Signaling Pathways: The role of the EGFR gene in Lung Adenocarcinoma

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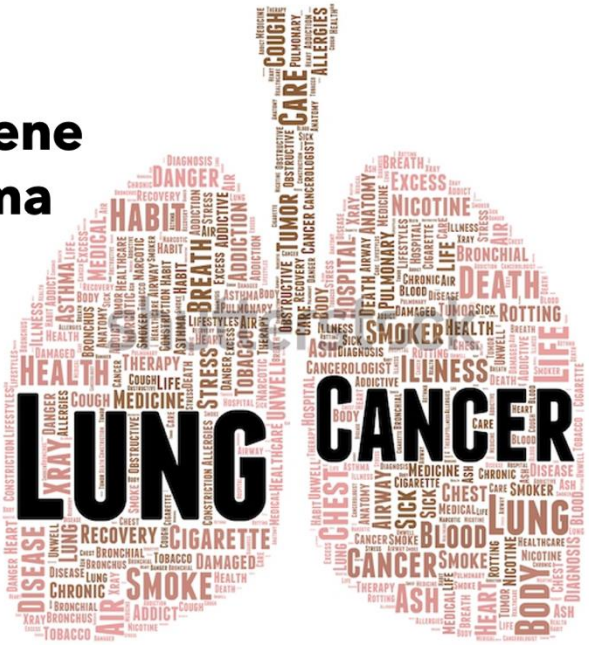
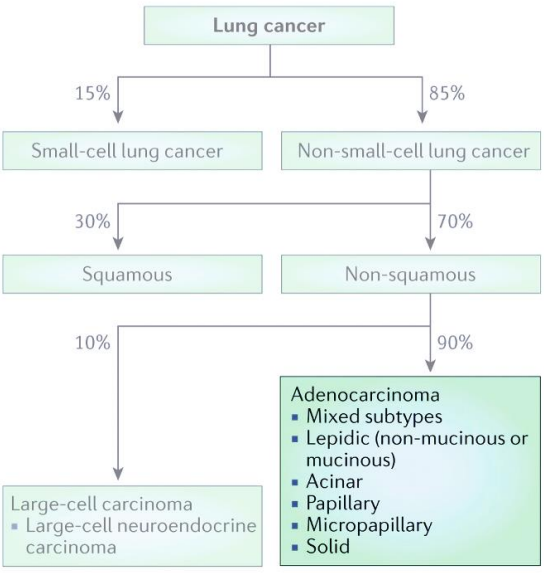
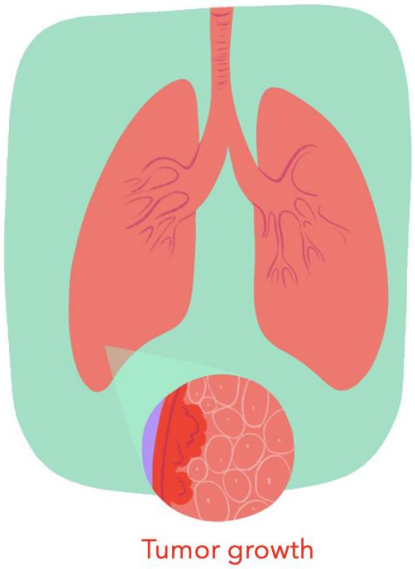


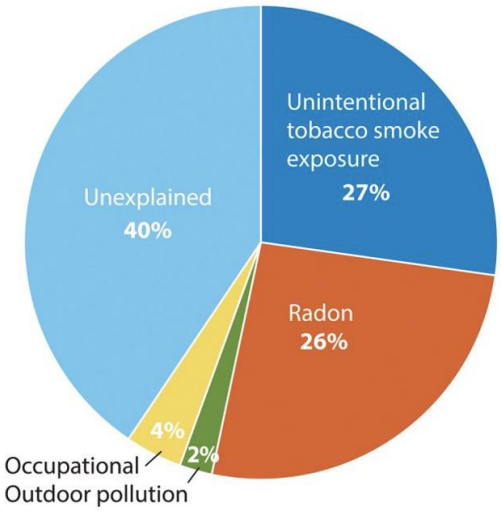
The EGFR gene is involved with signaling that ultimately affects tumor progression in LADC. Associated words are shown in the graphic.

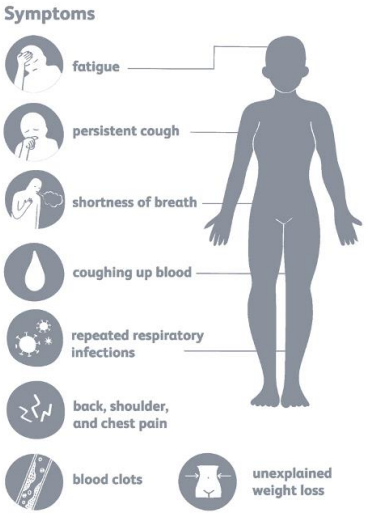
What is Lung Adenocarcinoma?





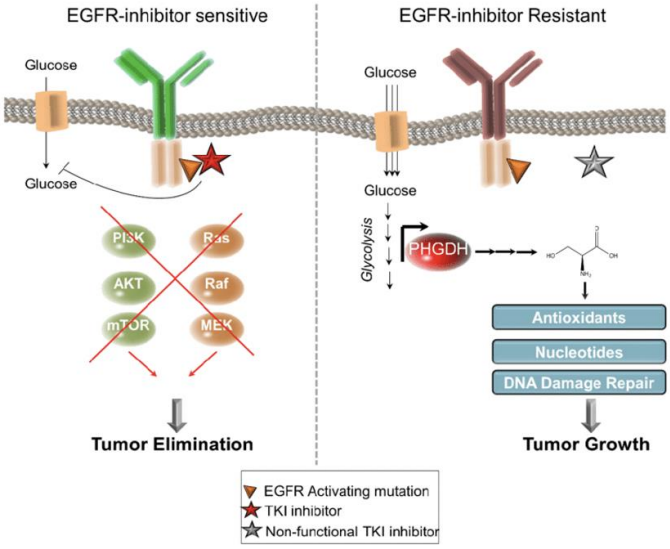
What are risk factors and symptoms of LADC?





Radon is an important risk factor of lung cancer as well as Tobacco smoke, which puts people at the most risk. It's important to get a Radon detector. This is especially important for older people who are prone to cellular damage.

What are current treatments for LADC?



