im excited to introduce todays lecture

in biology from mit and received an md

degree from the university of california

san diego he then completed residency in

surgery at ucsd before coming to the

surgery branch of the nci as a medical

staff fellow

after fellowship he joined the senior

staff of the surgery branch

during his tenure at the nci he has been

involved in the earliest clinical

experience with interleukin therapy

gene therapy and adoptive tcell therapy

for cancer

hes participated in numerous

immunotherapy trials for melanoma and

other cancers with emphasis on renal

cell cancer and currently lung cancer

and rasputation

malignancies hes authored over 00

manuscripts and a dozen book chapters on

tumor immunotherapy i know you will

enjoy todays lecture

hi my name is jim yang and im a senior

investigator in the surgery branch at

the national cancer institute today

were going to talk about immunotherapy

and in particular t cells as cancer

therapy the field of immunotherapy has

enjoyed an explosive expansion in recent

years and this is largely because it is

a new modality that carries the

potential to cure patients of advanced

cancers previously not curable

and one of the methods by which this can

be accomplished is by the administration

of the tumor reactive t cell as the

therapeutic reagent so the title of

todays talk is t cells as cancer

therapy and the modality is adoptive

cell therapy or act

my first slide

talking about the principles of adoptive

cell therapy

preclinical studies in mice have shown

that organ rejection and tumor

immunotherapy are mediated by t cells

these are the primary mediators of

tissue destruction

the goal of adoptive therapy is to

administer

sufficient tumor reactive t cells to a

patient to trigger the rejection of

their tumor

vaccines have not been able to induce

sufficient activated t cells in vivo to

reject metastatic cancers so this

approach uses direct administration as

the route to achieving this goal the

main obstacle to tcell adoptive therapy

has been finding tcells that react with

safe and effective antigens present on

the tumor

now in this talk were going to be

talking of two main sources of tumor

reactive t cells the first one was an

endogenous

population that was discovered in the

tumor infiltrating lymphocytes or till

of human cancers

human melanomas and some other cancers

can contain resident t cells that

recognize tumor but are often present in

small numbers these can be expanded in

vitro and administered to the patient

the other source of tumor reactive t

cells are gene engineered t cells these

are cells whose

that contain receptors introduced

genetically that recognize tumors these

receptors were cloned from tumor

reactive t cells and genetically

engineered into the

peripheral blood of any patient with

high efficiency and then can be

administered

now

there are several antecedent principles

for t cells transfer and the first is

preparation of the host to receive a t

cell infusion

the host doesnt need or want too many t

cells my lymphocyte count and your

lymphocyte counts are approximately the

same and fairly constant

and and so there is a control of the

number of lymphocytes in your body

another principle is that there are

immunosuppressive t regulatory cells

that are abundant in tumors and may

inhibit immune function

and therefore transiently lympho

depleting the host before giving t cells

leads to better engraftment and function

of those cultured t cells

the benefits of preparative host

immunosuppression come from the

following

effects immunosuppressing that recipient

prior to transfer removes those resident

t regulatory cells

the absence of lymphocytes promotes the

host to produce homeostatic cytokines

such as il and il in order to

restore their own lymphocyte count but

incidentally to also support the cells

that you give

it also reduces competition from the

endogenous population for those

supportive cytokines

and it also nonspecifically increases

toelike receptor ligands such as

lipopolysaccharide

this is a schematic of how

almost all of the t cell administrations

im going to talk about today are given

theres a lymphodepleting regimen of

cyclophosphamide and fludarabine that is

nonmyeloablative thats given to

patients prior to t cell transfer

you can see that beginning approximately

a week before the t cell transfer

theyre given hydrocyclophosphamide and

five doses of flu dairybean

at that point their lymphocyte count

falls to zero and the t cells we have

cultured are given to them and then

several doses of supportive systemic

interleukin a growth factor for t

cells is given after the t cell transfer

the end of that period the patients

allowed to recover spontaneously and at

that point in many of these patients you

can show that the t cells administered

have been grafted that patient durably

this is an illustration of those

homeostatic cytokines that are produced

in response to the lymphoid depletion

these are primarily two cytokines

interleukin in interleukin which

are produced from nonlymphoid sources

and you can see that to the left of

these various grafts using three

increasingly intense lympho depleting

regimens that there is very little

detectable il in the patients and

varying levels of il but following the

lymph depletion you can see these levels

rise dramatically

up to the day

here day zero immediately prior to the t

cell administration and these growth

factors for t cells can support the

administered cells

another principle which was a surprise

discovery was the state of t cell

differentiation affects their efficacy

naive t cells are more effective in vivo

than highly differentiated cells now in

the old days of t cell immunotherapy the

only way you could find a tcell

reactive with the tumor was by

repetitive stimulation to identify those

cells

once you had transgenic mice that

expressed a single known

tcell receptor in all of the tcells of

their body you no longer had to worry

about finding the t cell and you could

study the biology of those t cells from

their naive state to their highly

differentiated and stimulated state and surprisingly there was an inverse relationship in efficacy between these states

