

**MID-ATLANTIC BIOSAFETY ASSOCIATION
35th ANNUAL BIOSAFETY SYMPOSIUM**

**Application of Biosafety Protocols
for the Study of Cancer**

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Molecular Biochemistry**

June 15, 2023



Lewis Katz School of Medicine

Colorectal Cancer (CRC)

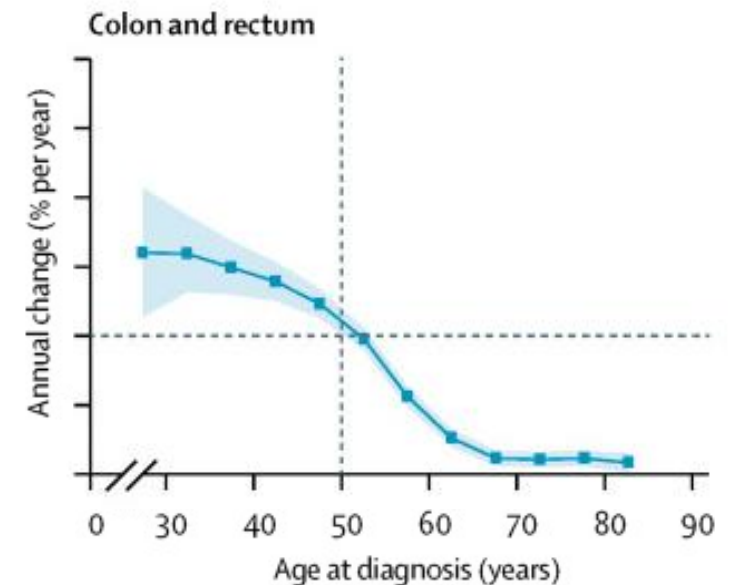
3rd deadliest cancer affecting both sexes

Estimated New Cancer Cases in the US in 2023

Male			Female		
Prostate	288,300	29%	Breast	297,790	31%
Lung & bronchus	117,550	12%	Lung & bronchus	120,790	13%
Colon & rectum	81,860	8%	Colon & rectum	71,160	8%
Urinary bladder	62,420	6%	Uterine corpus	66,200	7%
Melanoma of the skin	58,120	6%	Melanoma of the skin	39,490	4%

Estimated Cancer Deaths in the US in 2023

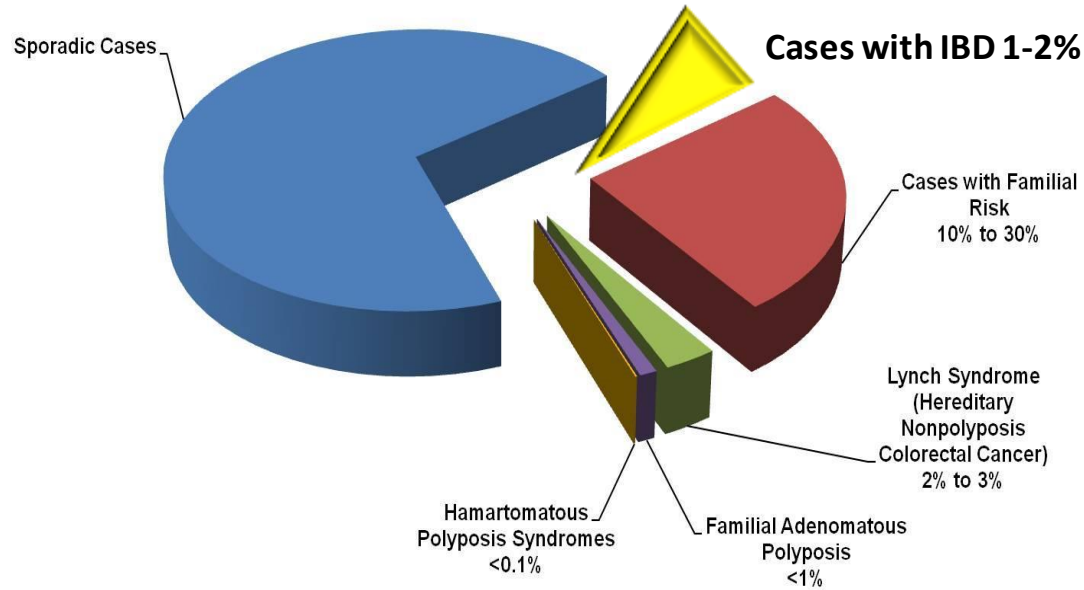
Male			Female		
Lung & bronchus	67,160	21%	Lung & bronchus	59,910	21%
Prostate	34,700	11%	Breast	43,170	15%
Colon & rectum	28,470	9%	Colon & rectum	24,080	8%
Pancreas	26,620	8%	Pancreas	23,930	8%
Liver & intrahepatic bile duct	19,000	6%	Ovary	13,270	5%



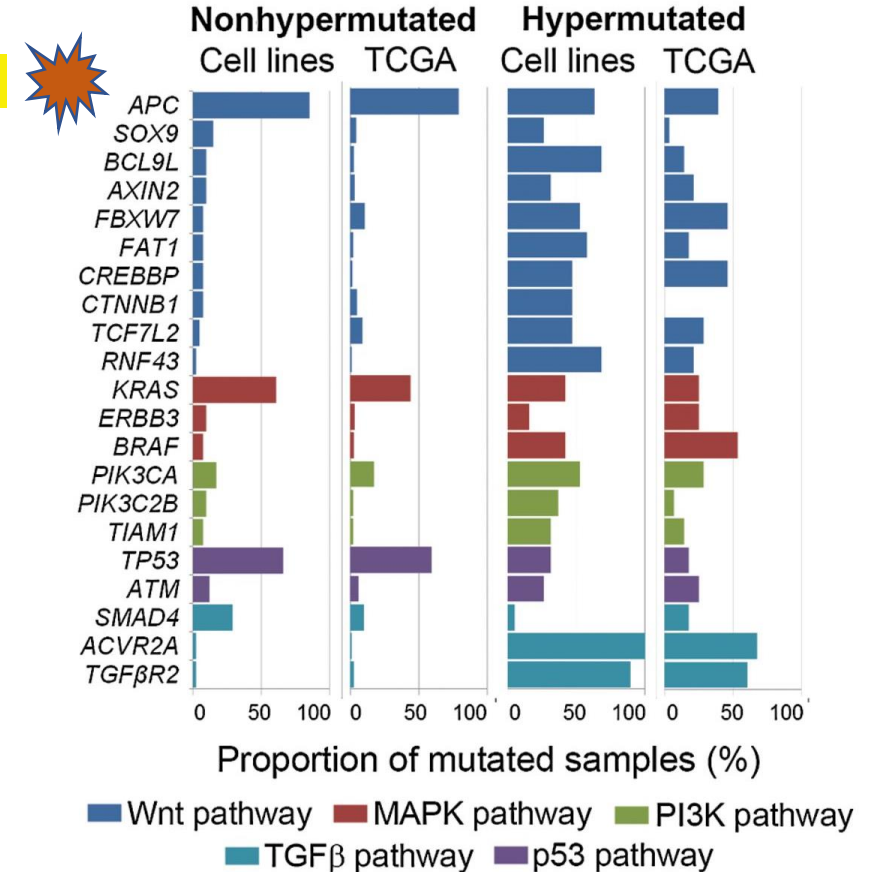
**** Number of cases of individuals younger than age 45 is on the rise**

