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, $A2780^{cisR}$. 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.

Answer: (i) Test multiple sgRNAs targeting different sites in SHB, or test shRNAs targeting SHB. (ii) Rescue SHB-deficient cells with an sgRNA-resistant SHB cDNA.

(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.

Keys: Perform SHB immunoprecipitation using extracts of cisplatin-treated or control ovarian cancer cells. Analyze co-immunoprecipitated proteins by mass spectrometry. Alternatively, perform time resolved-Forster resonance energy transfer (TR-FRET) protein-protein interaction assays.

(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.

Keys: Knock out or knock down endogenous SHB, then ectopically overexpress sgRNA- or shRNA-resistant W332A or WT SHB in cells. (Alternatively, perform CRISPR-mediated gene replacement). Monitor cell viability and cisplatin sensitivity in cells with W332A or WT SHB. If the binding is essential, wild type but not W332A SHB-expressing cells will demonstrate cisplatin resistance.

(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.

Keys: Use patient-derived xenograft. There is a minimum of four groups, which receive (i) vehicle control, (ii) cisplatin, (iii) peptide mimetic, (iv) cisplatin and peptide mimetic. If disruption of SHB-Lyn interaction by the peptide mimetic sensitizes cisplatin treatment, you will see further decrease in tumor growth in combination (iv) treated group compared to treatment with either agent alone groups (ii) and (iii).

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
Group 1: negative controls (vehicle, isotype control Ab)
Group 2: single agent anti-PD-1 Ab
Group 3: single agent ERK inhibitor
Group 4: combination of ERK inhibitor + anti-PD-1 Ab

Expected Results: Mice treated in the combination group will have smaller tumors at the study endpoint, or a slower rate of tumor growth as compared to all other groups.

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?
ERK inhibition had an on-target effect on T cells, resulting in their decrease.

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?

All possible answers below

- **Function:**
  Nitroasprin, DFMO, A2AR antagonism, Tasquinimod, VX15/2503
- **Transcription Factors:**
  STAT3, STAT6, Bv8, IRF8, C/EBPβ
- **Trafficking:**
  CXCR2 antagonist, CSF1R antagonist
- **Differentiation:**
  ATRA, 1,25(OH)2 D3
- **Cytokines:**
  IL-6, GM-CSF, VEGF, IL-4R, IL-13
- **Viability/Depletion:**
  Chemotherapy, PDE5 inhibition, Sunitinib, Zolendronic Acid, Antibody against one of their surface markers.

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.

Fecal transplant experiments, Co-housing of mice, Administration of antibiotics

Question 3 (Bernard Mainou) Oncolytic viruses.

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?

Positive targeting – make Benzovirus replication E2F dependent.
Negative targeting – encode a miRNA191 targeting sequence in Benzovirus.

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?
Antibodies neutralize the virus and minimize its oncolytic efficacy.

c. (3 points). Describe an approach to circumvent this problem and an experiment to demonstrate whether the approach is successful.

Engineer a virus that can bypass neutralizing antibodies, or add a peptide that neutralizes antibodies. Set up an experiment in tumor bearing mice with four groups: (i) untreated control, (ii) Original Benzovirus only, and (iii) Modified Benzovirus, (iiii) standard therapy or one that is (i) untreated, (ii) benzovirus alone, (iii) peptide alone, (iiii) peptide plus benzovirus.

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.
Potential answers: 1) Translocation and Whole Genome Sequencing, 2) altered methylation and CpG methylation arrays or whole genome bisulfite sequencing, 3) altered miRNA expression and miRNAseq, 4) overexpressed mRNA and RNAseq, or 5) gene amplification/deletion and copy number arrays (although you could probably figure that out from WES, so this is only partial credit).

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?
Potential answers: 1) Translocation and Whole genome sequencing, 2) altered methylation and CpG methylation arrays or whole genome bisulfite sequencing, 3) altered miRNA expression and miRNAseq, 4) overexpressed mRNA and RNAseq, or 5) gene amplification/deletion and copy number arrays (although you could probably figure that out from WES, so this is only partial credit).

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.
Transfect or infect obscura cells with vector that overexpresses DRIVERX and perform colony formation assays and soft agar growth assays.

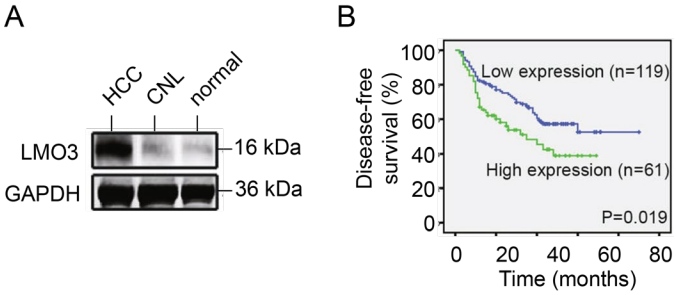
d. (2 points) Describe an experiment that would test whether DRIVERX can alter the ability of obscura cells to self-renew. Include all necessary controls.
Transfect or infect obscura cells with DRIVERX and perform tumorsphere assays and/or limiting dilution xenograft assays in immunocompromised mice.