if you

as you stimulate a t cell with a known specificity with its antigen youll demonstrate the appearance of effector functions

as they become more lytic they secrete more cytokines but paradoxically those cells become less and less effective in adoptive transfer and the finding was that the more naive and less different differentiated t cell was the better it would and graph the the host and function in vivo highly stimulated cells with

with market effector functions are often near the end of their life they have short telomeres and theyre destined to die or apoptosis and so theyre less effective and so this was demonstrated in these transgenic mice and is now a concept that is well established

established in the literature

the requirement for effector

t cell functions in mouse models was

initially concealed this finding but

those transgenic tcr mice were allowed

this to be discovered

this is an example of that this is uh

from a paper in 00 by dr gattinoni

showing that the lysis of a

t cell population transgenic for a an

antigen on the b melanoma and mice

was increased as you repetitively

stimulated it and they then

evolved from the naive state to the

early effector state to the intermediate

effector and then late effector stage

and you can see the lysis of those shown

by the dark circles is in the effector

cells is increasing as you stimulate

them repetitively but then when you

transfer them to mice bearing the b

tumor you can see the only ones that are

truly effective are the

mice are the t cells that are either

naive or early effector cells and so

this as i mentioned is a paradox of

increased activity in the face of

decreasing therapeutic efficacy

and this is something that

that

is potentially

adaptable to

modifications in therapy

this shows that they survive

differentially the early effector cell

shown in the dark black boxes is the

best survivor when transferred into a

mouse you can see that the number of

cells you can recover from the spleen is

increases dramatically at day four or

five it then collapses and disappears

fairly quickly but the only cells that

maintain

a persistence in the host were those

early effectors the effector and

intermediate factor cells which have

much better in vitro function actually

do not proliferate well in

in the short term and do not persist in

the long term

so as we go through the history of t

cell transfer

there

it progressed from the use of tumor

infiltrating lymphocytes largely from

melanoma initially but as ill show you

now from other tumors

and it also include the included the use

of geneengineered peripheral blood

lymphocytes into which receptors had

been put that were reactive with a

variety of tumorassociated antigens

these included normal tissue antigens or

socalled differentiation antigens

reflective of the tissue of origin of

the cancer and it also

could be against a class of antigens

known as tumorgermline antigens

and finally there are tcell receptors

that can recognize tumorassociated

specific mutations

im going to talk initially about the

experience using tumor infiltrating

lymphocytes

melanoma till were used in were grown

from melanoma lesions and used in two

clinical protocols

we use different

lymphodepleting regimens in these

protocols but a total of 9 patients
were ultimately treated with some sort
of lympho depleting regimen a t cell
transfer of melanoma till cultured in
vitro and some systemic supporting
interleukin
we did not find differences in the
efficacy of these treatments using
preparative lymphodepleting regimens of
varying intensity and so at this point
those are no longer being
studied the main findings though in all
of these trials were that there was a
very high overall response rate and that
longterm cures in patients with widely
metastatic disease were possible or
complete responders
in studying the in vitro function of the
cells we were given
the we found that tumor antigens could
be identified in the laboratory by
expression cloning and they fell into
three major groups
one was where differentiation antigens
associated with melon melanomas and
cells of melanocytic lineage those were