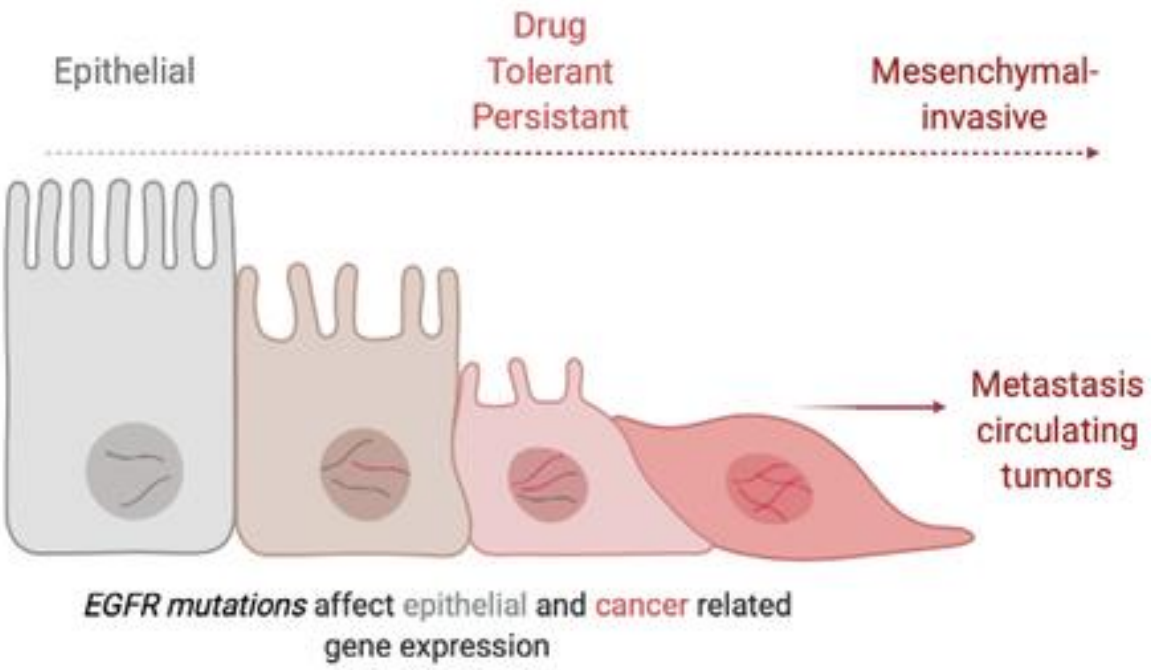
Targeted Therapy

Surgery

Chemotherapy

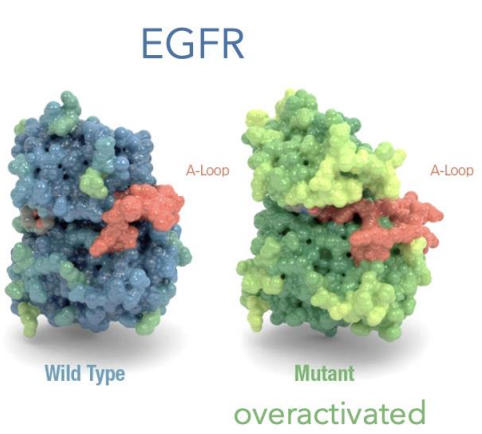
Immunotherapy

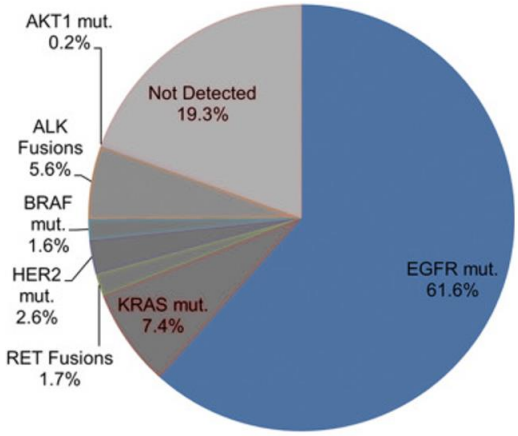
LADC treatment includes immunotherapy, chemotherapy, surgery and specific inhibition of cancer driving genes, in some cases EGFR. In these cases, Tyrosine Kinase Inhibitors can be used to reduce cell proliferation and hopefully tumor growth. However, there are cases where TKI treatment doesn't result in effective inhibition of the EGFR signaling pathway.



Epithelial transitions can result in pluripotent mesenchymal cells that can transform into various cell types, including cancerous phenotypes. Understanding the role of this process in LADC is important to understand the reasons behind tyrosine kinase inhibition and EGFR signaling in cell proliferation and migration.

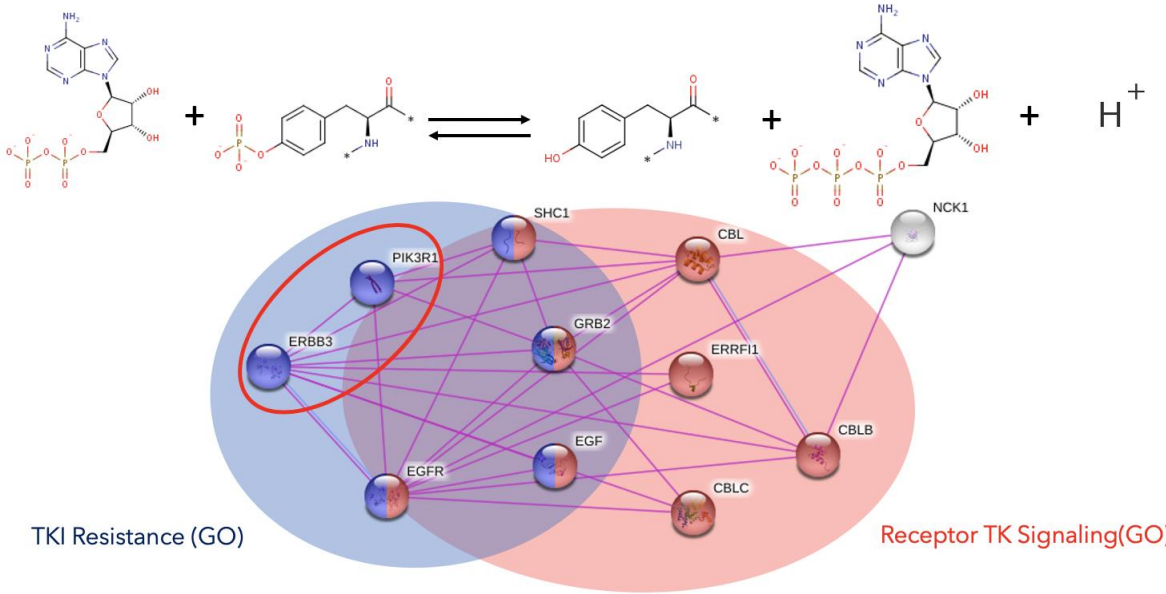
What are genetic causes Lung Adenocarcinoma?



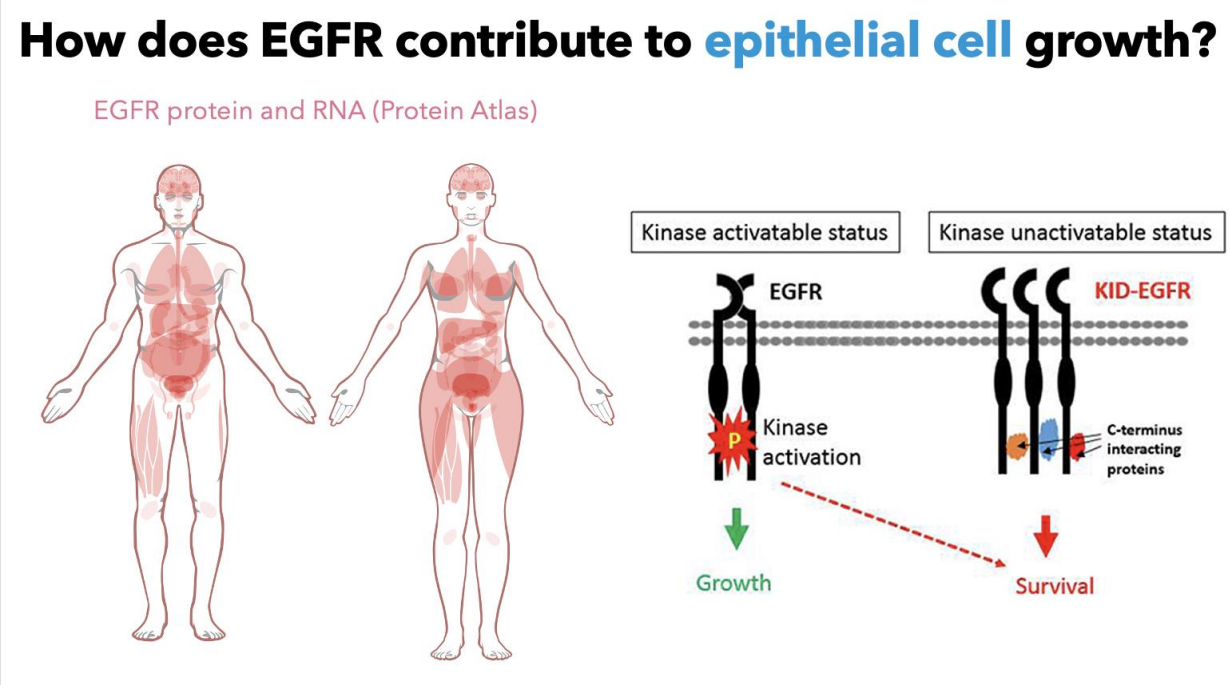


The A-loop resides within the Tyrosine Kinase domain and undergoes a conformational change upon ligand binding, or possibly other stimuli to ultimately signal other molecules in the cell.

