Cancer Biology Graduate Program

Part I Qualifying Exam

June 1, 2018

You must answer **8** of the **10** questions. Each question must be answered in a single MS Word .doc file. **The name of each file you submit must be Q#-codename – Make sure to put the question # followed by your chosen codename at the top of each page (example: Q1. Supastar).**

**If you are submitting figures, make sure to indicate see Figure 1, 2, etc. in your response and include Q#-codename.**

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

Good Luck!

Question 1: (Sumin Kang)

Tumor metastasis, a major contributor to deaths from nearly all types of cancers, is a multi-step cascading process that is influenced by a number of cell signaling proteins. Initiation of metastasis requires invasion. In addition, metastatic tumor cells must acquire migratory potential and resistance to anoikis (extracellular matrix detachment-induced apoptosis) to survive during circulation before forming metastatic foci in distant organs. Through a genome-wide expression profiling comparing circulating tumor cells to primary tumor cells, your lab recently identified that expression of the gene for an enzyme called N-acetyltransferase 2 (NAT2) is increased in disseminated breast cancer cells.

a. (3 points) Design two cell line-based *in vitro* experimental approaches and one *in vivo* xenograft model study to test whether NAT2 plays an important role in maintenance of invasive, anoikis resistant, and metastatic properties of breast cancer cells. Interpret the anticipated result.

b. (2 points) You performed a proteome-wide acetylation profiling in breast cancer cells with NAT2 knockout to identify any potential downstream effectors to confer anoikis resistance or invasive potential and found that the acetylation level of matrix metallopeptidase 1 (MMP-1) at lysine 72 in cells are dramatically decreased when you eliminate NAT2 in breast cancer cells. Design one set of *in vitro* and *in vivo* experiment to examine whether NAT2 promotes invasion and/or anoikis resistance *in vitro* and tumor metastasis *in vivo* by signaling through MMP-1 at K72 in breast cancer cells.

c. (2 points) You wish to explore how NAT2-mediated acetylation of MMP-1 regulates MMP-1 to promote invasion and/or anoikis resistance in breast cancer cells. Considering that MMP-1 is involved in proteolytic cleavage in cancer cells, design an *in vitro* study to explore the molecular mechasnim how acetylation of MMP-1 at K72 by NAT2 contributes to proteolytic activity of MMP-1. Interpret the anticipated result.

d. (3 points) You identified that NAT2 signals through MMP-1, which provides pro-invasive and anti-anoikis property to breast cancer cells to metastasize. You have a panel of primary and lung metastasized paired breast cancer patient tumor tissues. Design three correlation studies you would perform to clinically validate your functional finding.

Question 2: (Bernard Mainou)

a. (6 points) You've discovered a new virus that you have named hawkavir. You are intrigued about the ability of hawkavir to act as an oncolytic virus. When you add hawkavir to normal epithelial cells, the virus establishes infection poorly and the cells remain viable. In contrast, when you add hawkavir to transformed epithelial cells the virus infects the cells efficiently and rapidly induces programmed cell death.

