MCCRP

Uniformed Services University

MURTHA CANCER CENTER RESEARCH PROGRAM (MCCRP)

Uniformed Services University (USUHS)

Student Presentations

MCCRP Suite 310 Conference Room

July 28, 2026

MCCRP/USUHS Student Presentations

Tuesday, July 28, 2026
MCCRP Suite 310 Conference Room

meet.google.com/jur-anvg-kjn

Agenda

11:00 - 11:15 am	**Welcome Remarks:** Dr. Craig Shriver and Dr. Geeta Upadhyay
11:15 - 11:30 am	**Group 1:** Ms. Emily Pangaro, Ms. Alyssa Belen **Title:** Elucidating the Role of LY6E in ER+ Breast Cancer Therapeutic Response
11:30 - 11:45 am	**Group 2:** Mr. Ben Auerbach, Ms. Alivia Lombardo **Title:** Preparation for Confirmation of SANT-CTNNB1 Relationship
11:45 - 11:55 pm	**Group 3:** Ms. Sara Botwinik **Title:** Structure & Function of Immunoglobulins
11:55 - 12:05 pm	**Group 4:** Mr. Michael Valerio **Title:** Synergistic Growth Inhibition by LY6D Antibody, LY6K Antibody, and LY6K Small Molecule Inhibitor NSC243928 in Diverse Cancer Cell Models
12:05 - 12:15 pm	**Closing Remarks:** Dr. Craig Shriver and Dr. Geeta Upadhyay
12:15 - 12:30 pm	Group Photo
12:30 - 1:30 pm	Lunch, followed by a site tour

Students

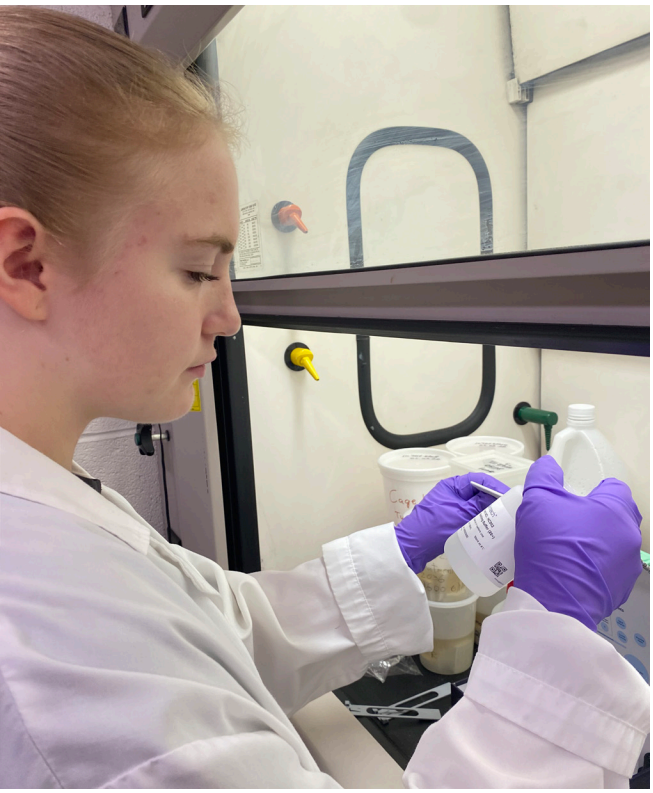
Ms. Emily Pangaro
Ms. Alyssa Belen
Mr. Ben Auerbach
Ms. Alivia Lombardo
Ms. Sara Botwinik
Mr. Michael Valerio

Mentors

Dr. Changqing (George) Yuan
Dr. Yong Chul Kim
Dr. David Wisniewski

Guests

Dr. Aaron Auerbach
Mrs. Eddie Pangaro
Dr. Jamie Lombardo
Mr. Miguel Belen
Mrs. Gina Valerio
Mrs. Heather Botwinik
Mr. Philip Botwinik
Dr. Craig Shriver
Dr. Geeta Upadhyay
Mr. Jaime Boone
Ms. Danielle Jateng
Ms. Denise Wright
Dr. William (Drew) Comrie



Alivia Lombardo inspecting the reagent

Michael Valerio retrieving therapeutic agents from -80

Student

Alivia Cadence Lombardo

Biography

Highschool Junior, Biomed program. Expected graduation date: Highschool and Biomed program 2027. I am a high school student, interested in the study of diseases. I joined a four-year biomed program called PLTW (project lead the way biomedical sciences). During this course I have studied forensics, HIPPA, human body systems and how they work together, injection techniques, different bone breaks and ways to fix them, blood transfusions, organ transplants, genetic diseases/disorders, genetic tests done on fetuses to find genetic disorders, what different regions of the brain control, Renee and Webber tests, dissected a sheep's heart, lungs and kidney, a cow eye, did a PCR lab and ELISA lab.

Title: Preparation for Confirmation of SANT-CTNNB1 Relationship

Abstract

Sclerosing angiomatoid nodular transformation (SANT) is a rare, benign fibrovascular lesion of the spleen. It is most common in middle-aged adults with a slight female predominance. It is also typically asymptomatic and discovered incidentally during imaging studies or abdominal surgeries. There is radiologic overlap with other splenic vascular lesions, making histologic analysis critical for diagnosis. Some research has suggested SANT to be a non-neoplastic process, however a study identified the loss of the CTNNB1 exon 3 in 7/7 cases tested, leading the authors to favor SANT to be a neoplastic process. Given the lack of consensus on the neoplastic or nonneoplastic nature of this condition, we aimed to further evaluate SANT using genomic sequencing. Cases of SANT dating back to 1975 were identified within the Joint Pathology Center (JPC) repository. We performed JAK1 and STAT3 inhibitor staining to identify possible connections within the antibodies, and examined the results illuminating the expected connections.

Student

Michael Valerio

Biography

I am a rising sophomore undergraduate student at Duke University in Durham, NC, where I am studying biology with a minor in chemistry and a concentration in pharmacology. My associated lab, the McDonnell Lab at the Duke University School of Medicine, studies androgen receptor therapies in human prostate cancer. Beyond my education and lab work, I am an avid golfer and guitarist, a volunteer for the United Way of Central MD and the Red Cross, and I enjoy spending time with friends and family.

Title: Synergistic Growth Inhibition by LY6D Antibody, LY6K Antibody, and LY6K Small Molecule Inhibitor NSC243928 in Diverse Cancer Cell Models

Abstract

Targeted combination therapies have emerged as a promising strategy for improving cancer treatment by enhancing therapeutic efficacy while reducing resistance. This study investigated the synergistic potential of the monoclonal antibodies LY6D-2 and LY6K-3 in combination with the LY6K small-molecule inhibitor NSC243928 across diverse cancer cell models including breast, ovarian and pancreatic cancer. We hypothesized that combined treatment with these agents would produce greater growth inhibition than individual therapies by targeting complementary cellular pathways. To evaluate this hypothesis, several different cancer cell lines were treated with each antibody and drug at varying concentrations, either alone or in a two-drug combination. We also performed surface expression tests on these cells to determine what proteins were detected by an antibody stain, and thus could be targeted by our drugs. Cell growth was assessed over 72 hours using an incucyte plate reader, and drug interactions were analyzed using total viability numbers to determine synergistic effects. The combination treatments provided varying results in different cell lines depending on what agents and concentrations were used. In addition to testing the effects on cell growth, we performed caspase and annexinV/PI assays to assess whether the drug combinations were inducing apoptosis. We also discovered that cancer cells treated with a combination of LY6D and LY6K antibodies had less cells in the G1 population, indicating combined effects on the cell cycle. These findings suggest that the combined use of LY6D-2, LY6K-3, or NSC243928 in the right contexts may represent a promising therapeutic strategy for enhancing growth inhibition across multiple cancer types. Future mechanistic studies will focus on pathways affected by these drug treatments, and whether LY6D and LY6K expression can predict response to these combination therapies.

Student

Alyssa Belen

Biography

I am a Schreyer Honors College student at Penn State University majoring in Medical Microbiology with a minor in Diversity and Inclusion. This fall, I will return to Penn State for my sophomore year as a member of the Presidential Leadership Academy. I look forward to taking on new leadership roles and expanding my knowledge in the sciences.

Title: Elucidating the Role of LY6E in ER+ Breast Cancer Therapeutic Response

Abstract

LY6E has been shown to be correlated to poor overall survival and therapeutic outcomes in populations with breast cancer, specifically ER+ breast cancer. Patients with high LY6E expression have worse progression-free survival when treated with anti-estrogen therapies Anastrozole, Tamoxifen, or Letrozole. This study confirmed LY6E knockout (KO) compared to wild type (WT) in estrogen receptor positive MCF7 cells. This study characterizes the LY6E-3 targeting antibody because it was shown to be specific to LY6E by flow cytometry in WT and KO MCF7 cells. Additionally, when comparing the LY6E-2 to the LY6E-3 antibody, LY6E-3 was shown to have much higher toxicity in the MCF7 cell line. The anti-estrogen therapies exhibited more toxicity in the LY6E KO cells as compared to the WT cells, indicating that LY6E expression plays a role in sensitivity to these therapies, which is consistent with the patient data. Further, combination of the LY6E antibody and anti-estrogen therapies exhibited an improved combined effect, providing evidence that LY6E antibody or antibody-drug conjugates may be clinically combined with current anti-estrogen therapies. Overall, this study provides evidence that LY6E expression plays a role in the cytotoxicity of anti-estrogen therapies, and suggests that combination of LY6E antibodies or antibody-drug conjugates with anti-estrogen therapies may have a clinical impact.