Colorectal Cancer

Colon Cancer Cases Arising in Various Family Risk Settings



APC mutated in 85%



IBD: Inflammatory bowel disease: Ulcerative Colitis and Crohn's Disease

Colorectal Cancer Cell Lines Are Representative Models of the Main Molecular Subtypes of Primary Cancer

Dmitri Mouradov, Clare Sloggett, Robert N. Jorissen, Christopher G. Love, Shan Li, Antony W. Burgess, Diego Arango, Robert L. Strausberg, Daniel Buchanan, Samuel Wormald, Liam O'Connor, Jennifer L. Wilding, David Bicknell, Ian P.M. Tomlinson, Walter F. Bodmer, John M. Mariadason, and Oliver M. Sieber

DOI: 10.1158/0008-5472.CAN-14-0013 Published June 2014

CANCER RESEARCH

Risk Factors: Minimize by making lifestyle changes



SMOKING

Smokers have an increased risk of colon cancer. Stop smoking to lower your risk of colon cancer.



HEAVY CONSUMPTION OF ALCOHOL

Limiting the amount of alcohol you drink may reduce your risk of colon cancer.



LOW CONSUMPTION OF HIGH-FIBER DIET

Those with low intake of fibers have an increased risk of colon cancer.



FAMILY HISTORY OF COLON CANCER

You're more likely to develop colon cancer if you have a blood relative who has had the disease.



LACKING PHYSICAL EXERCISE

Getting regular physical activity may reduce your risk of colon cancer.

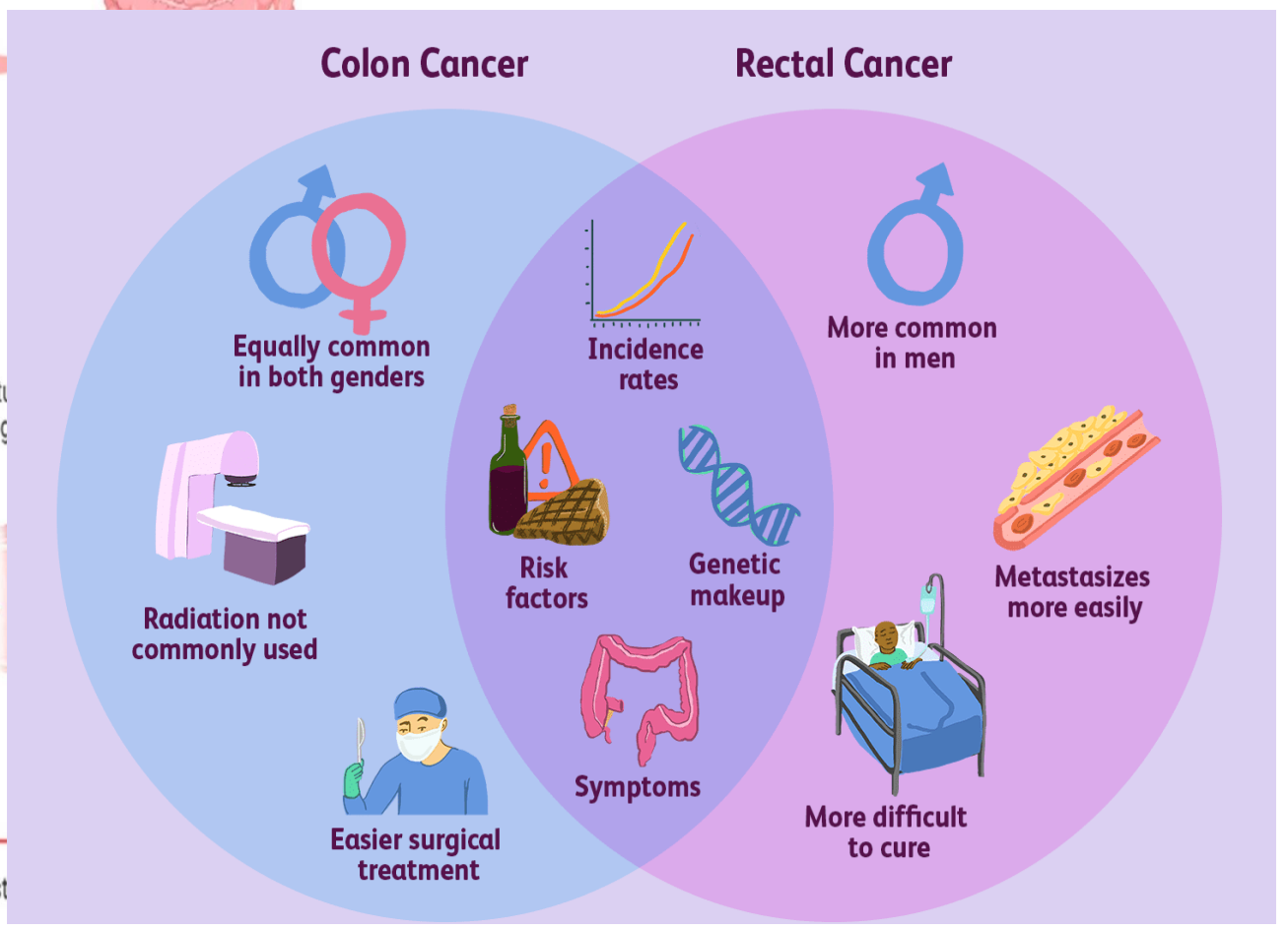
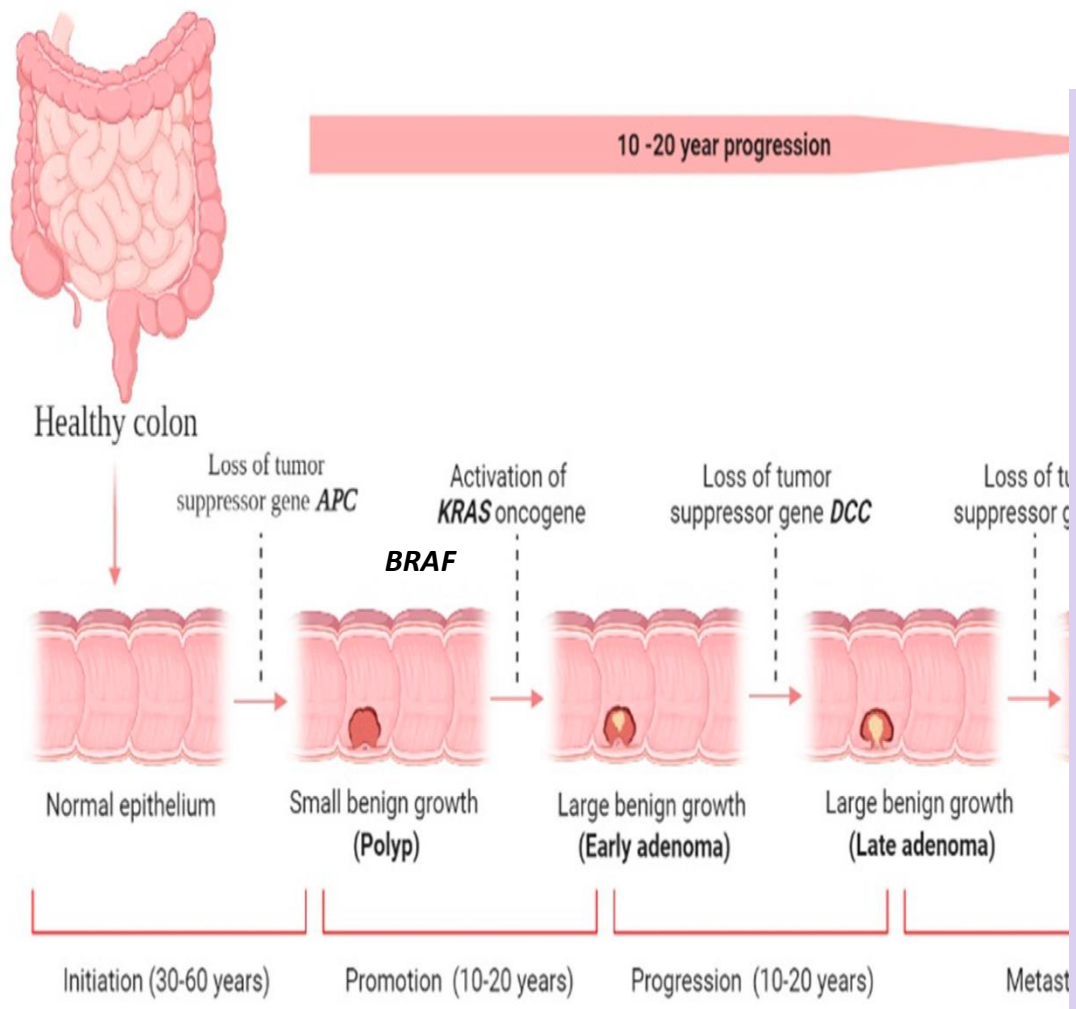