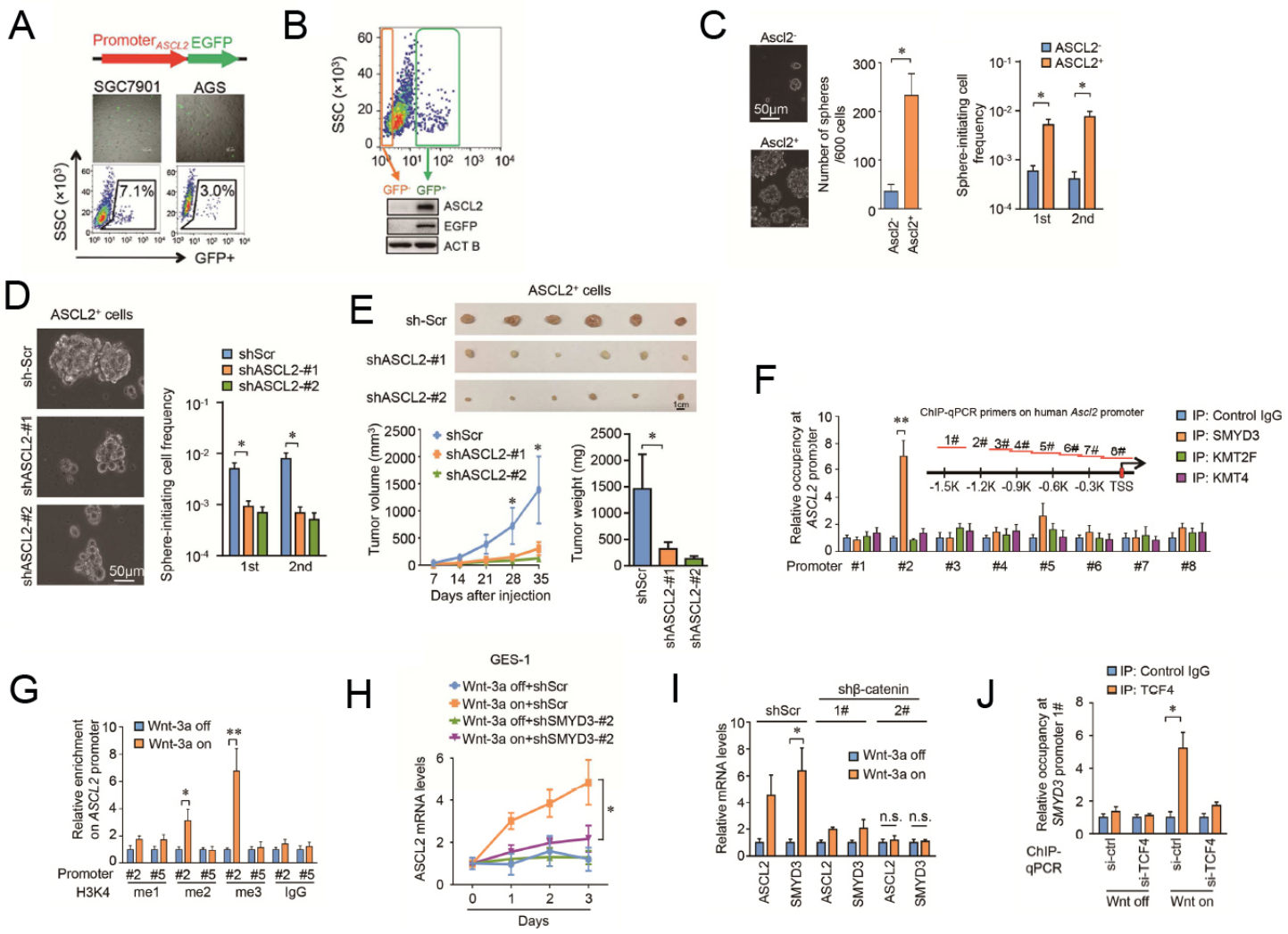
Name two features that might help hawkavir more efficiently infect and kill cancer cells? (Note that there may be more than two possible features of interest, but you should pick two and only two to discuss in your answer). Describe an experiment test if each of these two features is involved in hawkavir enhanced cytopathicity in cancer cells.

b. (4 points) You obtain tumor samples from patients that are part of a clinical trial to test the efficacy of hawkavir against an epithelial cancer. When you look at the histological sections of the tumors, you notice that patients that received hawkavir have substantially higher levels of dendritic cells, natural killer cells, and cytotoxic T cells than control patients.

How could hawkavir be inducing recruitment of these cells to the tumor? What experiment would you do to determine the role of each of these cells in hawkavir's oncolytic potential?

