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*Department of Chemistry*
*University of Florida*

*2001 Program*
*Investigator Initiated (2-year project)*

**Project Title: Synthesis and characterization of asparagine synthetase inhibitors**

**Project Summary:** Several clinical studies have identified a correlation between the levels of asparagine (a simple amino acid) in the blood and the susceptibility of leukemia cells to chemotherapy. Unfortunately, current treatments cause the appearance of tumor cells that become drug-resistant by turning on the synthesis of asparagine synthetase (AS), an enzyme that synthesizes asparagine inside the leukemia cells. Highly specific compounds that prevent AS from working inside cells are therefore potential drugs for drug-resistant leukemias and other types of solid tumor that might arise as a result of smoking. The goals of this project were to discover the first potent AS inhibitors. Bioactive compounds identified in this project will be significant for developing new drugs for treating childhood leukemia and solid tumors in adults that may be smoking-related. In addition, access to potent, and specific, AS inhibitors will provide new tools for exploring the pathways in cellular metabolism that underpin tumor formation and growth.

**Project Successes:** The research team was able to develop and optimize protocols for making multi-milligram (20-30 mg) amounts of pure human AS in insect cells. Prior to this study, the enzyme had to be purified from liver cells using procedures that gave only microgram amounts of pure AS. The results from these studies, in combination with other experiments aimed at understanding the chemical mechanisms used by human AS, were then used to develop the first nanomolar inhibitor of the enzyme. This level of activity is close to that required for compounds that can be used clinically. Very recent work, carried out in collaboration with Dr. Michael Kilberg (UF Cancer Center), has also shown that this new inhibitor, which is a 100,000 times more powerful than any other AS inhibitor that has been previously reported, stops growth of a drug-resistant leukemia cell line in the laboratory thereby providing powerful support for this approach to potential clinical treatments.

**Selected publications from BRP funded research in Peer Reviewed Journals:**

Tesson AR, Soper TS, Ciustea M, **Richards NGJ.** Revisiting the steady state kinetic mechanism of glutamine-dependent asparagine synthetase from Escherichia coli. *Arch Biochem Biophys.* 2003;413:23-31.

Koroniak L, Ciustea M, Gutierrez JA, **Richards NGJ**. Synthesis and characterization of an *N*-acylsulfonamide inhibitor of human asparagine synthetase. *Org Lett.* 2003;5:2033-2036.

Ding Y, Wang J, Schuster SM, **Richards NGJ**. A concise preparation of N-acylphosphoramidates. Synthesis of β-asparaginyladenylate. *J Org Chem.* 2002;67:4372-4375.

**Selected presentations from BRP funded research:**

Wang X., **Richards NGJ**., Roitberg A. *Computational studies of ammonia channeling in amidotransferases.*New York: 226th National Meeting, American Chemical Society; September 2003. (Poster)

Abbatiello S, Eyler J, **Richards NGJ**, Powell DH, Gutierrez JA, Koroniak L. *Characterization of human asparagine synthetase by FT-ICR MS.* Montreal, Canada: ASMS Annual Meeting; June 2003. (Poster)

**New grants based in part on BRP-funded work:**
National Institutes of Health/National Cancer Institute
Title: Measuring asparagine synthetase expression in leukemia
Project period: June, 2004 – May, 2006
Award amount: $279,532