im very honored to introduce dr ed

dr sausal is currently clinical

professor of medicine an adjunct

professor of pharmacology and

experimental therapeutics at the

university of maryland

prior to joining the university of

maryland in 00 dr saussfield was

associate director of the division of

cancer treatment and diagnosis at the

national cancer institute

dr sausville earned a bachelor of

science degree in biochemistry from

manhattan college

and md phd from albert einstein college

of medicine

he completed internal medicine training

at brigham and womens hospital

and fellowship in medical oncology from

the national cancer institute

dr sausals research focus has been on

early phase clinical trials of novel

anticancer therapeutics and the

screening and preclinical evaluation of

those drugs

please enjoy todays lecture

good day my name is ed sauceville

and im happy to be

talking to you today about an overview

of the drug discovery process

im on the faculty of

medicine and pharmacology at the

university of maryland school of

medicine in baltimore

so

on an overview of our presentation today

we will be having first a general

introduction to the topic

and then over the next

hour or so

discuss the definition of drug targets

how we generate diversity for screening

molecules as potential drug candidates

the definition of lead structures

and then how we qualify

the

lead structure for transition to early

clinical trials

my background

is in

cancer drug discovery and development

having served for many years as a head

of ncis developmental

therapeutics program prior to my current

faculty role

so

while there will be other lectures in

the series that will potentially

drill down into different aspects of

these

targets todays lectures are going

lecture is going to focus on an overview

of the process

so historically

drug discovery at least i from my point

of view is a succession of styles that

have been brought to beer as to defining

new agents for clinical use

throughout history until the middle part

of the last century

these were generally driven by mixtures

of

natural products or even folk remedies

potentially qualified in bioassays

that

defined activity of the agent in a

biological system that might be

therapeutically relevant

examples of this might include digitalis

the rao wolfia alkaloids were

hypertension

familiar penicillins

as antiinfectives and even as

from a cancer perspective

natural products that gave us

anthracyclines vinca alkaloids and

others

from the 90s to the present time there

has been an increasing emphasis in the

use of

pure compounds

or collections of pure compounds again

using a bioassay of some type to define

activity in a preclinical sense

examples of these would include sulfa

drugs many diuretics

first generation hypoglycemics and

antihypertensive medications

from the 90s to the present

the use of pure compounds against

purified

enzymes in particular or purified

macromolecules

that might represent the drug target

generated ace inhibitors the cholesterol

lowering statins

and more recently reverse transcriptase

and protease inhibitors as antihiv

agents and more recently there has been

an increasing focus on bringing

combinatorial methods to bring mixtures

of compounds

actually as

the basis of a screen

potentially against many targets

this process

has historically been viewed as

relatively inefficient and unfortunately

from many points of view remains that

way

the reason why compounds fail or slow

down in their development

include

toxicity that becomes appreciated

in a large animal or

even

first emerging in early clinical trials

and in this regard ambiguities in

toxicology studies are a basis uh for

reconsideration of a molecular uh lead

lack of efficacy ultimately for the goal

uh uh

in that is intended uh and this may come

from low potency

uh or

failure of a molecule to have

appropriate selectivity

uh market reasons that is to say

considerations why what might be a good

idea scientifically doesnt quite turn

out to be practical may relate to

synthetic complexity

the relatively time sensitivity of an

indication with emergence of competing

products

considered better ideas even though a

prior molecule may be very far far along

in its development

prominent however remains poor

biopharmaceutical properties

where the compound may be very active in

vitro or in a dish

but ultimately perform poorly in humans

remains a very

also prominent basis for potential

difficulties

so if one wants to look at a road map so

to speak of drug discovery

if one considers

many screens that examine many compounds

again across a number of

potential therapeutic areas

there are thousands of potential hits in

these screens that typically are

winnowed in the preclinical screening

process to a smaller number of uh lead

compounds of which a

fraction ultimately emerge as drug

candidates to enter preclinical

development and then proceed on to phase

one phase two and phase three trials

leading to a single drug candidate to

emerge as an approved candidate

and the basis for winnowing and how to