Question 3: (Carlos Moreno)

Below is a figure adapted from a recent paper on gastric cancer:




The *Achaete-scute homolog 2* (ASCL2) gene is highly expressed in gastric stem cells and in gastric carcinoma (GC) cells. Although it is known that Wnt-3a can induce ASCL2 mRNA, previous ChIP-seq studies have shown that β-catenin does not localize to the ASCL2 promoter. The authors of this paper cloned the endogenous ASCL2 promoter upstream of a GFP reporter gene and stably infected a GC cell line with this reporter as shown in Panel A. They then sorted the cells into $GFP^+$ and $GFP^-$ cells which were shown to be $ASCL2^+$ and $ASCL2^-$ by western blot in Panel B.

a. (3 points) What conclusions can you draw about the function of ASCL2 from the data in Panels C, D, and E? Why are there two shRNAs used in Panels D and E?

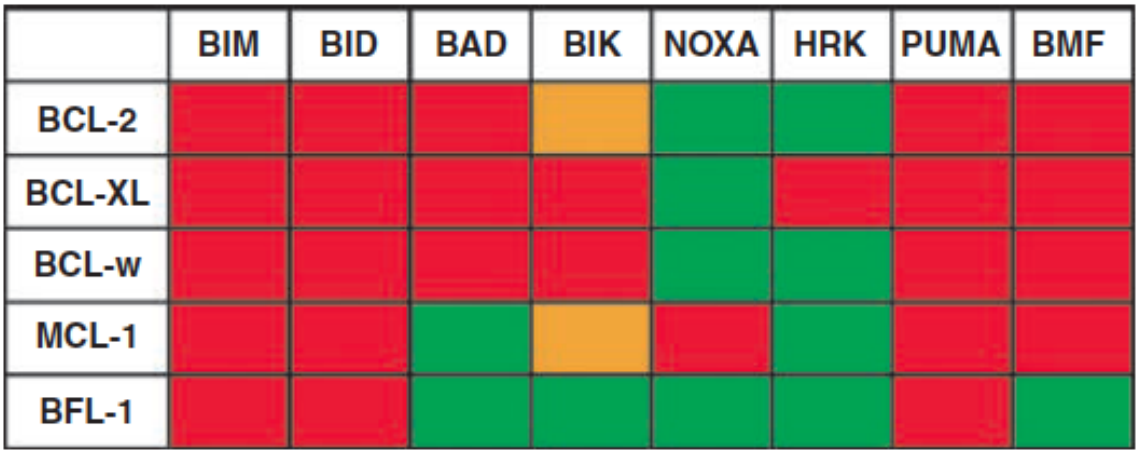
b. (2 points) KMT4, KMT2F, and SMYD3 are lysine methyltransferase enzymes. What can you conclude from the data in panels F and G about the chromatin changes at the ASCL2 promoter in response to Wnt-3a? What controls are included in these panels and why?

c. (3 points) What can you conclude from Panels H, I, and J?

d. (2 points). Draw a model that summarizes the data from this figure.

Question 4: (Larry Boise)

The diagram below illustrates the affinity of the 5 anti-apoptotic BCL2 proteins with 8 BH3-only BCL2 family members. Red indicates high affinity, orange is intermediate affinity and green is low affinity (not physiologically relevant).