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Warning Signs and Symptoms of Colorectal Cancer

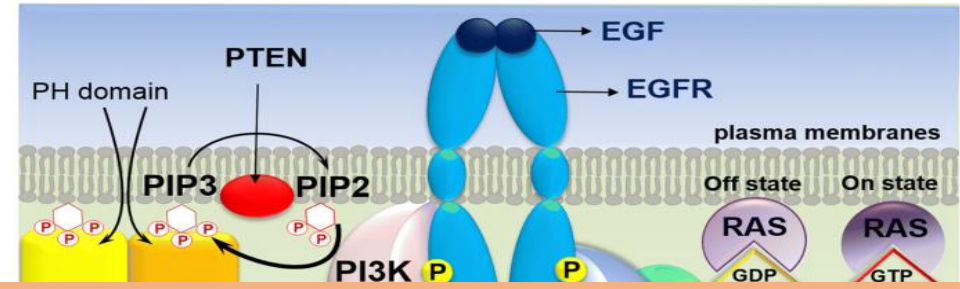


****Get screened if you are 45 or older**

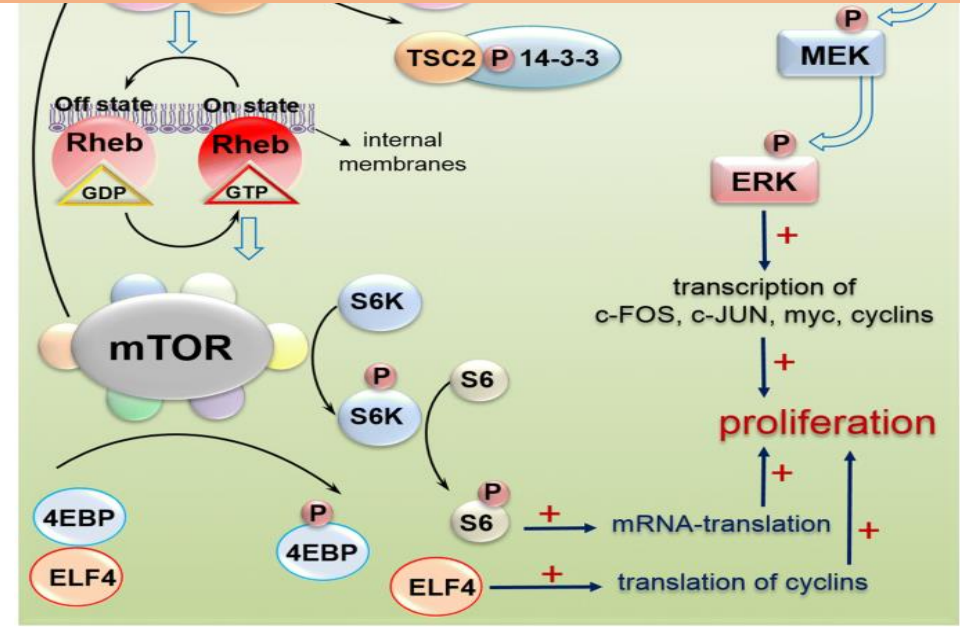
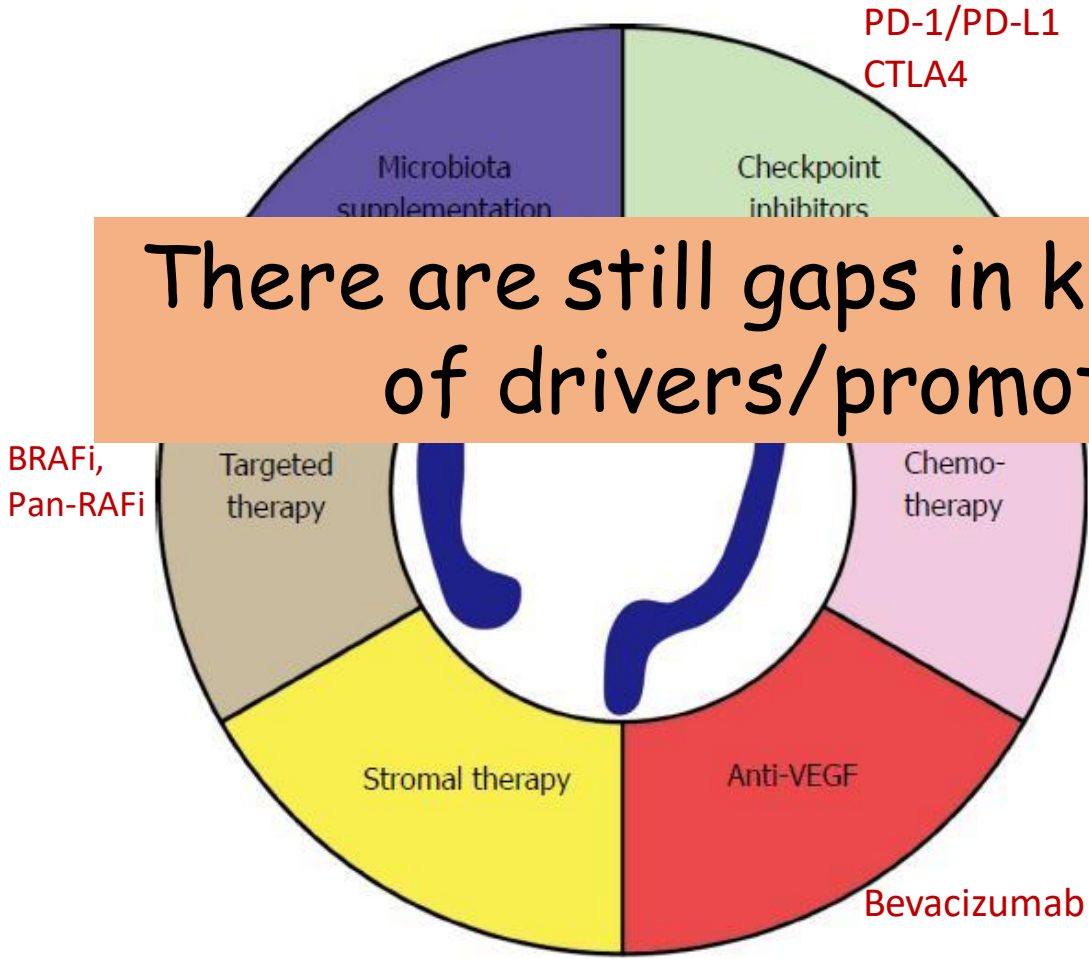