qualify drugs is a matter that we will

consider uh in

this lecture and in others in this

series

so how do we define drug targets that

may be relevant to

the

bringing

molecules forward that would be relevant

to a disease

there are two contrasting if you will

drug discovery philosophies

as might be apparent from my

historical overview

a socalled empirical process whereby

one it recognizes the initial drug lead

by a functionally useful effect did

dominate

the landscape

until the middle of the last century

and indeed up until close to the current

one

examples would be penicillin being found

by their antibacterial effect famously

with

dr flemings

bacterial clearing action

rao wolfias had antihypertensive in

effects in model systems

and in cancer of course antitumor

activity of

extracts ultimately defined the activity

of for example taxol

as i mentioned digoxin ultimately

derived from the use in folk medicine of

foxglove to treat

the dropsy

the problem of course is that any

empirical approach

really is potentially quite divorced

from the biochemistry and biology

that ultimately is responsible for

clinical value

in the past

generation the emergence of rational

drug discovery

themes and approaches

are

driven by the desire to recognize a

value of a drug or a valuable structure

either by de novo design understanding

the molecular structure of the relevant

target

or screening against a very precise

process

that

has a critical uh target to the

pathophysiology of the

uh of the disease and which is a

putative uh target for the drugs action

examples of these that have been

successful have been the emergence of

hiv protease inhibitors

where the initial target really wasnt

defined until relatively late in the

last century and within a relatively

short period of time this has changed

the landscape in that disease and even

in for example antitumor

activity methotrexate emerged from a

very considered understanding of the

importance of folates in uh cellular

metabolism and so for its day it was

certainly a basis of a rational uh drug

candidate for rationally derived drug

candidate against

cancer

in this case how to recognize the most

disease relevant targets

is the key aspect in designing a

screening for a rational drug discovery

program

so if one looks at the cancer

perspective which is what i have most

personal experience

one can derive potential

molecularly targeted approaches of value

fundamentally by considering biology

which in

the

cancer arena

is given by clues from the

cancer cells cytogenetics leading to

break points leading to specific

molecules such as bcrabl that well

consider in

a little bit greater detail later

positive selection from tumor dna to

define socalled active oncogenes which

derive tumors which drive tumors in

model systems

tumor gene expression profiling and si

rna for example induce modulation of a

phenotype can pinpoint quite precise

targets that are of potential value in

this arena

one can certainly try and retrofit

active molecules known to be

antiproliferative by defining the

binding partners of the molecules and

these then become a potential basis

for developing screening strategies

important in this effort has been the

development of computational

algorithms which one

tries to link the activity of a molecule

to bind to a particular target

as a basis for assisting in this effort

i alluded to

the relative success

with the antifolate structures and

these could be considered a

classical

use of a knowledge of cellular

metabolism or biochemistry to suggest

important enzymes in

the

progress of that metabolism and

therefore allow screening against single

targets

however while it may be relatively

inefficient as many targets select

themselves

detailed medicinal chemistry is possible

against these

against these targets

and more recently the on the advent of

chemical genetics using libraries

molecules against uh precisely defined

in some cases organisms or cell types

is a way of greatly increasing the

efficiency with which lead structures

can be tied to particular

molecules

one example

that was sponsored

by

nih

and which has been highly

used in the cancer drug discovery

process

was pressaged by the cancer genome

anatomy project where archival tumor

material was the basis for micro

dissection of tumor cells from defined

section to create cdna libraries and

these can be sequenced and then the

results of those sequences with respect

to

the sequence itself or the expression

level of the relevant genes deposited in

the public domain

and in this type of information data set

a given tumor

has a number of different genes arrayed

with some that are underexpressed in

cancer cells or over expressed in cancer

cells and clearly the ones that are over

expressed in the cancer cells are a

basis for potentially developing a

strategy to direct that particular type

of

a cancer to a drug

and these can be

searched through for example

the nih website mentioned here but now

there are numerous

public

and private agencies that have

collections of gene expression data that

is a basis

for

