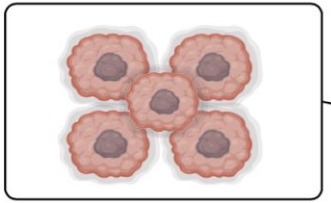
Dual Antibody Conjugated Silk Nanoparticles as a Targeted Delivery System for GBM Therapies

Names: Maddie Yost, Sabrina Zhang, Olivia Zeiden, Elysia Chang
Faculty Advisor: Dr. David Kaplan
Mentor: Sunny Shaidani

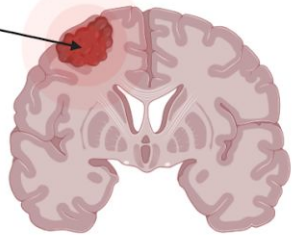
Dual Antibody-Conjugated Nanoparticles for Targeted GBM Treatment

1 Glioblastoma Multiforme (**GBM**) is an aggressive tumor initiated by mutated astrocytes that can be found in the brain and spinal cord.



Like many tumors, gliomas contain biomarkers of certain receptor and protein mutations.

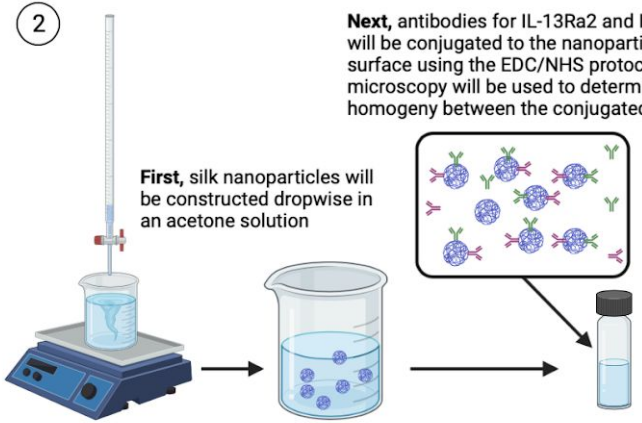
These mutations can be used as targets for drug delivery systems, like antibody conjugated nanoparticles



2

First, silk nanoparticles will be constructed dropwise in an acetone solution

Next, antibodies for IL-13Ra2 and EGFRviii will be conjugated to the nanoparticle surface using the EDC/NHS protocol; microscopy will be used to determine homogeny between the conjugated NPs



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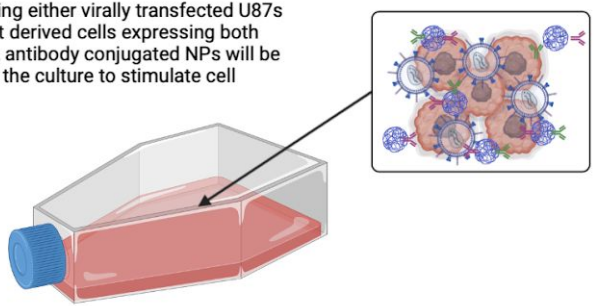
Then, using either virally transfected U87s or patient derived cells expressing both antigens, antibody conjugated NPs will be added to the culture to stimulate cell uptake



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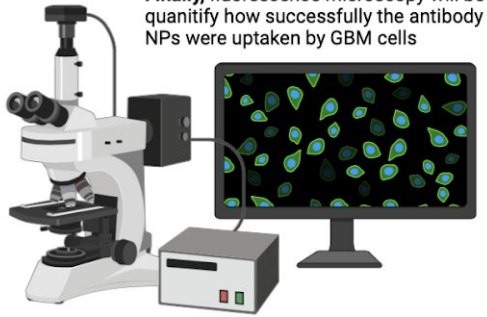
Finally, fluorescence microscopy will be used to quanitify how successfully the antibody conjugated NPs were uptaken by GBM cells



Project Goals

- ✓ **Determine the best receptor for targeting**
- ✓ **Formulate the appropriate nanoparticle size for dosing and tumor uptake**
- **Induce successful dual antibody conjugation**
- **Determine the proper antibody quantity**

Specific Aim 1:
Develop dual antibody conjugated silk nanoparticles

- Determine appropriate size and target receptors via lit review
- Create product:
  1. Process silk and form nanoparticles (100-120nm)
  2. Conjugate IL-13Ra2 and EGFRviii to loaded NP surfaces using EDC/NHS crosslinking
- Characterize nanoparticles:
  1. Quantify ratio of antibodies on NPs via fluorescent imaging or flow cytometry
  2. Ensure successful crosslinking of antibodies