Student

Emily Pangaro

Biography

I am currently a senior at the University of Maryland, College Park, majoring in neuroscience. This is my second summer working with the Upadhyay Laboratory on research relating to cancer. My immediate plans for the fall are to continue with my undergraduate degree while studying for the MCAT to pursue a future career in medicine.

Title: Elucidating the role of LY6E in ER+ breast cancer therapeutic response

Abstract

LY6E has been shown to be correlated to poor overall survival and therapeutic outcomes in populations with breast cancer, specifically ER+ breast cancer. Patients with high LY6E expression have worse progression-free survival when treated with anti-estrogen therapies Anastrozole, Tamoxifen, or Letrozole. This study confirmed LY6E knockout (KO) compared to wild type (WT) in estrogen receptor positive MCF7 cells. This study characterizes the LY6E-3 targeting antibody because it was shown to be specific to LY6E by flow cytometry in WT and KO MCF7 cells. Additionally, when comparing the LY6E-2 to the LY6E-3 antibody, LY6E-3 was shown to have much higher toxicity in the MCF7 cell line. The anti-estrogen therapies exhibited more toxicity in the LY6E KO cells as compared to the WT cells, indicating that LY6E expression plays a role in sensitivity to these therapies, which is consistent with the patient data. Further, combination of the LY6E antibody and anti-estrogen therapies exhibited an improved combined effect, providing evidence that LY6E antibody or antibody-drug conjugates may be clinically combined with current anti-estrogen therapies. Overall, this study provides evidence that LY6E expression plays a role in the cytotoxicity of anti-estrogen therapies and suggests that combination of LY6E antibodies or antibody-drug conjugates with anti-estrogen therapies may have a clinical impact.

Student

Sara Botwinik

Biography

I am a rising senior at Mercer University in Macon, GA majoring in biochemistry and molecular biology. For the past three years in my undergraduate biochemistry lab, I have worked on the production of nanodiscs with MSP1D1. I co-authored a paper on determining alternative media for emerging model system marine bacterium Vibrio natriegens. I am a math TA and chemistry TA at my university. This past year, I have also worked at a non-human primate facility as a technician assistant where I helped provide care for cynomolgus macaques and assisted in diagnostic lab processing. I plan on pursuing a career in the medical field after finishing my undergraduate degree.

Title: Structure & Function of Immunoglobulins

Abstract

Immunoglobulins are an incredibly essential component of our adaptive immune system. Immunoglobulins consist of light chains and heavy chains. These chains fold into characteristic antiparallel beta strands and are composed of variable and constant regions. Gene rearrangement such as somatic recombination allows for variability and diversity across antibodies. Variable domains are composed of three complementarity-determining regions. Aside from the variable regions, the stem of antibodies has the constant regions. The constant regions will ultimately determine the effector function of the antibody. The first type of antibodies produced on the surface of naive B cells are IgM and IgD. Isotype switching allows B cell receptors IgM and IgD to differentiate into IgA, IgG, and IgE by class-switching recombination. Antibodies such as IgM and IgA are multimers. These dimer and pentamer structures allow for neutralization, complement activation, and opsonization. Understanding the relation between structure and function of antibodies is key for the production of treatments such as immunotherapies and vaccines.

Student

Ben Auerbach

Biography

I am high school graduate entering freshman year of university. I have spent many years working with the Joint Pathology Center both on the Walter Reed and Glen Echo campus working directly with the pathologists in their work. This is my first year working at USUHS and I intend to continue my research efforts into the foreseeable future.

Title: Preparation for Confirmation of SANT-CTNNB1 Relationship

Abstract

Sclerosing angiomatoid nodular transformation (SANT) is a rare, benign fibrovascular lesion of the spleen. It is most common in middle-aged adults with a slight female predominance. It is also typically asymptomatic and discovered incidentally during imaging studies or abdominal surgeries. There is radiologic overlap with other splenic vascular lesions, making histologic analysis critical for diagnosis. Some research has suggested SANT to be a non-neoplastic process, however a study identified the loss of the CTNNB1 exon 3 in 7/7 cases tested, leading the authors to favor SANT to be a neoplastic process. Given the lack of consensus on the neoplastic or nonneoplastic nature of this condition, we aimed to further evaluate SANT using genomic sequencing. Cases of SANT dating back to 1975 were identified within the Joint Pathology Center (JPC) repository. We performed JAK1 and STAT3 inhibitor staining to identify possible connections within the antibodies, and examined the results illuminating the expected connections.

Mentors

David Wisniewski

Mentees: Michael Valerio, Alyssa Belen and Emily Pangaro

Biography

I am an associate scientist in the laboratory of Dr. Geeta Upadhyay. I am working on several projects, broadly focused on characterizing the LY6 family of proteins with novel therapeutics. This includes combining LY6E antibody or antibody drug conjugate with tamoxifen or anastrozole in ER+ breast cancer, as well as synergistically combining LY6D and LY6K antibodies in various cancer models. Outside of work, I enjoy spending time with my family, going to the beach, running, reading, playing video games and watching movies.

Changqing Yuan

Mentees: Sara Botwinik

Biography

2005, Ph.D in China.

2005~ 2009, Postdoc, NCI-Frederick, MD

2009 ~ 2018, Senior specialist, Johns Hopkins University, Baltimore, MD.

2018~ 2020, Research fellow (contract), Astrazeneca, Gaithersburg, MD.

2020~2025, Senior Scientist, Evolveimmune Therapeutics, Branford, CT.

2026~ present, Scientist II, HJF, Bethesda, MD

Yong Chul Kim

Mentees: Ben Auerbach and Alivia Lombardo

Biography

Dr. Yong Chul Kim received his PhD in microbiology from Kyoto University, Graduate School of Medicine. Dr. Kim received his postdoctoral training at the National Institute of Health, Bethesda, MD. Dr. Kim is a Research Associate in CBCP labs at USUHS. His research focuses on the molecular mechanism of breast cancer progression and chromatin biology in the context of targeted therapeutics including small molecules. When Yung-Chul is not in the lab, you can find him biking around the DMV area and enjoying the outdoors.

Presentations

Group 1: Ms. Emily Pangaro, Ms. Alyssa Belen

Title: Elucidating the Role of LY6E in ER+ Breast Cancer Therapeutic Response

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Group 2: Mr. Ben Auerbach, Ms. Alivia Lombardo

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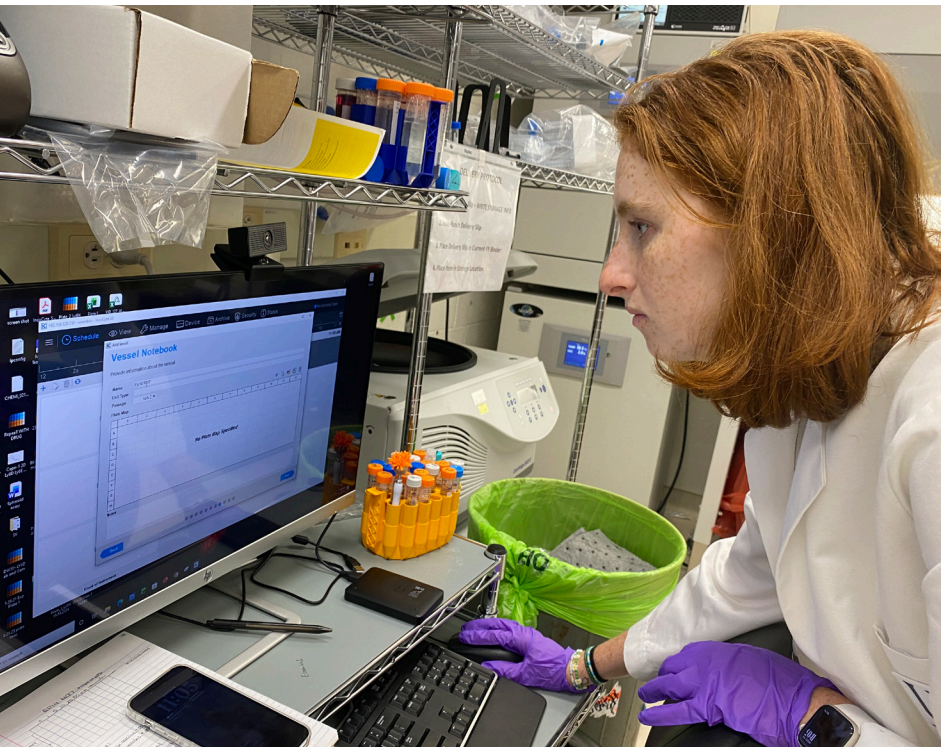
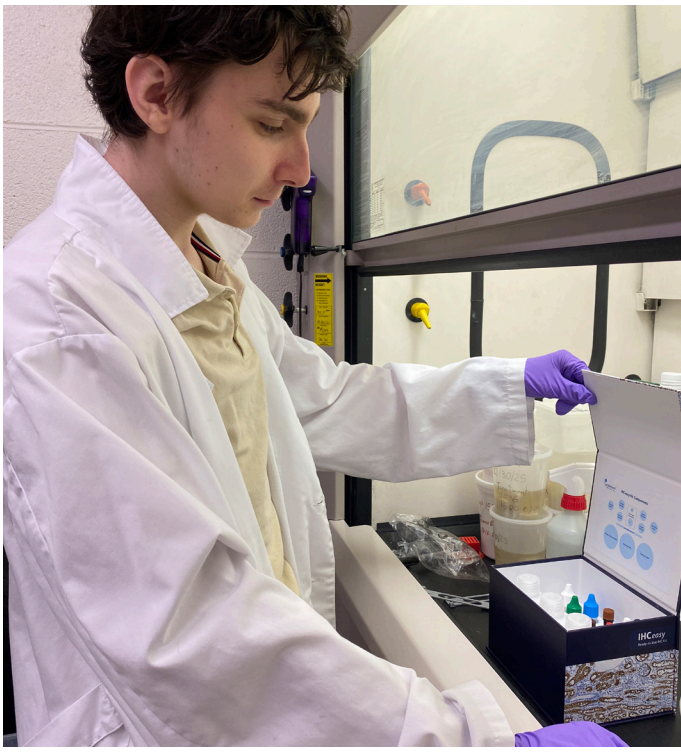
Title: Synergistic Growth Inhibition by LY6D Antibody, LY6K Antibody, and LY6K Small Molecule Inhibitor NSC243928 in Diverse Cancer Cell Models