uh defining potential candidates

relevant to drug discovery

and a relevant website is again shown

here

so another

basis

for

defining relevant targets is to take

active molecules and then to define for

example

the basis

of that activity by looking for the

binding partner

of

that entity in the relevant cell types

so this is a benzoquinoid ansamycin

exemplified by gildanamycin which has a

relatively unique ring structure that is

shown here

linked to a benzoquinone

and uh this is produced by a number of

bacterial species and was found to have

antiproliferative activity in empirical

screens

however what was uh most interesting was

the observation that these compounds

could

adopt the phenotype of socalled

reversing

aspects of a transformation driven by

oncogenes in a variety of model systems

and colleagues in japan defined the

ability

of this class of molecules to decrease

tyrosine phosphorylation of critical

oncogene targets

however

they really didnt inhibit the complex

for example kinase

immune complex kinase directly but the

target was inhibited in drugtreated

cells and this actually led to the very

early speculation

by japanese investigators that somehow

the intracellular environment

of

the

target was being altered

working

at the nci len neckers and colleagues in

the early 990s

using a knowledge of the pharmacology of

active species derivatized

the

structure to produce a solid phase

derivative that when incubated

with

tumor cells or even nontumor extracts

defined the existence of an

approximately 90 kilodalton protein that

was bound uh by the geldanamycin

competed by excess nonbeadbound

galdanomycin

not competed by an inactive derivative

and of course didnt have the bead

themselves didnt recognize this

on characterization of this entity

it became apparent that the target of

the drug was not the kinase but actually

the heat shock protein 90 which was

critically involved in the normal

maturation

of

the onco oncoprotein product and the

basis for

the apparent reversion of the

transformed phenotype was the inability

to produce an active properly folded

oncoprotein

hsp90 is also recognized to have a

critical chaperone role for a number of

other

molecules such as steroid hormone

receptors and exemplifies

the need to deconvolute

the

role of

molecules that appear to have an effect

in complex cellular systems

to understand their potential basis

for drug activity

so once you have a particular target in

mind

how do you actually

attempt to

generate diversity

in the molecules that are considered to

screen for active agents that might be

relevant to the target

if one is in an empirical drug discovery

arena or even if one has

purified products that would be active

in a bioassay a historically important

source of natural of diversity is

socalled natural products

the term refu refers to entities derived

from plants animals bacteria

may even have the use of socalled

ethnofire mecognosy

to suggest use

you could have pure compound collections

but more frequently these are extracts

either aqueous organic

and one can

look for

biologically interesting

enrichment of such extracts by using

producer organisms that have been

engineered to

augment

the useful effect

and this would be one source of

diversity one can also have a compound

libraries either peptide or nonpeptide

or you can have

target derived libraries that are

folded into or considering

the structural characteristics of the

relevant target that you are considered

these may be actually lead structures

that have emerged from

theoretical docking of

chemical structures defined

by their molecular features again into

structural

information of the target

returning to natural products uh

approximately a quarter to a third

of all

drugs uh at least as uh by the turn of

the century

did ultimately derive from actual uh

natural product uh uh initial um

extracts or were synthesis synthetic

derivatives theyre from

an example

is shown here

taxol

which is

derived from the pacific u tree

is of interest because natural product

scaffolds contain

the

a diversity of precise orientation of

acidic basic uh aqueous and

uh and organic functional groups in

space

and these therefore have a basis for

having extremely selective binding

features uh to uh to target molecules

uh an example of uh ethnopharmacognosy

is provided by actually lidocaine the

currently used anesthetic

which

ultimately came to attention by the

observation that certain camels didnt

like to eat a certain type of reed

and this led to the characterization of

this structure grameen

as the if you will antifeden principle

in uh the uh grain and this led to this

synthesis of isogramine which had uh on

taste tested humans abundant numbness

which then led to the production of

lidocaine which is used clinically to

this date

problems with natural production of

course is that you have to deconvolute

from the mixture

pure compounds

that allow

a precise definition of the biologic

effect

there is much interest and there

continues to be interest in

complementary and alternative medicine