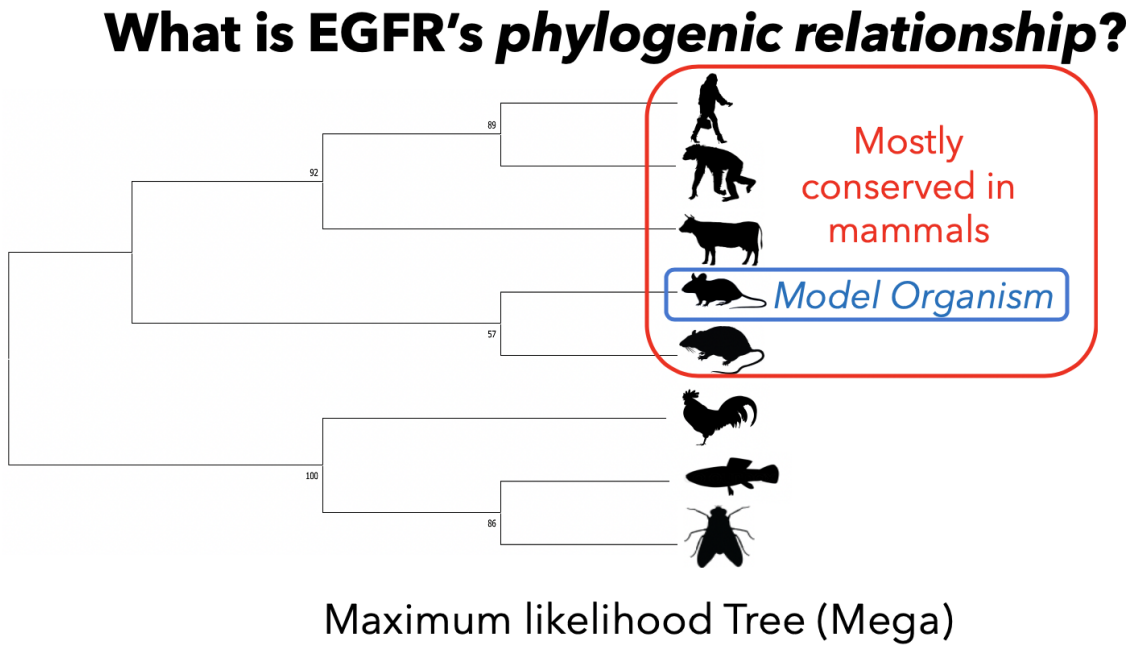
What is EGFR's molecular function?



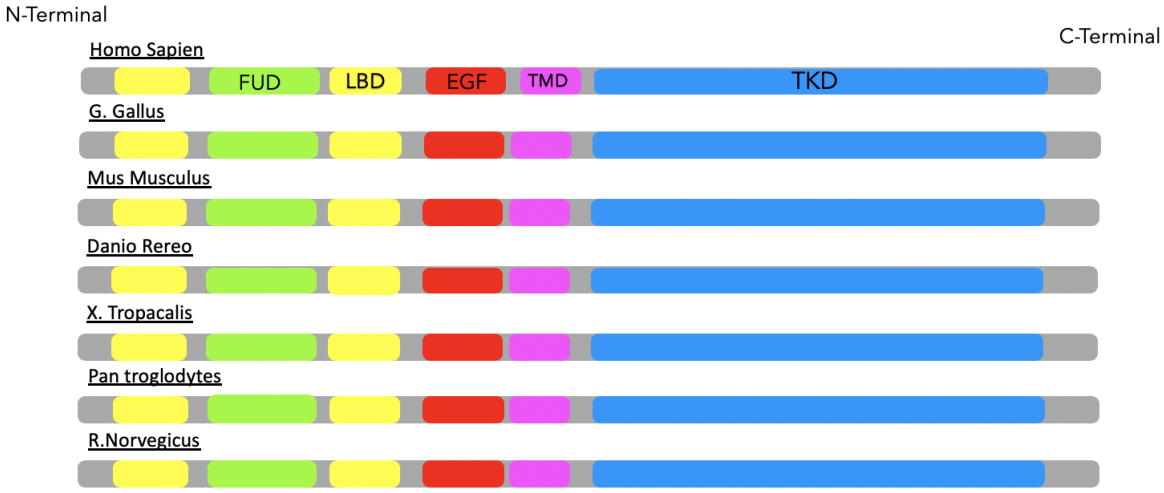
TK independent mechanisms may be important to the genes outlined in red. EGFR may play a more diverse in signaling than people have previously thought.



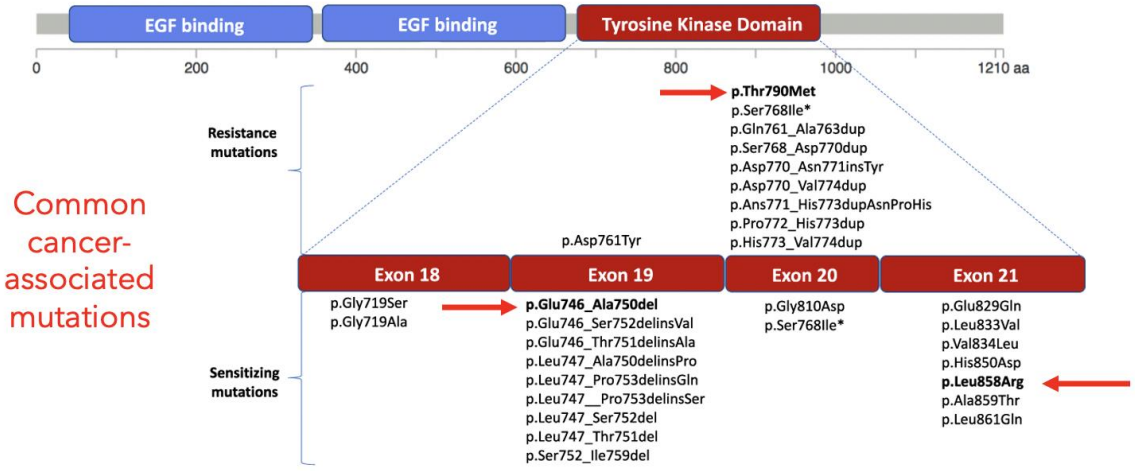
The EGFR gene is expressed in epithelial tissue throughout the body. There seems to be a difference between men and women which may be important to our understanding of cancer.



What are important domains of EGFR?

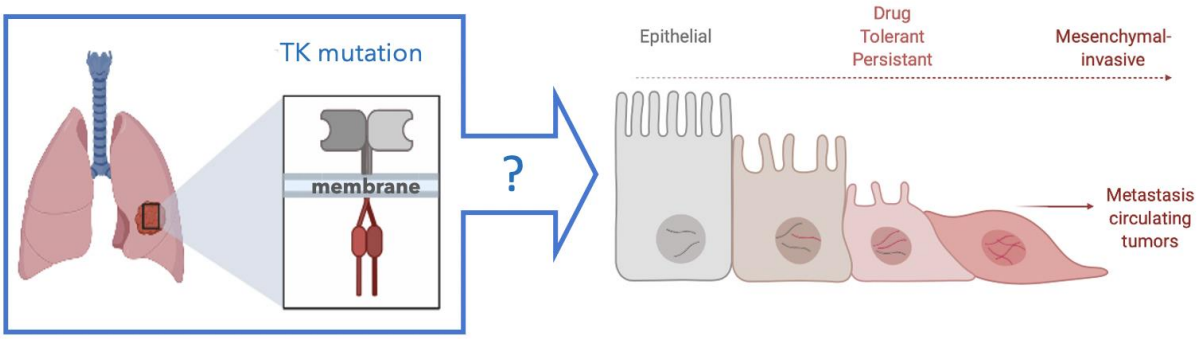


What EGFR mutations lead to cancer?



The epithelial–mesenchymal transition is a process by which epithelial cells lose their cell polarity and cell-cell adhesion and gain migratory and invasive properties to become mesenchymal stem cells; these are multipotent stromal cells that can differentiate into a variety of cell types.

