INTRODUCTION
Gastric cancer is a frequent cause of death worldwide. It has been linked to various environmental factors that differ around the world. In the United States, risk factors vary from smoking to eating salty to heavily processed foods, to the H. pylori bacteria. Gastric carcinomas are often therapy resistant and due to their cellular heterogeneity, this makes it difficult to establish a “one size fits all” therapeutic approach in patients.

miRNAs are small non-coding RNAs that found to be implicated in gene expression and regulation. One miRNA in particular, miR-10b, has been found to be highly oncogenic on metastatic cancers, with its overexpression linked to proliferation, migration, and tumor size. Oncogenic miRNAs operate via targeting tumor suppressor genes, silencing expression and permitting abnormal cells to divide uncontrollably, contributing to the growth of cancerous tumors. Increased levels of miR-10b are associated with poorer overall survival and serve as a contributing prognostic factor.

The limited treatment options around gastric cancer has led Calin’s group to develop a novel therapeutic binding miR-10b. This study reports on the effect of Compound 25, a derivative of the FDA-approved therapeutic linifanib. This compound was developed in collaboration with the Institute for Applied Cancer Sciences (IACS) at MD Anderson Cancer Center.

HYPOTHESIS
We hypothesize that miR-10b drives cancer through binding tumor suppressor genes and that Compound 25 decreases binding of miR-10b. Therefore, high miR-10b expression in gastric cancer cells will be impacted by the compound, leading to malignant cell death.

METHODS
The learning step: to gain a strong foundation on my project, I reviewed over 50 papers on miRNAs and miR-10b in PubMed. After my first week in the laboratory, I was able to perform cell culture and by the second week, I was maintaining several cell lines across three tissue types. I shadowed senior colleagues on RNA extraction, cDNA preparation, Western blots, and PCR techniques. I then learned to plate cells, use the microscope, and start new cell lines. By the sixth week, I did my own PCR to generate data for an ongoing publication. Afterwards, I was taught to evaluate and analyze the data.

The experimental step: For our study we cultured three different cell lines. GES-1 is the human gastric epithelial cell line of normal origin. AGS is an adherent stomach cancer tissue cell derived from a 54-year-old female. SNU-1 is a metastatic suspension stomach cancer tissue cell from a 44-year-old male. These cells have been cultured, passaged and split every 2-3 days.

Our experiments began by plating cells into wells and inserting a sequential dosage of Compound 25 to find our optimal dosage. For best results, the cells were incubated for up to 48 hours. Pictures were taken at 24 and 48 hours at 4x to compare cell density and morphological differences between the control wells and the wells treated with 5µM of Compound 25. After 48 hours, we performed RNA extraction, cDNA preparation and conducted a miR-10b quantitative PCR.

RESULTS
After treating the three cell lines with Compound 25, we used microscopy to assess morphological and phenotypic changes within the SNU-1 and AGS cells (gastric cancer cell lines). Fortunately, we were able to see that there were minimal differences to the GES-1 cell line (normal epithelial gastric line) validating that there was low miR-10b levels in normal cells, confirming it as a targeted therapeutic. By evaluating RNA expression using PCR, we tested miR-10b expression in both cell lines and patient samples derived from a collaborator at MD Anderson Cancer Center, supplementing our cancer cell line data. We identified a reduction of miR-10b after the treatment with Compound 25 specifically in malignant cells, correlated with the death of malignant cells but not of normal cells.

CONCLUSION
Oncogenic miR-10b is upregulated in gastric cancer cell lines and has limited to undetectable expression in a normal gastric line. Compound 25 target miR-10b in cancer cell lines and has the potential for translational application in a deadly type of cancer, the gastric cancer.

FUTURE DIRECTIONS
More research will be conducted regarding the specific mechanism of miR-10b in gastric cancer metastases and the specific genes that Compound 25 is targeting.

REFERENCES

*Diagrams generated in BioRender