Table of contents

Draft questions

Draft question #	Contributor	Topic area
D14	Grossniklaus	Uveal melanoma metastasis
D15	Henry	Aging and immunotherapy
D31	Rafiq	chimeric antigen receptor (CAR)
D39	Waller	Immunotherapy
D43	Yun	Autophagy

Question D14
(Grossniklaus)

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. Treatment, either by enucleation (removing the eye) or brachytherapy (irradiating the tumor in the eye) almost always leads to successful local control. However, there is a 40% to 50% mortality rate from liver metastases of this tumor. These metastases are diagnosed by imaging (MRI, CT) at about 3 years after the primary tumor is treated.

a. (3 pts) What are possible mechanisms regarding why UM metastasizes virtually exclusively to the liver? How can you test these possible mechanisms?

b. (4 pts) One early hypothesis was that enucleation spread the tumor to the liver. However, the Collaborative Ocular Melanoma Study (COMS) found that the rate of metastasis and death was identical in a large cohort of patients who were randomized to either enucleation or brachytherapy. What do these results tell us about the original hypothesis? What alternative hypotheses can you develop to explain the findings? How can you test your hypothesis?

c. (3 pts). Thus far there are no systemic medications that prolong life significantly for patients with metastatic uveal melanoma to the liver. Neither conventional chemotherapy nor immune checkpoint inhibitors have been effective. What are possible reasons why these therapies haven't worked? What are some strategies that may be tested to overcome these problems? What experiments would you design to test these strategies?

Question D15
(Curtis J. Henry)

Non-Small Cell Lung Cancer (NSCLC) has a median age at diagnosis of 70 years. Unfortunately, chemotherapy treatment is largely ineffective in older patients with NSCLC.
The emergence of immune checkpoint inhibitors (ICI), particularly αPD-1 antibody, has shown promise in patients with NSCLC. However, age-related disparities persist, as shown in the Kaplan-Meier plot below (from Lichtenstein et al., J Thorac Oncol. 2019 Mar;14(3):547-552).
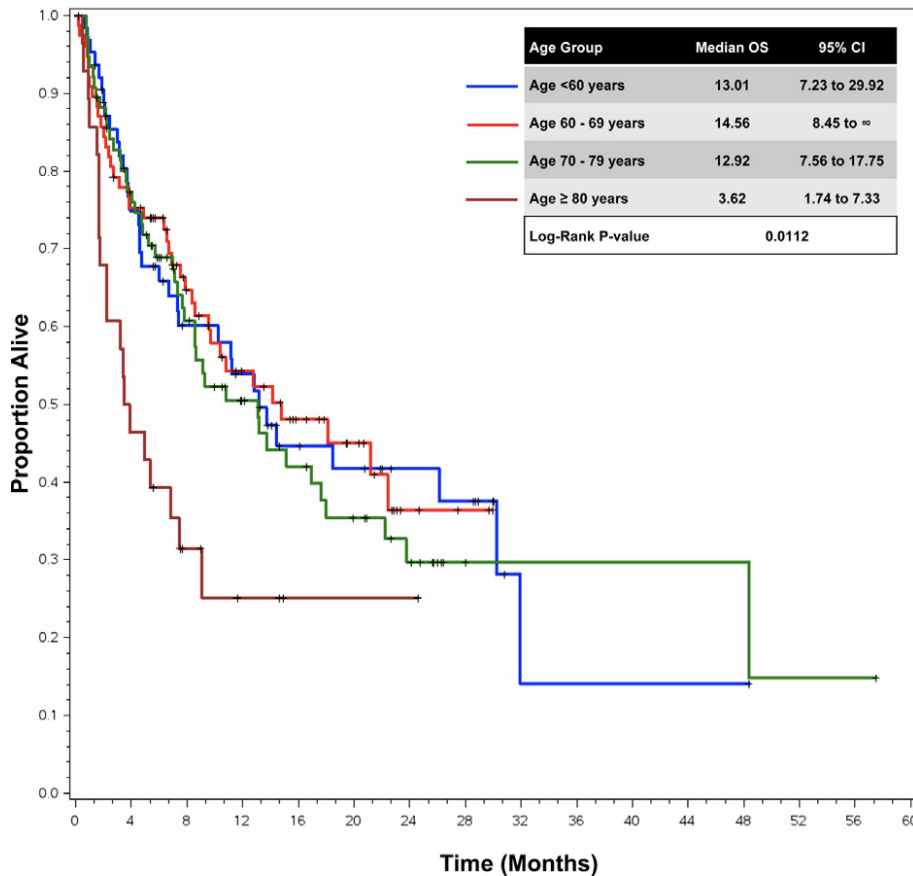
BRIEF REPORT

Impact of Age on Outcomes with Immunotherapy in Patients with Non-Small Cell Lung Cancer

Morgan R. L. Lichtenstein, MD, Ryan D. Nipp, MD, MPH, Alona Muzikansky, MA,
Kelly Goodwin, CNP, Danyon Anderson, BA, Richard A. Newcomb, MD,
Justin F. Gainor, MD*

Overall Survival

Age Group	Median OS	95% CI
Age <60 years	13.01	7.23 to 29.92
Age 60 - 69 years	14.56	8.45 to ∞
Age 70 - 79 years	12.92	7.56 to 17.75
Age ≥ 80 years	3.62	1.74 to 7.33
Log-Rank P-value		0.0112

a. (**2 pts**) What factors might account for the observation that *chemotherapy* is largely ineffective

in older patients?