strategies in various diseases to

actually use uh the originating uh

natural product extracts

but a continuing problem is the

definition

of the basis for potency and activity

and extracts

and therefore uh there is uh if theyre

going to be practically useful they uh

generally have to result in pure

compounds as a basis uh for uh biologic

effects

so turning to

chemical compound

libraries

the potential value of

compound collections that are

constructed according to precise

algorithms

can be can be exemplified

by considering uh a uh a simple tri uh

peptide that well consider

we can put four different amino acids in

each of the positions

uh therefore there are potential uh

peptides that would emerge if we were to

select for example alanine arginine

threonine and tryptophan

and

one can see that by increasing the

length of the peptide

one can uh and considering additional

uh

examples from the naturally occurring

amino acids

that very soon one has a huge number of

potential

precise molecular entities all of which

represent the variation of

amino acids at that position

in using such peptides to screen

one runs into

the practical problems of how

dilute so to speak a single molecule can

be and yet still

expect to see a useful signal

consider a peptide that has

activity uh in an assay with an ic0 of

one nanomolar

it becomes apparent that you really

cant get much more than 0 000 entities

before you push it to

a activity if thats the only active

principle

of between 0 to the minus th and 0 to

the minus th molar which is about where

youre going to start running into

solubility

problems

so

most

mixtures of free peptides

are pretty much

capped in their usefulness at about 0

000 members

different approaches to this

which can be potentially discussed in

other lectures in the series are to use

solid phase or other strategies

to have

ways of having in essence more

than 0 000 members in the mixture

so combinatorial libraries if you were

going to compare them to natural product

extracts as a source of diversity there

are pros and cons while definitely both

can have

allowed direct screening of compound

mixtures

and both can allow the discovery of very

active compounds

the problem with extracts is that by

definition

extract to extract is going to vary with

respect to

concentrations of compounds theres

going to be a relative

lack of understanding of the chemical

structures that are possible or the

synthetic pathways that would be

relevant to deconvoluting or working on

an active agent

and

its going to be rather difficult to

interpret from the data a structure

activity relationship whereas synthetic

combinatorial mixtures

are potentially going to be informative

in each of those areas even at the

screening stage

its certainly possible and schemes have

been defined to produce nonpeptide

combinatorial strategies where the

different substituents arise around a

common scaffold or backbone as uh

exemplified uh here

and in this uh capacity

uh it one commonly approac applies

different rules in constructing the

molecules to maximize the potential

value of the outcome as a as a as a bona

fide candidate

uh among the common algorithms or the

socalled rule of five

that uh compounds with two or more of

the following uh properties around

hbond donors molecular weight

oil to water coefficient

and

some of

nitrogen on oxygen subunits

are flagged as likely to have poor oral

absorption if it is viewed that the

successful drug candidate would require

frequent

administration

and this allows one to substitute or

select

side groups that are valuable in that

regard

so as an example of how

one applies these types of

mixtures you can make start out with

peptides

undergo chemistry to convert them into

nonpeptide molecules that have side

chains for example r r r in this

series

and uh within a relatively uh small

number of candidate side groups derive

hundreds of thousands of compounds for

screening

and

then one can use

these molecules

in different bioassays

against soluble acceptors membrane bound

receptors one could use them as

screening to live organisms or look for

effects on different cellular functions

and one can

fairly rapidly define

positions r r and r that have more or

less in this case of binding to a

particular

target substrate and then

iterate the screen to select molecules

that have the most effective binders to

hopefully derive molecules with

increased affinity

for the target

so

turning to

the next

aspect of drug discovery

once you have lead structures which

well define as a

pure compound or compound series

how do you actually

begin to try and qualify

for

subsequent development these lead

structures and

here

there are a number of

ways of

looking at devising

drug screens to apply to molecule

connection collections

if you choose for example a pure target

screen such as a biochemical screen

binding or functional or even structural

the advantage is that the binding

becomes in and of itself or the

functional success at the definition of

a socalled hit

the disadvantage is that one is looking

