MICROBIOTIX, INC. RECEIVES R01 GRANT TO CHARACTERIZE TYPE III SECRETION TARGETS WITH CHEMICAL PROBES

3 April 2012. Microbiotix, Inc, a privately held biotechnology company, announced that it was awarded a R01 grant from the National Institutes of Health/NIAID. The grant, entitled "Validating targets in *P. aeruginosa* type III secretion using chemical probes" provides four years of support to identify and validate targets in the type III secretion (T3SS) pathway that are potentially useful for future drug discovery.

The increasing prevalence of antibiotic-resistant strains of bacterial pathogens represents an unmet medical need. Type-three secretion is a mechanism used by many pathogenic bacteria to increase their virulence in human infections. Bacterial toxins secreted by this method reduce the protective effect of the infected individual's own innate immune system. Results of this project will provide detailed information on the identity and function of components of the type-three secretion apparatus that are vulnerable to attack by drugs, and thus, facilitate new drug discovery.

Pseudomonas aeruginosa is the leading cause of ventilator-associated pneumonia (VAP), and current antibiotic treatment strategies exhibit failure rates as high as 18%, even when the organism is susceptible to the antibiotic being administered. The goal of this project is to address this critical medical need by validating targets in the type III secretion (T3SS) pathway that are susceptible to inhibition by small molecules and determining their roles in the T3SS host-pathogen interaction. T3SS is the major *P. aeruginosa* virulence determinant contributing to the establishment and dissemination of infections (e.g., VAP, bacteremia, urinary tract infections). It is utilized by the bacterium to secrete and translocate toxin effectors into host phagocytes, thereby weakening the host's innate immune defenses. The presence of a functional T3SS is significantly associated with poor clinical outcomes and death in patients and markedly reduces survival in animal infection models. The strategy employed in this project is to use existing chemical probes to determine which components of the complex T3SS machine are susceptible to inhibition by small molecule compounds. Then, probes and strains carrying mutations in the probe targets will be used to define the roles of those vulnerable components in the host-pathogen interaction. Results will provide up to four well-validated, functionally-annotated, drugable targets in the T3SS host-pathogen interaction. Four published T3SS inhibitors with unrelated chemical structures that are inhibitory to *P. aeruginosa* T3SS at non-cytotoxic concentrations have been selected as probes. Two of the probes are potent inhibitors of both T3SS-mediated secretion and translocation of effector toxins while the other two probes inhibit only secretion or translocation. Thus, these four chemical probes likely inhibit at least three distinct targets or distinct regions within one or more targets. In Aim 1, two parallel approaches will be used to identify the molecular targets of these four probes – (a) addition of photo-reactive and molecular handle moieties to permissible sites on the probes, application of photo-affinity probes to modify the target(s), recovery and identification of modified proteins; (b) application of molecular genetic tools to enrich for probe escape mutants followed by identification of the mutated gene(s) by deep sequencing. Finally, target identity will be confirmed by mutation analysis, and target gene mutant libraries will be prepared to facilitate understanding the role of each target in T3SS. In Aim 2, the probes and mutant libraries will be used to define the role of each probe target in the T3SS host-pathogen interaction. Effects of the probes and mutants on twelve distinct steps in the T3SS pathway within the broad categories of regulation, assembly, secretion, translocation, and cytotoxicity will be used to dissect the roles of the targets in the T3SS machine and in host-pathogen interactions. Results will provide drugable, disease-relevant T3SS targets with characterized escape mutants for use in drug-discovery screening. Ideal targets will be prioritized as highly sensitive to rapid inhibition by probes and involved in critical roles in host-pathogen interactions.

We will accomplish the following specific aims: (1) Identify the molecular targets of four T3SS chemical probes; (2) Define the roles of each probe target in the host-pathogen T3SS interaction.

Donald Moir, Ph.D., Chief Scientific Officer, will serve as the Principal Investigator of the grant.

About Microbiotix

Founded in 1998, Microbiotix, Inc. is a product-focused biopharmaceutical company engaged in the research and development of novel, small-molecule, anti-infective drugs that address commercially significant medical markets. The company currently has several active research programs in the fields of anti-bacterial and anti-viral discovery, with three compound series in pre-clinical development. More information can be found on the company's web site, www.microbiotix.com.