melanocyte proteins such as mart and

gp00

we also did find members of the tumor

germline antigen family such as ny eso

and mage

and finally found some t cells being

administered were capable of recognizing

mutations that occurred exclusively in

the patients melanoma but not in the

normal cells of that patients body

these are the overall results of those

9 patients in two protocols you can

see that ive plotted here the

nonresponding patients in blue the

partially responding patients in red and

the completely responding patients in

black and this timeline goes out over

years what you see here is that the

overall response rate of this group was

approximately percent so more than

half of the patients had an objective

clinical response to the transfer of

these t cells

most interestingly though were the

patients who had complete responses that

is disappearance of all evidence of

their metastatic disease and all of

these patients had

demonstrated metastatic disease

those patients

amongst those patients were only two

that ever have relapsed now and

followup that extends out beyond 0

years and so we consider many of these

patients almost certainly cured of their

widely metastatic cancer

the

the striking finding that patients

in whom all evidence of tumor disappears

on an initial xray will not relapse in

the long term is really one of the main

reasons why immunotherapy now has

enjoyed such an intense interest its

one of the few

tools and one of the main new modalities

that can actually make a patient with

widespread cancer

reject their cancer completely and have

some assurance as you can see in these

completely responding patients that its

not coming back and there are almost no

other treatments systemic treatments

that can cure a patient with the common

adenocarcinoma adenocarcinomas once they

have widely metastasized and cannot be

removed and so thats one of the main

interests and probably the strongest

single factor that recommends

immunotherapy to patients with advanced

cancer the overall response rate of

percent is also one that that is

is quite uh

striking

were also going to talk about gene

engineered peripheral blood lymphocytes

these are ones in which we we have

constructed them from the peripheral

blood lymphocytes of any patient so the

patient is getting back their own

peripheral blood lymphocytes but theyve

been genetically reengineered to target

their cancer

this development began in 990 with the

publication of paper where the first

genetically manipulated cells were

administered to a human this was not a

therapy trial this was a trial to

demonstrate and investigate the safety

and feasibility of engineering

peripheral blood lymphocytes and

administering to

administering them to a patient

this used a marker gene or a trafficking

gene and we could show that you could do

this with high efficiency give them to

patients and we did not detect in a

small number of patients any

consequences or adverse events from this

manipulation

since then

hundreds if not thousands of patients

have received genetically modified t

cells from our group and many other

groups and have not demonstrated any

longterm consequences to the administer

administration of genetically modified

mature human t cells and thats an

important

differential point because in the

manipulation of bone marrow there have

been

reports of childhood leukemias in some

patients

and so the toxicity and the dangers of

manipulating human bone marrow and

administering them that in lieu of human

peripheral blood t cells is quite

striking and the human mature tcell

population has to my knowledge never

generated any secondary malignancies

after genetic manipulation

the way it works

is to use retroviruses to package up

your gene of choice and introduce it

into the cell in the upper left corner

is a replication competent gamma

retrovirus the genetic

material necessary to construct that

protein capsule is shown in blue and

contained in the competent retrovirus

on the right is a replication

incompetent retrovirus it has the same

viral capsule

but its genetic material has been

removed and replaced with a genetic

payload that you wish to introduce into

a cell

at the bottom is the method by which

that occurs we put the genes for

constructing the viral

protein capsule into a packaging line

and then we introduce the genetic

payload with a packaging signal into

that same cell that cell can then make

viruses using its

own

genetic material

encoding the protein capsule but

packages only the payload that has the

packaging signal attached to it and then

you get a t cell then you get a

retrovirus that can infect a cell

normally inject the

payload but cannot make more copies of

itself

when this is done with a t cell receptor

thats a two chain protein receptor so

we have a bicystronic

genetic payload in this replication

incompetent retrovirus this is usually

done by linking the alpha and beta chain

of the t cell receptor with either an

iris site that allows them to be

separately translated or a selfcleaving

peptide that allows from one message to

protein products to be made these then

associate together and are expressed in
the t cell that has been infected and
can then generate a new t cell receptor
alongside the endogenous t cell receptor
of that cell and
theres there is a possibility of
recombination of the two different alpha
and beta chains and producing new
receptors and that has been an area of
concern but in humans at this point has
not demonstrated any adverse events its
something that people are attempting to
deal with in this situation but even
without such precautions the engineering
of peripheral blood t cells and
administering two patients in large
numbers has not been associated with any
consistent new toxicities
for those who are not immunologists this
is a way that t cells recognize their
target their recognition is mediated by
the two chain t cell receptor
interacting with a peptide antigen this
is a small peptide excised from the
entire protein that is presented on a
specific major histocompatibility or mhc

molecule so a small cleaved processed

peptide from the antigen is then mounted

like a

like a gem on a ring and presented to

the t cell receptor and it has to engage

the entire complex the mhc molecule

which is a specific one that binds the

peptide fragment and that peptide

fragment so its called the peptide mhc

complex is the true ligand for the t

cell receptor

so

once we had discovered that the t the

till we were administering were

recognizing an array of antigens the

next step was to take some of those

receptors from those till and engineer

them into the blood of other patients

who had melanoma and the same mhc

molecules or type as the donor patient

and that was done using these

retroviruses that ive described and

initially the first antigens found were

melanocyte and melanoma specific

proteins that is proteins present in

both the normal melanocyte and the

malignant melanocyte or melanoma these

were administered to patients

and

they caused both tumor regression and

dramatic toxicities this is a patient

who had received a t cell engineered

to recognize the mart melanocytic

protein

you can see on the upper righthand side

that in her chest ct scans she had

several tumors that regressed with this

tcell transfer but at the same time

melanocytes in every other side of her

body were severely affected you can see

dramatic skin inflammation and peeling

you can see inflammation of the eye

which can

the uvula body does contain melanocytes

as well and they also had toxicity of

the inner ear where a very small number

of melanocytes also reside and so this

proved to be a limiting toxicity to

targeting this entire class of antigens

that if you mounted a really potent

attack on these proteins that could

cause the rejection of melanoma you also

injured the normal tissues that contain
melanocytes and so this is a a class of
proteins that illustrated a very
important principle of autoimmunity when
you target normal self proteins and is a
class of antigens that we are no longer
pursuing actively for therapy
this is another example of that where we
made peripheral blood lymphocytes with a
t cell receptor targeting the chorio
embryonic antigen or cea present in
colon cancers but also present in a few
cells in the normal colonic crypts you
can see on the upper left the colon of a
patient was treated this way which has
lost all of its epithelium its theres
severe colitis and stripping off of all
the lining of that colon with
granulation tissue being formed this
patient had liters of diarrhea and
severe colitis you can see on the upper
right that this patient also had
regression of some of the
metastases from their colorectal cancer
but again this proves to be a a limiting
autoimmunity a dangerous autoimmunity