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.
Make a transgenic mouse with an obscura-specific promoter driving high expression of DRIVERX. Alternatively you could use an obscura-specific CRE with a LOX-STOP-LOX cassette in front of the DRIVERX gene. Look to see if obscurinoma tumors are formed.

Question 5 (Robert Schnepp)

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 (1 point) Please explain your reasoning (1 point).



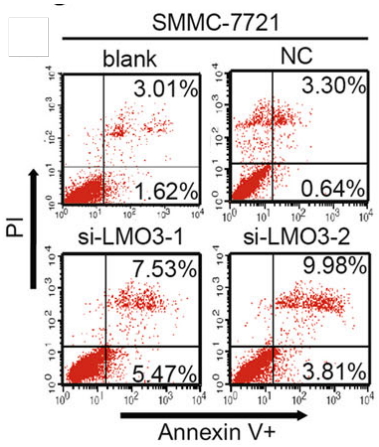
Based on these data, I hypothesize that LMO3 functions as an oncogene. Its expression is increased in HCC compared to surrounding normal tissues, likely driving certain aspects of the malignant phenotype. In addition, high *LMO3* expression is associated with worse overall survival.

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 (2 points). In addition, please describe 2 endpoints/outcome measures you might examine (1 point total, 0.5 points per endpoint).

I could engineer an HCC cell line model to have higher/lower levels of LMO3 by (at least) two different means: 1) Overexpress LMO3 in HCC line via transfection/infection; 2) Deplete LMO3 using shRNAs. CRISPR approach. I could then inject cells into the liver and monitor tumor progression/survival of mice. In addition, I could engineer cells to express luciferase construct, allowing for in vivo imaging (though not necessary for credit).

Endpoints: 1) Overall survival of mice; 2) Tumor burden, assessed via necropsy/imaging, 3) size of primary tumor

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 (1 point).

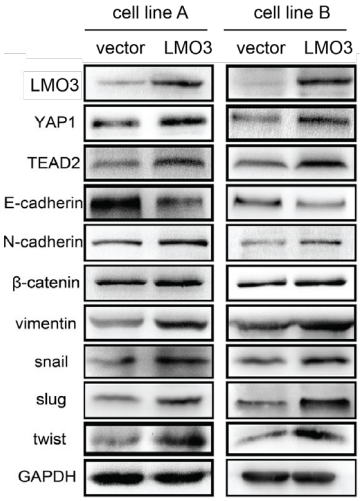


a) With depletion of LMO3, I see increased Annexin V/PI staining, consistent with increased apoptosis. Therefore I think that LMO3 inhibits anoikis (or promotes resistance to anoikis), conferring a survival advantage.

- Any additional, commonly accepted marker of apoptosis: caspase 3/7 activity, PARP cleavage, etc.

d) (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.

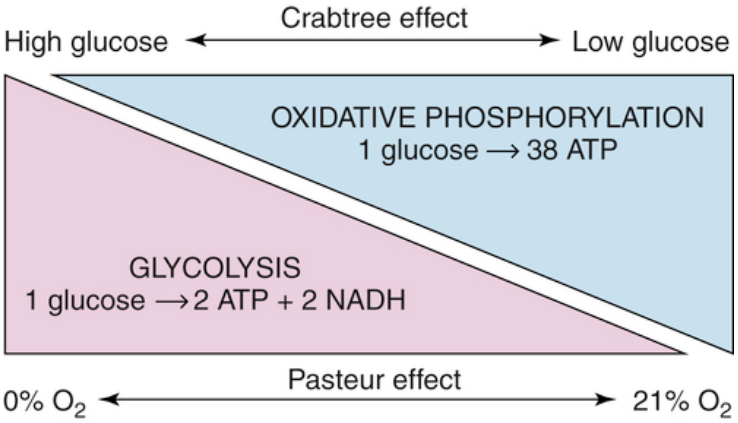
i) Please provide a working definition of EMT, briefly describing what happens to epithelial cells as they undergo this process.



ii) 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.

- EMT, epithelial mesenchymal transition, is a process in which epithelial cells lose polarity, sever adhesion junctions, degrade the basement membrane, and become migratory.
- LMO3 promotes EMT.
- E-cadherin decreases and N-cadherin increases. E-cadherin is an epithelial marker and N-cadherin a mesenchymal marker. Vimentin increases and is a mesenchymal marker.

Question 6 (Mala Shanmugam)



(From: The Metabolism of Cell Growth and Proliferation | Oncohema 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).

