

Cancer Biology Graduate Program

Part I Qualifying Exam

Exam date: June 7, 2019

Please use the paper provided to write out your answers. You must answer 8 of the 10 questions.

To pass you must score 70% overall and 7 or higher on 6 questions.

Write the question number at the top left, and your student number on the top right of the first page of each answer.

Please be concise and limit your answers to what has been asked. Do not include extraneous information in your answers.

Good Luck!

### Question 1 (Sumin Kang)

Platinum-containing compounds remain the single most important drug class employed for the treatment of a wide range of solid tumors. However, resistance limits their curative potential. To identify critical factors that drive resistance to platinum compounds in human cancer, your research team performed a whole human genome CRISPR screen in a cisplatin-resistant ovarian cancer cell line, A2780<sup>cisR</sup>. Next-generation sequencing of libraries recovered from cells treated with a sublethal dose of cisplatin revealed a loss of a single-guide RNA (sgRNA) that targets a gene encoding an adaptor protein, SHB. This observation establishes SHB as a candidate cisplatin-resistance gene.

- (a) (2 points). You want to confirm whether SHB is indeed a cisplatin-resistance gene. Any given sgRNA target sequence is likely to have partial similarity to additional sites throughout the genome, which may cause off-target effects. Describe two experimental approaches to demonstrate that cancer cells become sensitive to cisplatin mainly via on-target disruption of SHB rather than off-target effects on other genes.
- (b) (2 points). Only a few proteins have previously been reported to bind to SHB. You want to identify a novel interacting partner of SHB that may contribute to cisplatin resistance. Describe a laboratory-based experimental approach (not a computational approach) to identify novel SHB binding proteins that mediate cisplatin resistance in ovarian cancer cells.
- (c) (3 points). Via the experiment performed in (b), you identify a tyrosine kinase, called Lyn, that binds specifically to SHB. Mutational analysis shows that the C-terminal domain of Lyn binds to SHB residue tryptophan-332. Mutation of this residue to alanine (W322A) abolishes the SHB-Lyn interaction. Design an experiment to demonstrate whether the interaction between SHB W332 and Lyn is critical for development of cisplatin resistance in ovarian cancer. When designing your experiment, keep in mind that ovarian cancer cells contain substantial amounts of endogenous SHB.
- (d) (3 points) The experiment in (c), above, confirmed that the binding interaction between Lyn and SHB is essential for cisplatin resistance. You identify a cell-permeable peptide mimetic that disrupts SHB-Lyn interaction. Design an in vivo xenograft experiment to validate whether targeting SHB-Lyn interaction using this peptide mimetic is a promising therapeutic approach to overcome cisplatin resistance. Describe the treatments given to each experimental group of mice in this experiment, the anticipated results, and their interpretation.

## Question 2 (Greg Lesinski)

Your chemistry collaborator has made a small molecule inhibitor of ERK that induces apoptosis in cancer cells at concentrations that can be achieved in vivo as determined by prior pharmacokinetic studies. Your post-doc would like to combine this ERK inhibitor with an antibody that blocks PD-1 on T cells. Design a basic experiment to test the hypothesis that combined therapy with the ERK inhibitor and PD-1 targeted antibody will have a superior therapeutic effect against tumor growth as compared to either agent alone.

- (a) (4 points). Please provide details of each treatment group and expected results
- (b) (2 points). Once the study is completed, you decide to analyze the spleen from the mice to determine immunologic effects. You notice a dramatically lower number of T cells present in groups of mice that had received the ERK inhibitor. How would you interpret this result?
- (c) (2 points). One other thing you observed is a significant increase in myeloid-derived suppressor cells (MDSCs) present within the tumor, regardless of treatment group. Suggest two approaches (with different mechanisms) that you could use to target the MDSCs. What is the key mechanism involved in each approach?
- (d) (2 points). You are also interested in combining PD-1 blockade with a method to alter the microbiome, as you feel this may be a more natural approach to modulate immunity. Name two methods you could use to do this.