that prohibits the pursuit of in our

hands uh the further pursuit of this of

this antigen for the treatment of colon

cancer

now it doesnt mean that all cell

advantages are inevitably unsafe

im going to digress for a moment to

discuss a different type of receptor

that has been used recently this is the

socalled chimeric antigen receptor or

car what you see in this schematic is on

the left side the two chain t cell

receptor the alpha and beta chain that

associates with the zeta complex of the

t cell to trigger t cell signaling

you can see in the middle is a new

construct that was described in which an

antibody the variable portion of a heavy

and light chain of an antibody

or a single chain variable fragment of

an antibody reacted with something on a

tumor

outer membrane surface

has been covalently coupled directly to

the signaling molecules of the cd

complex here the cd zeta moiety and

surprisingly that this cut and paste

operation which really

would

you would be suspect would not function

uh properly does function it activates a

t cell based on the binding of that

antibody to its cell surface antigen and

can trigger the activation of a t cell

and these are now called cars they were

initially called t bodies but are now

cars or chimeric antigen receptors and

you can modify them to introduce other

potent costimulatory

moieties into that covalent chain shown

here you can introduce the costimulator

cd or cbb or even both in second and

third generation cars and so this is

another type of now artificial receptor

that can be used to target antigens but

importantly only antigens on the

exterior of the cell membrane of the

cancer thats a very limited number of

candidate antigens and it has to have an

antibody against that antigen and of

course that takes a

significant amount of time to develop

and

it also

is

something that can only be used against

constant self proteins but not against

novel proteins on the cell surface of a

cancer

now this has been used then to target

another self antigen successfully this

is a cd9 marker which is a b cell

marker its a marker of both normal and

malignant b cells but cars against cd9

have been developed multiple groups have

reported dramatic responses in

chemotherapy refractory lymphoma cll and

all

the constructs vary they use different

antibodies they use different

costimulatory constructs but when put

into the peripheral blood of a patient

administered they potentially

can cause rejection of those bcell

malignancies they also induce bcell

aplasia because this marker is as i

mentioned on normal b cells but bcell

application proves to be tolerable and

manageable plasma cells which are

actually the ones that make

immunoglobulin do not express cd9 so

patients can still make residual

immunoglobulin from their preexisting

plasma cells and eventually bcell

aplatia may wear off and b cells may

return

but this is an example of a normal

autoimmunity from targeting a normal

self protein b cd9 that is acceptable

now it turns out that the fda has just

approved the first

t cell therapy in humans which is this

cd9 car construct and the treatment of

chemotherapy refractory pediatric al

another clinical trial has been strongly

positive targeting diffuse large bcell

lymphoma in adults and is now before the

efda for approval so these products

targeting

b cell malignancies with a cd9 car

are the first products that are going to

be approved for use in humans and thats

based on very high response rates in

patients who have no other therapeutic

options as well as some very longterm

complete responses demonstrated in

patients with lymphoma and cll that

again are evidence of dramatic uh

benefit for using this type of cell even

administered a single time in these

patients

now in patients doing this another

concept has come to the fore which is

the concept of t cell persistence in the

host

these are data from the university of

pennsylvania treating patients with a

cd9 car and the upper two rows show

eight responders who have plotted the

number of car expressing cells found in

their blood at various times out to a

year after

administration and you can see that all

of the eight responders show significant

persistence of their t cells at delayed

time points after administration of

these car t cells the bottom six plots

are nonresponders and you can see they

have very erratic and most cases very

poor persistence of the t cells so this

has led to the concept that t cell

persistence is important or even

necessary in achieving good tumor

regressions but this is very

controversial and that another group we

are led by dr kochendorfer has treated

patients with a different construct

using the cd costimulator in a cd9

car and treating diffuse large bcell

lymphoma

and they had seven patients that were

treated four of whom achieved durable

complete responses now extending from

about three years to almost five years

and only one patient in this entire

experience showed any significant

persistence of the administered cells

beyond days and so

its clear from this experience that

persistence high levels of persistence

of t cells in these patients after

administration is not necessary to get

durable complete responses and so the

reasons for these differences in data

are at this point not clear but they

likely have to do with the

costimulatory function of the two

receptors and it is not yet clear

whether persistence is necessary or just

an epiphenomenon of different receptor

function

this is a patient this is actually the

first patient ever treated and

responding to a cd9 car this is a

patient treated at nih in may of 009

who had uh

chronic lymphocytic leukemia shown on

the left side are his ct scans with

diffuse bulky axillary adenopathy

mediastinal

adenopathy retroperitoneal adenopathy

and and bulky pelvic adenopathy at the

upper right are is his bone marrow with

the cd9a marker a marker of his bcell

lymphoma shown in his bone marrow and

then you can see that

in march 0 all of this lymphoid

enlargement has disappeared in this

patient and weeks after his treatment