at molecules that are acting outside of

a cellular biochemical and ultimately

organismic context and therefore you run

the risk that something that looks very

good

in a structural or functional aspect is

going to perform poorly

in the

cellular milieu

one can use a cell or even organism

based

readout the advantage uh hearkening back

to the empirical observations is that

the readout occurs in a living system

something that if you observe an

interesting enough uh effect becomes

worthy of pursuit uh in its own regard

the disadvantage as we saw from for

example the geldanomycin example is that

you ultimately must deconvolute the

mechanism if one is going to optimize

compounds against a particular relevant

target and you also run the risk that

you may actually have a combination of

different targets that become

relevant

and that

having the activity

disappear as you further deconvolute the

mechanism is certainly something that

can happen in this in this regard

lets take an example tyrosine kinases

clearly an important uh target in uh

many

cancer related indications

emerged from the fact that various

tyrosine kinases are overexpressed or

activated in cancer

these can be activated by mutation or

translocation

and in a variety of contexts these have

been defined with advanced stage or an

inferior prognosis

the proposed enzymatic mechanism for

tyrosine kinase is rather similar the

enzyme

ultimately makes labile a phosphate

group at the end of an atp in the

context of an acceptor tyrosine and so

as a

potential target its relatively

straightforward

so if you look at the initial molecules

that were considered

as potential uh relevant to this

transition state

a number

of things that kind of sort of looked

like tyrosine

if one could imagine for example in this

lavendestine based advantage emerged in

screens of either natural product

collections

or pure compounds

if one considers the initial application

of our attempted application of these

molecules

to a highly relevant clinical target the

b cerebral fusion protein which emerges

from the linking of sequences from one

chromosome

to a kinase on a distinct chromosome to

produce the translocation that is

important in the pathogenesis of chronic

myelogenous leukemia

these initial tyrophostones certainly

did have evidence of activity in

inhibiting directly in complex kinase

assays the bcrabl oncoprotein

kinase function however they were very

difficult to develop from a

pharmacologic perspective

and that

both

ag9 herb statin examples of such

molecules basically didnt have useful

activity in vivo

subsequent refinement of this structure

by considering

in particular

molecules

that had some basis for binding to other

kinases

can that were available from a

structural standpoint

led to the definition of this molecule

here

initially called

sti which was a second generation uh

synthetic species directed against bcr

able

and in models

that were relevant to leukemia it had

the property of decreasing the

phosphorylation of the target b cerebral

protein in tumor cells

in tumors and animals when either given

in by the intraperitoneal or oral root

if one looked at animals afflicted with

tumors that had the bcrable target

protein these animals survived in

contrast to

animals bearing tumors that did not have

the bcrable

target sequence and therefore this was

an argument for the specificity of this

agent against the bcrabl target

and

this led to an initial experience in

humans which was highly rewarding its

one of the few examples where a phase

one trial was used as a basis for

ultimately drug approval

where many patients experienced an

improvement in white blood cell count

and

many patients had disappearance of that

translocation chromosome

within

several months of treatment with an oral

pill and this led to the approval of

imatinib

after an initial

report of the clinical experience

and a phase iii trial of imatinib as a

single agent clearly showed value when

compared to

the combination chemotherapy that was

considered standard for the time

and is a poster child so to speak of

lining up a molecule with a target

thats relevant to the biology of the

disease however

unfortunately in a minority of patients

there was not a good response or there

was the emergence of growth

of

the

leukemia

as a function of time and this was

heralded by the socalled blast crisis

that it can occur in patients

uh treated uh with

the imatinib as well as emerging after

other treatments

and when

this was sought to be understood it

became apparent that the wildtype

kinase had a binding pocket that could

easily accept imatinib

but a number of resistant variants

exemplified uh here

uh by the uh socalled threonine

isoleucine

mutant

it didnt basically fit into the binding

pocket and was a potential basis for

its not for its lack of value directed

against uh the leukemia on the other

hand that became the basis for screening

campaigns to derive subsequent

derivatives and in this case dissatinib

which has activity against many

but not all of the resistant mutants

went on to be an improved agent and is