Question 3 (Bernard Mainou)

- (a) (4 points) Your lab works on an oncolytic virus called Benzovirus, which you can genetically alter to express different foreign sequences. When doing transcriptional profiling of normal epithelial cells (NEC) and squamous cell carcinoma (SCC) cells, you learn that micro RNA (miRNA) 191 is expressed in normal epithelial cells but is not expressed in SCC cells. Conversely, you learn that the transcription factor E2F is expressed at low levels in NEC but at high levels in SCC cells. Using your knowledge of oncolytic viruses, list two approaches for modifying Benzovirus to enhance its oncolytic capacity of SCC cells while minimizing toxicity on NECs?
- (b) (3 points). When you look at data from patients that receive Benzovirus, you notice that patients that have high levels of pre-existing antibodies against the virus do **not** respond to the oncolytic virus. Why does the presence of antibodies against the virus impact the efficacy of Benzovirus?
- (c) (3 points). Describe an approach to circumvent this problem and an experiment to demonstrate whether the approach is successful.

Question 4 (Carlos Moreno)

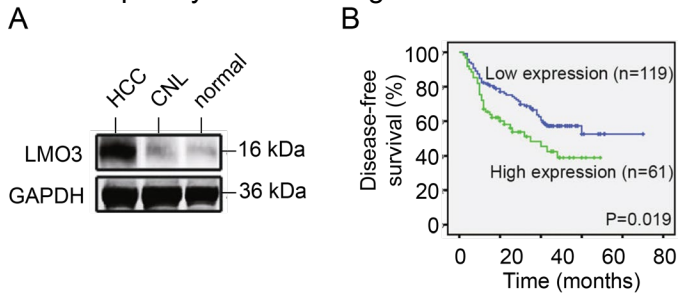
You are studying a very rare form of cancer, obscurinoma, which arises from a unique cell type called the “obscura cell.” There is almost nothing known about the molecular nature of this disease. Through years of dedication, many collaborations, and much hard work, you have managed to assemble tissue samples from 100 patients and obtained funding to perform genomic characterization. Unfortunately, your whole exome sequencing (WES) data does not uncover any point mutations, frameshifts, or indels in known driver genes, and in fact very few point mutations overall.

- (a) (2 points) Propose a mechanism by which these tumors could arise and another genomic experiment (different from WES) that would test your hypothesis.
- (b) (2 points) Oh no! Your hypothesis didn’t pan out. With funding and time running out, you propose a second hypothesis that could be tested by yet another type of whole genome characterization. What is the hypothesis and the method to test it?
- (c) (2 points) Congratulations! This time you were right. You have identified what appears to be a key driver gene (DRIVERX). Describe two independent *in vitro* experiments that would allow you to test whether or not DRIVERX can transform normal obscura cells. Include all necessary controls.
- (d) (2 points) Describe an experiment that would test whether DRIVERX can alter the ability of obscura cells to self-renew. Include all necessary controls.
- (e) (2 points) Propose an experiment using mice that would demonstrate that DRIVERX is an oncogene that drives obscurinoma *in vivo*. Assume you have all the reagents and funding needed. Include all necessary controls.

Question 5 (Robert Schnepf) (Note that this question spans two pages).

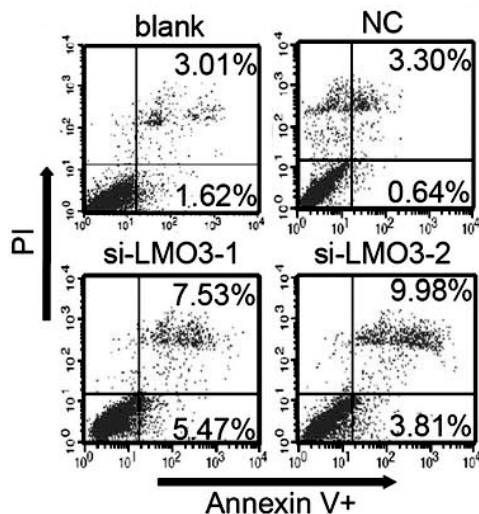
You are studying hepatocellular carcinoma (HCC), an aggressive malignancy that continues to cause significant morbidity and mortality in patients. Your laboratory is investigating a possible role for LMO3 in hepatocellular carcinoma.