theres no evidence for persisting

lymphoma in his bone marrow this patient

is treated in may 009 he had a second

treatment due to an incomplete response

in in 00 and hes had no further

treatment since then he remains a a

responder to this day

this is a young woman who had

a very very bulky and aggressive

diffused large bcell lymphoma she had

had 0 other systemic therapies for this

and progressed and you can see in this

pet scan on the left with the yellow

illustrating the size of her lymphoma

that she had extensive liver disease

involvement of her stomach wall

adenopathy renal involvement and you can

see months later in the pet scan on

the right she has no evidence of

lymphoma at that point and has had a

complete response to a single

administration of these car t cells

so once we found though that normal

tissue antigens by and large induced

unacceptable autoimmunity we then looked

at a series of receptors that targeted a

class advantage known as tumor germline

antigens

these are found by the cerex technique

which is serologic analysis of

recombinant tumor cdna expression

libraries at memorial sloan kettering

they took patients serum and looked for

binding to the patients tumor

by high affinity igg

and this

revealed a family of proteins now over a

hundred members most encoded on the x

chromosome

of proteins that were expressed during

fetal development but not in adult

tissues except with the exceptions of

the germline tissues such as testes

ovaries and placenta but could be

reexpressed on some human tumors

one of them that was initially found

that we found a receptor against and

targeted with gene engineered cells was

the ny eso antigen

we treated patients with metastatic

melanoma or synovial sarcoma a very high

expressor of nye so in 0 percent of

cases

with t cells

that had

derived from peripheral blood

lymphocytes and transduced with the t

cell receptor recognizing ny eso in

the context of hla a the mhc molecule a

very common mhc molecule in humans

that presented the epitope from nyu cell

these patients all expressed the

antigen and had the correct restricting

elements and overall the response rates

were

objective partial and complete responses

for patients with melanoma and in

patients with synovial sarcoma five of

these patients achieve complete

responses with four of them ongoing at

one to five years and no autoimmune

toxicities were seen targeting this

particular tumor testis antigen

this is a patient with melanoma shown

with bulky liver disease on the upper

two panels on the left as well as

multiple lung

metastases some illustrated on the lower

left you can see this patient

was treated in 009 and to this day

remains a complete responder with

regression of all of that liver disease

as well as all of those pulmonary

metastases

this is a remarkable case of a woman

with synovial sarcoma in her pelvis

destroying the right side of her pelvis

shown on the left panels unable to walk

in a wheelchair

and she was treated in 00 she got a

single administration of t cells and

although all evidence of her tumor has

not regressed uh has not disappeared she

continues to regress to this day and she

has had

a dramatic regression of her primary so

that she can now walk normally

and she had hundreds of pulmonary

metastases that had failed resection and

chemotherapy shown on the left here that

have almost all disappeared there are

small residuals left of a few of these

and so this shows the power of a single

tcell transfer if you give the right

tcell to the right patient you can

cause the rejection of kilograms of

malignant tissue and so this shows the

power of a t cell transfer in the immune

system to reject bulky advanced

metastatic disease that cannot be

treated with any other modalities

the problem with the tumor germline

family of antigens is theyre not common

on the common cancers im showing you

here a review that was done looking at

the expression of nyuso and mage a on

three classes of tumors in melanoma the

expression of the mage a family of

antigens is present about a third of

those melanomas not all consistently nor

at high levels but detectable on those

melanomas but only about percent

express nyu so one squamous cell cancers

mage a can be expressed but very rarely

can nye cell one and in the most common

class of cancers the ones that which the

vast majority of people die of lung

cancer breast cancer colon cancer

theres extremely low levels of these

target antigens limiting their utility

as a general cancer reagent

so

we were looking for better

and more

pertinent antigens and so the question

came up how often are

mutated nonself antigen recognized if

you compare the advantages and

disadvantages of a normal unmutated

selfantigen as a target versus a

mutated nonself tumorassociated

antigen you can see that on the left the

main advantage of targeting a normal

self protein is you can make an off the

shelf reagent its the same in every

patient you need one reagent for all

patients but the dangers are the

potential for autoimmune toxicity and

the t cell repertoire against normal

selfantigens is limited by the thymus

which to prevent autoimmunity in

normally by deleting the most avid and

most active receptors from the

repertoire so you dont attack your

normal tissues

the mutated nonself antigens

unfortunately are totally patient

specific every patient will have a

different set of tumor mutations in

their cancer and they have but they have

very low potential for autoimmunity

because theyre not present on the

normal tissues of the body and theyre

also foreign proteins now not present in

the previously in the life of that

patient and so theres no central thymic

tolerance or deletion

against the repertoire to limit their

immunogenicity and so these are

socalled neoantigens generated by the

new mutations in a cancer that made it a

cancer

so these are the three advantages that

you would like to have but unfortunately

each is associated and accompanied by a

disadvantage

now this class of antigen is known to

exist though because piraculi and the

group in brussels described the first