Emily Pangaro setting up Incucyte



Ben Auerbach getting ready for IHC assay

Elucidating the Role of LY6E in ER+ Breast Cancer Therapeutic Response

By: Alyssa Belen (Summer Student), Emily Pangaro (Summer Student), and Dr. David Wisniewski (Associate Scientist)

Background

Breast Cancer

- A disease where abnormal cells grow in the breast tissue causing tumors
- Affects 2.4 million women globally in 2024 according to WHO
- Treatments include surgery, radiation, and medication

Estrogen Receptor Positive (ER+) Breast Cancer

- When high levels of estrogen in the body help the cancer spread and grow faster
- Makes up about 70% of all breast cancer cases in the United States

Background

Lymphocyte Antigen-6 (LY6) Gene Family:

- LY6 locus is frequently amplified in human cancer

LY6E

- Glycosylphosphatidylinositol (GPI)-anchored cell surface protein - a lipid anchored protein on the cell surface
- Has been linked to malignant tumor growth

Background

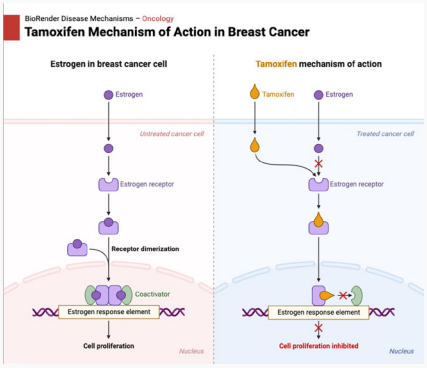
Hormone Therapies

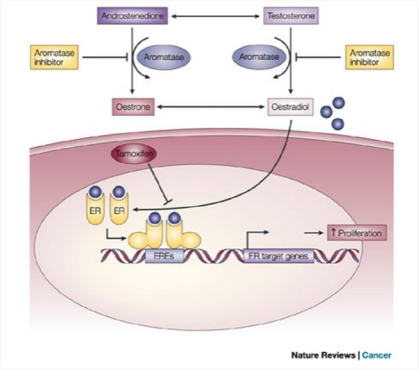
- Tamoxifen: Blocks the estrogen receptor
- Anastrozole: Prevents the body from making estrogen (aromatase inhibitor)

Aromatase Inhibitors

- Aromatase= enzyme that helps make estrogen
- Aromatase Inhibitor= drug that blocks the estrogen making enzyme

Drug Mechanisms

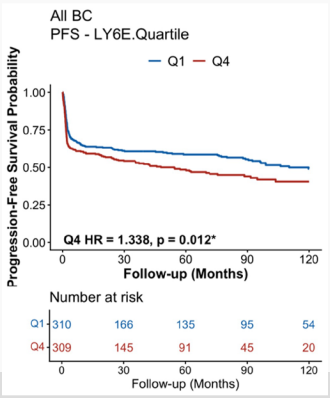




Significance of Study

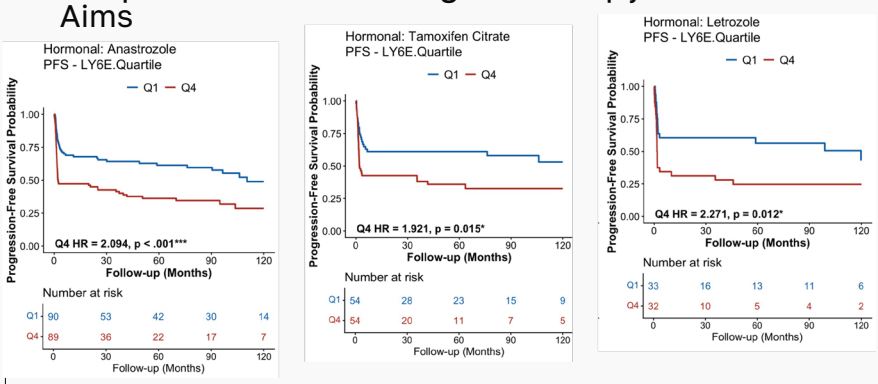
- Problem: Patients with high LY6E expression appear to respond poorly to treatments
  - LY6E is a protein found on the surface of many cancer cells. High LY6E levels are linked to worse patient outcomes.
- Can we confirm this relationship between LY6E and estrogen receptor therapy?
- Can we therapeutically target LY6E with an antibody to improve the efficacy of estrogen receptor therapy?

High LY6E Expression Predicts Poor Patient Outcomes in Breast Cancer



Unpublished Data (Nam Nguyen)

Patients with High LY6E Have Worse Response to Anti-estrogen Therapy Aims



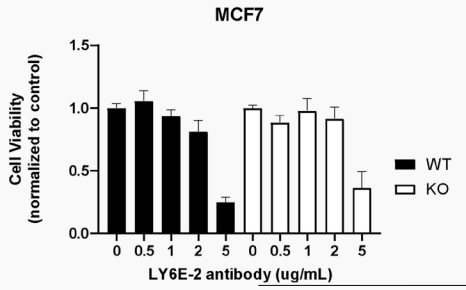
Unpublished Data (Nam Nguyen)

Project Aims

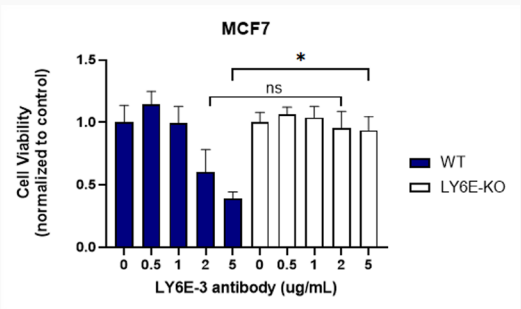
- Determine whether LY6E knockout affects response to tamoxifen and anastrozole
- Confirm LY6E antibodies are specific to LY6E
- Test whether LY6E antibodies increase tamoxifen or anastrozole toxicity

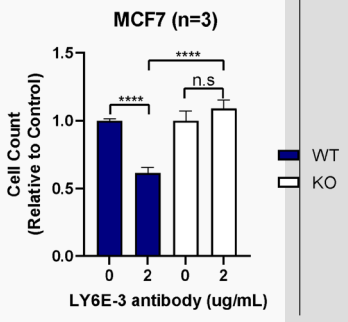
Does LY6E-2 or LY6E-3 work more effectively to target surface LY6E?

LY6E2 Killing in MCF7 WT vs KO Cells at Varying Concentrations



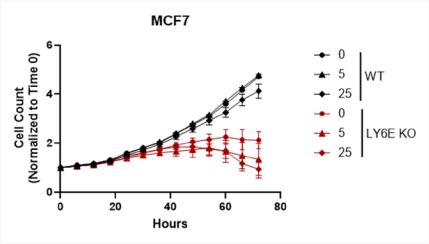
LY6E3 Killing in MCF7 WT vs KO Cells at Varying Concentrations

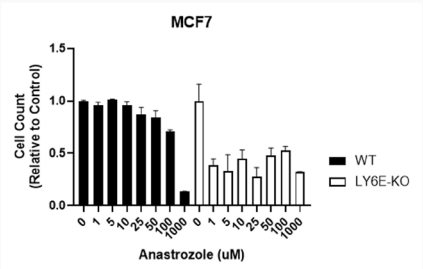




Does LY6E KO affect Anastrozole or Tamoxifen toxicity?

Anastrozole Drug Curves

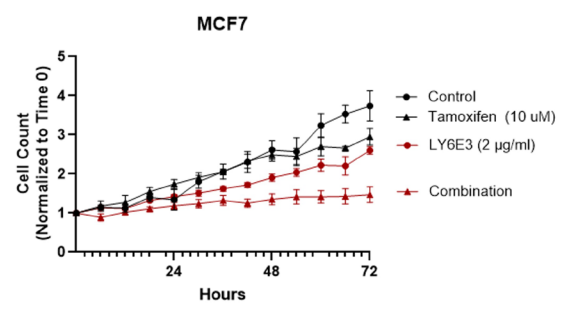




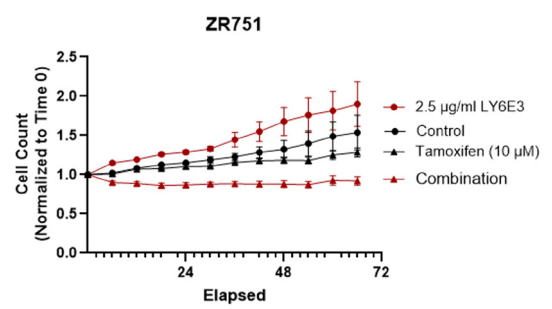
WT IC50: 218 uM
LY6E KO IC50: 4 uM

Does LY6E KO affect Anastrozole or Tamoxifen toxicity?

