Structural and functional studies of engineered and chimeric plant-type asparaginases as novel antileukemics

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and the second most common acute leukemia in adults. L-Asparaginase has been used in ALL therapy since 1967. Currently, only enzymes of bacterial origin are clinically utilized. Unfortunately, these asparaginases contribute to serious toxicity and allergic reactions and their strong side effects indicate an urgent need for better therapeutic enzymes. Some new and promising candidates for antileukemic agents can be found among plant-type asparaginases. Plant-type asparaginases are present not only in plants but also in microorganisms, insects, and mammals.

This project aims to design, produce, and characterize new plant-type asparaginases. As a far-reaching goal, these novel enzymes should have good pharmacokinetics for efficient but gradual hydrolysis of asparagine and minimal side effects. The novel enzymes should have increased stability for efficient distribution, prolonged circulation in the blood, and reduced immunogenicity to avoid allergies and premature clearance by the immune system.

L-Asparagine (L-Asn) is one of the endogenous amino acids, while asparaginases are enzymes hydrolyzing L-Asn to L-aspartic acid (L-Asp) and ammonia (NH_3). In healthy cells, L-Asn is produced by asparagine synthetase, but most of leukemia cells are unable to produce L-Asn, therefore, these cells are solely dependent on exogenous circulating L-Asn. Therapeutic asparaginases work by depleting the pool of L-Asn circulating in the blood, thus inducing cancer cells starvation, and ultimately death (Fig.1).

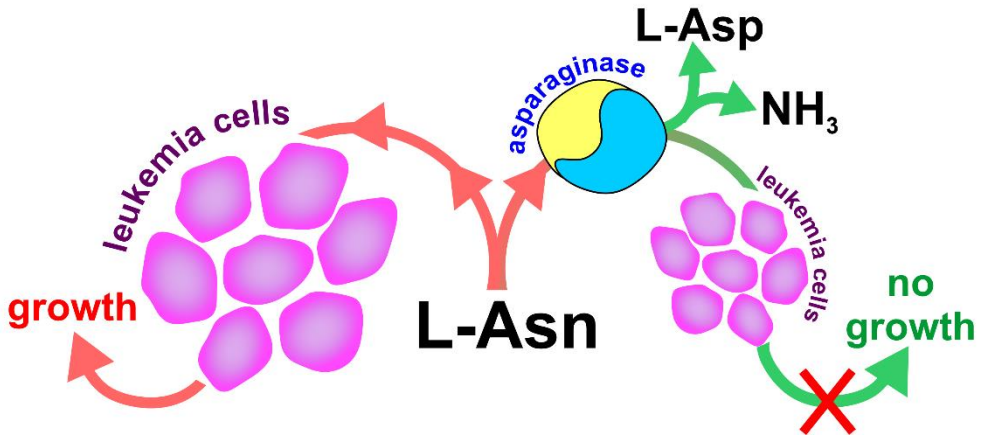

Fig.1. Dependence of leukemia cells on exogenous L-Asn: asparaginase works by depleting the circulating pool of L-Asn, resulting in starvation of cancer cells, inhibition of their growth, and finally death.

It would appear that finding therapeutic enzymes of human origin should be the best strategy, but several previous attempts at making human enzymes suitable for clinical applications have been unsuccessful so far. Similar trials of improving the currently utilized bacterial enzymes have been reported as well, albeit also without success. My preliminary experiments have revealed that the plant-type enzymes are highly susceptible to modifications; however, simple site-directed mutagenesis is insufficient to generate enzymes with improved activity. Therefore, in this project, more advanced approaches will be used to modulate the enzyme action, including elements of directed evolution and the production of chimeric proteins.

The molecular architecture of plant-type asparaginase can be described as a fusion protein built of two structural subunits (α and β) connected by a flexible linker. Such an architecture offers the possibility of designing chimeras. Chimeric proteins are built from sequences taken from two or more proteins, joined together into one polypeptide. Construction of chimeric proteins opens the possibility of designing protein folds with novel functions, derived from each component. In the case of plant-type asparaginases, joining subunits α and β from different organisms should affect the catalytic mechanism by local and global structure rearrangements.

The research planned within this project will be divided into several experimental steps. At each step, different aspects of the enzyme properties will be tested, such as stability, activity, (auto)proteolysis, and atomic structure. The novel, fully characterized enzymes will serve as prototypes for creating new therapeutic proteins in the future. The results will be important for biochemistry, enzymology and structural biology, and will promote the advancement of protein engineering and rational approaches to drug development.