human tumor antigen to be identified and

cloned in a melanoma in 99

it was a mutated intron sequence that

was

abnormally being translated and

presented

by a human melanoma and they found a t

cell against it cloned the energy and

demonstrated it was the target of that t

cell

so the question then arose how often are

melanoma till recognizing these mutated

class of antigens and the way this was

initially looked at by paul robbins was

to perform dna sequencing on melanomas

so we either did whole genomic or whole

exomic sequencing on the patients

melanoma we identify we grew tumor

reactive t cells from those melanomas

and then we displayed the mutated

proteins that were identified by

sequencing

to those tills from the same tumor and

looked for reactivity

the way this was done

was to

look at every single

amino acid substitution found in a tumor

the illustration here is a patients

melanoma with 0 point mutations

nonsynonymous point mutations that

generates

0 000 potential mutated epitopes

because the most common class one

epitopes are either nine or ten amino

acids long so each mutation has 9

possible epitopes that would contain the

mutant amino acid

instead of making all 0 000 we only

made the top 0 that were predicted to

bind to the patients mhc molecule and

by looking at only 0 out of 0 000 we

got two hits in the top 0

predicted binders to the mhc molecule

and these proved to be antigens mutated

antigens found in the melanoma that were

being recognized by the patients bulk

tumor infiltrating lymphocytes and this

demonstrated that this was not an

unusual phenomenon

it shouldnt have been surprising

because if you look at the burgeoning

field of sequencing tumor

exons and genomes you can see in these

000 sequences of human tumors melanoma

is the most mutated human cancer as a

class thats likely due to the

ultraviolet radiation that

that causes most melanomas and it

results in the highest frequency and

number of mutations in the tumor genome

and so there is a plethora of targets

present in melanoma for t cells to

recognize as foreign

this has also been found in the field of

checkpoint blockade immunotherapy where

antibodies that block ctla or pd which

are inhibitory receptors on t cells can

unleash a t cell response against

certain tumors and cause

tumor regressions and when you look at

the tumors that were responding to

either antictla or antipd they

tended to be the tumors with the

greatest number of mutations in them but

the data is a little bit unclear this is

the actual frequency of either mute

total mutations in melanomas being

treated with epilimumab or their

predicted uh epitopes binding to an mhc

allele our socalled neoantigen load or

mutational load and you can see that the

patients who had clinical benefit from

ipolimumab in green

have

have highly overlapping numbers of

mutations or neoenergy predicted

neoantigens compared to nonresponders

or longterm survivors without clinical

benefit and so although the principle is

probably correct the

utility of this is a predictive marker

of response is very poor

the most convincing experiment was was a

tiny fraction and size of this one this

is a

small study from hopkins johns hopkins

in which patients with mismatch repair

deficiencies in their tumors were

studied this results in huge numbers of

mutations in those patients cancers you

can see a mismatch repair deficient

colorectal cancer in the second column

here has 00 mutations on uh

as amine

if you have mismatch repair normal

colorectal cancer its only and so

those with mish match repair have huge

numbers of potential neoantigen targets

if you treat them with antipd antibody

you can see that colorectal cancer far

the that were treated here none of

them responded objectively but in the

ones who had the deficiency in large

numbers of mutations 0 out of 0

responded this is an example where a

very small study can lead to a dramatic

conclusion because the only real

difference that you consistently found

in these tumors was the fact that they

could not repair their dna and had many

more mutations on the right is a series

of noncolorectal cancers with the same

deficiency same large number mutations

and percent of those patients its

only five out of seven but dramatically

again demonstrating that

high numbers of mutations can be

associated with response to

immunotherapies and so this is proving

to be

probably the most significant class of

tumor antigens to target with

immunotherapy to cause rejection

so we decided to look at whether this

could be exploited actively and so we

had a patient a year old woman with

chlangiocarcinoma we sequenced her dna

she had only coding mutations

her till were grown from the tumor and

screened against those mutations as

potential antigens and only one

reactivity was found which was a t cell

against mutated herb b interacting

protein

we did were lucky to find

a

culture of hertil that was highly

enriched for these cells we expanded

them specifically and gave them to her

now she had had a previous treatment

with just her whole bulk till and she

had a very minor response that lasted

only a few months but during that time

we were able to complete these studies

find a highly enriched population of t

cells and administer those

exclusively

this is the way we do this screen we do

identify mutations we make long peptides

around the mutated amino acid or a mini

gene encoding

the sequence around that mutation so now

we have a mini gene expressing the

mutation in its flanking sequence or a

peptide encoding that is overlapping

amino acid run

and we can introduce those into the

patients own dendritic cells or antigen

presenting cells either by in vitro

translated and electroporated rna or

just simply incubating and loading them

onto their dendritic cells in the form

of a peptide we then coculture this

avatar this representation of the tumor

mutations on the patients own antigen

presenting cells as the target for their

t cells

this is necessary because the vast

majority of common cancers cannot be

cultured in vitro and used as an immune

reagent the number of lung cancer colon