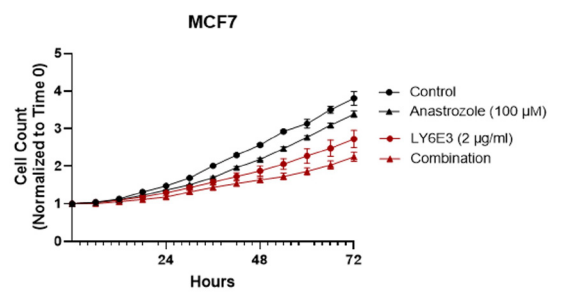
MCF7 Tamoxifen and LY6E3 Antibody Combinations



ZR751 Tamoxifen and LY6E3 Combinations

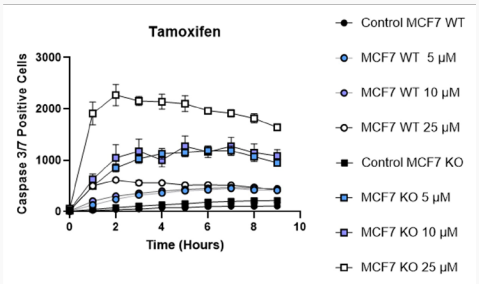


MCF7 Anastrozole and LY6E3 Combinations

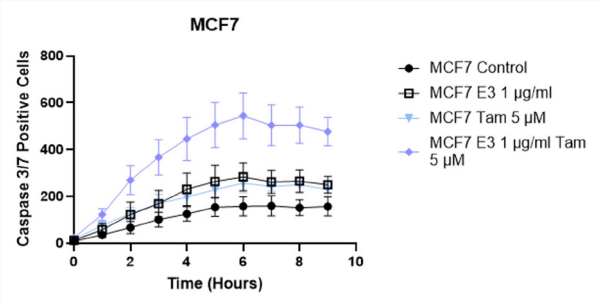


Is the Decrease in Cell Count Due to Apoptosis?

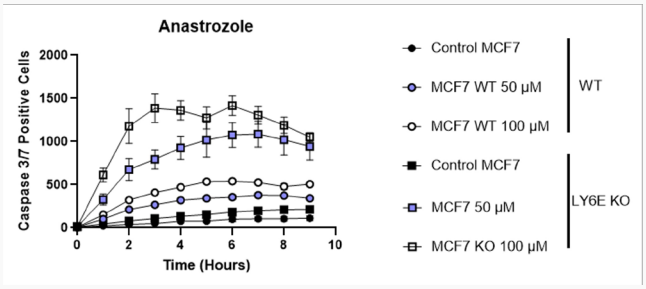
Tamoxifen MCF7 Caspase Data



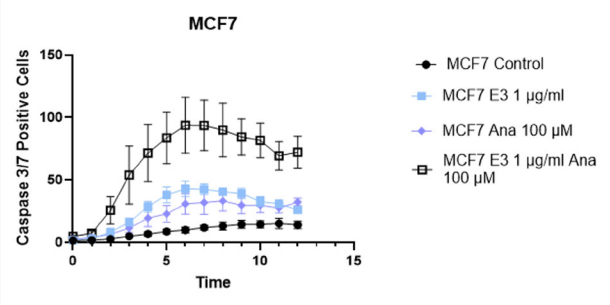
E3 and Tamoxifen MCF7 Caspase Data



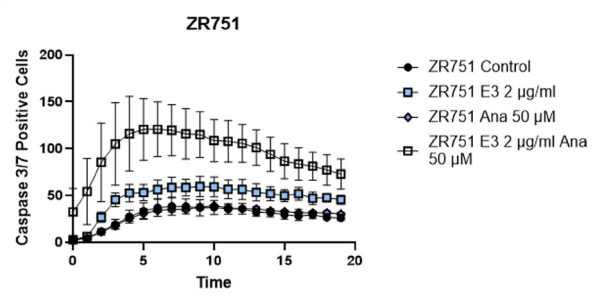
Anastrozole MCF7 Caspase Data



E3 and Anastrozole MCF7 Caspase Data



E3 and Anastrozole ZR751 Caspase Data



Conclusions

- Confirmed LY6E KO in the MCF7 cells
- LY6E3 antibody is more effective than LY6E2 antibody
- Anastrozole and tamoxifen kills cells more effectively in the MCF7 LY6E KO
- LY6E antibody treatment increases effectiveness of anastrozole and tamoxifen in ER+ breast cancer cells

Future Directions

- Statistical analysis of data
- Calculate synergy scores for drug combinations
- Determine mechanism by which LY6E KO or antibody affects estrogen receptor therapy
- Expand study to more cell lines
- Test whether LY6E antibody drug conjugate also affects tamoxifen and anastrozole toxicity

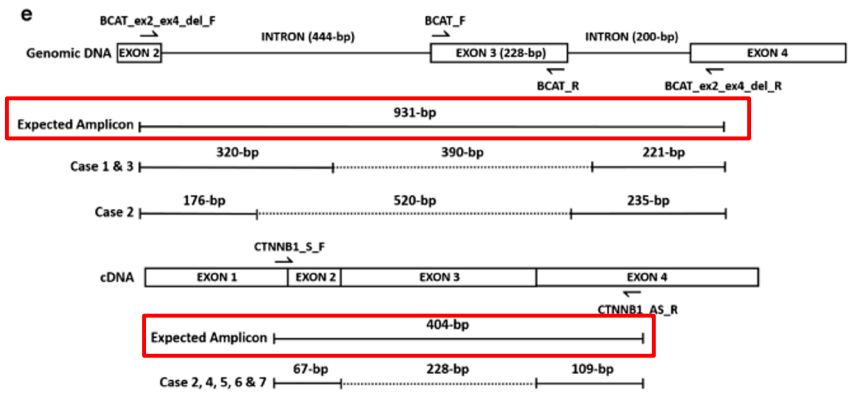
Acknowledgements

We would like to thank Dr. Geeta Upadhyay, Dr. Shriver, the Murtha Cancer Center, and the Henry M. Jackson Foundation for making all of this possible.

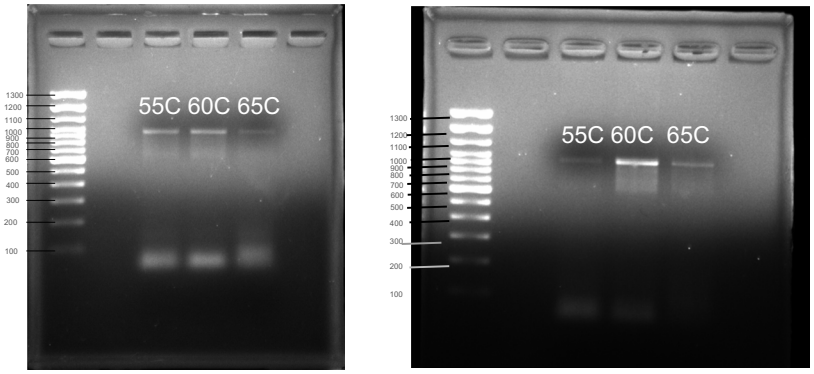
Week of July 13

Preparation for Confirmation of SANT-CTNNB1 Relationship

Primer Preparation



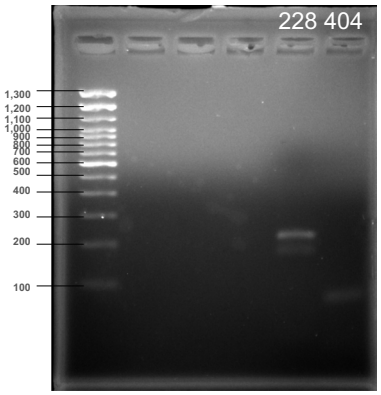
Primer set 1, 931bp results:



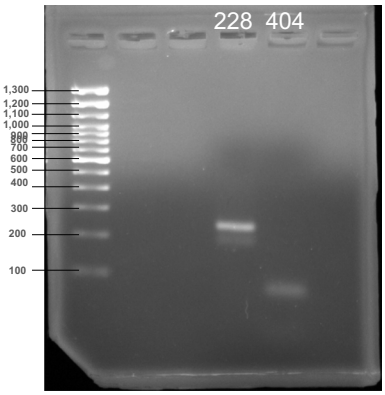
Breast cancer cells Hs578t

Ovarian cancer cells Ovsaho

Testing primers BCAT(228bp) and CTNNB1_AS(404bp)



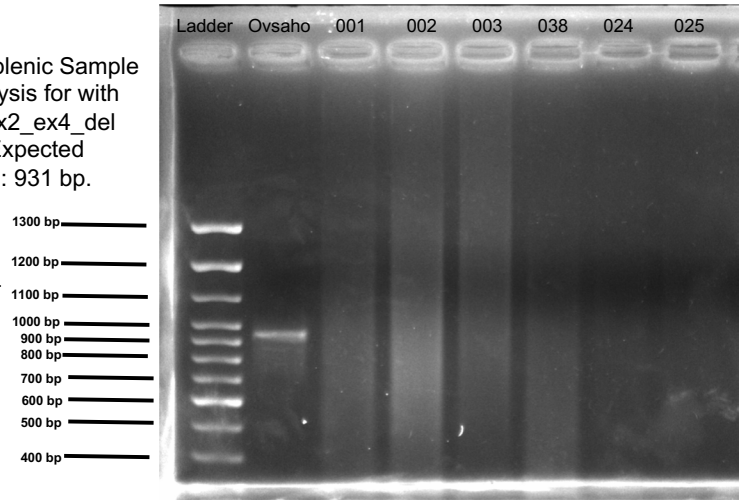
Breast cancer cells Hs578t



Ovarian cancer cells Ovsaho

SANT Splenic Sample Gel Analysis for with BCAT_ex2_ex4_del primer. Expected amplicon: 931 bp.

