We suggest reading the paper in this order:

1. Introduction
2. Results: Computational and experimental modeling of ErbB2/3-driven tumor growth (Figure 1)
3. Materials & Methods: Cell lines and reagents
4. Materials & Methods: Multiplex Luminex protein assays (Figure S1)
5. Materials & Methods: Computational methods -- Approach and quantitative logic
6. Materials & Methods: Simplifying assumptions (Figures S9, S10)
7. [Materials & Methods: the rest of the model description]\*\*
8. Results: Calibration of ErbB-mediated signaling…. (Figure 2A), (Figures S2, 2B), (Figure S3)\*\*\*
9. Results: Regulation of tumor growth by PI3K and… (Figure 3A, S6, 3B) (Figure 3C)
10. Results: In vivo translation: Assessing drug combination…(Figure 4A, 4B) (Figure 4C, D)
11. Discussion (We will not discuss Figures 5 - 8 in class)

\*\*While it is not necessary for you to completely understand the model that has been built, it is good practice to read these sections and it will help you to follow the rest of the text.

\*\*\*These figures are tricky. Please see Shannon with questions and do your best to make an explanatory slide.

**Figure 1**

Team:

Read the first subsection under Results, titled "Computational and experimental modeling of ErbB2/3-driven tumor growth", and the "Computational methods -- Approach and quantitative logic" subsection in the Materials and Methods (which is located at the end of the paper). Answer the following questions to guide you:

(1) What is the system being modeled and what specific pathways are represented?

(2) What do the authors state is the key insight to required to accurately related clinical response to cell stimulation?

(3) How do the authors take advantage of this insight to simplify their model -- what is the name of the modeling technique that they used?

(4) Under simplifying assumptions -- what is the most important assumption included in the model construction?

**Figure S1**

Team:

Read the first paragraph under the Results subsection "Calibration of ErB-mediated signal transduction and feedback regulatory circuits". Answer the following questions to guide you:

(1) How are luminex-based total and phosphoprotein measurements completed? A good read of the Method section covering this assay and a Google search will help you make a good slide.

(2) How is a z-score calculated?

(3) Why is this an important control experiment?

**Figure S9 & S10A**

Team:

These figures are referenced in the Material & Methods: Simplifying assumptions section. Answer the following questions to guide you:

(1) How do MM-111 and Lapatinib work? (You may need to read ahead to the Ligand-receptor interactions section to find this).

(2) How were the experiments performed? Where did you find that information? (If you can't, how do you feel about that? Can you find enough information to provide an explanation to the class)

(3) How would the model be changed if the assumptions experimentally verified in S9 & S10 did not work out?

**Figure 2A\*\*\***

Team:

Read the Results subsection titled Calibration of ErbB-mediated signaling….and the Materials & Methods sections: Approach and quantitative logic, Simplifying assumptions, Ligand-receptor interactions, Intracellular signal transduction and Transcriptional feedback regulation. Think about the following points/questions to guide you:

1. How do MM-111 and Lapatinib work?
2. Draw a box, label the corners with (low, low), (low, high), (high, low), and (high, high) according to the inhibitor concentrations. Use this to orient yourself while interpreting the figure.
3. Remember the following: High MM-111 = low Erb3 (E3) activity, High Lapatinib = low ErbB2 activity (E2).
4. What experiments were used to populate the left hand column of response surfaces?
5. How did the authors make the right hand column?

**Figure S2, 2B\*\*\***

Team:

Read the Results subsection titled Calibration of ErbB-mediated signaling….and the Materials & Methods sections: Approach and quantitative logic, Simplifying assumptions, Ligand-receptor interactions, Intracellular signal transduction and Transcriptional feedback regulation. Think about the following points/questions to guide you:

1. What connections in Figure 1 best describe the data in Figure S2?
2. If a phosphorylated Akt leads to decreased ErbB3 levels (Figure S2) and activated ErbB3 leads to significantly more phosphorylated Akt than activated ErbB2 (see Figure 2A, middle response surface on left side) – what should happen to ErbB3 levels if Akt phosphorylation is decreased due to MM-111 treatment?
3. Think about revisiting Fig1 or drawing your own feedback loop.

**Figure S3\*\*\***

Team:

Read the Results subsection titled Calibration of ErbB-mediated signaling….and the Materials & Methods sections: Approach and quantitative logic, Simplifying assumptions, Ligand-receptor interactions, Intracellular signal transduction and Transcriptional feedback regulation. Think about the following points/questions to guide you:

1. If a phosphorylated Akt leads to decreased ErbB3 levels (Figure S2) and activated ErbB3 leads to significantly more phosphorylated Akt than activated ErbB2 (see Figure 2A, middle response surface on left side) – what should happen to ErbB3 levels if Akt phosphorylation is decreased due to MM-111 treatment?
2. Think about revisiting Fig1 or drawing your own feedback loop.
3. Do agree with the authors that their ErbB3 feedback loop is real evidenced by 50% of the cancer cell lines showing some signs of it?
4. Is this figure easy to interpret? How did they obtain the data? How would you improve the visual ease?

**Figure 3A, S6, & 3B**

Team:

Read the Results subsection titled "Regulation of tumor growth by PI3K and MAPK pathway integration". Consider the following to guide you:

(1) How is the timescale of the experiment in Fig 3A different from Fig 2A? How do the authors use that to motivate their logic-based modeling technique? What is a logic gate?

(2) What do the authors do to verify that their final model (Fig 3B) is the best one?

(3) According to figure 2B, what two things contribute to tumor growth and (according to the Materials & Methods: Ligand-receptor interactions section) what is the driver in this cell line?

**Figure 3C**

Team:

Read the Results subsection titled "Regulation of tumor growth by PI3K and MAPK pathway integration". Answer the following to guide you:

(1) What do MM-111 and Lapatinib target? (You may need to read the Materials & Methods:Ligand-receptor interactions section to find this).

(2) What do UO126 and MK2206 target?

(3) What data was used to 'train' the logic model? How would you use the logic model to predict the effect of MMM-111 and Lapatinib?

**Figure 4A, 4B**

Team:

Read the Results subsection titled "In vivo translation:…" Answer the following to guide you:

(A) What is the major modeling assumption the authors are making when going from *in vitro* to *in vivo*?

(B) What is a syngeneic cell line? What is a xenograft?

(C) Why was it required to estimate the rates of proliferation, cell death, and [HRG] before utilizing the logic model parameterized using *in vitro* data to predict tumor growth *in vivo*? A visit to the Materials & Methods: Simplifying assumptions section might help here

(D) Would you consider the logical model approach successful here?