Investigator initiated study (IIS)

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1. **Title of the proposed study: Clinical Biomarkers of Disease Activity and Treatment Response in Patients with CNS Sarcoidosis Treated with H.P. Acthar® Gel**

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2. **Hypothesis/Hypotheses:**

   a. Treatment with H.P. Acthar® Gel will result in the improvement and long-term stabilization of clinical and radiographic abnormalities that occur in patients with CNS sarcoidosis.
   b. Treatment will be also associated with improvement in measures of quality of life.
   c. Specific immunological abnormalities will be observed in patients pre-treatment that will serve a biomarkers of disease activity. These abnormalities will also improve with treatment.

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3. **Rationale for the study:** Sarcoidosis is a chronic and frequently progressive systemic disease that affects the central nervous system (CNS) in approximately 5% of patients (Stern et al., 1985). The hallmark of the disease is the development of chronic inflammation with formation of non-caseating granulomas that can involve the brain parenchyma and meninges and appear as contrast-enhancing mass lesions on magnetic resonance imaging (Miller et al., 1988). The granulomas are primarily comprised of proinflammatory T cells (Th1 cells and Th17 cells) and macrophages which accumulate during the early stages of granuloma formation (Agostini et al., 2002; Co et al., 2004). The inflammation that is generated by these cells is modulated by anti-inflammatory responses mediated by Th2 cells and regulatory T (Treg) cells (Agostini et al., 2002; Miyara et al., 2006) that later appear and populate the outer regions of the granuloma. The presence of Treg cells are of particular interest since these cell are also detected in high numbers in peripheral blood and the immune suppression that results may underlie the occurrence of anergy in patients with the disease (Miyara et al., 2006). The treatment of CNS

sarcoidosis involves the use of either corticosteroids or potent immunosuppressive agents, both which can induce severe long-term side effects (Moller, 2003; Stern et al., 2010). The adverse effects of steroids may be avoided by treatment with adrenocorticotropic hormone (ACTH) (Arnason et al., 2013), which is available for patient use as H.P. Acthar® Gel. The efficacy of H.P. Acthar® Gel in the treatment of CNS sarcoidosis and the impact on quality of life have not been previously examined. In addition, little is known regarding the expression of immune markers in CNS sarcoidosis and the association of such markers with disease activity and response to treatment. These issues, therefore, will be explored in the context of this proposal.

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4. Patient population and estimated sample size:

a. Patients with a diagnosis of CNS sarcoidosis (n=10) will be recruited for these studies.

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5. Study design:

All patients will be recruited from the inpatient and outpatient services of the University of Maryland School of Medicine Department of Neurology.

Criteria for enrollment

a. A highly probable diagnosis of sarcoidosis, as determined using the WASOG Sarcoidosis Organ Assessment Instrument (Judson et al., 2014), with involvement limited to the central nervous system.
b. At the time of enrollment, a history of clinical deterioration based on the development of new symptoms or worsening previously present symptoms with confirmation by clinical examination and objective clinical testing.
c. If on steroids, on a stable dose of the medication for at least 1 month~~3 months~~.

Assessments

a. Administration of questionnaires
    1. Patient Determined Disease Steps (PDDS): a disability measurement
    2. Montreal Cognitive Assessment (MoCA) and Symbol-Digit Modalities test (SDMT): cognitive assessments
    3. Fatigue Assessment Scale (FAS)
    4. Short Form-36 Health Survey (SF-36): measures quality of life
    5. Work Productivity and Activities Impairment—General Health (WPAI-GH): measures health-related work productivity loss
    6. Beck Depression Inventory-II (BDI-II): a depression scale

b. Brain MRI with and without gadolinium
c. Phlebotomy
d. Lumbar puncture

Treatment

a. Acthar 80 units subcutaneously per day for 10 days, followed by Acthar 80 units subcutaneously 3 times per week through month 12
b. Continuation of the patient's previously prescribed CNS sarcoidosis maintenance regimen, as appropriate.
c. Acthar dose may be adjusted based on the patient's tolerability at the discretion of the investigator.