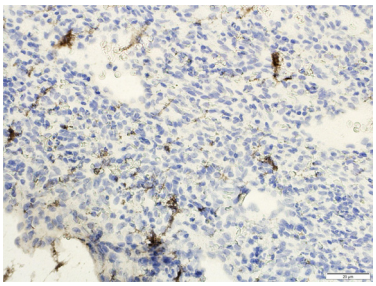
Ovsaho cells used for control.



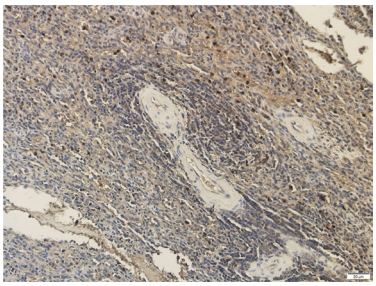
JAK1 and STAT3

- Activation of the JAK1/STAT3 pathway is a strong indicator of a neoplastic nature of SANT.
- We hand-stained 12 cases of SANT with JAK1 through IHC with positive and negative controls. The following are some of our results.
- Brown indicates the presence of the JAK1 Antibody.

Case Example 1

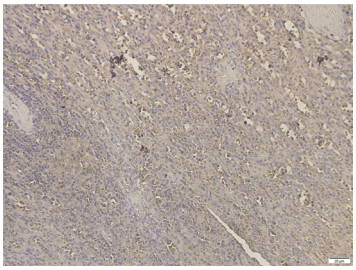


Negative JAK1 Control Stain

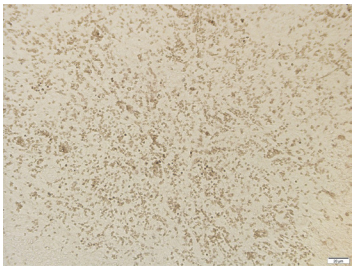


Positive JAK1 Stain

Case Example 2

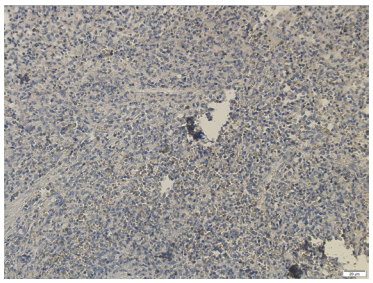


Negative JAK1 Control Stain

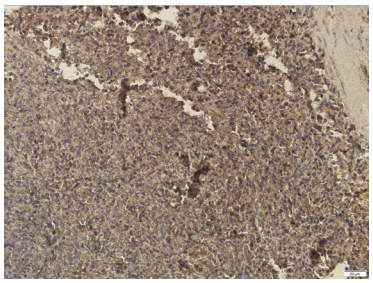


Positive JAK1 Stain

Case Example 3

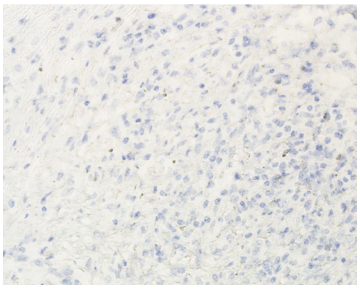


Negative JAK1 Control Stain

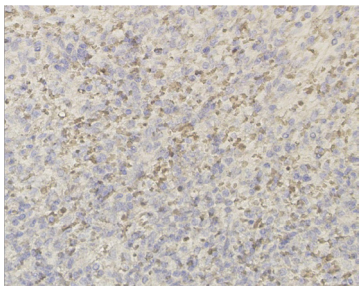


Positive JAK1 Stain

Case Example 4

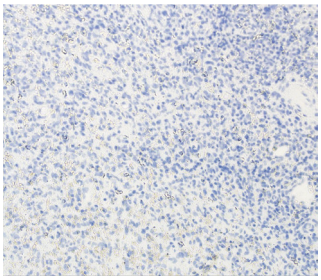


Negative JAK1 Control Stain

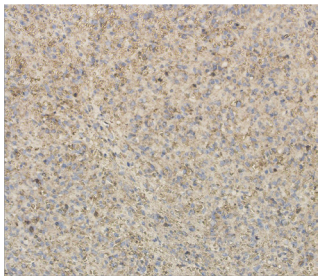


Positive JAK1 Stain

Case Example 5



Negative JAK1 Control Stain



Positive JAK1 Stain

Moving forward

These are promising results, we intend on performing more IHC to confirm.

Next week we plan to scrape slides for genomic DNA

We will also be able to stain for STAT3 to further look into the full JAK1/STAT3 Pathway connection.

Structure & Function of Immunoglobulins

Immunoglobulins

- Abs are heterodimers (antiparallel β strands) composed of a heavy chain and a light chain.
- The heavy & light chains have constant and variable regions.
- Constant domains specify effector functions and determine the class of Ab.
- The antigen-binding fragment (Fab) contains 1 complete L chain (V_L and C_L) and the V_H and C_H1 portion of 1 H chain.

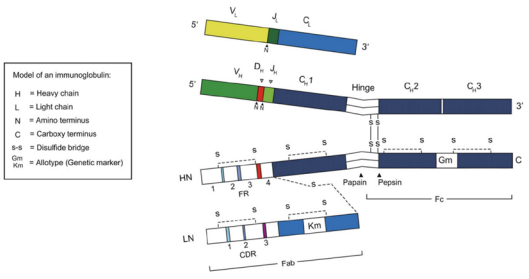
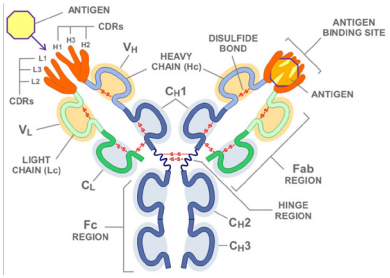


FIG 1. Two-dimensional model of an IgG molecule. The H and L chains at the top deconstruct the antibody at a nucleotide level. The chains at the bottom deconstruct the protein sequence. See the text for further details.

Heavy Chain

- 80 V_H gene segments
- During early B cell development, H chains form a complex with unconventional **λ** light chains, known as "surrogate" or "pseudo-light" chains (ΨLC), to form a pre-B cell receptor.
- The region of the ΨLC gene that corresponds to CDR-L3 covers CDR-H3 in the pre-B cell receptor, allowing the pre-B cell to avoid antigen-specific selection.



Light Chain

- L chain has no Diversity gene segment, only Variable and Joining → Relies on recombination.
- **λ Locus:** 30-36 potentially functional Vλ gene segments and an equal number of pseudogenes.
- **κ Locus:** 5 Jκ and 75 Vκ gene segments
- TdT can introduce random N nucleotides to either replace some or all of the lost Vκ or Jκ nucleotides. Also can add to the original germline sequence.
    - Initial diversification of the κ repertoire is focused at the VJ junction of the light chain CDR3 (CDR-L3).

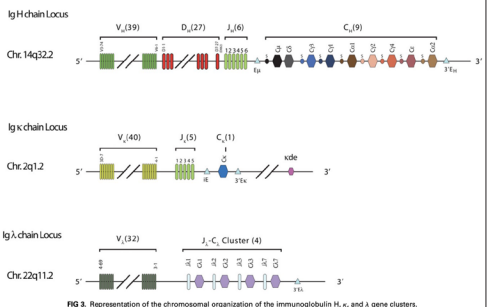
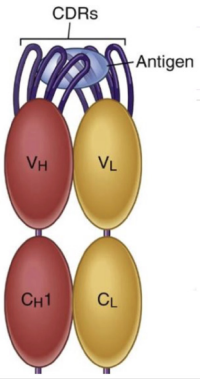


FIG 3. Representation of the chromosomal organization of the immunoglobulin H, κ, and λ gene clusters. The typical numbers of functional gene segments are shown. The κ gene cluster includes a κ-deleting element that can rearrange to sequences upstream of Cκ in cells that express λ chains, reducing the likelihood of dual κ and λ light chain expression.

CDRs

- The primary sequence of the V domain is functionally divided into 3 hypervariable regions: CDR1, CDR2, & CDR3.
- Complementarity-determining regions (CDRs) are situated between 4 regions of framework regions (stable sequences).
- CDR3 is the most variable region (easiest to mutate for Ab production).



V(D)J Recombination

- 3 CDRs of the H chain and the 3 CDRs of the light chain.
- H chain rearrangement can yield as many as 38,000 different VDJ combinations
- Random nucleotides are added in recombination → Randomness contributes to our ability to have diverse TCRs and BCRs.