Therapeutic advances in Colorectal Cancer

Signaling Pathways involved in colorectal cancer progression



There are still gaps in knowledge on our understanding of drivers/promoters of colorectal cancer



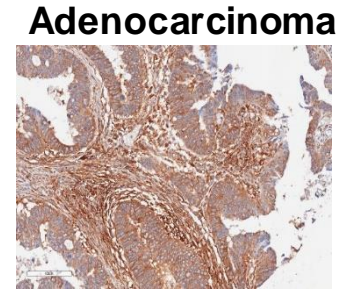
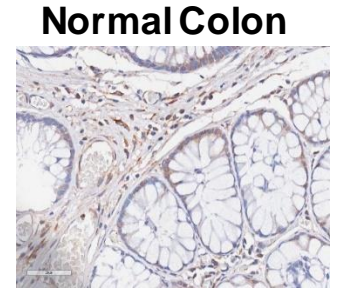
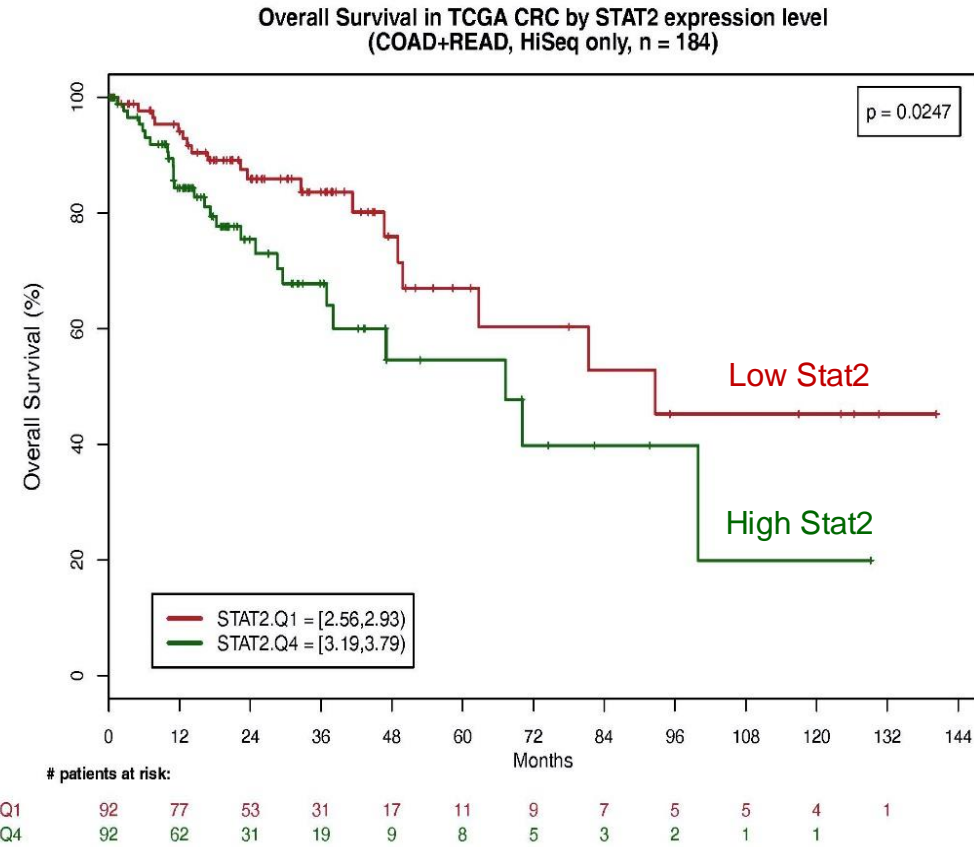
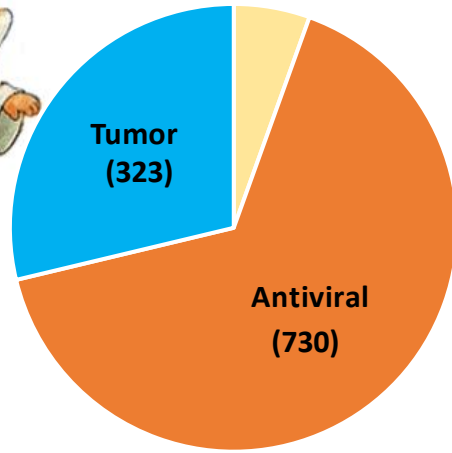
*****Patients continue to relapse**

STAT2



Is this gene important in cancer And how to study it?