b. (**3 pts**) State a mechanism-based, immunological hypothesis why *immunotherapy* is less effective, specifically why the therapeutic outcomes shown in the figure above are significantly worse in the age $\geq$ 80 years cohort. Before contemplating experiments to test this hypothesis, what confounding factors might you wish to rule out.

c. (**5 pts**). Assume that you have been granted IRB approval to use blood and tumor samples from these patients. Propose a set of experiments using these sample to test your hypothesis. This should include one *in vitro* and one *in vivo* approach. Provide an experimental design, specifying the experimental groups including control groups. Discuss potential outcomes and whether they support or refute your hypothesis.

D31
(Rafiq)

Your lab designed a novel chimeric antigen receptor (CAR) against a tumor-associated antigen found on solid tumor X. In preclinical testing, these CAR T cells were effective in killing tumor cells *in vitro* cytotoxicity assays as well as eliminating established tumors engrafted in immunodeficient (NSG) mice. Phase I trials conducted in patients with relapsed/refractory solid tumor X treated with the CAR T cells demonstrated that the CAR T cells were well tolerated and there are no dose-related toxicities. However, no objective clinical response was seen.

a. (1 pt) Propose two reasons why the preclinical data did not accurately predict or correspond to the patient data.

b. (3 pts) Correlative analysis performed on tumor biopsies from the trial show that CAR T cells are trafficking to the tumor but are only found on its periphery. You hypothesize that solid tumor X creates a microenvironment that is functionally suppressive to the CAR T cells. Design an experiment with proper controls to identify a factor by which the tumor cells are suppressing the CAR T cells. Include anticipated results and how you would interpret them.

c. (3 pts) After identifying a factor by which the tumor could be creating a hostile microenvironment in (b), you next want to validate this factor's effect on CAR T cell function and determine the mechanism of suppression in T cells. Design an experiment with proper controls to test this and include anticipated results and how you would interpret them.

d. (3 pts) Given what you have learned in questions (b) and (c), hypothesize a therapeutic strategy to overcome CAR T cell suppression in the solid tumor X microenvironment and design an experiment with proper controls to test your hypothesis.

Question D39
(Ned Waller)

Describe an experimental approach to test a new cancer immunotherapy.

a. (2 pts) What is the mechanism of action for the new immunotherapy that you will be testing?

b. (4 pts) Provide a set of preclinical experiments to test the new immunotherapy. What considerations inform your choice of a model system? What would be the primary end-point to evaluate this new therapy? What secondary end-points would you use to evaluate efficacy? How would you evaluate toxicity? Provide an experimental design, showing experimental groups including control groups. Discuss the anticipated results and their interpretation, including how results from the control groups would inform your analysis of the results with the other experimental groups.

c. (4 pts) Based upon promising results in the model system, you wish to conduct a clinical trial. What would be your concerns regarding about translating these results into a human clinical trial?

Question D43
(Yun)

Activation of the transcription factor TRIM1 has been documented in a wide range of tumors, including colorectal cancer (CRC). Your goal is investigate the role of TRIM1 in tumorigenesis using a loss-of-function approach in intestinal epithelial cells (IECs) in mice. For this purpose, you have crossed Trim1$^{f/f}$ mice with villin-cre to generate Trim1$^{\Delta IEC}$ mice, which lack Trim1 expression in IECs. Trim1$^{\Delta IEC}$ mice were then treated with the somatic mutagen, azoxymethane, (AOM) ,and tumor development was examined 20 weeks later. AOM induced tumors in wild type (wt) mice, but not Trim1$^{\Delta IEC}$ mice. Consistent with this, AOM-treated Trim1$^{\Delta IEC}$ mice survived much longer than AOM-treated wt mice.

a. (2 pts) Histological examination of AOM-challenged Trim1$^{\Delta IEC}$ mice revealed a pronounced accumulation of lymphocytes in mouse intestinal mucosa. You suspect these are T-lymphocytes, but based on the morphological features under a light microscopy you are not sure if these are CD4+ or CD8+ cells. Describe an experiment to determine if these are CD4+ or CD8+ T cells.

b. (4 pts) Based on your experiment, you found out that these are CD8+ T cells. However, you are not sure whether the survival advantage of Trim1$^{\Delta IEC}$ mice is dependent on the adaptive immune response rather than on Trim-dependent IEC autonomous effects such as proliferation. Describe an experiment how you will test this. Provide an experimental design showing experimental groups and control groups. Discuss anticipated results and their interpretation.

c. (4 pts) In order to find the underlying mechanism, you consulted a pathologist friend to look at the histochemical feature of tumors of wt and Trim1$^{\Delta IEC}$ mice treated with AOM. Your friend found double membrane-bounded cytosolic vesicles in IECs of Trim1$^{\Delta IEC}$ mice but not in those of wt mice. Based on your knowledge, the presence of double-membrane vesicles is a feature of autophagosomes, which is formed by autophagy. Propose a hypothesis how Trim1 loss prevents tumor formation. Describe one experimental approach that you could use to test your hypothesis. Provide an experimental design showing experimental groups and control groups. Discuss anticipated results and their interpretation.