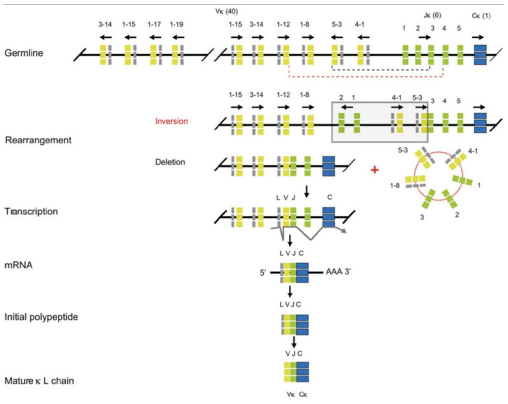
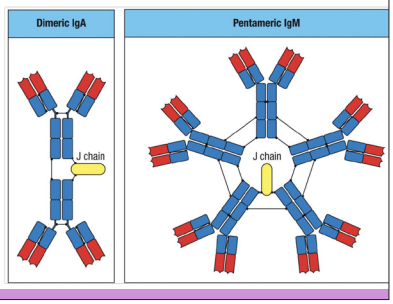


FIG 2. Rearrangement events in the human κ locus. See the text for further details.

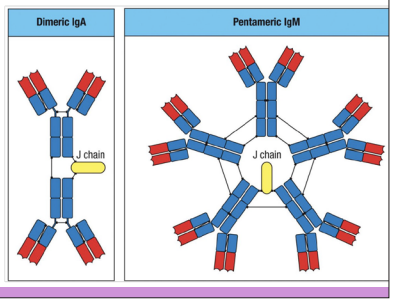
Heavy Chain Isotypes

- Class-switching recombination results in isotype switching. IgM, IgD → IgA, IgG, IgE
- IgD that is membrane-bound is involved in signaling. Circulatory IgD effector functions are unclear.
- IgM: first Ig produced during B cell development.
  - Pentamer upon maturation.
  - Avidity compensates for its low affinity.



Heavy Chain Isotypes

- IgA: in mucosal layers and secretions. Secretory IgA (sIgA) is a dimer with a J chain & is more efficient at damage prevention to epithelial cells.
- IgG: predominant isotype in the body. Has longest serum half-life.
  - IgG1 & IgG3: induced in response to protein Ag.
  - IgG2 & IgG4: associated with polysaccharide Ag.
- IgE promotes upregulation of FcεR expression (associated with hypersensitivity responses).



References

Schroeder H.W. Jr, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol.* 2010;125(2 Suppl 2):S41-S52. doi:10.1016/j.jaci.2009.09.046.

Synergistic Growth Inhibition by LY6D Antibody, LY6K Antibody, and LY6K Small Molecule Inhibitor NSC243928 in Diverse Cancer Cell Models

Michael Valerio
Dr. David Wisniewski & The Upadhyay Lab
USU Summer Scholars Program
Duke University, Class of 2029

Background on LY6K & LY6D in Cancer

Genes of the LY6 family have been found to serve as **prognostic biomarkers** and **potential therapeutic targets** for cancer progression

- Often associated with **poor patient outcomes**
- Upregulated in several cancer lines

Significance of Study

Using LY6D and LY6K as therapeutic targets could help provide better **individualized treatments**

- Observing synergistic effects in these drugs would mean that the combinations can be given to individuals with more aggressive cancers
- Classifying each cancer cell is also helpful for determining patient outcomes

Study Aims

My study investigated the **synergistic potential** of the monoclonal antibodies **LY6D-2 and LY6K-3** in combination with the LY6K small-molecule inhibitor **NSC243928** across diverse cancer cell models

- Hypothesized that **combined treatment** with these agents would produce **greater growth inhibition than individual therapies** by targeting complementary cellular pathways
  - Cells treated with each antibody and drug at varying concentrations, alone or in two-drug combo
  - Before treatment, we performed surface expression tests on some of these cells to determine what proteins were detected by an antibody stain, and thus could be targeted by our drugs

Methods & Results Overview

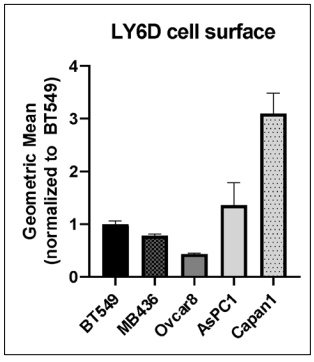
- Cell growth and/or viability were assessed using an **incucyte** plate reader over 72 hours
- Drug interactions were analyzed using **total viability numbers** to determine synergistic effects
  - Synergistic effects observed in numerous lines

This study provides evidence supporting further investigation of this combination as a **potential targeted anticancer therapy** and establishes a foundation for future mechanistic and preclinical studies

LY6D Surface Staining

Identifying the presence of LY6D protein on the surface of different cancer cell lines

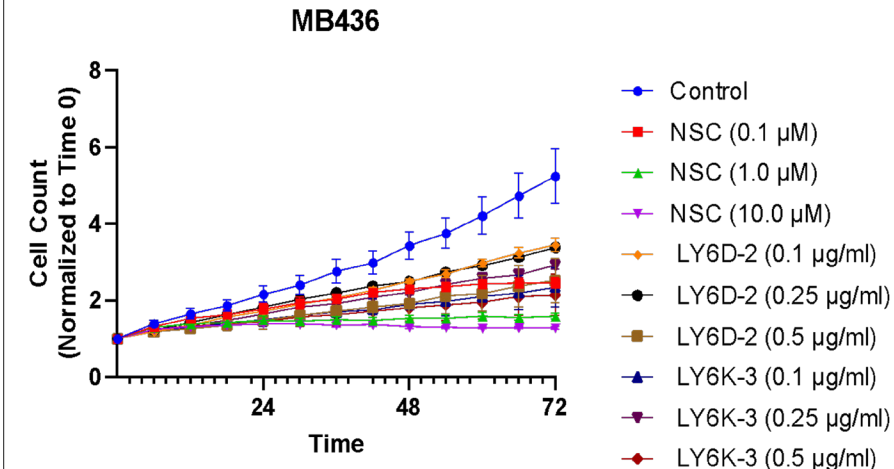
- Ensuring that the LY6D-2 antibody has a target to bind
- Some cells have much higher expression than others
  - We hypothesized that these cells would show the best response to LY6D-2 in our synergy experiments
  - E.g., Ovcar8 has low LY6D expression → poor response to LY6D-2

