib and

key signaling pathway nodes that were

part of

the already defined

signaling elements that drive the

proliferative

nature of diffuse bcell lymphomas

as defined by the stout lab and others

for instance we say remarkable synergy

between ibrutinib and inhibitors of the

pi kinase

class of enzymes

good strong synergies between

inhibitors of

anything really that stimulated the nf

kappa b pathway

strong synergy between

bcl

inhibitors and ibrutinib

these were

i would say

not totally surprising to the stout lab

or others who have studied

this particular signaling pathways that

govern this cancer

we also noticed a number of synergy

synergies and antagonisms between

ibrutinib and more classical

chemotherapeutics

the bar charts represent strong on the

left strong synergy and green between

ibrutinib and

classical chemotherapeutics like

doxorubicin topocyte cytobin

uh we also noticed a significant number

of ibrutinib antagonisms

antagonisms with classical

chemotherapeutics

most predominantly the antifolate class

so drugs like methotrexate

building upon this knowledge

the stout and wilson labs

designed a new clinical regimen for the

treatment of primary central nervous

system lymphomas

that included a pretreatment with a

brute nib followed by the combination of

established chemotherapies like

temozolomide topocide

dexamethasone

the name of this was a

teddy r

this this particular

combination regimen

notably missing from this was

methotrexate based upon the work that we

had found and that the stout lab had

confirmed uh that methotrexate combines

in an antagonistic fashion with a brute

nib

uh we decided uh or the wilson uh team

uh wilson clinical team decided to

remove this from this particular

combination

uh uh clinical combination

um the results were

staggering really um uh

0 some percent uh complete uh remission

um with a number of uh remissions going

on today of the patients which were

evaluatable from the study which was

reported in the general cancer cell in

0

so i hope that this this lecture uh

provided some background uh historical

on how drug combinations have been

discovered over the years

some of the modern methods and some of

the ways that we can define synergy

versus

additivity versus antagonism

what remains for me to do is to

acknowledge and thank the individuals

who have been part of

ncats and the nci team which has really

spearheaded this work over the years

um

special

mention for leslie matthews who did all

the original work uh uh

on our platform raj guha who i already

mentioned uh built the data processing

and web interface for the evaluation of

the data that we generate paul shin

who worked out the compound management

details

xiaohu zhang who does a significant

amount of the current work

that our team pursues in this domain

mindy davis who did the

study in ewing sarcoma that i

highlighted uh krista mcknight who does

the daytoday compound management

operations

sam michael mark ferrar who really was a

key element of uh really establishing

this platform

at ncats

many others uh who are listed here and

many others who are not

uh i also want to acknowledge and thank

uh key collaborators like lou stout and

wendell molson tom waldman

christine heskey and lee hellman who i

had mentioned were part of the ewings

study java khan whos done a number of

the studies with us whos at nci and

many others

and finally its very important for me

to acknowledge the incredible scientists

and clinicians who over the past several

decades have defined the science of

drug combinations

thank you for watching this lecture i

hope it was of use

if you do have questions please contact

the course coordinator

and

we can probably resolve them thank you

you