- (a) (2 points). You perform immunoblotting analysis on tumor (HCC), corresponding non-cancerous liver (CNL) and normal liver. You also have the survival curve data available. Based on these data, generate a hypothesis as to whether LMO3 likely functions as an oncogene or a tumor suppressor in HCC. Please explain your reasoning.

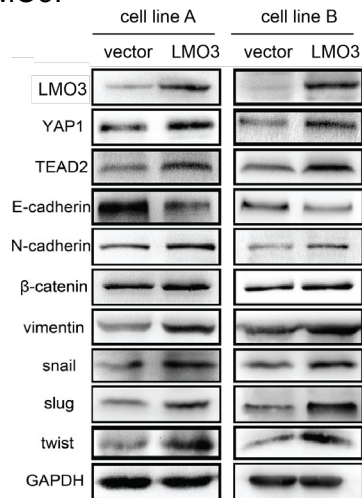


- (b) (3 points) You are interested in determining whether LMO3 promotes the metastatic phenotype in an *in vivo* setting. Describe an *in vivo* experiment that would allow you to determine whether LMO3 affects HCC dissemination. For this experiment, assume you have access to HCC cell lines, any plasmids/ vectors (shRNA, CRISPR, etc.) that you may need, and immunocompromised mice. In this model system, also assume that cells injected into the liver will metastasize to the lung. In addition, please describe 2 endpoints/outcome measures you might examine.

- (c) (2 points). To complement the above analysis, you determine the impact of LMO3 on anoikis, which is apoptosis that normal cells undergo when detached from their normal environment/matrix. You perform an experiment and obtain the following results. NC is control and si is short interfering RNA directed against LMO3. First describe how LMO3 affects anoikis and why you draw this conclusion (1 point). Next, describe one additional test/marker you could evaluate to further describe the effect of LMO3 on anoikis.

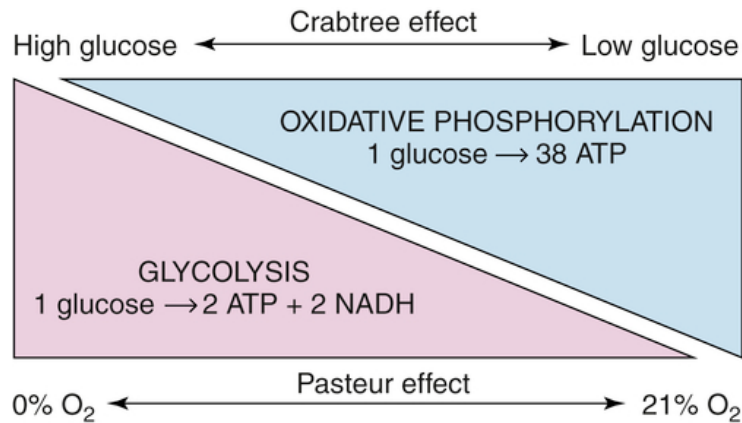


(e) (3 points) You wish to determine whether LMO3 impacts EMT (epithelial mesenchymal transition). You take a gain of function approach and therefore develop two cell lines (A and B) that express control or LMO3.



- Please provide a working definition of EMT, briefly describing what happens to epithelial cells as they undergo this process.
- Based on the immunoblots, how do you think that LMO3 influences EMT in these cell line models? In doing so, please comment specifically on what happens to E-cadherin and N-cadherin, and vimentin and classify these markers as either epithelial or mesenchymal.