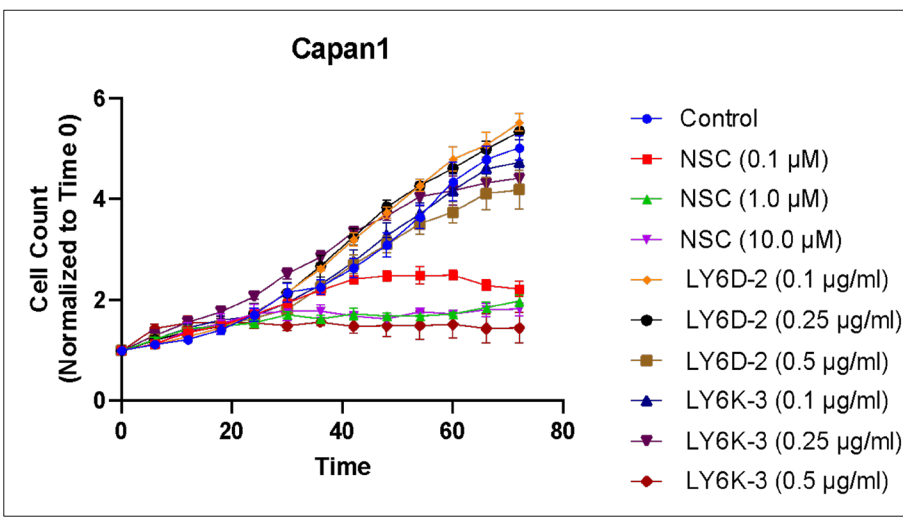


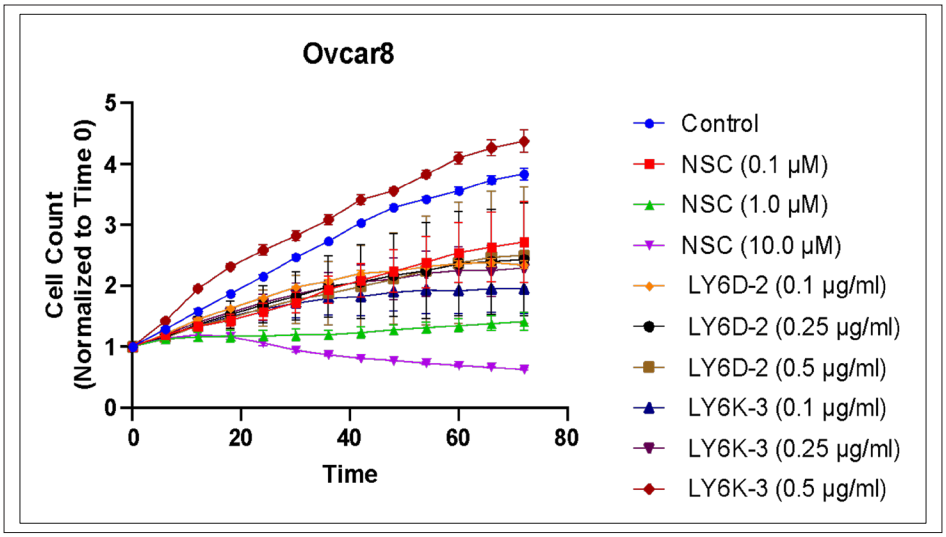
Single-Drug Treatments

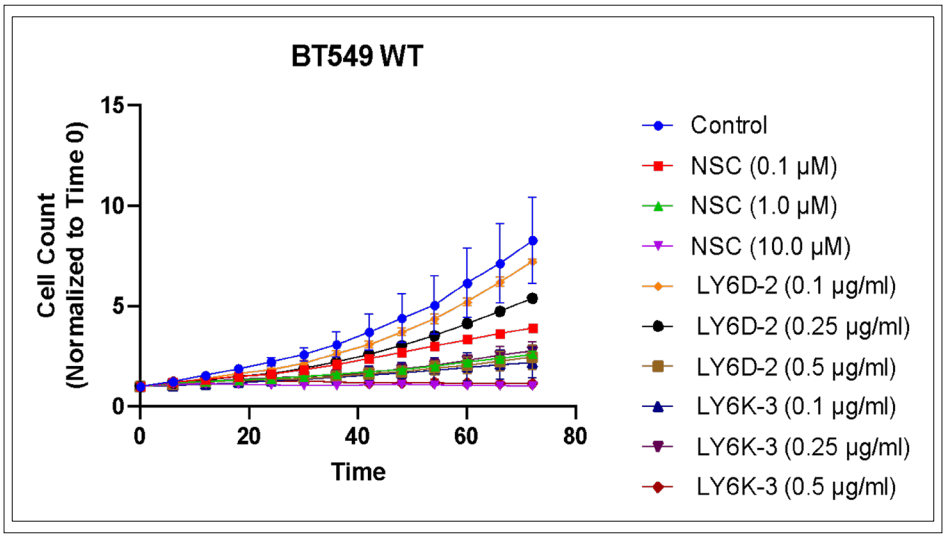
Started with treating cell lines with three concentrations of each drug in isolation

- Blue line is control (no antibody/drug)
- Different cells = different responses
  - NSC appears to be relatively effective across all cell lines; more effective at higher concentrations
- These tests allowed us to establish a concentration range for synergy experiments









Synergy Scores

We used an incucyte to quantify cell growth in different treatment conditions, normalized to time zero control

- Two examples of matrices on right

Graphs are calculated using four different scoring systems

- An average score of **10.00** or more across the four tests indicates a combination having good synergy
  - Keep in mind - **red** is **good** synergy, **green** is **poor** synergy

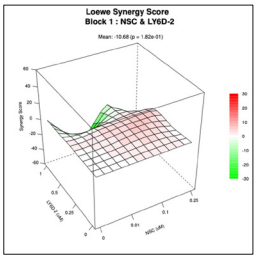
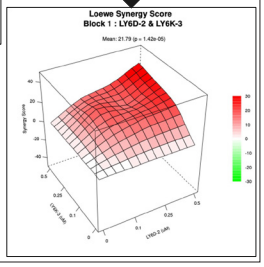


Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	26.03	21.79	22.4	27.21



Group 4: Mr. Michael Valerio

Title: Synergistic Growth Inhibition by LY6D Antibody, LY6K Antibody, and LY6K Small Molecule Inhibitor NSC243928 in Diverse Cancer Cell Models

Ovcar8 NSC vs. LY6D-2 Data

24h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	NSC	LY6D-2	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	7.12	4.79	4.14	7.5

48h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	NSC	LY6D-2	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	3.55	-0.3	-0.19	6.01

72h

Table 1: Drug Combiantion Meta Data

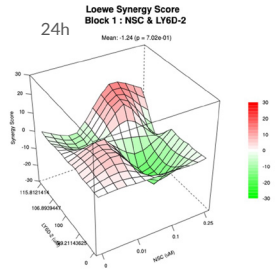
Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	NSC	LY6D-2	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	3.24	10.82	-5	-5.77

Ovcar8 NSC vs. LY6D-2 Graphs

24h



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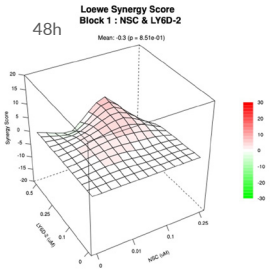

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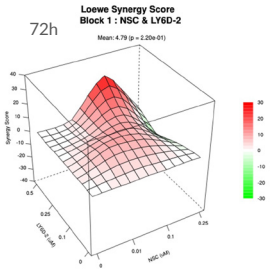


MB436 LY6D-2 vs. LY6K-3 Data

24h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	26.03	21.79	22.4	27.21

48h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	29.33	24.68	27.94	30.22

72h

Table 1: Drug Combiantion Meta Data

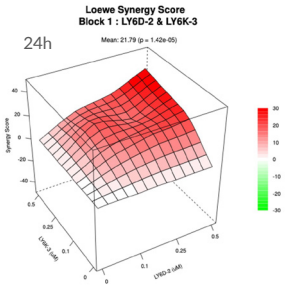
Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	36.79	24.78	33.36	39.17

MB436 LY6D-2 vs. LY6K-3 Graphs

24h



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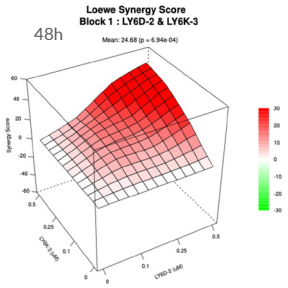

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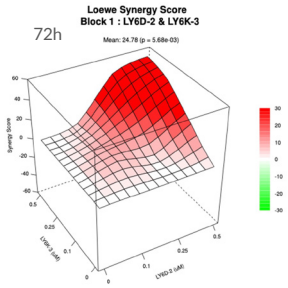


MB436 LY6K-3 vs. NSC Data

24h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6K-3	NSC	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	29.49	27.65	30.99	33.73

48h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6K-3	NSC	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	36.56	30.49	39.21	38.36

72h

Table 1: Drug Combiantion Meta Data

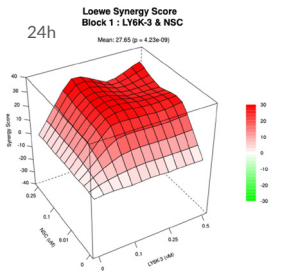
Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6K-3	NSC	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	32.98	24.84	37.96	37.05

MB436 LY6K-3 vs. NSC Graphs

24h



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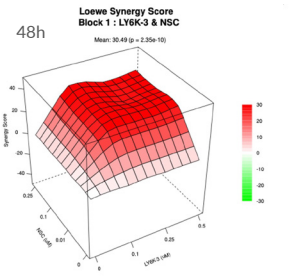

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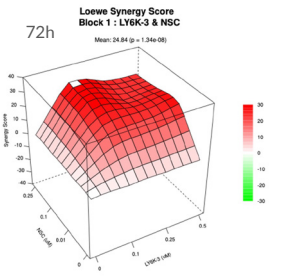


BT549 WT LY6D-2 vs. LY6K-3 Data

24h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	25.98	18.72	17.35	32.44

48h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	74.79	19.66	48.13	83.13

72h

Table 1: Drug Combiantion Meta Data

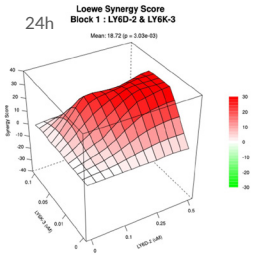
Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	LY6D-2	LY6K-3	uM	uM

Table 2: Synergy Score Summary Table

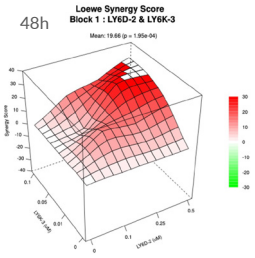
Block ID	ZIP	Loewe	HSA	Bliss
1	64.23	25.7	45.35	69.94

BT549 WT LY6D-2 vs. LY6K-3 Graphs

24h



48h



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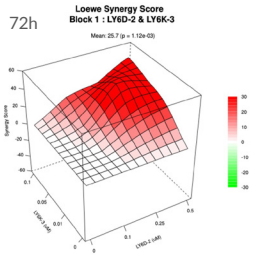


Capan1 NSC vs. LY6D-2 Data

24h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	NSC	LY6D-2	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	4.91	4.96	8.13	5.28

48h

Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	NSC	LY6D-2	uM	uM

Table 2: Synergy Score Summary Table

Block ID	ZIP	Loewe	HSA	Bliss
1	11.63	16.78	16.01	10.46

72h

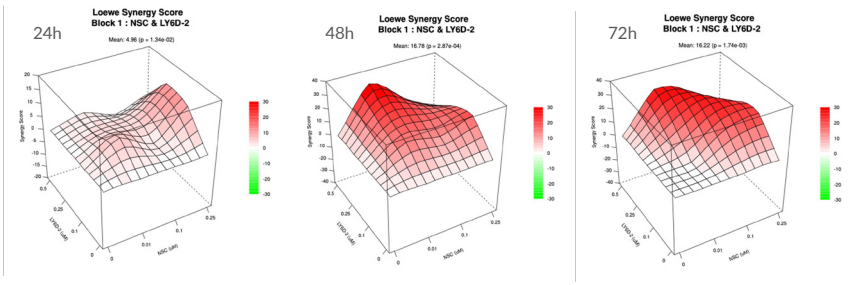
Table 1: Drug Combiantion Meta Data

Block ID	$Drug_1$	$Drug_2$	$Conc\ Unit_1$	$Conc\ Unit_2$
1	NSC	LY6D-2	uM	uM

Table 2: Synergy Score Summary Table

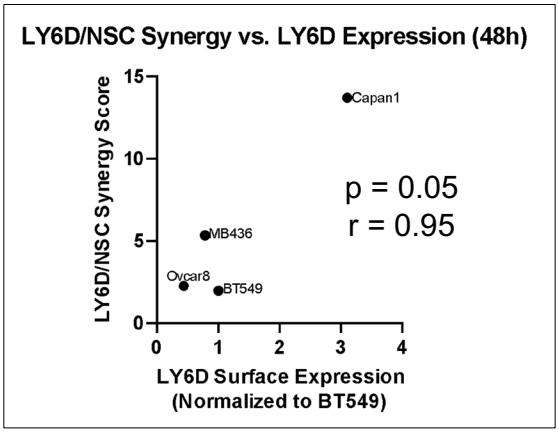
Block ID	ZIP	Loewe	HSA	Bliss
1	9.83	16.22	16.37	7.42