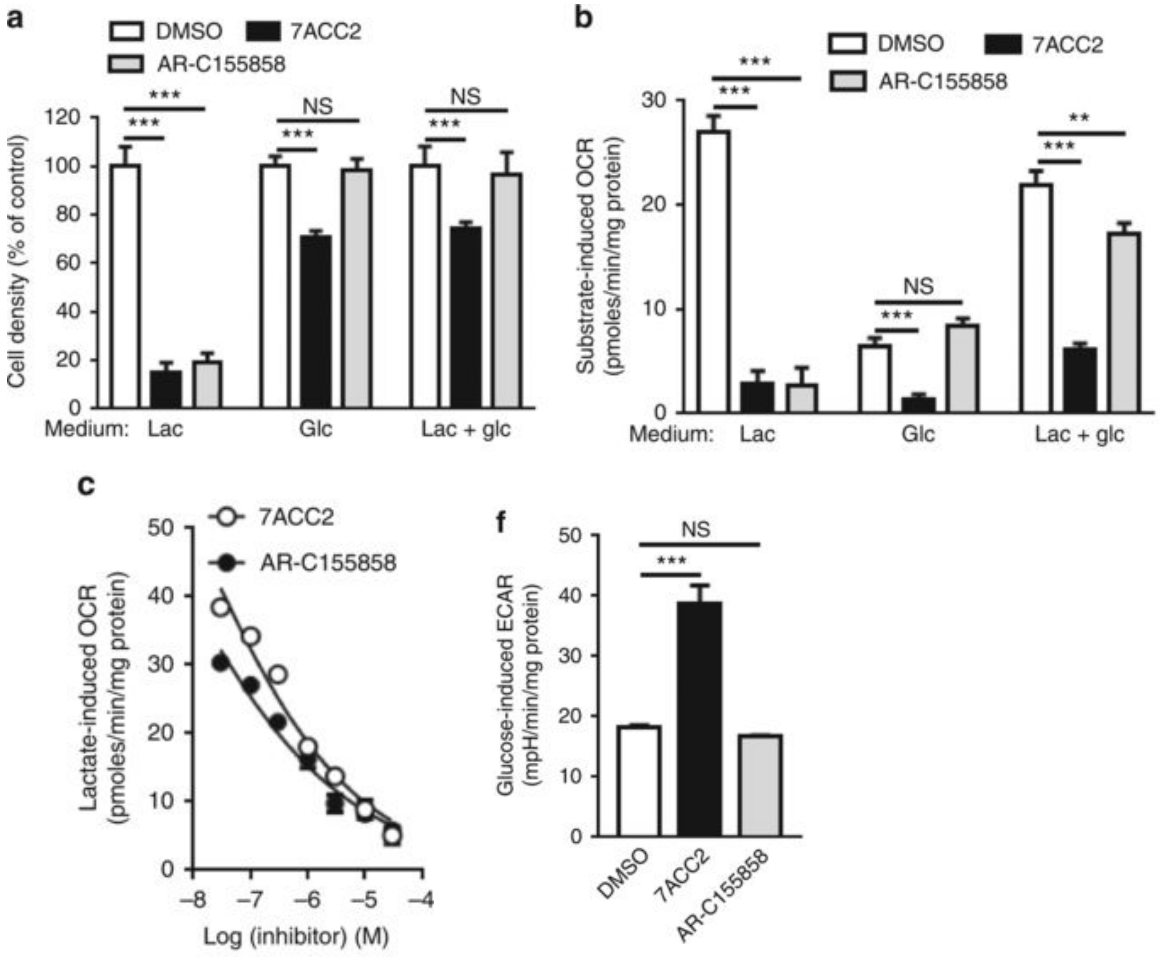

a. (2 points) Based on what is presented here, which BH3-only proteins would you predict to be BH3-only activator proteins and which ones would be BH3-sensitizer proteins?

b. (3 points) Explain the rationale for why you assigned proteins as BH3-only activator proteins? As part of the explanation be sure to include the function of BH3-only activator proteins and how that guided your decision (diagrams are always acceptable as part of your answer).

c. (3 points) If you were going to design a drug that mimicked the function of a BH3-only protein to selectively kill cancer cells, would you pick a BH3-only activator or sensitizer protein? Please explain why you picked one over the other and in your explanation be sure to include the molecular basis for why your drug would work to selectively kill cancer cells.

d. (2 points) Design an experiment that demonstrates that your drug is working by the mechanism that you predict. Be sure to include what your readout will be and how it will measure drug activity.

Question 5: (Mala Shanmugan)




One broadly applicable characteristic of cancer cells is their increased avidity for glucose and preferential use of aerobic glycolysis that has come to be known as the Warburg effect. Glycolysis produces pyruvate, which can be reduced to lactate or oxidized to acetyl-CoA to support the TCA cycle and oxidative phosphorylation (OXPHOS) in the mitochondria.

