Distribute: May 4, 2026 Due: May 18, 2026, 3:00 PM via email to David McDonagh

Problem Set 2 – Cancer Biology – Spring 2026

1. From Dr. Adrienne Boire's Lecture:

Choose the best modeling system for each mechanistic question and provide a brief explanation for your choice: Include what the main experiment might look like. Time and Money are not constraints in this magical hypothetical universe!

Question	Modeling System(s)*	Explanation/Experiment you would set up
How to cancer cells enter the brain?		
How do cancer cells metabolically adapt to the brain microenvironment?		
How do cancer cells alter blood vessels in the brain?		

*Choices include:

Intracranial Implantation

Intra-arterial injection by ICA

Iterative in vivo selection

In vitro co-culture

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2. From Dr Mara Sherman's lecture (Submitted by Kate Ryan):

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a prominent stromal compartment composed of extracellular matrix, cancer-associated fibroblasts (CAFs), and immunosuppressive myeloid populations. CAFs arise from heterogeneous cellular origins and can exhibit both pro- and anti-tumorigenic functions. Their activation is often driven by reciprocal interactions with tumor cells.

Analysis of human single-cell RNA sequencing (scRNA-seq) data from PDAC patients reveals a gene of interest (YFG) that is enriched in a subpopulation of malignant epithelial cells. Notably, tumors with high YFG expression are associated with an increased abundance of pancreatic stellate cell (PSC)-derived CAFs. **You aim to design a research project using both in vitro and in vivo models of pancreatic cancer to investigate whether a functionally significant interaction exists between YFG-expressing tumor cells and PSC-derived CAFs.**

In your answer, address the following (each worth 5 points):

1. **Role of YFG in PDAC progression**
   How would you determine whether YFG contributes to tumor initiation, progression, or maintenance?
2. **Tumor–stroma signaling interactions**
   How would you test whether a signaling interaction exists between YFG-expressing tumor cells and PSC-derived CAFs? How would you assess whether YFG is required for this interaction?
3. **Characterization of PSC-derived CAFs**
   How would you define and characterize this PSC-derived CAF population? What approaches would you use to determine whether it represents a distinct or unique fibroblast subtype?
4. **Model systems**
   Discuss the advantages and limitations of using:
   - Autochthonous mouse models
   - Orthotopic transplantation models
   - In vitro systems (co-culture, organoids)

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3. From Dr Kayvan Keshari's lecture:

Low grade glioblastomas have been shown to express a mutation in a metabolic enzyme, isocitrate dehydrogenase (IDH) which results in the formation of a neomorphic metabolite 2-hydroxyglutarate (2-HG). Small molecule IDH inhibitors have been developed and translated to humans, with recent clinical trials showing some promise though responses can be variable.

How would you determine what nutrient source of carbons is responsible for the generation of 2-HG? Using that approach, could you determine if and to what degree an IDH inhibitor in GBMs works?

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4. From Dr Kathryn Taylor's lecture:

Emerging evidence suggests that the nervous system can regulate cancer initiation, progression and dissemination in multiple tumor types. As part of you PhD project you are interested in understanding if neurons play a role in driving the cancer type you are studying.

Please design an experiment to investigate how neuronal activity regulates the behavior of cancer cell biology. You may choose the cancer type and experimental model system. Your answer should clearly state the biological question and hypothesis, and include:

1. the experimental model you would use
2. the key manipulations and controls
3. the main readouts / outcome measures
4. how the results would be interpreted
5. at least one limitation of your approach and one alternative strategy

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5. Based on Dr Sarat Chandarlapaty's lecture (submitted by Elizabeth Benitez):

Evaluating transcriptional regulation and therapeutic resistance in breast cancer

In class, we discussed the paradoxical response of neurofibromin $(NF1)^{low}$, ER+ breast cancers to endocrine therapy (Zheng et al., Cancer Cell, 2020). In breast cancer, estradiol (E2)-bound ER recruits co-activators to estrogen response elements (EREs) and drives cancer progression, whereas tamoxifen typically displaces co-activator binding and promotes repression. In this study, the authors show that in the $NF1^{low}$, ER+ setting, tamoxifen promotes tumor growth, whereas E2 induces cell death. NF1 is best known as a RAS GTPase activating protein, but the study suggests a novel function of NF1 as a transcriptional co-repressor independent of canonical RAS signaling.

Based on this study, are you convinced that NF1 acts as a co-repressor of ER-bound loci? What do the authors show that support this claim, and what additional experiment would you like to see to better establish the mechanism of NF1-mediated repression? For example, what evidence would be required to demonstrate that NF1 directly represses transcription at ER-bound loci rather than indirectly affecting ER signaling through upstream pathways? With these questions in mind, complete the following:

Provide two mechanistic models by which NF1 represses transcription and design an experiment to distinguish between them. Your answer should include:

- **hypothesis**
- **experimental system (e.g. cell lines, reporters, shRNAs,CRISPRi, dTAG, pharmacologic inhibitors, etc.)**
- **assay readouts (e.g., ChIP-seq, RIME, co-IPs, IP-MS RNA-seq, Hi-C, multiplexed IF imaging, CUT&RUN, etc.)**
- **expected results supporting each model**

Consider the following possible scenarios:

- A protein can repress transcription without stably binding DNA.
- Epigenetic repression may occur through recruitment, displacement, or sequestration of regulatory factors
- Conformational changes can affect protein interactions and transcriptional output