Capan1 NSC vs. LY6D-2 Graphs



Correlation

No other combinations at any time point showed a statistically significant correlation



Future Directions

Caspase analysis

- We are taking synergy combos in various cell lines deemed to work well and performing a caspase assay to determine if the cells are **actually undergoing apoptosis**
  - Currently testing MB436 (breast) with LY6D-2 + LY6K-3 and NSC + LY6K-3 combinations

Optimize LY6K cell surface expression + test correlation with synergy scores

- Determine whether expression affects the synergistic effect of the drug combinations

AnnexinV/PI stain

- More quantification of apoptosis in synergistic combos

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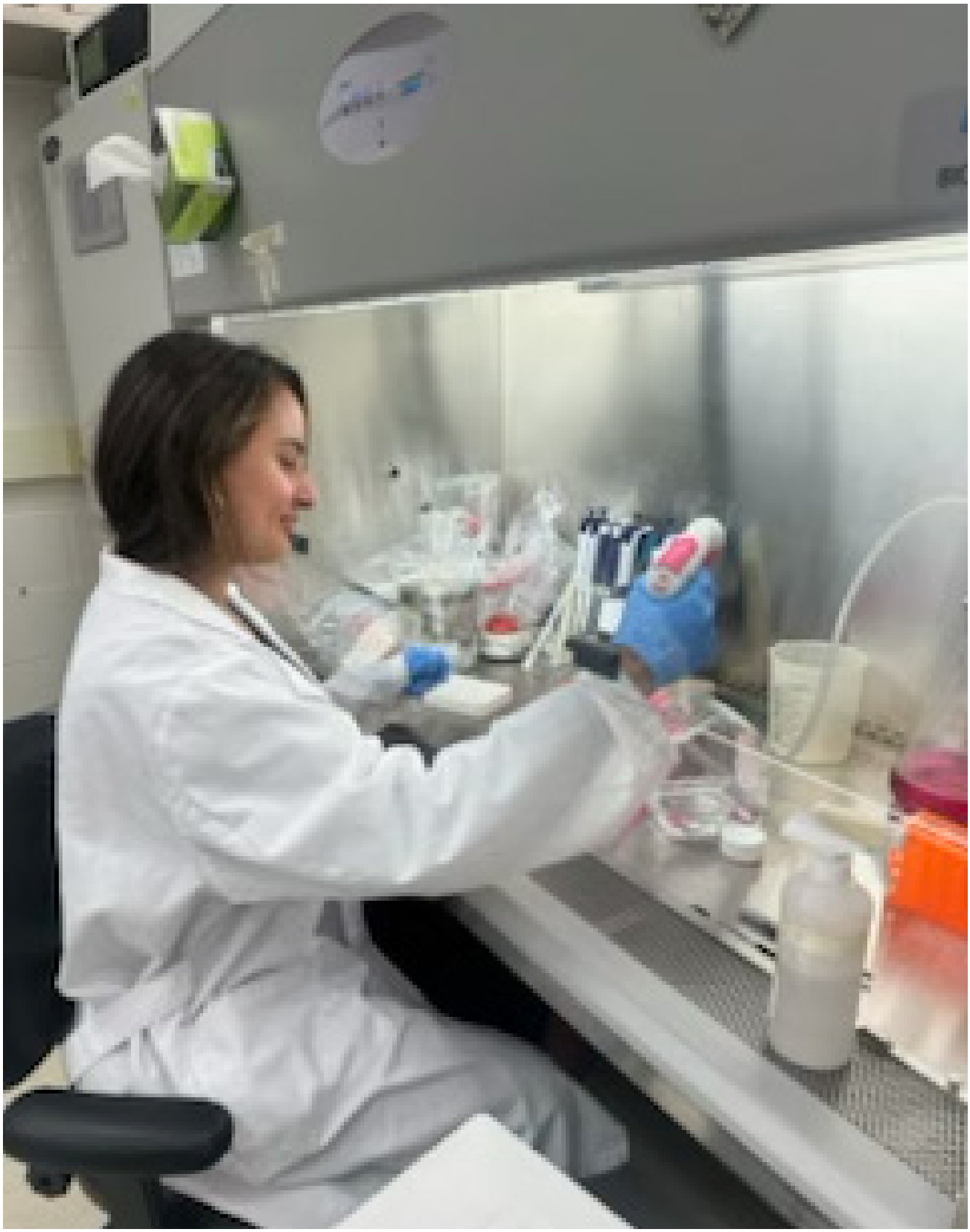
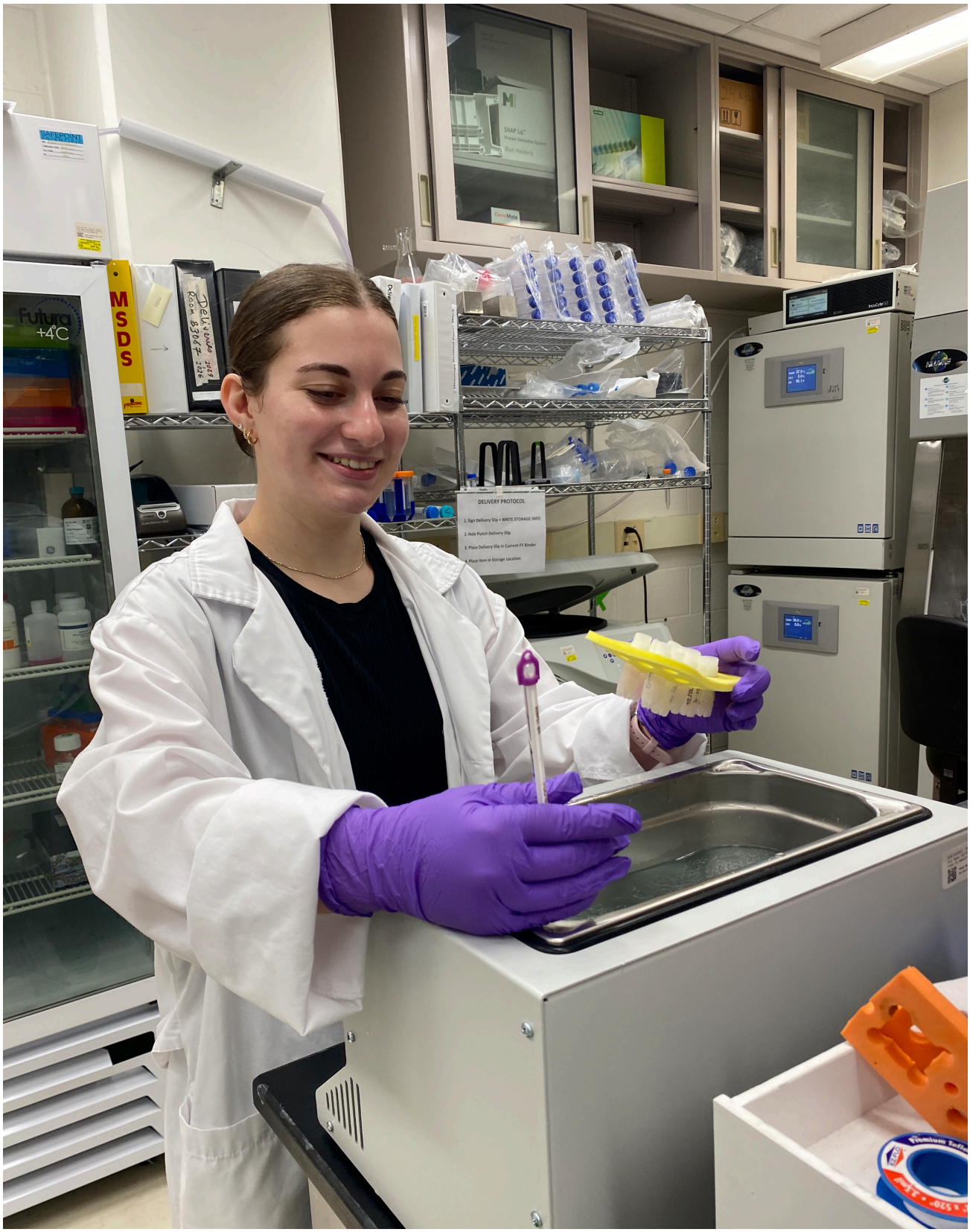
- Dr. Geeta Upadhyay - principal investigator
- The Upadhyay Lab - affiliate lab
- The Uniformed Services University Summer Scholars Program
  - 2026 Undergraduate Scholar
- The Henry M. Jackson Foundation for the Advancement of Military Medicine

Thank you to all!



Alyssa Belen performing cell culture



Sara Botwinik thawing cells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