exemplary of where

drug resistance as defined in molecular

terms can be a basis for screening

additional

useful

molecules

a

second case

in terms of screening is to use

socalled interfering rna technology in

cellbased train to develop synthetic

lethal drugs

synthetic lethality refers to the

concept that arises in drosophila

genetics where the loss of one gene may

be tolerated

owing to an overreliance on another

genes function in a redundant or

partially overlapping pathway

synthetic lethality occurs when the gene

from the redundant pathway

it also is mutated or becomes inhibited

potentially by a drug

deletion of neither gene alone is lethal

but together the organism cannot survive

this

state is potentially important

in

tumors where tumor suppressor genes

are proposed to induce a state of

dependence on genes replacing their

functions and therefore looking for

inhibitors of the second gene would be

potentially valuable in the clinical

context of cells bearing the mutated

suppressor gene

an example where this

has been utilized in designing drug

screens

derived from the knowledge that the

enzyme polyadp ribose polymerase

contributes to successful dna repair

pathways in a variety of contexts

the breast cancer associated tumor

suppressor gene

abbreviated braca and bracket

are tumor suppressor genes responsible

for familial breast and ovarian cancers

as well as a subset of socalled

sporadic tumors in these organs

these are important for a particular dna

repair pathway called homologous

recombination

preclinical studies suggest that braca

defective uh cells

were very sensitive to parp inhibition

by

relatively

nonpotent compounds

this raised the possibility that better

parp inhibitors would be synthetic

lethal with bracha one or bracket two

mutated tumors

and therefore this uh allowed the design

of an uh synthetic uh socalled uh

interfering uh irna screen to define

novel parp inhibitors

so uh synthetic

or interfering rna screening utilizes

uh

short rnas that

activate degradation of target rna

splice systems through the interfering

rna system active in a wide variety of

cellular types

one can precisely eliminate a target rna

and this allows

the cells that are resulting to be a

basis for identifying new targets to

develop screens looking for compounds

that are active

more in the context of the deleted rna

and thats what were were going to

focus on and they have other uses that

can either

be allow target validation or

modifiers of cytotoxics

and uh what was done was to create

panels of cells that have independent

ways of knocking out bracha one or

bracket two and these cells could be

screened against compounds looking for a

phenotype

of greater activity

in the knocked out cells than in the

control

which did not have loss of bracha one or

bracket

and what emerged was a series of

compounds

that had

abundant activity against polyadp ribose

polymerase

and have gone on in early clinical

trials

to have abundant activity in patients in

this case

with bracha or bracket mutated

ovarian cancer and

these drugs have recently been

approved for use in those clinical

conditions

of great interest is the fact that other

results from these parp inhibitor

screening

indicated that the same compounds had

potential activity in different contexts

and a variety of different dna repair

pathways implying that a certain

bracketness might allow their activity

and how we define that is now very much

an area of interest in clinical

oncology and may reveal other ways to

use these compounds

another case

of

devising screens that are informative

in advancing the cause of the series

are the cd phosphatases

these phosphatases are overexpressed in

many cultured cancer cells

they suppress

cell death and over expression of

the phosphatase has been detected in a

variety of different cancers

its also to show that they can formally

qualify as an oncogene by cooperating

with

ras alleles

in

in causing focus formation

in certain cellular backgrounds

particularly those that lacked rb

and the role of this phosphatase is to

convert

cyclindependent kinases from an

inactive state to an active state and

thus promote

cellular division

so a method

was uh designed by laszlo and colleagues

to identify

cdc phosphatase inhibitors uh by

partially expressing uh the target

protein

using a fluorescentated derivative that

when the phosphate is removed the

fluorescence is um is augmented and this

allowed the definition of a series of

molecules that again

all had a range of uh quinone structures

that could act as potential cdc

phosphatase inhibitors

so in qualifying compounds for potential

development

an important

step is to socalled develop counter

screens to use for example a other

phosphatases and define those that are

most inhibitory against the target as

opposed to other phosphatases

one can then use

engineered cells to validate

the target and in this capacity uh a

temperature sensitive uh

system of uh that employed a mutant cdk

that at the nonpermissive temperature

showed no functional cdk activity

and what this allowed is