The Crabtree effect (in yeast) suppresses respiration by elevating fermentation (substrate dependent).

Promotion of glycolysis even in the presence of elevated oxygen can occur by 1) increased PDHK repression of PDH 2) Genetic mutations say in Kras or activation of AKT elevates hexokinase and GLUT expression to force use of glycolysis or a dependency on the pentose phosphate pathway creating an elevated the need for glycolysis taking away glucose from OXPHOS.

Elevated OXPHOS in the presence of elevated glucose may occur when you have suppression of PDHK, elevated TCA substrates like glutamine entering the cell, increased ATP demands or need for TCA for other epigenetic regulation.

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.

They should have shut down OXPHOS (under high glucose conditions, i.e. Crabtree effect) but have switched to using OXPHOS. Do Seahorse to evaluate oxygen consumption rates and ECAR (indirect readout of glycolysis). Treat cells with a glycolytic vs OXPHOS inhibitor and determine effects on proliferation/viability. The cells should be more sensitive to an OXPHOS inhibitor.

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.

Say the inhibitor you identify is a Complex I inhibitor. Show reduced OCR potentially elevated ECAR, effects on viability/proliferation and do metabolomics to show that OXPHOS is impacted

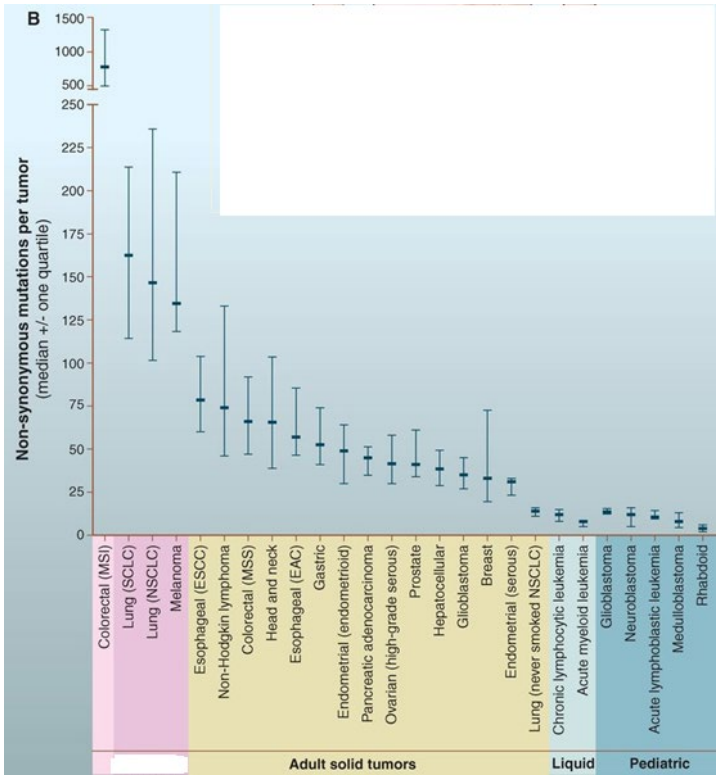
i.e. TCA cycle metabolites are reduced. KD target and show lack of additional effects of the inhibitor.

d) (2 points) How does the Warburg effect contrast from the Crabtree effect?

The Warburg effect proposed that cancer cells avidly consume glucose and exhibit glycolysis despite presence of oxygen because of defects in mitochondrial OXPHOS. The Crabtree effect on the other hand demonstrates that under elevated glucose exposure cells will shift to use glycolysis by shutting down use of OXPHOS.

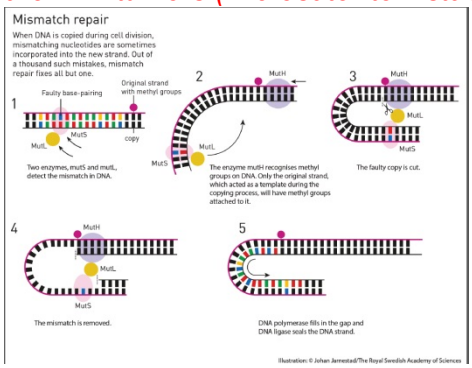
Question 7 (Wei Zhou).

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 identifed 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?

Answer: *MSI stands for microsatellite instablility. These tumors are defect in mismatch repair, and they are MIN tumors (microsatellite instable tumors)*



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?

***Answer**: MSS stands for microstatellite stable. These tumors are usually CIN (chromosome instable) tumors. Some of these tumors have defects in mitotic checkpoints which resulted in alteration in chromosome numbers or structures.*

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. (i). Can you provide an explanation for this difference?)