Assessments baseline/screening, 4 weeks, 6 months and 12 months post-treatment

a. Administration of questionnaires
    i. Patient Determined Disease Steps (PDDS)
    ii. Montreal Cognitive Assessment (MoCA) and Symbol-Digit Modalities test (SDMT)
    iii. Fatigue Assessment Scale (FAS)
    iv. Short Form-36 Health Survey (SF-36)
    v. Work Productivity and Activities Impairment—General Health (WPAI-GH)
    vi. Beck Depression Inventory-II (BDI-II)
    vii. Treatment Satisfaction Questionnaire for Medication (TSQM)
b. Brain MRI
c. Phlebotomy

Assessments at baseline/screening, 3 months and 12 months post-treatment

a. Lumbar puncture- 3 to 4 teaspoons(15 to 20 ml) of CSF fluid per lumbar puncture
b. Immunological assessments

Laboratory Assessments

a. The following immunological assessments will be performed
    i. Flow cytometry of mononuclear cells in blood and cerebrospinal fluid (CSF)
    ii. Activation of peripheral blood mononuclear cells in culture to assess for T cell and monocyte activation responses and for secreted cytokines and chemokines
    iii. Bio-Plex analysis of levels of secreted cytokines and chemokines in blood, CSF and in cell culture supernatants

Safety Assessments

a. Labs: specific monitoring of serum calcium, potassium, creatinine, cortisol responses and levels, glucose and HbA1c, 1, 25-dihydroxyvitamin D levels
b. Symptoms and signs: specific monitoring for gastrointestinal symptoms (abdominal pain, bloody or black stools, nausea, vomiting or hematemesis), fatigue, edema, hypertension, mood changes, infectious complications

b. Table 1 lists the testing that will be performed on blood (plasma) and CSF samples

Blood	CSF
CBC with differential	Red blood cell count
Comprehensive chemistry panel	White blood cell count
Angiotensin converting-enzyme level	Protein
C-Reactive Protein	Glucose
Cortisol (checked 4-24 hours after the Acthar dose)	Angiotensin converting-enzyme level
Hemoglobin A1C	IgG index
1, 25-dihydroxyvitamin D	Oligoclonal bands
25-hydroxyvitamin D	Myelin basic protein
	Protein electrophoresis

c. Immune phenotyping of peripheral blood and CSF mononuclear cells, including analysis of Treg cells, and analysis of specific proinflammatory and anti-inflammatory subsets by staining for intracytoplasmic cytokine will be performed using flow cytometry as previously described (Royal, III et al., 2009; Royal, III et al., 2007). The cell markers and cytokines for which the cells will be labeled are described in table 2.

Table 2: Markers for Analyzing Mononuclear Cells by Flow Cytometry

Cell type	Function	Markers
T Cells		
1. CD4+		
a. Th1	Proinflammatory	CD3+CD4+Interferon (IFN)-γ
b. Th17	Proinflammatory	CD3+CD4+Interleukin (IL)-17+
c. Th2	Anti-inflammatory	CD3+CD4+IL-4+
d. Treg	Anti-inflammatory	CD3+CD25+CD127^{Low}
2. CD8+ (cytotoxic)	Proinflammatory	CD3+CD8+
Monocytes (Shi and Pamer, 2011)		
1. Classical	Proinflammatory (antibacterial)	CD14++CD16-
2. Intermediate	Proinflammatory (more differentiated phenotype)	CD14+CD16+
3. Non-classical	Proinflammatory (antiviral)	CD14+CD16++

d. In vitro activation assays will be performed to assess peripheral blood mononuclear cell proliferative responses and secretion of cytokines, chemokines and other soluble immune factors using approaches that are well established in the lab of the PI (Royal, III et al., 2012), including analyses soluble plasma markers of monocyte activation (Royal W III et al, Clinical and Laboratory Assessments of Monocyte Function and Associations with Cognition and Gender among HIV Infected Individuals in Nigeria, Annual Meeting of the International Society of NeuroVirology, Washington, D.C. October 31, 2013). The cytokines and chemokines are listed in table 3 and will be detected in the University of Maryland Cytokine Core Laboratory by Bio-Plex Assay (Bio-Rad).