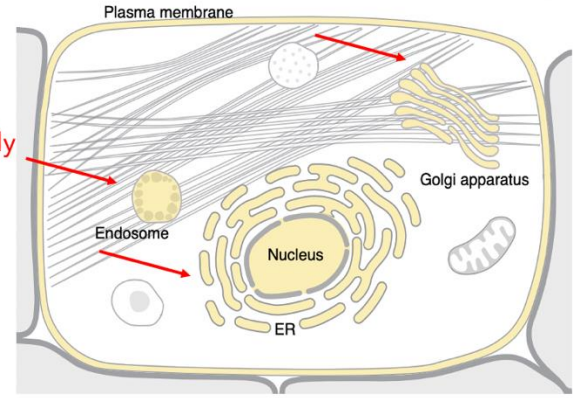
What is the Gap in knowledge?

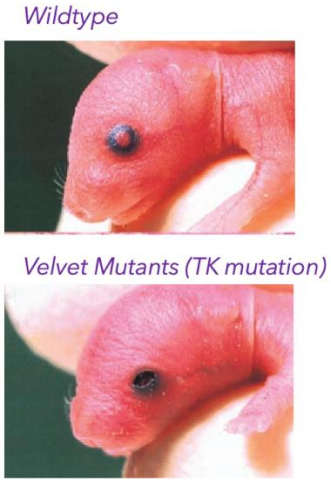


It's not completely understood how EGFR mutations affect epithelial transitions

Mice as a LADC model

Drosophila and Zebrafish EGFR only localize to PM

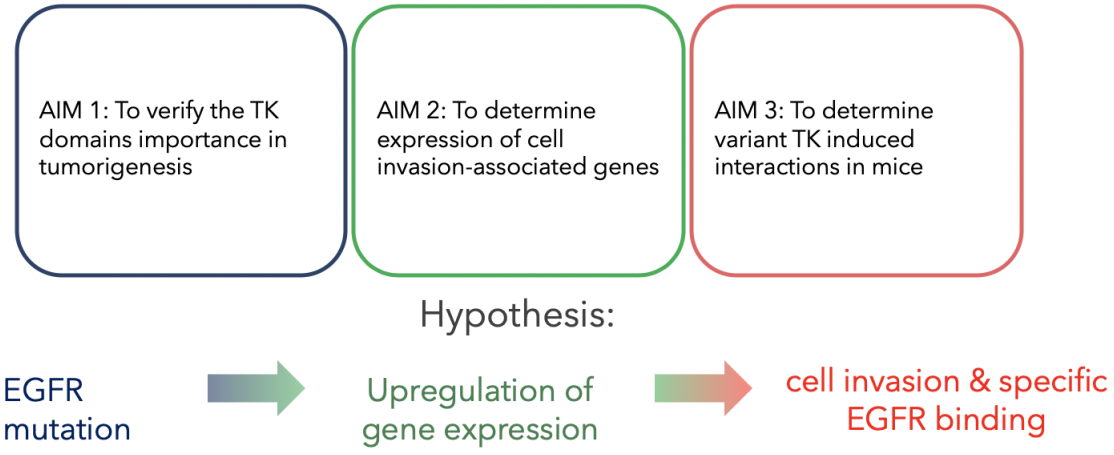




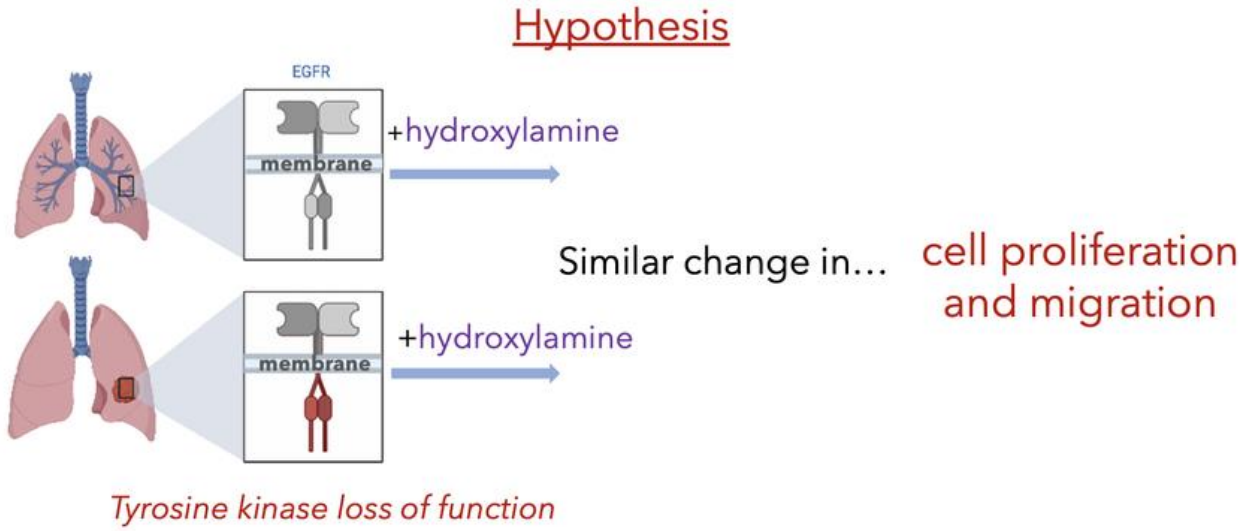
Translocation to the nucleus is important for treatment sensitivity

Velvet is the sole dominant representative of the *Egfr* allelic series described to date. a point mutation within exon 21 (of 28 exons in the gene)

Specific Aims

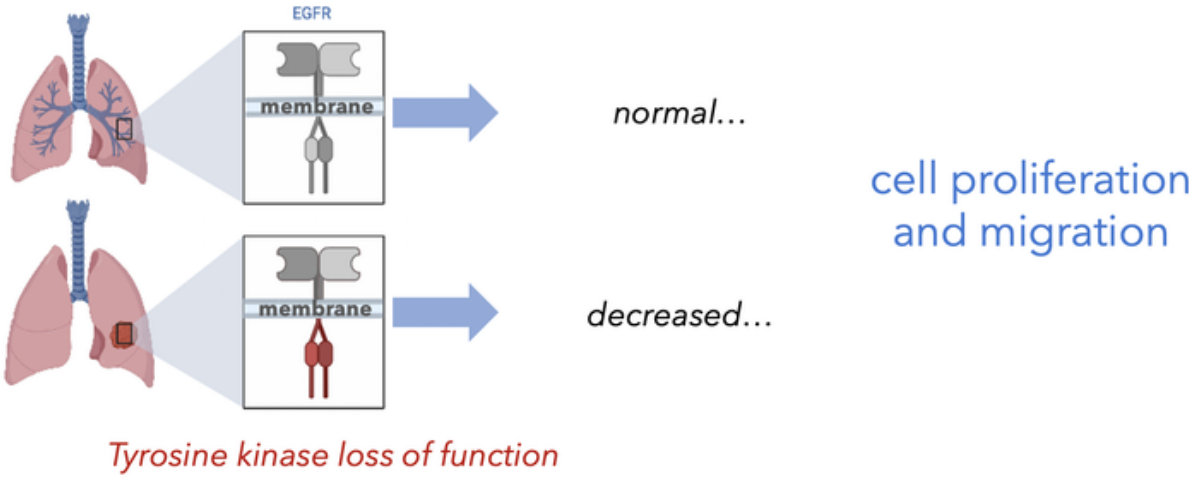


Tyrosine kinase mutations have a known association with cancer, but the prevalence of EGFR signaling hasn't been fully characterized. The following aims outline a method to understand cell proliferation and migration in the context of the EGFR gene.