Specific Aim 2:
Create 2D cell culture model using transfected U87 cells

- Transfect U87 cells to express the two receptors of interest:
  1. Infect cells with lentivirus carrying genes for receptors
  2. Characterize cells to ensure receptor expression using western blot, ELISA, or flow cytometry
- Culture cells on 2D plate

Specific Aim 3:
Characterize therapy efficacy

- Load nanoparticles onto the 2D model:
  1. Evaluate binding efficacy and nanoparticle uptake
- Compare efficacy of dual-antibody, antibody A, and antibody B nanoparticles

Project Design Chart

Characteristic	Target Value	Why This Value	How We Will Test
Nanoparticle size	100-120 nm	Appropriate size for entering tumors via leaky vasculature and for tumor cell uptake	DLS/SEM imaging
Nanoparticle antibody expression	**TBD** → enough to have efficient uptake in GBM cells	Throughout various experiments, we will determine the target value for nanoparticle antibody expression based on which values optimize cellular uptake	FTIR Analysis, Fluorescence microscopy with secondary antibody
Silk concentration	6%	6% silk has been determined by past studies to result in 100-120 nm particles	Concentration calculations by weighing 1000ul of silk solution, leaving overnight in 60°C oven, and weighing remaining silk
Uptake efficiency	**TBD** → enough to have efficient uptake in GBM cells	This value will be dependent on the various experiments we conduct to test nanoparticle antibody expression uptake efficiency (uptake is changed a lot by cell line & nanoparticle size	FITC and lysosomal fluorescent microscopy or flow cytometry
Cell receptor expression	Cells express one of each receptor	This is important to test the efficacy of dual antibody conjugation, making sure both biomarkers are expressed whether we transfect cells with both, or receive IL-13Ra2 cells and transfect with EGFRviii	Flow cytometry and/or Western blot

What Has Been Done so Far?

01

LITERATURE REVIEW

Glioblastoma Multiforme (GBM) vs Breast Cancer vs HCC

02

ANTIBODY RESEARCH

IL-13Ra2 vs EphA2 vs EphA3 vs EGFRviii

03

LAB WORK AND RESEARCH

Silk processing, cell culture, and research on purchasing antibodies and conjugation kits

04

SILK NANOPARTICLES

Created silk nanoparticles with diameter of ~76 nm
(but could be closer to 100-120 nm as the DLS needs to be serviced)

05

SECONDARY RESEARCH

Consulted Dr. Priceman from City of Hope about GBM research and cells he could give to us

06

WEBSITE

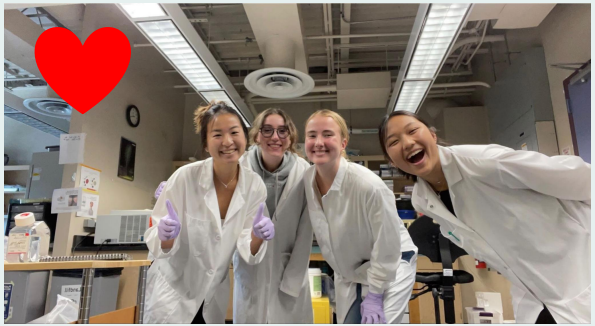
Designed team website to update as project progresses

Aims	Sub-Aims	Completion	September	October	November	December	January	February	March	April	May
Define Project		100									
1st Biweekly Report		100									
Antibody Lit Review	Choose Antibody for Targeting Research	100									
Silk Processing		100									
Silk Nanoparticles		100									
2nd Biweekly Report		100									
Cell Culture Training		50									
Technical Proposal Report Draft		100									
Project Presentations		100									
Preparing Nanoparticles		100									
Start Website	Add in Home Page, People Sections, References, and Project Update	100									
3rd Biweekly Report		100									
Risk Assesment Analysis		100									
Update Website	Update Project Section and References	100									
Order Materials Necessary for Antibody Conjugation		50									
4th Biweekly Report		100									
Update Website	Update Project Section and References	100									
Project Presentations		100									
Finalized Web Site		100									
Technical Report		100									

Project Next Steps

1 Receive City of Hope cells

Confirm U87s endogenous expression of both IL-13Ra2 and EphA2



EXPRESSION

NO EXPRESSION

2 Culture New Cells

Practice cell culture on U87 cell line expressing receptors of interest

2 Submit Amended Lentivirus Registration

Determine proper transfection protocol for U87 cells and send to Biosafety Office

3 Order Antibodies

Order IL-13 and either EphA2 or EGFRviii antibodies