Question 6 (Mala Shanmugam)



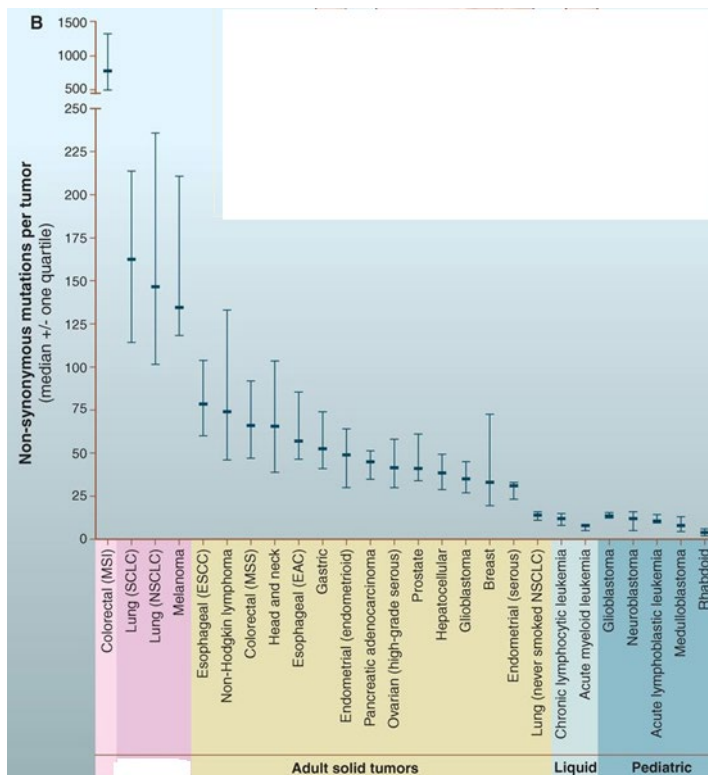
(From: *The Metabolism of Cell Growth and Proliferation | Oncohematoma Key*)

- (a) (2 points). The diagram above illustrates how yeast adapt their metabolism to extrinsic glucose and oxygen concentrations. Provide a mechanistic basis for how cancer cells circumvent each of these two normal sensing mechanisms (one example for oxygen sensing and one for glucose sensing).
- (b) (4 points). You have been sent a panel of aggressive metastatic breast cancer cell lines that have successfully adapted to high glucose conditions and do not exhibit the Crabtree type of metabolism. What is the type of metabolism they are exhibiting? Design two experiments to identify the type of basal metabolism they are *dependent* upon. Please include rationale and interpretation of your anticipated results.
- (c) (2 points). You are able to identify a small molecule that inhibits the basal metabolic pathway you identified in part (b). What further studies will you perform to ensure its metabolic specificity and lack of off-target toxicity? Outline two experiments with proper controls, rationale and interpretation.
- (d) (2 points) How does the Warburg effect contrast from the Crabtree effect?



Question 7 (Wei Zhou).

This is a figure taken from a review article by Vogelstein et al (Science 2013;339:1546-1558). It summarizes the number of non-synonymous mutations identified per tumor using data generated by the cancer genome project (TCGA).



- (a) (2 points). Colorectal (MSI) tumors contain the highest number of mutations per tumor (~500 to 1400). What is the genetic instability mechanism responsible for such a high mutation rate? What are the key players and mechanism involved?
- (b) (2 points) Colorectal (MSS) tumors contain an average of ~50 to 90 mutations per tumors. What kind of genetic instability mechanisms are usually involved in the formation of these types of tumors?
- (c) (2 points) Based on existing literature, the adult tumors that are highlighted in pink and yellow in this figure have the same type of genetic instability mechanism. However, the number of mutations found in the pink group is significantly higher than the ones in the yellow group.
- Can you provide an explanation for this difference?)
  - Pediatric tumors have the lowest number of mutations per tumor. Why?
- (d) (2 points). Based on your understanding of immune checkpoint therapy, which tumors listed in this figure are likely to be have a favorable response and why?
- (e) (2 points). Most of mutations identified in the figure above are passenger mutations. If these mutations does not provide growth advantages, why are they present?