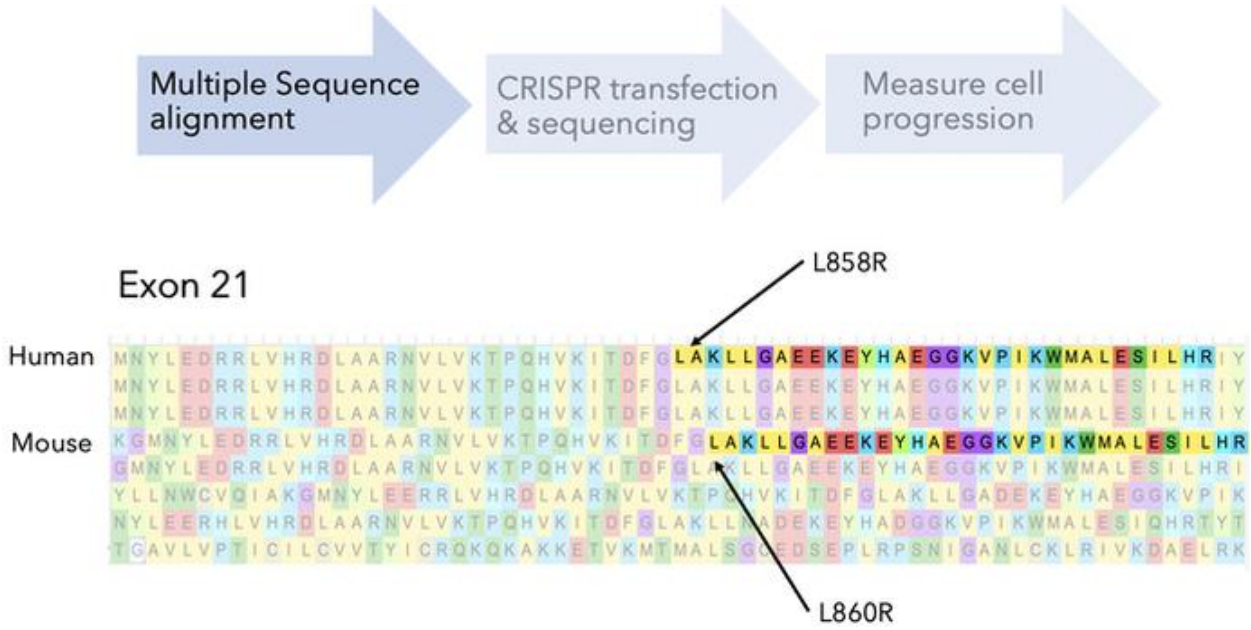


Tyrosine kinase mutations have a known association with cancer, but the prevalence of EGFR signaling hasn't been fully characterized. The following aims outline a method to understand cell proliferation and migration in the context of the EGFR gene.

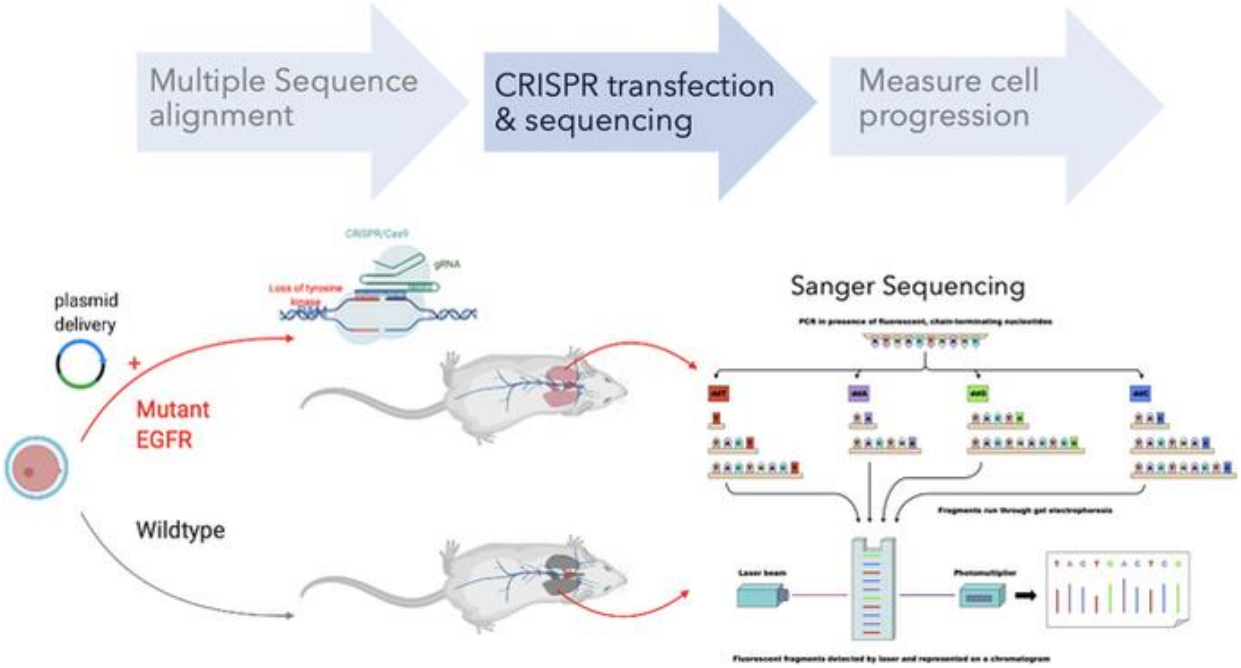
Aim1



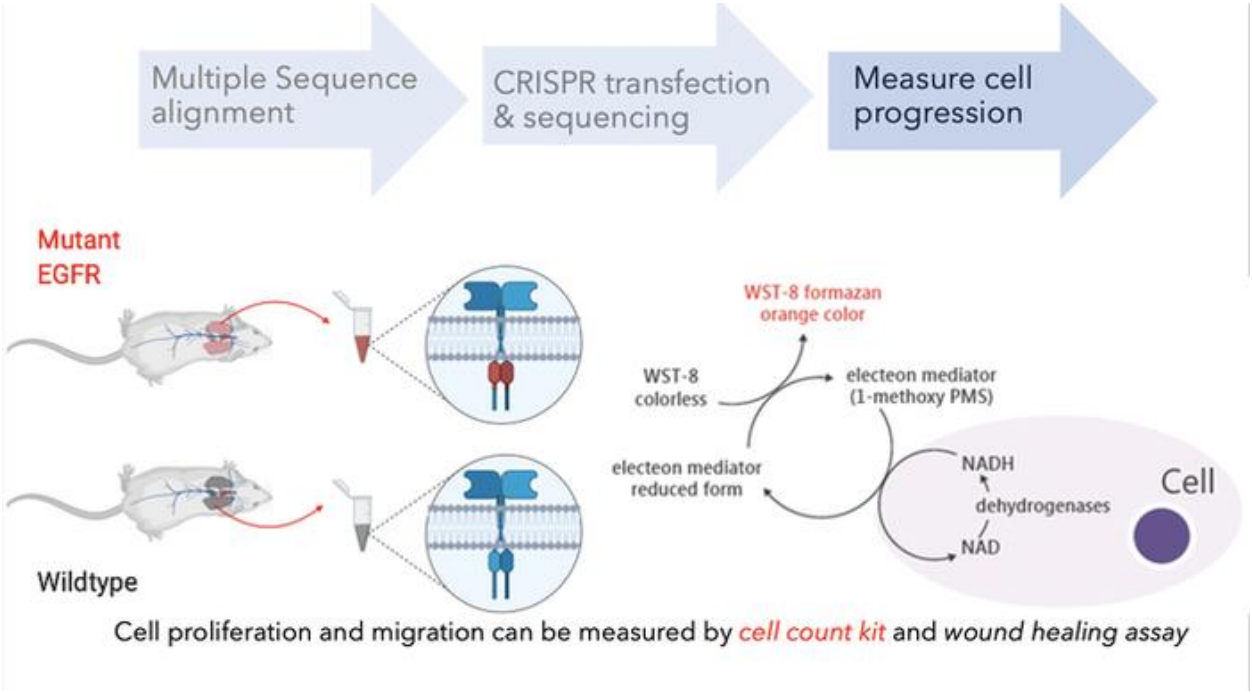
The goal of the first aims is to successfully mutate the tyrosine kinase domain so that its catalytic activity is altered. Sequencing of mutated tissue will discover whether the desired result has been achieved. Studying cell proliferation and migration will demonstrate the function of EGFR is tyrosine-independent pathways.



Ensemble and ClustalOmega will be used to align known homologs of the EGFR gene and I will identify unique Amino acids in the Tyrosine kinase domain that might be important for cell proliferation and cell adhesion in the lung.



