

BIOL 485 Cell Biology Workshop Summer 2018

Website: Blackboard

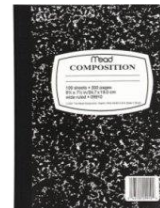
Instructor: Geraldine Grant PhD **Office:** 207 Discovery Hall, Manassas

Contact: Email: ggrant1@gmu.edu **Office Hours:** By Appointment only.

Schedule June 18th – 29th 2018
9:30am -6pm, Discovery Hall Rooms 223 and 303W

EXAMS: Friday June 29th at 2:00pm lab exam (50%)
Lab notebook (10%) and a full laboratory report in the style of a research paper (40%) due
Monday July 23rd by 5pm via Blackboard Assignments.

Items required for this class: Lab coat and a hard backed notebook similar to the one in this picture



composition

**THE HONOR CODE IS STRICTLY ENFORCED.
Attendance each day is REQUIRED**

Aim of this class:

This class will consist of a full week of 9am to 6pm laboratory exercises and lectures-June 18th – June 29th 2018.

The aim of this class is to:

1. Introduce you to and/or expand your ability to think and operate in the context of the Scientific Method i.e. how to ask and answer scientific questions,
2. How do design a series of experiments to answer a scientific question
3. How to execute these experiments and interrupt the data generated.
4. How to discuss and present this data in a meaningful manner

You will learn mammalian cell/tissue culture, molecular and biochemical techniques and their practice.

During this class we will be addressing a scientific question – designing experiments to answer this question and interrupting and presenting the data correctly.

Scenario: In non-small cell lung cancer, the epidermal growth factor receptor (EGFR) is often mutated. EGFR is receptor tyrosine kinases (RTK). Gefitinib is a RTK inhibitor. Gefitinib works best in cancers that harbor the L585R mutation. Even so, cells can develop resistance to this drug, which often goes hand in hand with development of epithelial to mesenchymal transition (EMT) and metastasis. The ability of a cell to easily undergo EMT may be an indication of its ability to develop resistance.

In our workshop we will be taking a personalized medicine approach towards a patient's tumor.

In order to do this we need to test if the cells from this patient with lung cancer – A) have the L585R mutation – this will tell us if they can take this drug B) Do these cells undergo EMT easily – which will tell us if they might

be predisposed to develop resistance. These results COMBINED will allow us determine IF the patient should be given the drug or not.

No mutation – do not give drug (why?). Yes Mutation but ease of EMT – Do not give the drug (Why?).

We will be running a series of experiments¹ that will allow us to answer these questions.

Assessment: Via Assignments on Blackboard and in Person exam

Assignment

1. Laboratory/lecture based **exam Friday June 30th**. This exam will account for **50%** of your grade and is based on the lectures and lab manual. (Look for the questions posed in the lab manual)
2. Your laboratory notebook (**10%**) in which you will keep a running account of your days in the lab – the keeping of a detail accurate lab notebook **is essential to laboratory practice**. In this notebook you will DAILY detail your experiments AND your results.
3. A full written laboratory report (**40%**) detailing your analysis of the data that you collect over the week. This report is to be written in the style of a **primary** research paper
 - Title
 - Abstract
 - Introduction
 - Materials and Methods (short and NOT detailed)
 - Results
 - The results section will include ALL your ANALYZED data from class including:
 - Morphology images, movies and ICC - images and explanation
 - Protein analysis
 - Bradford assay - graphed and include how you calculated your protein concentration
 - Images and analysis (densitometry) of western blot gels
 - Toxicity data – analyzed and graphed
 - Gene expression
 - Real time PCR data **normalized** and graphed as FOLD DIFFERENCE from control.
 - Mutation analysis
 - Gel image and explanation
 - Discussion (Did the compound cause EMT or not. If yes HOW if NO why not – explain)
 - References (at least 5, you will have used these in your introduction AND discussion).

You may submit your laboratory report through safeassignment on or before July 23rd 2018.

¹ As we only have 2 weeks to complete our investigations some of our experiments will be out of logical order and we will discuss the correct order (how you would run these experiments in real time).

Due to time constraints we are doing these experiments *out of order*.

	Monday	Tuesday	Wednesday	Thursday	Friday
Week 1	Lectures Overview Cell culture and cell counting EMT Experiment Planning	Lectures Planning Toxicity Testing	Lectures Western Blotting	Lectures PCR and real time PCR ICC	Lectures Today what are we doing?
	In the lab: Serum Starve Cells	In the lab: Seed and count cells	In the lab: Treat cells with TGFbeta Toxicity Test	In the lab: Observe Cells	In the lab: Toxicity test down. Flash Freeze dishes- RNA and DNA Extract Protein
Week 2	Lectures Today	Lectures Today	Lectures Today	Lectures Today	EXAM 1-3pm
	In the lab: Extract RNA and DNA and Quantify Make cDNA from RNA Restriction digest DNA	In the lab: Western blot 1 Gel-transfer-block-incubate Realtime Q-PCR	In the lab: Western blot 2 Washes-develop-visualize-scan ICC	In the lab: ICC	