ND*nano* Summer Undergraduate Research 2017 Project Summary

1. Student name & university

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2. ND faculty name & department

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3. Project title

Targeted Asparaginase for Improved Treatment of Pediatric Leukemia

4. New skills acquired during summer research

During my summer research, I learned techniques to attach polyethylene glycol (PEG) chains to a currently used protein drug. Also, I cultured lymphoblastic leukemia cells in media to analyze the toxicity of the protein drug and established a concentration dependent kill curve for a currently used chemotherapy drug. Last, I synthesized a molecule that has a high affinity for a macrocycle host, utilizing the rotary evaporator, lyophilizer, centrifuge, and magnetic stirrer.

5. Practical application/end use of research

Methods to treat acute lymphoblastic leukemia (ALL), the predominant form of pediatric leukemia, are constantly improving. Despite significant developments, ALL remains the leading cause of death for children in the United States. Furthermore, the treatment methods for ALL cause adverse side effects in children due to the toxicity and allergenicity of the therapeutic agents, and this is specifically problematic for a bacterially derived enzyme drug known as L-Asparaginase, a major component in treating ALL.[1] This project sought to target lymphoblastic leukemic cells to improve the therapeutic efficiency and reduce the dose and allergenicity of the enzyme drug L-Asparaginase.

6. Abstract of project

We sought to target the drug L-Asparaginase to lymphoblastic leukemia cells. Thus, we have explored routes to modify L-Asparaginase through bioconjugation for attachment of minimal supramolecular affinity motifs. Simultaneously, we are modifying tissue-specific labeling moieties with the complementary portion of these affinity motifs. Thus, a cell or tissue of interest could be labeled with one half of the affinity motif, which would subsequently serve to home the modified enzyme to this site through a supramolecular affinity axis.

7. References for papers, posters, or presentations of research

1. Pui, C. H.; Mullighan, C. G.; Evans, W. E.; Relling, M. V., Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood 2012, 120 (6), 1165-74.
2. Broome, J. D., Evidence That L-Asparaginase Activity of Guinea Pig Serum Is Responsible for Its Antilymphoma Effects. Nature 1961, 191 (479), 1114-&.
3. Muller, H. J.; Boos, J., Use of L-asparaginase in childhood ALL. Crit Rev Oncol Hemat 1998, 28 (2), 97-113.
4. Broome, J. D., Studies on the mechanism of tumor inhibition by L-asparaginase. Effects of the enzyme on asparagine levels in the blood, normal tissues, and 6C3HED lymphomas of mice: differences in asparagine formation and utilization in asparaginase-sensitive and -resistant lymphoma cells. J Exp Med 1968, 127 (6), 1055-72.
5. American Cancer Society: What Is Acute Lymphocytic Leukemia? Atlanta: American Cancer Society: 2016.
6. DeSantis, C. E.; Lin, C. C.; Mariotto, A. B.; Siegel, R. L.; Stein, K. D.; Kramer, J. L.; Alteri, R.; Robbins, A. S.; Jemal, A., Cancer treatment and survivorship statistics, 2014. CA Cancer J Clin 2014, 64 (4), 252-71.
7. SEER 9 Incidence & U.S. Mortality 1975-2015.

Project Summary

This project sought to develop a supramolecular affinity axis for the enzyme drug L-Asparaginase to cancerous cells for the treatment of pediatric acute lymphoblastic leukemia (ALL). The drug function of this enzyme arises through its catalysis of the reaction of the non-essential amino acid L-Asparagine to ammonia and L-Aspartic Acid. Lymphoblastic leukemia cells lose the ability to synthesize L-Asparagine and thus rely on circulating sources of this amino acid for their survival.[2-4] The enzyme depletes these circulating resources, contributing to cancer cell death. Targeting the enzyme to cancer cells would localize enzymatic activity to the immediate vicinity of the cell, reducing local L-Asparagine levels without needing to act systemically. This would reduce the dosage needed to observe an effect and decrease the allergenicity of the drug. Antibodies are the most common method to facilitate biological targeting; however, due to their large size (MW ~150,000 Da), attachment of antibodies to a protein drug is not feasible. High affinity small molecule motifs with affinity comparable to that of an antibody to its antigen, on the other hand, could facilitate the required targeting using a minimal construct. In this project, we have utilized bioconjugation techniques to attach minimal supramolecular affinity motifs, involving a host and a guest, to a labeling antibody and, separately, to L-Asparaginase. Using the high affinity of the guest for the host, the enzyme could be directed to the cancer site.

Three specific aims are identified for this project. First, bioconjugation techniques will be used to link L-Asparaginase to a supramolecular host and a targeting antibody to a high-affinity guest. To attach the supramolecular host to the enzyme, a polyethylene glycol (PEG) chain will first be conjugated to the enzyme, which will increase the stability, solubility, and circulation half-life of the enzyme in addition to providing a means for the attachment of the supramolecular host Cucurbit[7]uril (CB[7]). Separately, the labeling antibody will be modified with an adamantyl guest, which has a high affinity ($K_{eq} \sim 10^{12}$) for CB[7]. Second, the functional activity of the enzyme will be verified by an assay that detects the amount of ammonia, a direct product of the reaction catalyzed by L-Asparaginase, produced. Third, the therapeutic function of the supramolecular affinity axis will be evaluated in vitro by determining the cytotoxicity of the modified drug for cells in culture.

Throughout the course of this summer, the assay to detect the amount of ammonia produced by the reaction of L-Asparagine to L-Aspartic Acid through the drug L-Asparaginase was developed, and it quantified the potency of the drug. L-Asparaginase was successfully PEGylated, with the concentration of attached PEG chains varying from two to four chains, an initial goal of the project. This bioconjugation was most successful at a twelve molar excess of PEG. The functional activity of the PEGylated L-Asparaginase did not decrease after bioconjugation, which was a promising result, as PEGylation often inhibits the activity of enzymes. Independently, the adamantyl guest was synthesized, and its presence was confirmed by proton nuclear magnetic resonance (H NMR). An assay to determine the cytotoxicity of L-Asparaginase was made using a varied concentration of a chemotherapeutic drug. In the near future, the PEGylated enzyme will be modified with the host CB[7], and the adamantyl guest will be conjugated to the antibody. After the verification of the enzymatic activity, the affinity axis will be tested in vitro with Raji cells.

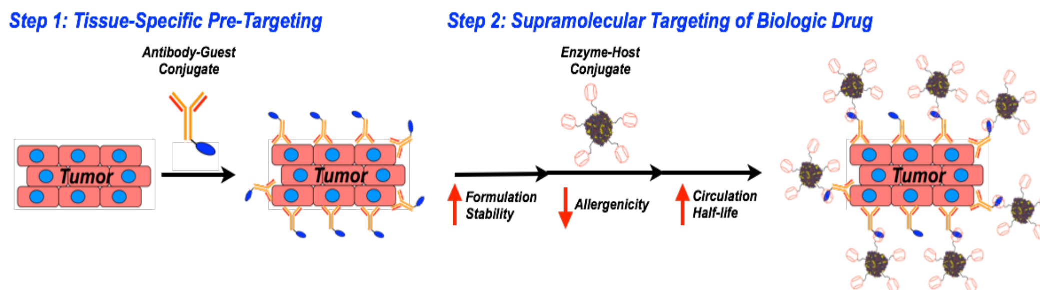

Figure 1: Depiction of our proposed method to target a tumor with a high affinity small molecule motif.
Photo Credit: Dr. Matt Webber