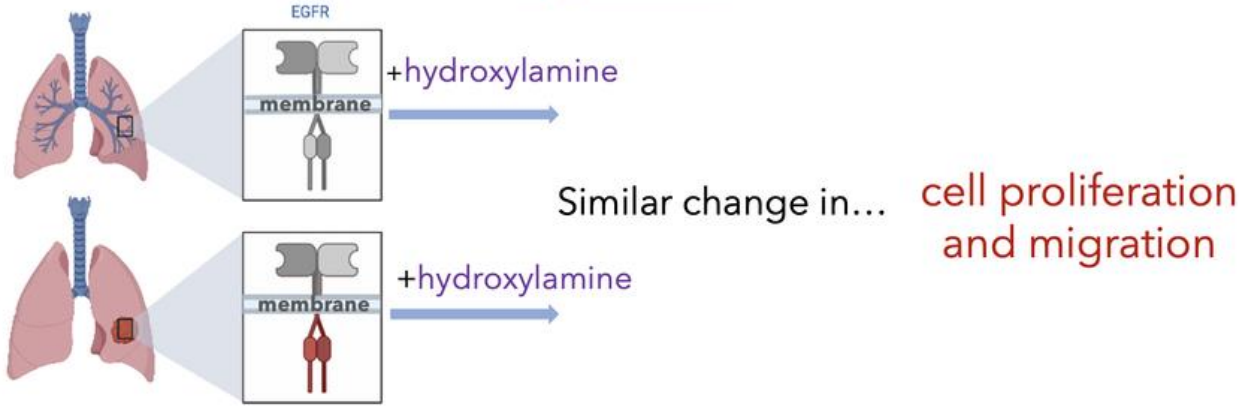
CRISPR/Cas9 plasmid delivery to mouse eggs will mutate the corresponding DNA segments in mouse eggs7, followed by DNA sequencing of wildtype and transfected mice.



To measure cell the rate of proliferation and migration in the lung, I would use a cell counting kit and a wound healing (scratch) assay, respectively.