STAT2 publications in PubMed
Total: 1116



STAT2 protein

Most of the cancer work has been done in vitro using tumor cell lines

Colorectal cancer models:



1) Chemical model:
Azoxymethane (AOM) + Dextran Sulfate Sodium (DSS)

2) Genetic engineered models: *Apc* deletion,

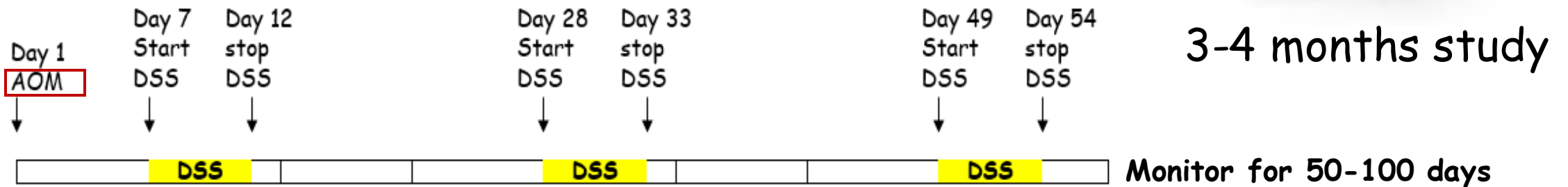
3) Combination of chemical+ GEM: *Il10* deficiency + AOM



Stat2 deficient

Model of AOM-DSS of Colorectal Cancer

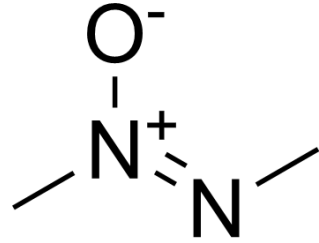
- Involvement of Environmental Health & Radiation Safety (EHRS)
- Requires approval by IBC and IACUC



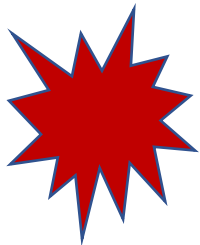
Single intraperitoneal injection of AOM

DSS (2-3%) is given in the drinking water (3 cycles)

Azoxymethane:



- Colon carcinogen
- Sold in liquid and powder form
- Do not want to inhale or ingest
- Delivered intraperitoneally in mice



Dextran Sulfate Sodium:



- Inflammation in the colon
- Sold in powder form
- Do not want to inhale or ingest
- Dissolved in water
- Not considered hazardous by OSHA



Safety Practices: **AOM** in the Lab



We have Standard Operating Procedures (SOP) of how to handle chemicals

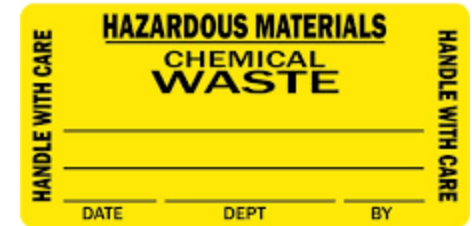
Wear protective protective equipment (PPE): Nitrile gloves, lab coat mask, goggles, closed toe shoes

- Do not work with **AOM** if skin is cut or scratched
- Pregnant women should not handle this chemical
- Transport in a secondary container
- Do not forget to decontaminate
- Have the appropriate sharps container to dispose of syringes, pipettes, pipet tips



Safety first: **AOM** in the Animal Facility

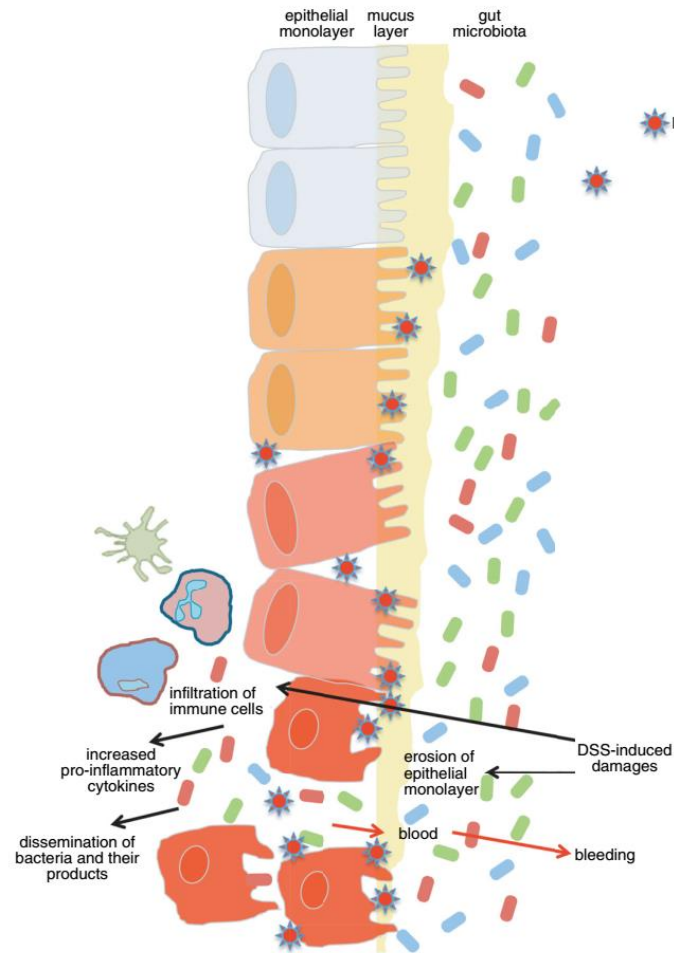
Wear PPE: Nitrile gloves, lab coat mask, goggles, shoe covers, head cover



- Place chemical hazard label on cages
- After injection, animal waste, bedding and cage are considered hazardous for at least 48 hours
- Always use a biological safety cabinet
- Dispose of needles in sharps container

Safety first: DSS in the lab

Wear PPE: Nitrile gloves, lab coat mask, goggles, shoe covers, closed toe shoes



- Classified as non-hazardous chemical
- Can cause irritation of eyes and skin, nausea
- Use a biological safety cabinet to minimize aerosolization
- Stable in solution for a week
- Its products of degradation are not toxic

Safety first: DSS in the animal facility



- Prepare freshly
- Place label on cage not to change water
- Use a biological safety cabinet to change water bottles
- Check volume in bottle daily

A hazardous studies door sign should be posted on the outside of the animal room

DANGER



HAZARDOUS CHEMICAL USED IN ANIMALS

NAME OF HAZARDOUS CHEMICAL AZOXYMETHANE

toxic if swallowed, skin and eye irritant, corrosive, mutagen, carcinogen

Minimum PPE Requirements: nitrile gloves, eye protection, face protection, skin protection, head cover, lab coat, shoe covers

Additional PPE or Other Requirements: Please refer to Manufacturer Safety Data Sheet

DANGER



HAZARDOUS CHEMICAL USED IN ANIMALS

NAME OF HAZARDOUS CHEMICAL DEXTRAN SULFATE (SODIUM SALT)

skin and eye irritant

Minimum PPE Requirements: nitrile gloves, eye protection, face protection, skin protection, head cover, lab coat, shoe covers

Additional PPE or Other Requirements: Please refer to Manufacturer Safety Data Sheet

What are the issues to be aware of?