Lactate itself, however, can also serve as a major carbon source to sustain the TCA cycle and OXPHOS, as lactate imported into the cell can be oxidized to pyruvate, freeing up glucose for other cellular functions. As a result, inhibitors that target glucose metabolism *or* lactate import alone are rather ineffective as single agents for inhibiting cancer cell growth.

Lactate import across the plasma membrane is primarily mediated via the monocarboxylate transporter MCT-1, whereas pyruvate import to the mitochondria is mediated by the mitochondrial pyruvate carrier (MPC). The inhibitors **AR-C155858** and **7ACC2** reduce lactate import significantly (data not shown). Recent investigations with these two agents have shown that they also impact proliferation (panel A) and oxygen consumption rate (OCR) (Panel B) when cancer cells are cultured in media containing lactate alone (no glucose). However, when cells are cultured in media containing either glucose alone or both glucose and lactate, only inhibitor **7ACC2** reduces cell proliferation (Panel A) and OCR significantly (Panel B).

Although both inhibitors reduce lactate-sustained OCR (Panel C) only **7ACC2** increases the extracellular acidification rate (ECAR) of cells cultured in glucose (Panel F). The investigators

hypothesize that **7ACC2** inhibits the mitochondrial pyruvate carrier (MPC) and that one of the effects is to inhibit lactate import.

a. (2 points) Which two panels/data above suggest that these cells rely on lactate import to sustain oxidative phosphorylation? Please provide a rationale for how the experiments support this conclusion.

b. (2 points) Which data/panels above suggest that the effects of **7ACC2** are indirect (i.e., not via direct binding of the drug to MCT-1) and likely on the mitochondrial pyruvate carrier and why? (2 points)

c. (2 points) Which inhibitor would you prefer to use to treat this cancer and why?

d. (2 points) How would you test whether **7ACC2** targets the mitochondrial pyruvate carrier MPC, and not MCT-1?

e. (2 points) If you were able to only administer the **AR-C155858** compound which other metabolic pathway would you target to elicit cytotoxic effects.

Question 6: (Renee Read)

You've started as a new PI at Winship. Your just isolated a new gene, named *tenure project*, that, based on genetic assays, regulates signaling downstream of the EGFR receptor tyrosine kinase in gut development, and, in your newly awarded NIH grant, you've proposed to determine the function of *tenure project* (TP) in colon tumor cells.

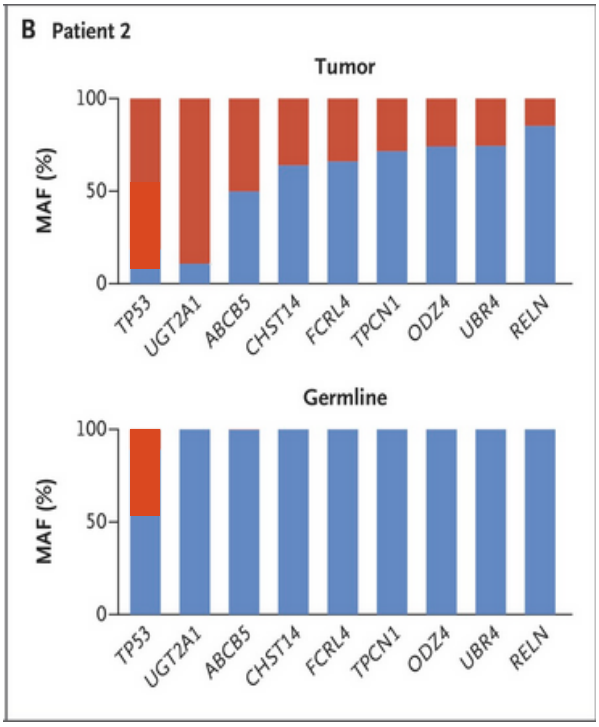
a. (3 points). Name three domains that you would immediately look for in the protein sequence of TP. (Note that there are more than three possible domains of interest but you should pick three and only three for your answer). Describe the functions of these domains.

b. (4 points) You transfect EGFR-negative colon cancer cells with EGFR and TP, and discover that, in response to EGF treatment, TP binds to EGFR in co-immunoprecipitation assays. What does this result indicate about the interaction between TP and EGFR? Draw a model showing the mechanism of TP binding to EGFR. Design an experiment using colon cancer cells to test your model of the mechanism of TP binding to EGFR.

c. (3 points) You do a second experiment to determine if TP binds to GTP-loaded Ras or GDP-loaded Ras. What is the difference between these two forms of Ras and how are they related to EGFR signaling? You find that TP only binds to GTP-loaded Ras. What would you conclude about TP's interactions with Ras and why?