### Question 8 (Bill Dynan)

Last year, a clickbait medical conspiracy blog, “Health Nut News” published an article under the chilling headline “60 Lab Studies Now Confirm Cancer Link to a Vaccine You Probably Had as a Child.” The article begins by mentioning contamination of 1950s/1960s-era polio vaccines with SV40, a monkey virus that causes cancer in lab studies of rodents. SV40 is a DNA virus that encodes two oncogenes. In rodent cells (which are not the natural host and cannot support viral replication), fragments of DNA encoding these oncogenes become integrated in the host genome, leading these cells to acquire hallmarks of cancer.

- a. (2 points). List two hallmarks of cancer that can readily be observed in cultured cells.
- b. (3 points). You are interested in whether children who received contaminated polio vaccine in the 1950s and 1960s developed cancer later in life as a result. Suggest a research approach to answer this question.
- c. (3 points). The blog post went on to suggest that the vaccine for human papilloma virus (HPV), which was introduced in 2009, also presents a cancer risk. (It also suggested that there is a conspiracy to cover all this up). What is the mechanism by which HPV causes cancer? Could a vaccine trigger this mechanism? Explain your reasoning.
- d. (2 points) Suggest a design for a study to determine whether vaccination increases or decreases the risk of human cancer(s) in patients who receive the vaccine.

Question 9 (Adam Marcus)

A lung cancer patient enrolls in a clinical trial that allows for longitudinal biopsies. On an initial visit, the patient has a biopsy at the primary tumor site in the lung and the metastatic site in the liver. Genomic analysis reveals that:

- The primary tumor has mutations in Genes A, B, and C
  - The metastatic site has mutations in Genes A, D, and E
- a. (2 points) Provide an explanation as to why the metastatic site would have both similar and different mutations as the primary tumor? What advantage could this provide to the metastatic lesion?
  - b. (2 points) Outline an experiment to test the hypothesis that mutations in genes D and E promote metastasis. Include proper controls.
  - c. (2 points) A literature search reveals that Gene E encodes a kinase that is necessary for cell adhesion. Based upon your knowledge of cell motility, explain how a mutation in gene E could be related to the aggressiveness of this tumor.

The patient is treated with cisplatin and shows a complete response, with the primary tumor and liver mass being undetectable 6 months later. Unfortunately, at a one-year follow up visit, metastatic disease is again observed in the liver. Another genomic test is done on the new metastatic liver site, which now contains mutations in Genes A, D, E, and F.

- d. (2 points) What potential benefit would the mutation in Gene F provide the tumor to allow it to survive during treatment?
- e. (2 points). Design an experiment to test your hypothesis. Include proper controls.

Question 10 (Anna Kenney)

The lab is studying mechanisms involved in the DNA damage response after irradiation of brain tumor cells. Brain tumor cells that can repair their DNA after irradiation survive and cause regrowth of the tumor, inevitably leading to death of the patient. A student in the lab has identified a novel cytoplasmic tyrosine kinase, GBMTK, that localizes to sites of DNA damage shortly after irradiation in cultured glioma cells. Separately, analysis of human tumor data genetic profiling data reveals that patients with activating mutations in GBMTK have aggressive disease with poor prognosis and limited therapeutic response. GBMTK is highly conserved between mouse and human, and good antibodies are available.

(a) (3 points) Describe experiments:

- To identify binding partners of GBMTK in cells with DNA damage.
- To determine whether any of these are GBMTK substrates.
- To determine whether GBMTK kinase activity is required for DNA repair.

(b) (3 points) Describe an in vitro experiment to determine whether mutant GBMTK has oncogene properties in brain tumor cells of origin (glia).

(c) (4 points). Assuming your results from part (b) indicate that GBMTK can drive transformation, describe an experiment to determine whether GBMTK can drive gliomagenesis alone or in combination with another common genetic aberration in GBM. Assume that time is limited, and that because of competition you need to design an experiment that can be initiated, completed, and analyzed all within a twelve-month period.