

Double Blinded, Placebo  
Controlled Trial of Alpha Interferon  
Therapy for West Nile Meningoencephalitis  
Protocol WN-102

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a. Specific Aims

The specific aim of this project is to determine whether interferon  $\alpha$ -n3 (IFN  $\alpha$ -n3) a leukocyte derived mixture of natural alpha interferons, is beneficial when administered to patients with, or at high risk of, acute meningoencephalitis caused by West Nile virus (WNV). The primary outcome measurement will be the effect of this agent on the change in neurologic function from drug administration to that at three months, as compared to placebo. A secondary outcome measurement will be the effect of this agent on functional score at 3, 6 months, and 1 year, as compared with placebo. Additional secondary endpoints will be the effect of this agent on length of hospital stay and mortality.

b. Background and Significance

WNV is a mosquito borne flavivirus and a human neuropathogen which first infected humans in the western hemisphere in the late summer of 1999 (1). During 2002, 284 deaths occurred among 4,156 patients, almost exclusively due to central nervous system infection. As of December 17, 2003; 8,912 cases of WNV infection with 211 deaths had been reported to the Center for Disease Control ArboNet System. In survivors there is often long term morbidity. Standard treatment of WNV infection is supportive care (2). There is an immediate need to find therapeutic agents which will improve the course and outcome of WNV infection. WNV transmission is usually through mosquito bites, however five novel transmission modes have been identified: transfusion, transplantation, breast feeding, transplacental and occupational exposure (3, 4). This suggests persistent viremia in certain patients or in asymptomatic individuals that leads to such transmission. Thus, agents which suppress viremia or central nervous system viral replication may have a positive therapeutic effect.

A study of  $\alpha$ -IFN-2b against flavivirus (Modoc virus) encephalitis of SCID mice showed a significantly increased mean survival time of treated mice. Treatment reduced the levels of viral RNA in serum, brain and spleen significantly (5). In contrast, treatment of West Nile infection of albino mice with the interferon inducer polyriboinosinic/polycytidylic acids (poly I:C) resulted in increased survival despite similar levels of virus in the blood and brain of treated and untreated mice (6). The author postulated that despite minimal penetration of  $\alpha$ -IFN to brain, its therapeutic effect may have been due to a protective action on the endothelial cells of brain capillaries, preventing passage of virus from blood to brain.

WNV is a single-stranded RNA virus of the family *Flaviviridae*. Interferon alpha-2b (IFN  $\alpha$ -2b) and ribavirin are active against hepatitis C virus which is also a member of the *Flaviviridae* family. We reported previously that IFN  $\alpha$ -2b and ribavirin are effective *in vitro* against WNV, and that IFN  $\alpha$ -2b inhibited WNV replication at a relatively low concentration (5.9 units/ml) when applied after infection of green monkey kidney cells. Ribavirin was not as potent and had a cytotoxic effect at a concentration close to that required for viral inhibition. Thus, IFN  $\alpha$ -2b possesses greater activity *in vitro* than ribavirin, with a potentially greater human therapeutic ratio (7). In humans, a dose of 3 million units of  $\alpha$ -IFN provides serum levels of 10 units/ml after 8 hours, and daily doses of 3 million units yield serum levels of 20-30 units/ml, which are above the concentration

required for *in-vitro* efficacy against West Nile virus (8). Although systemic administrations of  $\alpha$ -IFN produces low levels in cerebrospinal fluid and brain, beneficial effects against WN encephalitis may occur through suppression of viremia (if given early enough) and/or enhancement of cell mediated immunity systemically and in the central nervous system (9). A beneficial effect of interferon alpha on HTLV-1 associated spastic paraparesis has been demonstrated by a randomized, double blinded study (10). The benefit was associated with a significant decline in HTLV-1 virus load (11). These results provide evidence for activity of interferon alpha against viral nervous system disease by its antiviral and/or immunomodulating activity.