- A bad habit to recap needles



- Walking around the lab with gloves that have touched chemicals



- Disposing contaminated tubes, unused solutions and tips in the wrong sharp container



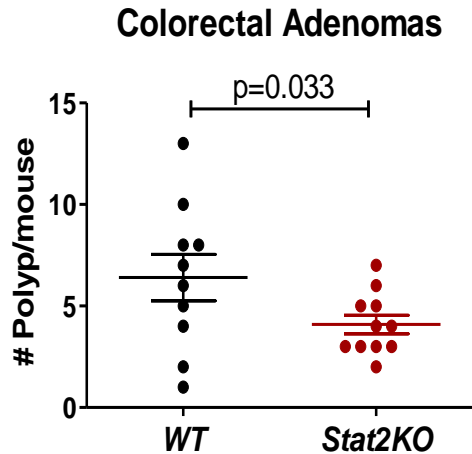
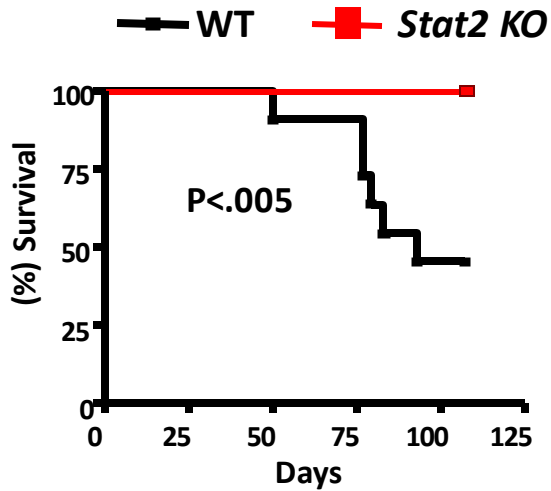
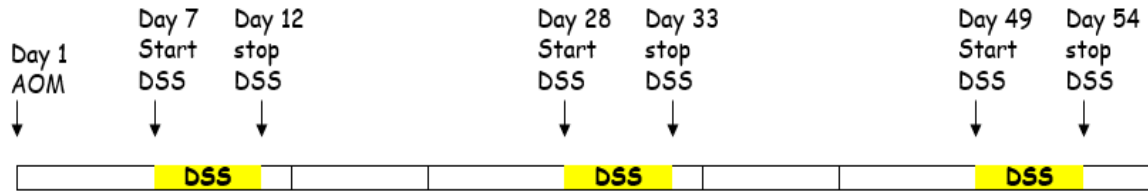
VS



HOW TO MINIMIZE RISK?

- Restrict access and follow safety practices
- Train personnel to change bedding/food /water
- Always wear PPE for those in contact with mice

STAT2 Promotes Colorectal Cancer



Wild Type



Stat2 KO
($<$ tumors)

What's next.....
In vitro studies will help us understand the importance of STAT2 in colon cancer and how it works.

Use of Tumor Cell Lines to modify gene expression (STAT2)

- 1) Overexpression
- 2) Silence expression (shRNA)
- 3) Delete gene of interest (Crispr/Cas9)



Bacterial plasmid, retroviral and lentiviral systems

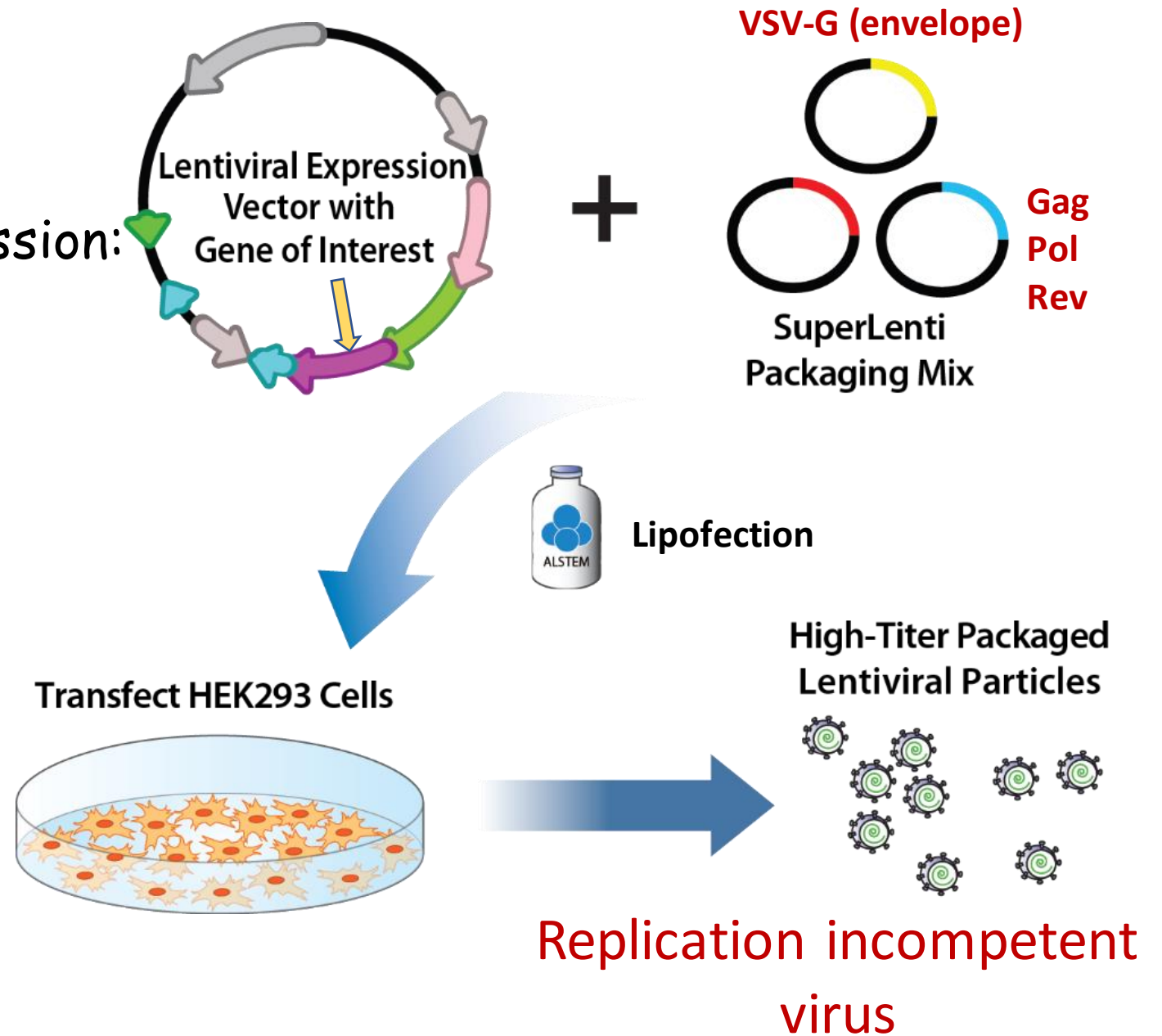
*Every cell line of human or mouse origin must be registered and approved by IBC