cancer breast cancer lines available for

such studies is very few and can almost

never be generated from individual

patients in a timely fashion

but when you make this mutational avatar

and incubate it this is an ellie spot

where each purple dot is a t cell that

recognized the antigen you presented to

it and made interferon gamma detected by

this ellie spot eliza type of assay you

can see tmg shown in duplicate was the

gene that encoded something that the

tumor was that till were recognizing

and when we finally parsed this out it

was herb b interacting protein and so

she was given this enriched population

you can see she had very bulky pulmonary

metastasis on the left when treated in

october 0 and she had a dramatic

partial response that went on for two

and a half years after a single

administration of these mutation

reactive t cells against a single

antigen

you can see at the on the right side

that her tumors have regressed uh

dramatically

she also had liver disease shown on the

left side to liver disease central liver

metastases that also shrank and at this

point are pet nonavid and and

completely indolent with no vascular

enhancement either and so she had

regression of both major organ diseases

with the transfer of these mutation

reactive t cells

this is a dramatic patient with

widespread breast cancer that had failed

hormonal and

chemotherapy

and she had bulky disease all over the

chest wall inside the chest and the

mediastinum and she had a dozen large

liver metastases shown on the bottom

left she was given till reactive with

two mutations on her cancer we dont

know the significance of these proteins

but it was a mutated

slca which is an amino acid

transporter and mutated kiaa which

is a proteosome associated protein and

she has had a dramatic complete

regression of all of her cancer that is

now ongoing in a year and a half and so

this shows again that mutation reactive

t cells can induce the regression of

very large amounts of metastatic cancer

when only administered one time now i

cant say that this occurs all the time

this mostly illustrates the

potential for this therapy but its not

something that we are able to achieve in

the majority of cases yet

when you look at a variety of cancers

for

the presence of mutation reactive t

cells in those

tumor till

this is a table showing the patients who

are evaluated with a variety of

different cancers on the left the number

of patients that have been evaluated

looking for mutation mutationreactive t

cells in their cancers and the number in

which they were found you can see

overall across all these different

histologies

percent of these patients you could

find a till in their tumor that reacted

with a mutation in their tumor

and so

the frequency with which this happens

really raises the possibility that this

could be a treatment for many many

different kinds of cancers and cancers

irrespective of their type this could be

a pan cancer therapy

a principle that can be applied to

almost any human malignancy because all

malignancies have mutations now not all

have

reactive t cells that we can demonstrate

but the method by which we do it now is

still very inefficient and ways to

improve that are in development now

so im going to show you one example

where we combine the best of both worlds

as i told you we would love to have

constant off the shelf

reagents to target

tumor antigens

but we want to avoid the autoimmunity of

that and we want to avoid central thymic

tolerance as a blunting mechanism for

the immune response we want to achieve

and so

what youd like to do is combine a

create a therapy that combines the

advantage on the left with the two on

the right

that brings us to the kras pathway or

the entire ras family of oncogenes this

is a family of

really the first family of human

oncogenes in which mutations led to

activations of the raf

eric pathway

and this is a mutation that is extremely

common as a matter of fact its the

second most common mutation in all of

human cancer second only to p

this is the cosmic database showing

across the top the site of all mutations

in the r and the k ras protein and you

can see theyre all clustered around a

very small region near the beginning of

the protein if you blow that up you can

see that the vast majority are present

at codon and the substitution for the

wild type glycine are very restricted as

well with veiling aspartic acid

dominating the substitutions that you

find in mutated mutations in ras and

human cancer and so this now represents

for us a constant target

against a mutated antigen so this is why

we consider this potentially the best of

both worlds

if you look at the utility of this

percent of pancreas cancers have a

rast mutation and the vast majority of

them are the g d substitution or the

g v substitution you can see at the

bottom theres almost 0 000 patients

who have one of those two mutations in

pancreas cancer

colon cancer approximately a third of

patients have a kras mutation and again

far and away most of them are the same

gd or gv substitutions and so the

estimation is theres over a hundred

thousand patients with mutations in k

ras that are either gd or gv

so

receptors recognizing mutation these two

mutations in kras have been identified

they were actually identified by

immunizing human

hla transgenic mice where you can

vigorously immunize those mice against

these antigens generate tcell receptors

and those tcell receptors can be put

into human tcells without modification

where they function extremely well and

uh that has been done both

experimentally and clinically in other

protocols and so two high avidity hla

a restricted receptors were found that

recognize these two common mutations

if you take pbl from any donor and

transduce them with these receptors they

will recognize an hla a positive tumor

line with one of these two mutations it

turns out all members of the ras family

share the same sequence and mutations

at that codon and can be recognized by

these t cells and so of the us

population is hla a but it is the most

common class allele in the 00 million