Question 7: (Paula Vertino)

Recent findings from the Pediatric Cancer Genome Project (588 children; median age 6.9 years) and a similar genome project of pediatric cancers in Germany found that ~8% of children and young adults with cancer carry a germline predisposing mutation. Unlike adult tumors, less than half of these cases showed any evidence of a family history of cancer. Data were derived by exome sequencing or whole genome sequencing of PBMCs (peripheral blood mononuclear cells) or buccal cells (cheek mucosa) and tumor tissue from the same patient. Results for a subset of genes in a single patient are shown below:



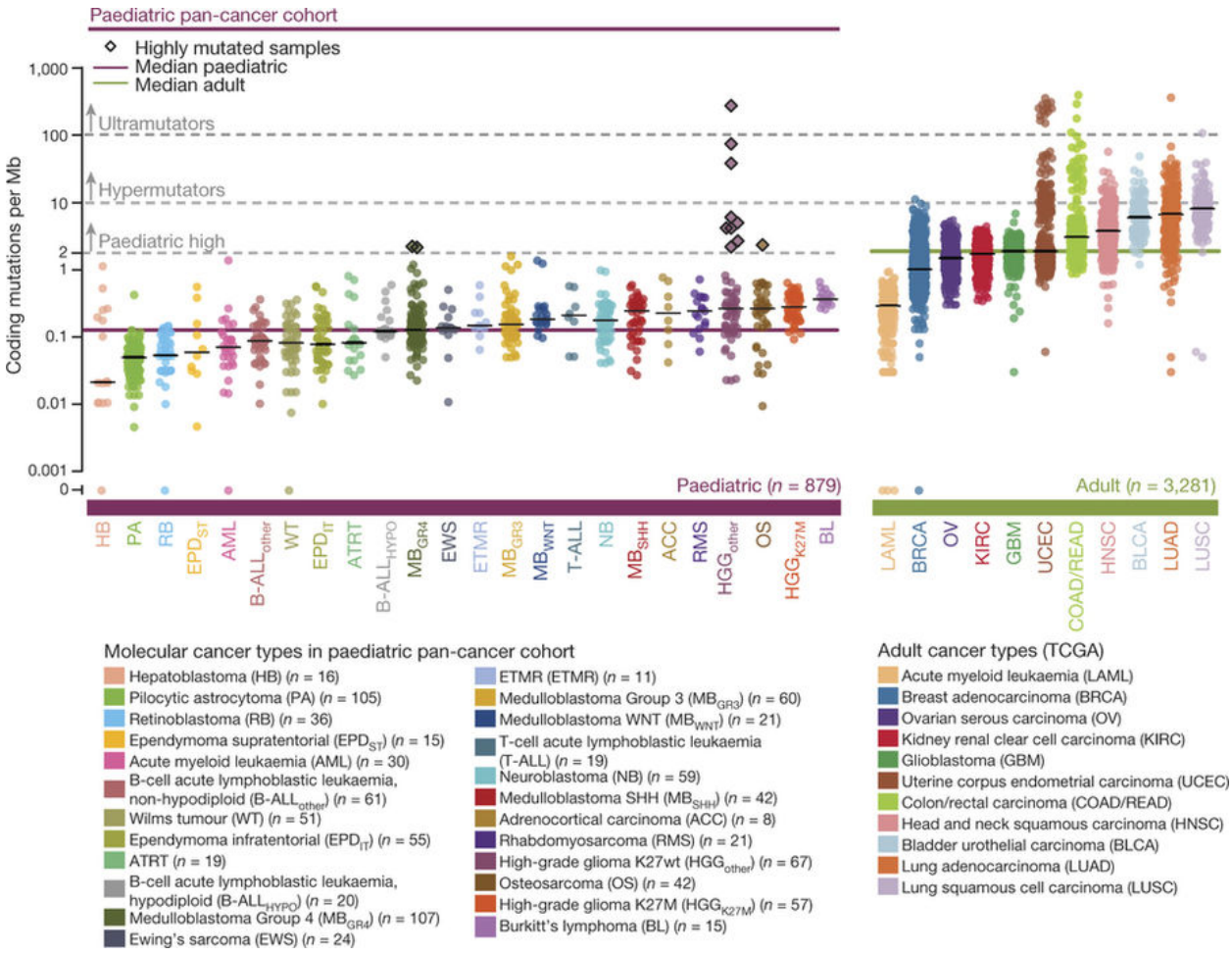


MAF= allele frequency

a. (2 points) What is your interpretation for how the genes differ ie. if you had to classify them as potential oncogenes or tumor suppressor genes, how would you do so? Justify your answer.

b. (2 points) With regards to patient 2, what is your interpretation of the differences observed between p53 (TP53) and UGT2A? As part of your answer explain the relationship between the germline data and the tumor data for each gene and a likely scenario for how each pattern arose. What critical concept in cancer genetic do these two examples illustrate?

c. (2 points) The Figure below shows the burden of somatic mutations among pediatric tumors (left side) relative to adult tumors (right side). What does the overall pattern suggest with regards to total mutation burden between the two and why is this the case?. If you plotted the same data within a particular tumor type how would you expect the data to stratify? …that is, within each pediatric tumor type the mutational burden is distributed across 1-2 orders of magnitude….what might account for this variability from low to high? (draw a graph if its easier)




d. (3 points) In spite of the above, certain pediatric tumor types appear to have just as high a burden as some adult tumors, including a subset of High Grade Gliomas and Group 4 Medulloblastomas.

Noting the similarity to some adult cancers, provide an explanation for what might account for the hogh mutational burden. How would you test your hypothesis given all the genomic information you have at hand for this cohort?