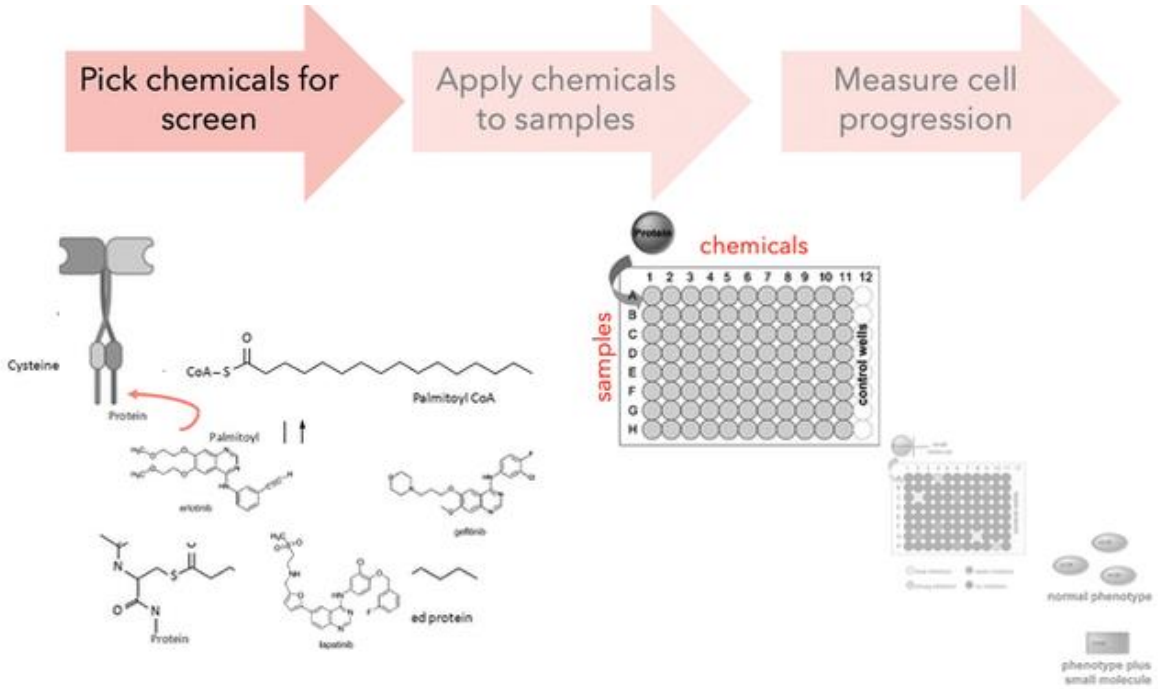
Aim 2

Hypothesis

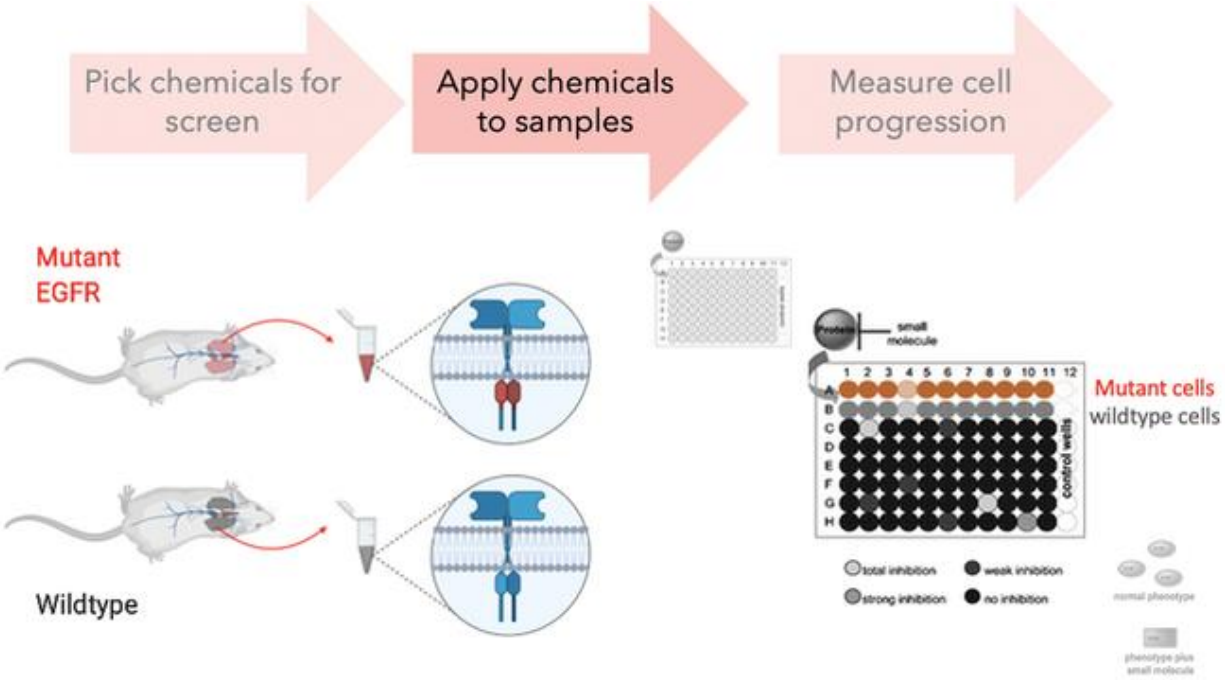


Tyrosine kinase loss of function

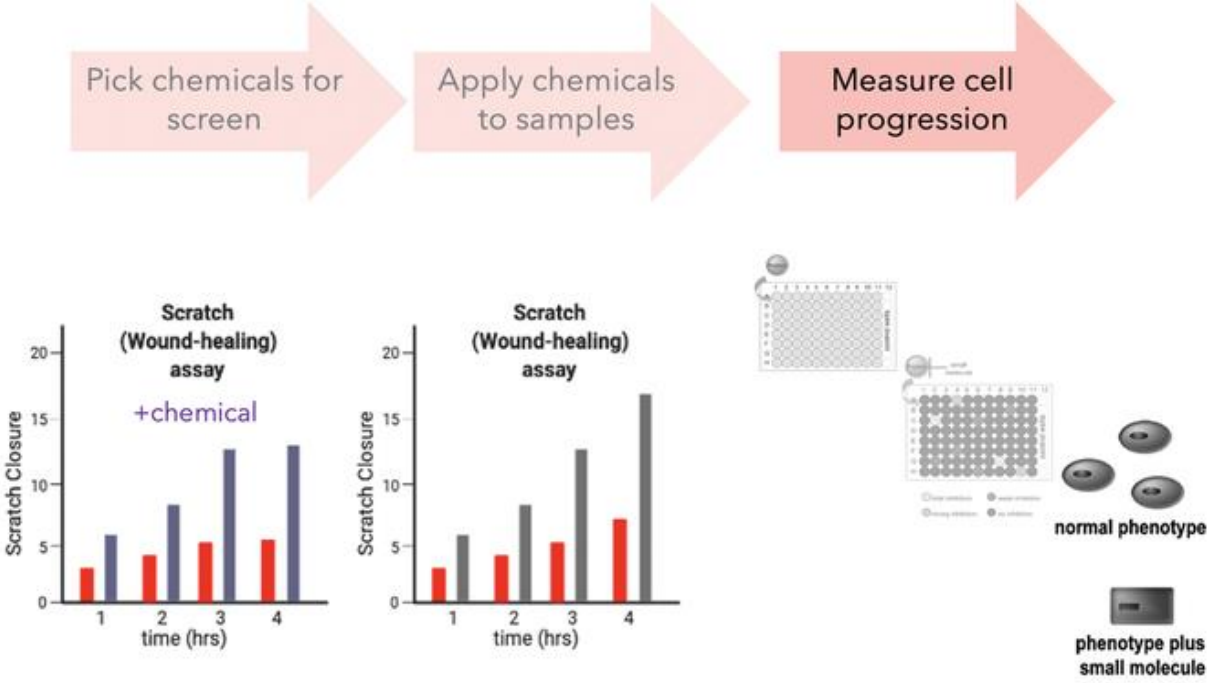
The second aims is meant to study tyrosine-kinase independent function when exposed to various chemicals, including tyrosine kinase inhibitors. Altering EGFR's function in the absence of fully function tyrosine kinase domains will hopefully affect cell procreation and migration in a way that contrasts aim 1.



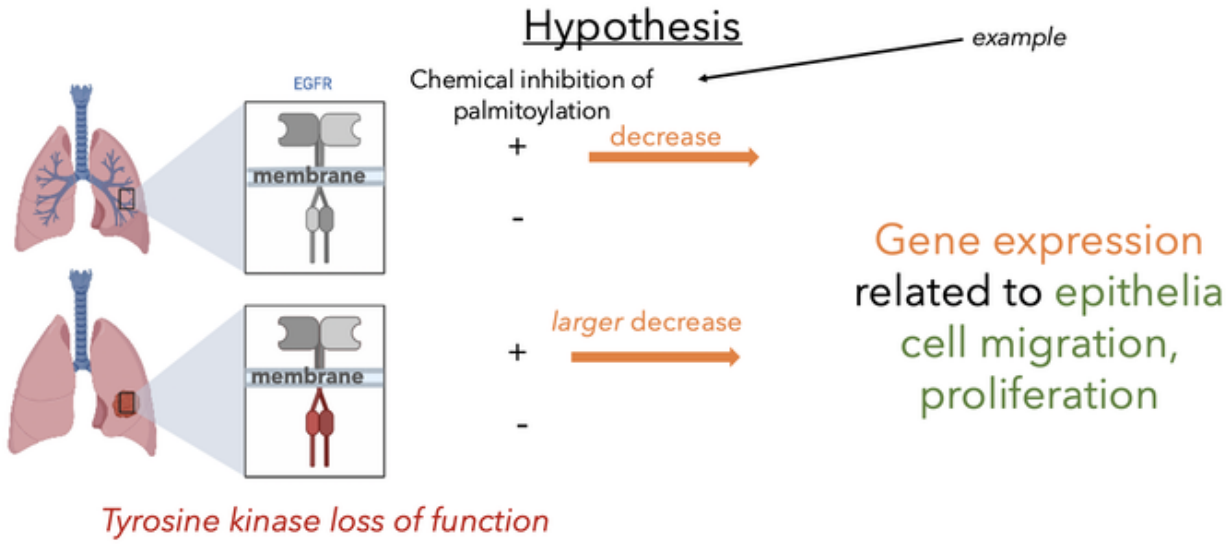
Samples will be subject to a variety of chemicals such as hydroxylamine that cleave cysteine- palmitoylation bonds



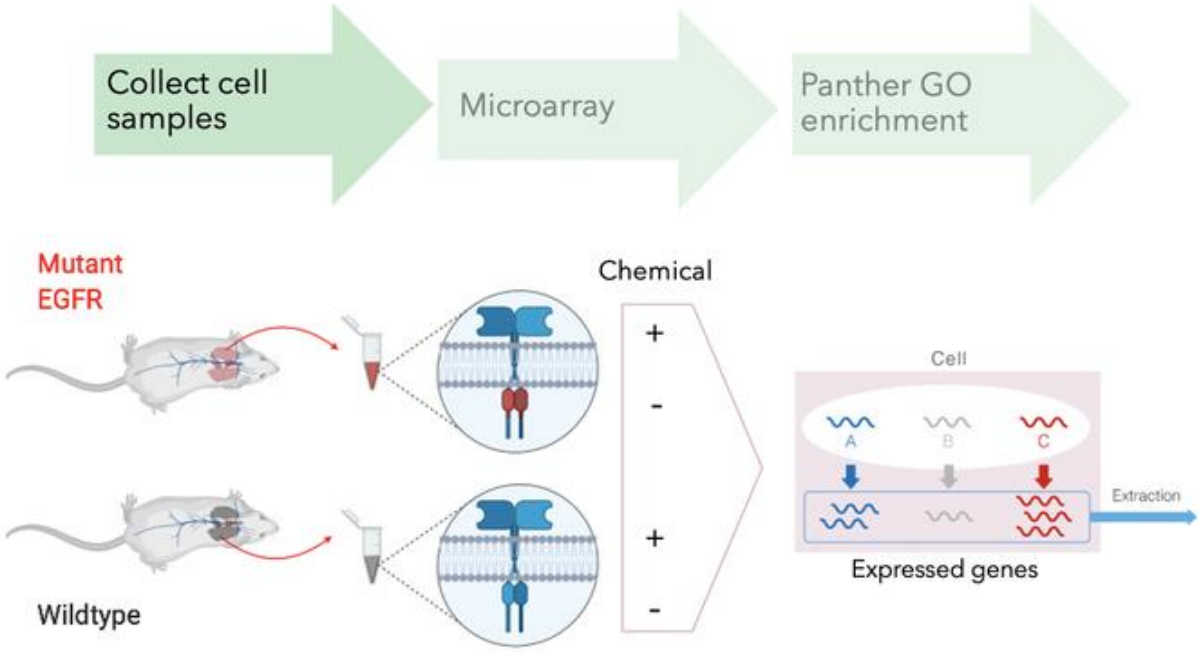
Reverse chemical genomic screening will quantify exogenous ligand to EGFR between wildtype and transfected mice. Mutant epithelial and control sample tissues will be subjected to these chemicals.