the

definition that compound by inhibiting

phosphates

does cause gm arrest

therefore preventing indicating that it

that it was inhibiting the phosphates

and therefore uh preventing uh the

arrest uh from occurring and uh this

therefore defined that in the cellular

system this molecule was acting as

anticipated

more recently structurebased design

approaches have become of great interest

in defining lead structures

this can uh usually come from two

different routes

one can have a protein that one can

crystallize

in the presence of the drug and

therefore uh defined from the resulting

structure

the potential relevance of the drug

target

another approach is to use nmr

spectroscopy to define fragments of

molecules with affinity for the target

of great interest

is

computational data that defines

potential binding pockets on the surface

of different molecules here shown for

the ras oncoprotein

one can therefore

look at putative

molecules

that dock into the binding process

pocket through computerized approaches

to therefore provide a basis of leads to

then move to

a biological screening system

and

this can also be done to reveal

different ligand confirmations such as

for example

targeting the atp binding pocket of sarc

kinase

nmr based screening

looks for fragmentlike molecules with

leadlike properties

generally small fragments

that uh bind to a portion of the

molecule in

of interest

uh ligands can be uh

with

weak affinities can be recognized by

this technique and thats an actually at

some levels an advantage

and that one can therefore pick a higher

affinity binding through iterative

screening

interestingly by by labeling the protein

of interest with different isotopes of

different sensitivity

to

nmr uh based screening you can define

the locus of binding

by effects on

the

molecules binding parameters

so nmr has long been known as a way of

defining binding sites this is an

example

from the older literature that shows the

wellknown antibiotic gliomycin which

binds to dna

in the bound state there is a

suppression of signals emerging from

these methyl groups and thats the basis

for defining

that portion of the molecule as

interacting with in that case dna

so using nmr fragment based screening

takes that further

by

understanding that the target of

interest

has potential pockets that if you have

in the presence of one lead that binds

here

uh

evidence of an interaction

and a presence of another lead that

binds here

evidence of a distinct interaction

when one puts together

these two binding fragments

if one remembers the multiplicative

properties of binding constants from

general chemistry you can get a powerful

augmentation of binding to allow a

definition of a more potent binding

constant

in a recent

series of molecules that was studies

that looked at the antiapocatic protein

bcl xl

nmr binding properties were used to see

a

a range of

of affinities that ultimately

resulted in the definition of a compound

that had the ability to

bind to the relevant uh target areas as

defined by chemical shift from labeled

protein of n versus

regularly protonated uh substances and

this uh led to a

evidence that each fragment was binding

to its appropriate pocket

linking these uh molecules then related

in a series that has

recently led to an approved drug

for

the treatment of chronic lymphocytic

leukemia

so to conclude

the discussion

having defined a lead structure that is

potentially qualified

for transition to clinical trials

what are the steps that are

conventionally undertaken

again reflecting my

primary background in the cancer

sphere

one wants to then look for evidence of

activity in animal models of cancer but

this could be animal models of any

relevant disease

and then importantly relate the activity

or lack thereof in animal models to the

concentrations and durations of drug

exposure that is to the pharmacology of

the agent which is uh the focus of many

other aspects of this course

um and an example uh here

uh a series of benzoyl phenylurias that

had antiproliferative activity and were

recognized as antitubulin binding

agents

which was studied by nci and many

members of the series had active

activity in a variety of cell lines

shown here as inhibiting cell growth as

a function of concentration

when these were studied in human tumor

xenographs there was evidence of

activity in a variety of cellular models

given by

intraperitoneal or oral roots

importantly

molecules differed with respect to

the concentration

that was achieved in

different degrees of methylation in the

series

with the

monomethylated and dimethylated having

shorter exposures

than the other member of the series and

that

pointed to the benzoyl phenouria

compound that was advanced to clinical

trials

one then defines in animals a safe

starting dose

which conventionally

looks at in the case of drugs two

species one rodent and nonrodent

according to a clinical route and

schedule thats relevant and

incorporating information from

pharmacokinetics where possible

biologicals follow a somewhat different

route and that a single most relevant