Provide one potential explanation for the difference between “ultra” high mutator HGGs and the regular hypermutators, keeping in mind that these tumor arise within the first 2 years of life typically.

e. (1 point) While not currently used in this population, immune checkpoint therapy is now being considered for some pediatric cancers based on these data. Given your understanding of immune checkpoint therapy, which pediatric tumors are likely to have a favorable response and why?

Question 8: (Melissa Gilbert-Ross)

A genetic mosaic screen was performed in a model organism to identify genes that restrict tissue growth. The goal was to identify novel conserved tumor suppressor genes. One of the genes that emerged from the screen was *sautéed potato-eye* (*stp),* which has four well-conserved human homologs. You are interested in in determining whether these homologs have a role in mammalian tumorigenesis.

a. (3 points) Prior to starting experiments, what bioinformatic approaches can you use to determine whether any of the four human homologs might be tumor suppressors in human cancer?

b. (2 points) Based on your answer to part a, above, you find evidence that one of the four homologs, STP2, may in fact be a tumor suppressor gene. A colleague offers you an STP2-deficient lung cancer cell line that forms tumors when injected into immunodeficient mice. Design an experiment to investigate whether loss of STP2 function indeed contributes to the tumorigenic phenotype in this line

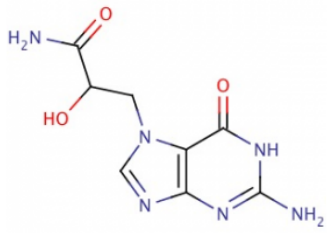
c. (3 points) Again based on your investigation in part a, above, you hypothesize that loss of a single copy of *STP2* in the germline *(*haploinsufficiency*)* would predispose to lung cancer. Design an experiment to test your hypothesis using a genetically engineered mouse model.

d. (2 points). While carrying out your experiment in part c, above you discover that homozygous loss of *Stp2* is lethal. Moreover, you have extended your analysis in part a and discovered that LOH of *STP2* commonly co-occurs with oncogenic KRAS mutations in lung cancer. You hypothesize that loss of *STP2* promotes the progression of KRAS-mutant lung cancers. Design an experiment to test your hypothesis in a genetically engineered mouse model.