A double-blinded placebo controlled study of interferon alpha 2a (IFN  $\alpha$ -2a) therapy of Japanese encephalitis, a member of the family *Flaviviridae*, in Vietnam resulted in no significant reduction in mortality or decrease in severe sequelae. That study was conducted with IFN  $\alpha$ -2a, and enrolled patients age 14 or younger, most of whom were in coma (71.4%) at randomization, with 29.5% exhibiting opisthotonus, or decerebrate or decorticate posturing. Delayed onset of treatment beyond the development of advanced cerebral infection may have negated a potential benefit of  $\alpha$ IFN-2a. However, there were no long-term side effects of this therapy (12). Harinasuta, et al, noted favorable clinical responses to IFN  $\alpha$ -2a in two cases of Japanese encephalitis in Thailand (13).

De Salvo, et al, reported WNV encephalitis in 2 immunosuppressed renal transplant patients. Immunosuppression was discontinued in both. One patient died within 2 weeks of admission, the second patient was treated with IFN  $\alpha$ -2b with neurological recovery 3 days after initiation of treatment. This patient has no residual neurologic deficit 10 months after WNV meningoencephalitis and his serum creatinine is at the pre-WNV infection level (14).

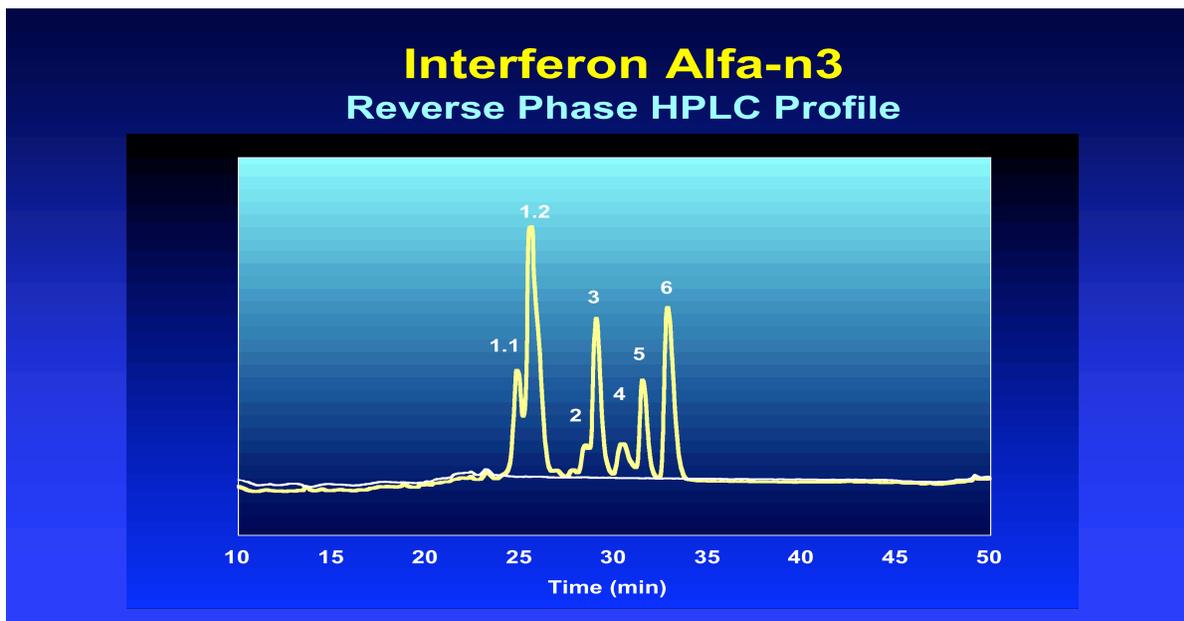
Alferon (IFN  $\alpha$ -n3) a product of Hemispherx Biopharma, Inc., is the first FDA approved natural source, human leukocyte derived mixture of at least seven species of  $\alpha$ -interferon. In contrast, other available FDA approved  $\alpha$ -interferons are single molecular species of  $\alpha$ -interferon made synthetically or in bacteria using DNA recombinant technology (Table 1).