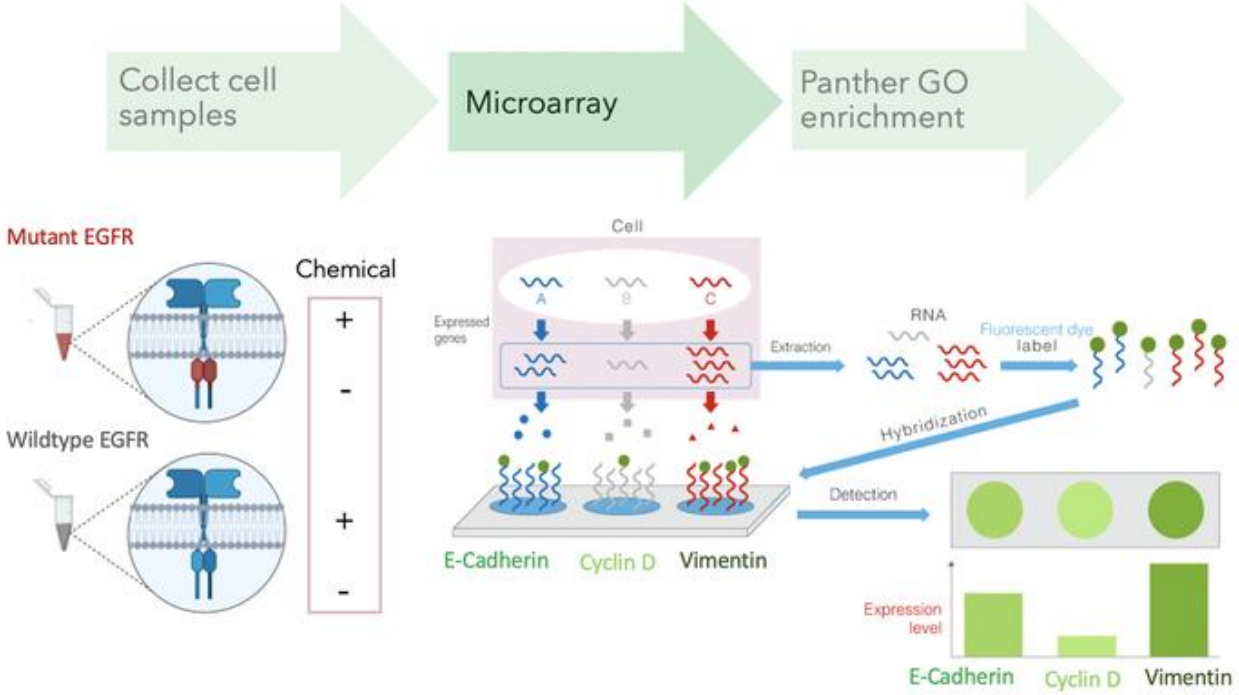
Once again, cultures will be measured for proliferation and cell migration by scratch assay and cell counting.



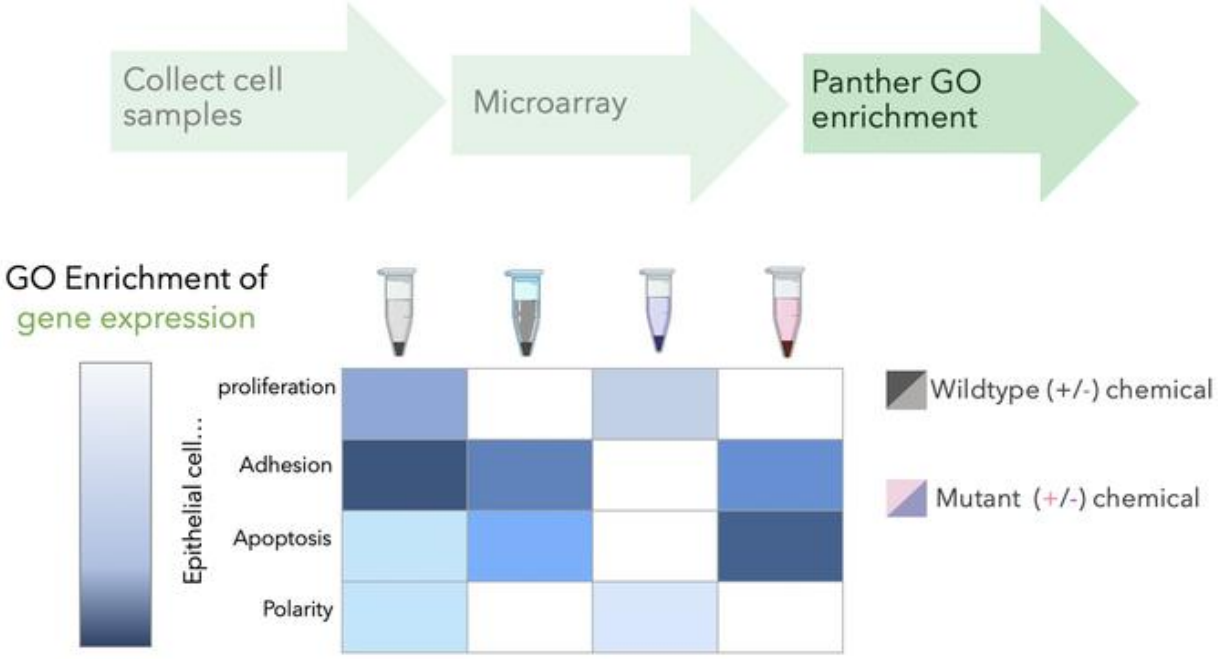
The last aim is meant to measure the gene expression of the cell cultures developed in the previous parts of the experiment. Since EGFR is mainly involved in transcription regulation, measuring RNA expression may prove fruitful.



Collect samples exposed to small molecules and those without. Comparing mutant tissue gene expression with wild type may implicate other domains of EGFR in signaling besides its main catalytic activity.

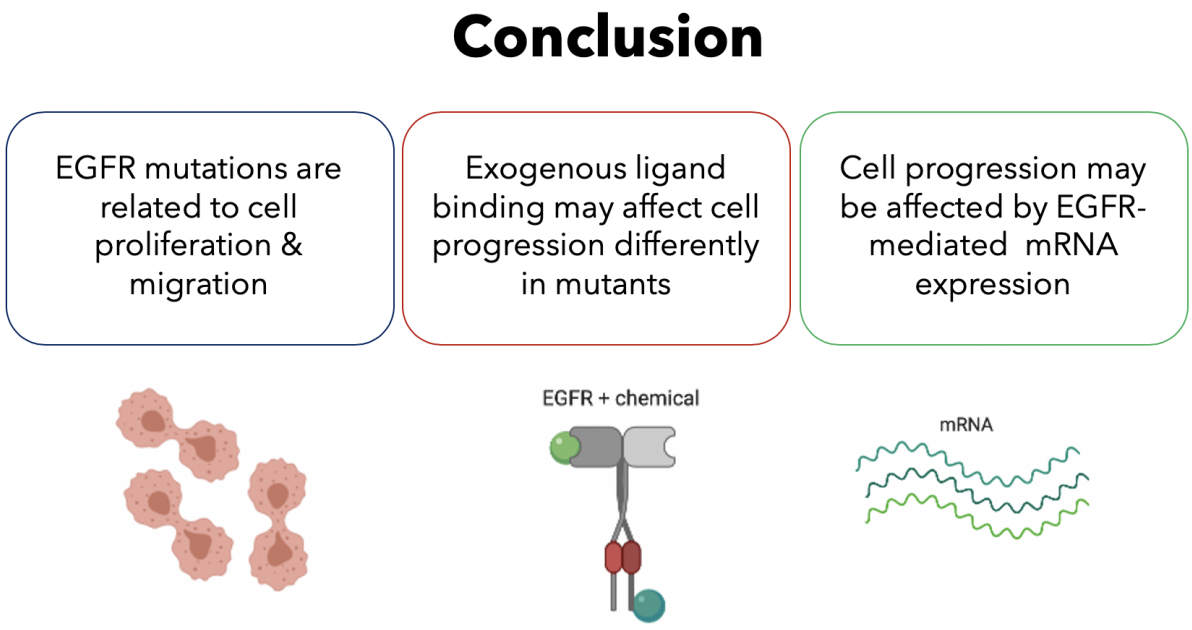


I will perform a microarray on wildtype and transfected mice mouse cells treated with chemicals from the screen.



Afterwards, Gene Ontology analysis using PANTHER can enrich gene expression with GO terms relating to cell growth patterns such as cell proliferation, migration and protein palmitoylation which are all implicated in epithelial transformations.

What are future directions for LADC research?



There are many ways to study mutations in EGFR as outlined in the flowchart bellow. This experiment mostly focused on cell viability and protein function, however clinical studies are necessary to understand Lung Adenocarcinoma. In vivo Zebrafish models with xenograft human tissue are an emerging method, also included in the graphic.

Future Directions