Table 3: Soluble Plasma and CSF Markers

Cell type	Cytokine/Chemokine Marker(s)
T Cells (Pan)	IL-2, soluble IL-2 receptor, CXCL8, CCL22
1. CD4+	
a. Th1	IFN-γ, IL-12, IL-18
b. Th17	IL-17
c. Th2	IL-4, IL-13, IL-10
d. Treg	IL-6, TGF-β
2. CD8+ (cytotoxic)	IFN-γ IL-12, IL-18
Monocytes	TNF-α, IL-1β, IL-10, CCL5, CCL2, CCL-3, CCL4, soluble CD14, soluble CD16, β2-microblobulin, neopterin, chitotriosidase

6. **Primary end-points**:

    a. Change in total number of lesions and in the number and size of enhancing lesions 1, 3, 6 months and 12 months relative to baseline

7. **Secondary end-points**:
    a. Change in PDDS, MoCA, SDMT, SF-36, WPAI-GH and BDI-II at 4 weeks, 6 months and 12 months relative to baseline
    b. TSQM scores at 4 weeks and change in TSQM scores at 6 months and 12 months versus baseline

8. **Exploratory endpoints**
    a. Change in levels of immune markers at 4 weeks, 6 months and 12 months versus baseline

9. **Estimated time line**:
    a. Months 0-15: Enrollment and assessment

    b. Months 14-18: Initial analysis of clinical laboratory data for presentation at scientific meetings and for publication

    c. Months 18-24: Manuscript preparation and submission

Schedule of Events (table 4)

Table 4. Schedule of Events	Screening/ Baseline	Follow-up (post treatment initiation) 1 mo.	3 mos.	6 mos.	12 mos.
Informed consent	x				
Medical history	x				
Physical exam	x	x	x	x	x
Neurological exam	x	x	x	x	x
Vital signs	x	x	x	x	x
Clinical laboratory testing	x	x	x	x	x
Pregnancy test	x				
Inclusion/exclusion criteria	x				
MRI	x	x	x	x	x
Phlebotomy	x	x	x	x	x
PDDS	x	x	x	x	x
MoCA/SDMT	x	x	x	x	x
SF-36	x	x	x	x	x
WPAI-GH	x	x	x	x	x
TSQM	x	x	x	x	x
KPSS	x	x	x	x	x
Lumbar puncture	x		x		x
Immune assessments	x		x		x

10. Budget (table 5)

	YR 1	YR 2	TOTAL
Clinical laboratory tests ($250)	4,500	1,500	6,000
Pregnancy tests (up to 10 tests; $18 per test)	180	0	180
Other clinical laboratory supplies (250)	7,500	2,500	10,000
Lumbar punctures (includes labs) ($1,200)	4,500	15,000	19,500
MRI scans (5 per pt.; 10 pts; $1,200 per MRI)	36,000	24,000	60,000
Immune assays			
Flow cytometry	20,000	10,000.00	30,000
Bio-Plex assays	22,000	11,000	33,000
In vitro activation assays	15,000	7,500	22,500
ELISA kits (sCD14: $700, sCD163: $900, neopterin: β-microglobulin: $910; chitotriosidase: $600): $2,510	3,110	1,555	4,665
Other Supplies			
Center For Clinical Trials fee	1,000		1,000
Patient reimbursement	2,400	600	3,000
Personnel (3% COLA for year 2)			
PI (W. Royal; 5% effort)	15,000	15,450	30,450
Co-Investigator (B. Stern; 1% effort)	4,000	4,120	8,120
Coordinator (15% effort)	20,000	20,600	40,600
Technical (15% effort)	9,500	9,785	19,285
Administrative (5%)	3,500	3,605	7,105
Total direct costs	164,690	123,610	288,300
Indirect costs (30%)	47,703	34,865	82,568
IRB fee	2,000	1,000	3,000
TOTAL	212,393	158,475	370,868

Please submit your curriculum vitae with the investigator initiated concept to your MSL and/or email to studyinfo@questcor.com.

11. References

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