***Answer**: External/enviornmental mutagens play important roles in the generation of mutations in tumor groups labeled in pink. Smoking is responsible for high mutation rate found in lung cancers, and UV light-exposure is responsible for the ones found in melanoma.*

(ii). Pediatric tumors have the lowest number of mutations per tumor. Why?

***Answer**: As a hallmark of cancer, genetic instability is an ongoing process throughout the entire tumor development process. Pediatric tumors have lower number of mitotic divisions compare to adult tumors. Therefore, they do not have sufficient time to accumulate high numbers of mutations.*

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?

***Answer**: groups 1-4 on the left. Immune checkpoint therapy request neo-tumor antigens presented on the tumor. Group 1-4 have high mutations per tumor, so they are more likely to generate such antigen.*

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?

***Answer**: Clonal expansion is a key feature of human cancer development. These mutations are likely to be present in the founder clones of tumors that were eventually sequenced by the TCGA project.*

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 pts). List two hallmarks of cancer that can readily be observed in cultured cells.

Any two of the following:

- Limitless replication potential
- Insensitivity to anti-growth signals
- Invasion (as a proxy for metastatic behavior)
- Self-sufficiency in growth signals
- Evading apoptosis

Also acceptable, functional endpoints such as immortalization, soft agar growth, etc.

b. (3 pts). 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.

SV40 causes cancer by integrating into the genome and expressing viral oncogenes. An SV40-related cancer would have viral DNA and viral RNA. You could test for this in tumor biopsies. Alternatively, you could take a population-based approach, examining cancer incidence in populations that received contaminated versus non-contaminated batches of vaccine.

c. (3 pts). 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.

Viral integration and expression of viral early gene products E6 and E7. A vaccine could work by this mechanism is it contained viral DNA encoding these genes. (Credit for any correctly reasoned answer. In actual fact, the vaccine is recombinant and is made up of virus-like particles that don't contain DNA)

d. (2 pts) 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?

Did incidence of HPV-related cancers decrease in a vaccinated versus unvaccinated group (i.e., efficacy)? Were there unexpected cancers at new anatomical sites?

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?

A is a driver for initial growth that is maintained in the metastatic clone since it likely occurred early on. Mutations D & E necessary for metastasis- either early invasion, metastatic site seeding, and/or growth at the metastatic site [B and C not present at metastatic site because? Passenger mutations? Confer growth advantage in lung but not liver?]- Also potentially multiple clones at the primary tumor site (with diff't passenger mutations), only one or a small number metastasize

b. (2 points) Outline an experiment to test the hypothesis that mutations in genes D and E promote metastasis. Include proper controls.

Could perform an in vivo experiment to test metastasis ; could disrupt genes D&E (in a PDX??) and look at metastatic disease. In vitro experiment would likely not receive full credit since the question is focused on metastasis and not cell motility. Just checking primary tumor growth and not metastatic disease in the mouse would also not receive full credit

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.

Active kinase, triggers increased adhesion to the matrix, allows it to invade better/remodel the ECM, other answers possible

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?

Encodes a gene that confers cisplatin resistance.

e. (2 points). Design an experiment to test your hypothesis. Include proper controls

Put mutation in WT cells and test for cisplatin resistance in vitro.
Knock out mutation in metastatic cells and test resistance in vivo.

Question 10 (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:

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

After irradiation, IP GBMTK and carry out mass spectrometry to identify binding partners, or western blot for potential partners known to localize to sites of DNA damage. Once potential partners are identified, carry out the reverse co-IP to see if the partner brings down GBMTK.

In vitro synthesize GBMTK and the partners, incubate with ^{32}P-ATP, resolve by SDS-PAGE, and analyze incorporation of radiolabel. Alternatively, incubate with non-radiolabeled ATP and perform immunoblotting using phosphor-specific BTK antibody.

Use site-directed mutagenesis to produce a kinase-dead GBMTK. Transfect into the cultured glioma cells. Irradiate, then assay the rate of DNA repair (comet assay, focus resolution). One could also generate activated mutant GBMTK and compare the rate of repair after irradiation to that of cells carrying wild type GBMTK, to determine whether repair is faster.

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

Transfect primary cultures of mouse or human astrocytes with wild type or mutant GBMTK constructs. Carry out colony formation assays, or any other transformation assay.

c. (4 points). Assuming your results from part (b) indicate that GBMTK can drive transformation, describe an experiment to determine whether the activated mutant 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.

Use the RCAS-TVa system to infect TVA-expressing (nestin or GFAP-driven) cells in the brain with RCAS-GBMTK or mutant GBMTK in vivo. Measure survival and brain tumor growth between the two groups.

If GBMTK alone is not sufficient, utilize the RCAS-TVA system to infect the TVA expressing cells with RCAS-GBMTK in combination with RCAS-shP53 or RCAS-PTEN. Could also get the TVA strain onto a P53 or PTEN-null background and use the RCAS-GBMTK construct.