Lentiviral System

Application to modify gene expression:

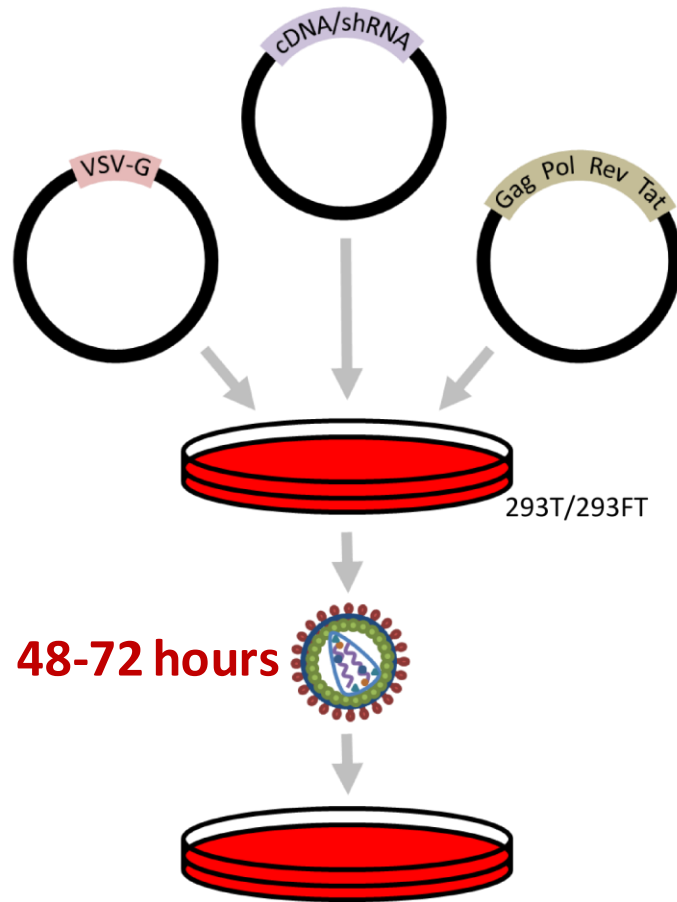
- Primary Cells
- Difficult to transfect cells

***Every plasmid or vector DNA must be registered and approved by IBC**



Minimize hazards:

Lentivirus (LV)



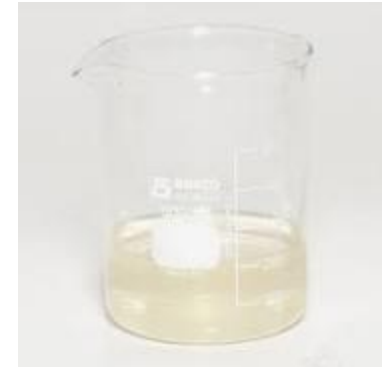
1) Transfect packaging cells

2) Collect virus particles

3) Transduce target cells

48-72 hours

**Antibiotic selection to
Generate stable cell line**

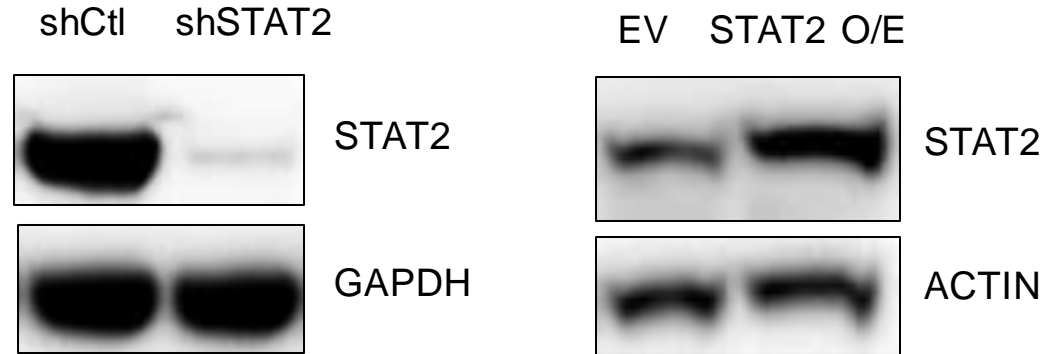
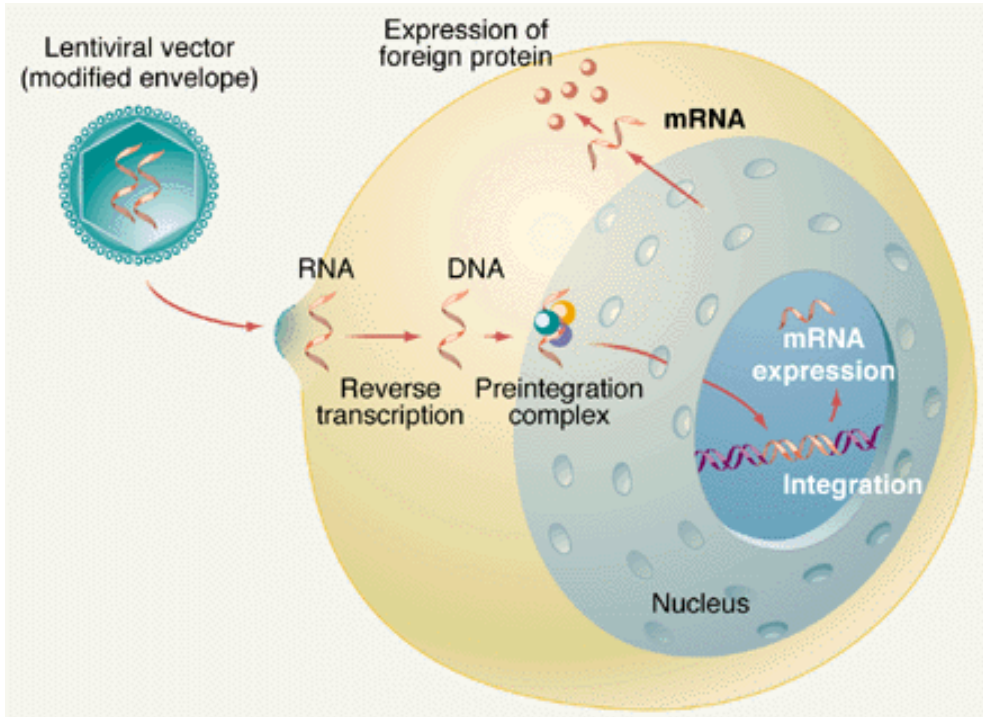


➤ Treat with bleach:

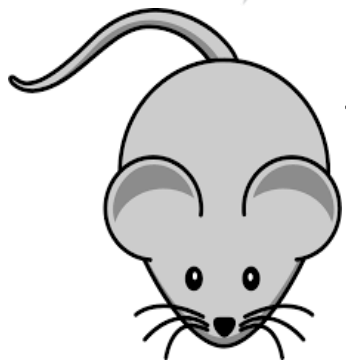
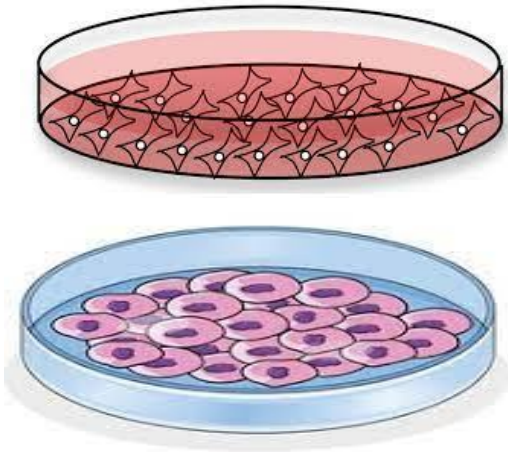
- media containing virus
- Pipettes, pipette tips and tissue culture supplies
- Dispose as biohazardous waste

Check expression of protein in modified colon cancer cell line

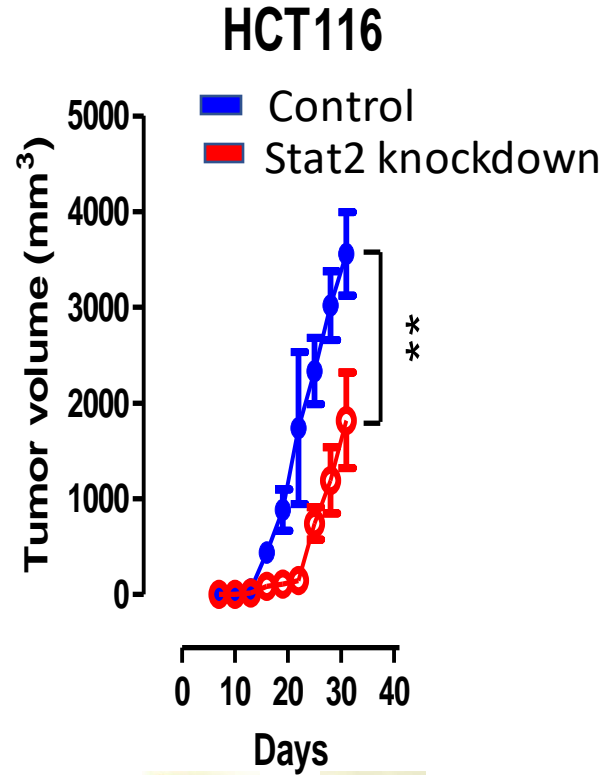
HCT116



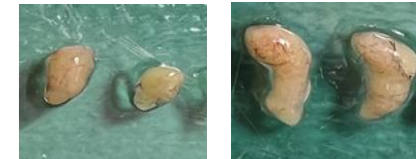
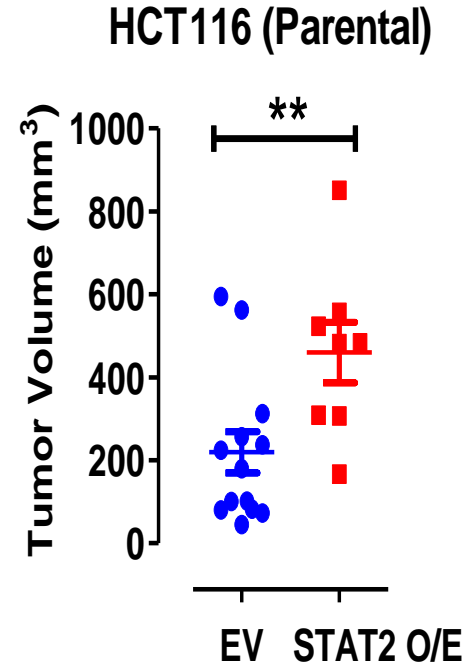
Effect of STAT2 in tumor growth



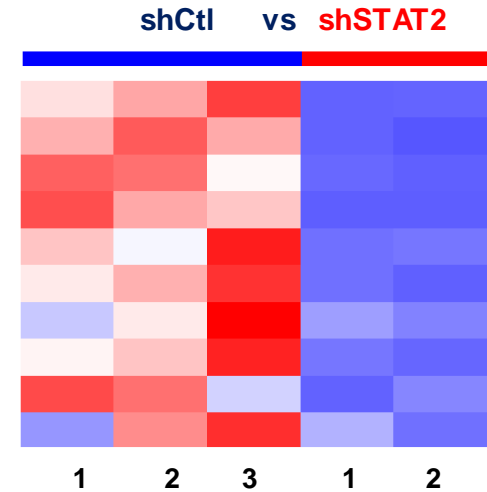
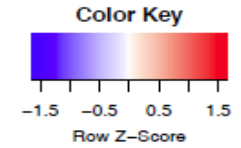
5 million cells



shCtl shSTAT2



Expression of genes
in lipid metabolism



So what's next?



SMOKING

Smokers have an increased risk of colon cancer. Stop smoking to lower your risk of colon cancer.



HEAVY CONSUMPTION OF ALCOHOL

Limiting the amount of alcohol you drink may reduce your risk of colon cancer.



LOW CONSUMPTION OF HIGH-FIBER DIET

Those with low intake of fibers have an increased risk of colon cancer.



FAMILY HISTORY OF COLON CANCER

You're more likely to develop colon cancer if you have a blood relative who has had the disease.



LACKING PHYSICAL EXERCISE

Getting regular physical activity may reduce your risk of colon cancer.



IBC



IACUC

In vitro

Chemicals

Biologicals

Tumor cell lines

DNA vectors

- Oncogenes
- Tumor suppressors
- Introducing mutations

In vivo

Tumor cell lines

Reduce pain/distress

Other factors to consider

Funding

Plastic vs glass pipettes

Recycling of plastic red biohazard waste containers

Insufficient training that affects labs with open design

- Sharing tissue culture rooms
- Incorrect disposal of broken glass/biologicals



SAFETY



IS PART OF SCIENCE .

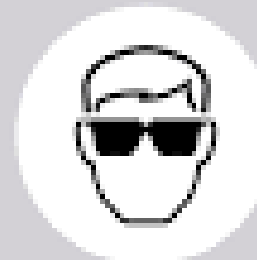
LAB SAFETY



Anyone not following the rules will be denied access to the lab room



Don't touch
the animals



Wear safety
goggles



Wear lab coat



Wear gloves
when necessary



Don't eat at your
workstation



Clean up your
workspace

ACKNOWLEDGEMENTS

Gamero Lab
Jorge Canar
Amy Alvarado
Kennedy Darling
Ryan Dadey
Maddie Bono
Cal Harper



STAT2



Pro-inflammatory
Pro-carcinogenic

