This project discusses the synthesis, characterization, and anticancer activity of Arsenoplatin-4, a new anticancer compound. A brief introduction of arsenoplatins starts with a background of two commonly used cancer treatments.

Platinum drugs are the most used widely cancer treatment today, currently included in nearly half of all chemotherapeutic protocols. While one of these compounds, cisplatin, is effective at treating a variety of solid tumor cancers, a resistance to treatment often develops. The only additional FDA approved inorganic drug for cancer treatment, arsenic trioxide ($As_2O_3$), is limited to the treatment of acute promyelocytic leukemia (APL) and some blood cancers including multiple myeloma. About 90% of APL patients are cured with $As_2O_3$, but due to its rapid clearance, it has been ineffective in the treatment of solid tumor cancers. In an effort to overcome the shortcomings of these two drugs, a new class of very potent anticancer agents named arsenoplatins have been synthesized containing both arsenic and platinum moieties. When the anticancer activity of Arsenoplatin-2 was compared to $As_2O_3$ in the National Cancer Institute human tumor cell line screen, a high Pearson correlation coefficient (r=0.96) was obtained (Figure 1). This high correlation potentially indicates that AP-2 serves as a delivery vehicle for $As_2O_3$.

Recent work suggests a diiodide analogue of cisplatin, cis-$PtI_2(NH_3)_2$, produces increased cytotoxicity levels and the ability to overcome platinum resistance[2].This has led to the development of an iodide analogue of AP-2, labeled AP-4. The purpose of this project was to synthesize and test the anticancer activity of this new compound, AP-4.

X-ray, NMR, and elemental analysis have been completed to determine the structure and purity of this new compound. The cytotoxicity of AP-4 in the triple negative breast MDA-MB-231 cancer cell line has been evaluated by MTS cell proliferation assay. Interaction of AP-4 with the biologically important molecule glutathione (GSH) was assessed by fluorescent detection assay.

Looking at our methods, an MTS cell proliferation assay was performed. First, MDA-MB-231 triple negative breast cancer cells treated with AP-4 and incubated for 72h at 37°C
A 20:1 MTS/PMS mixture was added to treated cells and incubated for 2h to quantify viable cells. Absorbance was then measured at 490 nm.

Then a Glutathione fluorescence detection assay Performed. MDA-MB-231 cells and MDA-MB-468 cells treated with 10µM and 30µM of AP-1 and AP-4. MDA-MB-231 cells incubated for 2h while MDA-MB-468 cells incubated for 4h. Cells were lysed with 5% SSA and a detection reagent added which binds to the free thiol group on GSH. The reaction mixture was incubated for 15 minutes at room temperature. Fluorescent emission read at 510 nm after excitation at 390 nm to determine free GSH concentration

To go over the results, AP-4 was successfully synthesized from the reaction of AP-2 and KI in a methanol-$H_2O$ mixture over 3 hours at 37°C. Purity of this complex was determined by proton NMR and elemental analysis. The structure AP-4, determined by single-crystal crystallography, showed a Pt-As bond length of 2.28343(4) Å compared to 2.2687(4) Å in AP-2. This difference in Pt-As bond lengths shows a weakened bond as a result of substituting chloride with iodide. Using ultraviolet-visible spectroscopy, it was determined that AP-4 reacts with GSH at a 1:1 ratio. The cytotoxicity AP-4 ($IC_{50}$ =15.75 (± 2.27) µM) was determined using colorimetric MTS assay. Glutathione levels were measured by Glutathione fluorescence

detection assay. Glutathione is a powerful antioxidant that protects against reactive oxygen species. High levels in tumor cells have been correlated with a resistance to platinum-based drugs and preliminary data on MDA-MB-231 cancer cells treated with AP-4 at a 30μM concentration show a decrease in free GSH concentrations. This decrease in GSH potentially sensitizes these cancer cells. The decrease in GSH concentration observed in cells treated with AP-4 is not apparent in cells treated with AP-1, in spite of its lower $IC_{50}$ value (9.5 ($\pm$ 0.1) μM).

As I mentioned earlier, the high Pearson correlation coefficient between $As_2O_3$ and AP-2 (r=.96) in the National Cancer Institute human tumor cell line screen shows the two compounds act in a similar manner. This indicates the potential for AP-2 to act as a delivery vehicle for $As_2O_3$. Similarities in $IC_{50}$ values between AP-4 and AP-2 ($IC_{50}$=17.5 μM) introduces the possibility that AP-4 may act as a delivery system as well. These potent compounds may be able to overcome rapid renal clearance associated with $As_2O_3$, one of the primary limitations of $As_2O_3$ as a drug to treat solid tumors.