Jan 12, 2010 **Immune Function in MS** AT Reder 10681A
NCT03344094

Study Design/Rationale:

Multiple sclerosis is an inflammatory disease that causes demyelination of the brain and spinal cord. In MS, 20-60 year-old patients, women more than men, develop episodes of neurologic dysfunction that last days to weeks and then partially resolve. Damage is cumulative and eventually becomes progressive. The immune system also evolves over time and becomes less sensitive to immune suppression. Demyelinating variants of MS such as neuromyelitis optica and CNS Sjogren's disease, have similar, but often more severe symptoms.

We will be studying proteins and RNA in white blood cells, and how these change during therapy of MS and demyelinating variants. White blood cells control some immune responses and may control the attacks, progression, and severity of MS. We will also analyze DNA polymorphisms of relevant genes identified in these studies. By studying these white blood cells, we hope to determine what causes MS and use this knowledge to design potential therapies for MS patients in the future.

More specifically, assays will evaluate the following:

Immune function: We will study immune function in multiple sclerosis, with assays of expression of costimulatory molecules and interferon receptors on the cell surface, also cytokine secretion, intracellular cytokines and signal transduction proteins, expression of mRNA for these proteins, NK function, and lymphocyte proliferation. This information is intended to determine the mechanism of action of various therapeutic agents in MS (unknown at this time).

Some examples of the cytokines to be investigated include interleukins, monokines, neuropeptides, interferons, TGF, and TNF; signal transduction molecules include JAK/STAT proteins, NF-kB, kinases, and phosphatases; costimulatory molecules include HLA-DR, B7.1, and B7.2.

The particular assay is dependent on the mechanism of action of the agent being studied in MS. All are variations on the same limited theme; all require a modest amount of blood; and all are subject to the same confidentiality arrangements. As examples, we will use ELISA and RT-PCR to measure lymphocyte production of cytokines (such as IL-10 and IFN-gamma) protein and mRNA because of its relevance to immune function in MS patients before and after interferon therapy; use flow cytometry to measure lymphocyte B7.1 membrane expression for comparison to patients receiving Copaxone therapy, use Western blots and intracellular flow cytometry to measure STAT-l levels for comparison to patients receiving interferon therapy, and use RT-PCR, micro arrays and next generation RNA sequencing to measure RNA levels and exon use.

Assays are done on blood from clinic population of MS patients and healthy age-matched normal controls, and from clinical trial patients. Blood from patients who are not part of trials, and also from patients who are in trials--before and after drug therapy, will be analyzed during the course of the study and compared to untreated patients and controls

Clinic visits typically occur at 3-12 month intervals. Trials of experimental or FDA-approved drugs last 1-3 years and typically require visits every 2-6 months. Blood will be drawn at regular clinic visits before therapy, at 1 & 3 mo, and at 1, 2, and 3 years after starting treatment, depending on duration of study. Research will take place in J218 and J220, SBRP.

Kinetics: Some patients with stable and active and progressive MS will be tested while on drug treatment. Here we will measure kinetic effects of immunomodulatory drugs. In the latter patients, blood will be drawn before, and at 4, 18, and 42 hours or 5 days after drug administration.

DNA: Blood from some patients with each form of MS and family members will be analyzed for genes linked to MS and controlling immune function. In some cases, DNA may be obtained from buccal scraping or other body fluids. Multiple sclerosis is caused by interactions of multiple genes. This is a broad screen of multiple genes to determine linkage to MS, and to determine responses to interferons. Separate data on immune function, also without identifiers, will be compared to genes of interest. These genes include, but aren't limited to, those regulating all aspects of immunity, cell survival, matrix metalloproteases and migration, kinases and phosphatases, and cell cycling, and pH regulation.

In addition, we will assay methylation of DNA (circulating in plasma). Some genes are methylated, a marker of their ability to be turned on that reflects cell regulation and death. When cells die, they release DNA. Sensitive assays can detect methylated forms of DNA from relevant genes (*i.e.*, methylation-sensitive restriction enzyme digestion, then PCR).

Al of these assays are directed at finding the immunologic cause of MS, how to predict exacerbations, and how to optimize MS therapy.