on chinese

this is the data showing on the left if

transduction of a target cell with

either uh wild type cost g d cost or

the g v target gene shown by the high

black bar and then coincubated with pbl

that were genetically modified with the

antigv receptor you can see those

cells now make 000 micrograms of

interferon gamma in response to the

mutation but not to any wild type

reactivity or other mutations

and then a panel of tumors shown in the

middle you can show if those ones uh

with the black bars are krest gv

mutated tumors that are hla a and they

all

accept one perhaps generate interferon

gamma in response to

those

correct antigen types and mhc types

the parental tumors which mostly lack

a showed no reactivity if they were

just mutation positive but mhc negative

you actually now put a human pancreas

cancer in an nsg immunosuppressed mouse

you can wait until it grows to six or

seven millimeters before you start the

treatment and you can still cause a

dramatic regression shown by the bottom

line cells with mock transduction on

transducer mice with no treatment show

progressive growth of this human

pancreatic cancer

in this model

this is the other receptor against gd

again restricted by hla a and again

very strong recognition of hla a

positive

cancers with human cancers in vitro with

the gd mutation but in the absence of

the gd mutation or hla a theres no

background recognition of those tumors

and so clinical protocols using these

two receptors are opening this year the

first one against gv is already open

and the other one is slated to open

later this year

now at the same time this was happening

an experiment of nature occurred a

patient with colon cancer was found to

naturally have a till that recognized

the gd mutation in kras

and this patient it was restricted by

co0 a relatively less common mhc

allele but in this case it was an

autologous till from the patients tumor

and it was grown and the patient was

treated with it and you can see here she

had initially seven pulmonary metastases

and you can see with the yellow arrows

here ive shown three of them in three

panels on our starting ct scan you can

see that at three months all of them had

regressed to some degree but with

continuing treatment out to nine months

several of them continued to regress but

some started to grow again

or at least one started to grow again

lesion number two

so we resected lesion number two

of our original seven lung lesions and

it was found to have lost the presenting

hla allele co0

but it was the only one that was

progressing at the time the other

lesions that remained behind and were

not resected

continued to regress and disappeared

completely and she remains diseasefree

now months after that surgery and

months since her tilt transfer so this

illustrates that a gd react

kras reactive t cell can be

administered to a patient and cause

meaningful tumor regression

and so ultimately

i think the direction in which

this field will go is to use

geneengineered peripheral blood

lymphocytes and target probably the most

important class of tumor antigens which

is tumor mutations and this may have to

be done on a patient individual basis

the ability to do that in a timely

fashion already exists we can make a

retroviral supernatant against a spec

with a specific t cell receptor in a

matter of months put it into the

peripheral blood of a patient and

administer that to that patient against

a whole variety of antigens once theyve

been identified the major restrictions

on this are regulatory and issues about

safety of genetic engineering i think

are becoming clearer and clearer and so

ultimately if we want to target

tumorspecific mutations which tend to

be different in almost all patients we

need to be able to tailor t cells and t

cell receptors against those on an

individual patient basis

and the regulatory requirements for

doing that

have to be simpler or we will not be

able to do it in a timely fashion to

treat patients with metastatic disease

and so the future directions i think for

t cell transfer are to gene engineer

peripheral blood lymphocytes with these

patientspecific t cell receptors to

combine t cell transfer transfer with

pharmacologic manipulations of the tumor

microenvironment or even tumor energy

and expression to enhance therapy and

ultimately probably the most important

and the most promising future direction

is are gene modifications of t cells

that dont merely redirect their

targeting but optimize their function we

know molecules are present in inhibit t

cells in their function and those can be

deleted or modified in ways that would

then unleash tcell functions or create

tcell functions and are not already

present that would enhance the rejection

of tumor and so i think this is the

future of tcell therapy its a living

reagent that we will be giving to

patients that is now already proven to

be capable of causing the complete

rejection the durable rejection and

likely the cure of widely metastatic

disease in some patients

and so

many people have contributed this talk

as well as to this effort and so id

like to thank you all for your time i

think that you will find that cell

therapies in general are a coming

modality it is a new

component of the armamentarium against

advanced cancer it currently is the main

new modality that can actually cure

patients with widespread malignancy from

the common adenocarcinomas and melanomas

and i think that this is something that

will only get better as we understand

better the mechanisms by which t cells

reject cancer and be able to construct

those or induce them and give the

therapy we want and so i think this

represents enormous promise and the

administration of a single

tcell transfer one time in a patient

that can cause this i think is really

the dream of really every immunologist

and tumor immunotherapist and oncologist

that i know of and thank you for your

time and thank you and if there are any

other questions or issues then feel free

to contact the course coordinator

and ill be happy

to assist in any

responses as well thank you