species is undertaken again adhering to

the clinical route and schedule

returning to our benzoyl phenyluria

in

studied in rats and dogs on a schedule

that would support either twice weekly

or once weekly

administration in humans it was possible

to define maximum tolerated doses as

shown here

and doselimiting toxicity in each case

was uh bone marrow and gastrointestinal

tract dysfunction

and conventionally onesixth to

onetenth of the dose uh in the most

sensitive species uh allows uh inception

of human clinical trials in this case

milligram per meter squared as the

initial dose

the problem however with

maximum tolerated dose driven endpoints

is that many of the drugs that are

important in oncogenesis and in many

other physiologic processes

are effective by combining with high

affinity binding sites

therefore using toxicity as the basis of

advancing a

drug class

is problematic particularly in

noncancer

related

contexts

whether dosing beyond the effect on the

desired target buys any value is

obviously not clear

therefore a great deal of interest

exists in preclinical studies

to define a biologically effective dose

as opposed to or at least parallel with

a maximum tolerated dose

using this biologic rather than toxic

endpoints in early phase one studies

another way to think of this is that if

one is pursuing a rational drug

discovery scheme

where one has uh

knowledge ahead of time of the target

and of the

presence and importance of the target in

the biological model of the disease

one can tailor both the toxicology

and the ultimate human development path

by affecting the target at every step

along the way

an example of where this was an

important contribution even in the

cancer sphere is provided by the 0s

proteasome inhibitors

boronic acids

these were received by nci as a series

of compounds with potential

antiproliferative agent

and

what you can see

is that the most potent members of the

series in this case ps or one of the

more potent ones

had a great degree of correlation of

activity

uh as a proteosome inhibitor

along with

the ability to inhibit cell growth

so called ps

emerged as a

convenient lead structure in terms of

synthetic properties it had evidence of

in vivo activity in a variety of tumor

types

with animals treated between 0 and

milligram per kilogram manifesting

evidence of useful antitumor activity

potentially

when this was correlated

with the activity on the proteasome both

in a surrogate tissue

white blood cells as well as in the

tumor tissue it was apparent that those

doses that uh

portended activity was associated with

an about an 0 percent inhibition of the

proteosome activity in peripheral blood

uh mononuclear cells

this led to the development of an assay

for proteosome

activity that

guided uh the uh

the drug dose escalation and that was

predicated on the fact that when one

looked at a series of toxicology studies

in

different species including nonhuman

primates although there was a

a 0 to 0fold variation

in the dose

that

was productive of a very common degree

of

proteosome inhibition this suggested

that escalation of dose beyond this

seventy to eighty percent proteosome

inhibitory inhibition uh is a basis for

uh calling a uh end to dose escalation

because as you can see going from 0 to

youre not getting any more effect on

the relevant target where there is

potentially a

basis for toxicity

this led to an initial clinical

experience

where

one escalated only to

the occurrence of about

0 to 0 percent inhibition

and this was accomplished

by pooling data from a number of

different

clinical sites and

right about here is where there was

first evidence of valuable activity in

patients with multiple myeloma was

observed and that led to ultimately a

development strategy

for what we now know as bortizomid as an

index proteasome inhibitor of great

value to patients with that disorder

so

to summarize

drug discovery is a sequence of

preclinical studies

ranging from a very early

recognition of lead structures

their

potential activity in

models of the disease of interest

and then an optimization of

those structures

by a variety of techniques ranging from

fairly

classical

medicinal chemistry to

modern molecularly

assisted

screens and ways of qualifying molecules

but all of these studies

in the drug discovery process

have the goal of aiding and promoting

clinical trials and assuring the likely

safety of the initially explored regimen

certainly provides a scientific basis

for assessing the clinical effects of

the agent

and there is going to be an increasing

focus on correlating the molecular

effects of these agents on the intended

targets

along with the more usual

pharmacologic and toxicologic endpoints

to refine and minimize hopefully the

risk of

failure of the agent

in clinical trials

i want to thank you for joining us today

and i hope this presentation was

valuable

in describing the process of drug

discovery

if you have any questions concerning the

presentation

please contact the coordinator of the

course

and thank you and have a good day