Question 9 (Bill Dynan)

California proposition 65, passed in 1986, mandates that businesses place a warning label on products containing any of 1065 chemicals that the present risks of “cancer, birth defects or other reproductive harm.” Proposition 65 has been in the news recently because a California judge ruled that Starbuck’s coffee must carry a warning label based on the presence of trace amounts of acrylamide, which is formed during the roasting process.

The mechanism of acrylamide carcinogenesis involves metabolic conversion to glycidamide, which forms DNA adducts including N7(2-carbamoyl-2-hydroxyethyl) guanine, (N7-GA-Gua), A structure is below. This adduct is present in the DNA of animals exposed to high doses of acrylamide. It is also known to be released from adducted DNA, as a free base, by a repair process.




a. (2 points). Briefly describe experimental approaches that might have been used to determine (i) that acrylamide is carcinogenic, and (ii) that the metabolite, glycidamide, and not acrylamide itself is the ultimate carcinogen form.

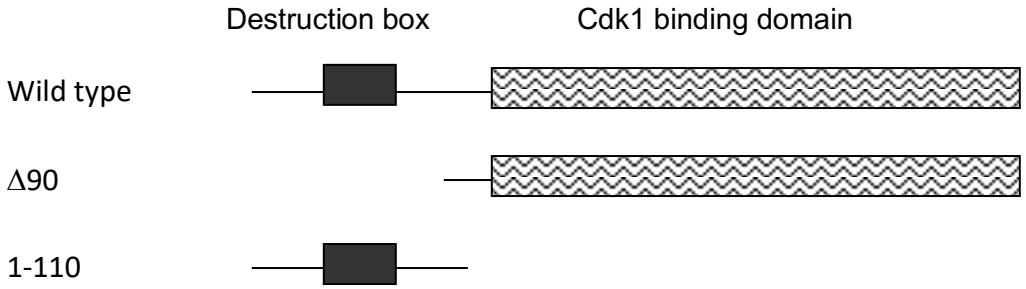
b. (4 points) Assume that base excision repair is responsible for repair of N7-GA-Gua modified DNA. List the sequential steps in base excision repair, including the step that releases the free base.

c. (2 points) In a cell culture model, what are the likely consequences of failure to repair the N7-GA-Gua lesion?

d. (2 points) Design an experiment in cultured fibroblasts to determine whether base excision repair is indeed the mechanism for repair of N7-GA-Gua lesions.

Question 10 (Maureen Powers)

While studying the requirements for sister chromatid separation in anaphase, you separately overexpress each of the following mutant versions of cyclin B in cells. Cyclin BΔ90 is missing the destruction box required for recognition by the APC. Cyclin B 1-110 retains the destruction box but cannot bind to Cdk1.




When either mutant cyclin is expressed, MPF activity remains high and cells do not exit mitosis. The two mutant proteins differ in the stage at which transfected cells arrest. When cyclin BΔ90 is overexpressed, sister chromatids separate normally and cells arrest in anaphase. When cyclin B 1-110 is overexpressed, sister chromatids do not separate and cells arrest in metaphase.

a. (2 points) Why does MPF activity remain high in the presence of cyclinBΔ90?

b. (2 points) Why does MPF activity remain high in the presence of cyclinB1-110?

c. (2 points) What does this experiment tell you about the respective contributions of cyclinB and the APC to sister chromatid separation?

d. (4 points) In cells with the following mutations, would the cell cycle be arrested, yes or no. If yes, at what stage of the cycle would arrest occur and why?

i. non-degradable mitotic cyclin

ii. overexpression of p21

iii. non-phosphorylatable Rb

iv. constituitive E2F expression