**Table 1. Characteristics of FDA Approved  $\alpha$ -Interferons**

Commercial Name	Generic Name	Number of Molecular Species	Source	Natural Carbohydrate Present
Alferon N	Interferon $\alpha$ -n3	Seven	Human leukocytes	Yes
Infergen	Alfacon-1	One	Synthetic	No
Intron A	Interferon alpha-2b	One	Bacteria, E.coli	No
PEGASYS	Pegylated Interferon alpha-2a	One	Bacteria, E.coli	No
PEG-Intron	Pegylated Interferon alpha 2-b	One	Bacteria, E.coli	No
Roferon-A	Interferon alpha-2a	One	Bacteria, E.coli	No

These single molecular species of  $\alpha$ -interferon lack an important structural carbohydrate component because a glycosylation step is not performed during the bacterial process. IFN  $\alpha$ -n3, on the other hand, is produced by human leukocytes which are able to glycosylate the multiple  $\alpha$ -interferon species. Reverse Phase HPLC studies show that IFN  $\alpha$ -n3 is a consistent mixture of at least seven species of alpha interferon ( $\alpha$ 2,  $\alpha$ 4,  $\alpha$ 7,  $\alpha$ 8,  $\alpha$ 10,  $\alpha$ 16,  $\alpha$ 17). A reverse phase HPLC profile of IFN  $\alpha$ -n3 consists of six well defined components, each or which corresponds to a major  $\alpha$ -interferon species. Figure 1, Table 2 (15).

**Figure 1**



**Table 2**

**Interferon Alfa-n3**  
IFN Species in RP-HPLC Peaks

RP-HPLC Peak #	Major IFN- $\alpha$ Species
1.1	2b >> 2c
1.2	2b >> 2c
2	4a, 4b, 16
3	10a
4	8, 17
5	17 > 7
6	8b

**Clinical Responses in Humans Using Interferon  $\alpha$ -n3**

Even though recombinant interferons mimic human proteins structurally, some difference still exists causing these molecules to become immunogenic. During therapy with the recombinant forms of interferon alpha and beta, anti-interferon antibodies develop [ $\alpha$ -2a (16-20),  $\alpha$ -2b (18-20),  $\beta$ (21) and  $\beta$ -1b (22, 23)]. In clinical studies using IFN  $\alpha$ -n3 injection, no neutralizing antibodies were noted in patients previously not exposed to other interferons (24, 25).

IFN  $\alpha$ -n3 is better tolerated than recombinant IFN  $\alpha$ -2b. A double-blinded study with 14 individuals randomized to receive IFN  $\alpha$ -2b and 15 individuals randomized to receive IFN  $\alpha$ -n3 at the same dose, showed that the incidence of body chills and fever was significantly lower in volunteers receiving the natural IFN  $\alpha$ -n3 (79% and 64% respectively) (Table 3 and 4), (15).

**Table 3**

<b>Comparative Safety Study of Alferon N Injection® and Intron® A in Healthy Volunteers</b>	
Study Design:	Double-blind, randomized, parallel group
Patient Population:	Volunteers 18-50 years old. No prior interferon treatment. No prior evidence of viral disease for at least 4 weeks.
Treatment:	5 MU, 5 times per week for two weeks of Alferon N Injection® or Intron®.
Objective:	Evaluate occurrence and severity of adverse reactions

**Table 4**

<b><u>Comparative Safety Study</u></b>					
Treatment	No. of patients	Body Chills %	P	Fever >100°F (%)	P
Alferon	15	1 (7)	<0.001	2 (13)	0.007
Intron	14	11 (79)		9 (64)	

Alferon N is approved by the F.D.A. for the intra-lesional treatment of papilloma virus induced condyloma acuminata in patients 18 years or older. It had been reported that Alferon N is active against recombinant interferon resistant multiple sclerosis and Hairy Cell Leukemia.

### Pharmaceutical Partner

Hemispherx Biopharma Inc. (HEB) licenses/owns the intellectual property rights to Alferon. It manufactures Alferon under GMP guidelines and FDA oversight.

Hemispherx Biopharma, Inc., is a public traded company on the American Stock Exchange. William A. Carter, M.D., is its CEO. Dr. Carter has a distinguished scientific background in interferons and their induction. David Strayer, M.D. is its Medical Director. Dr. Strayer has abundant experience in clinical trials and FDA regulatory affairs relevant to INDs and NDAs. The administrative offices are located in Philadelphia, PA. Alferon laboratories are located in New Brunswick, NJ.

### Significance

To date, no randomized double-blinded placebo controlled trial of alpha interferon for West Nile viral central nervous system infection has been conducted. A Phase II prospective, randomized, placebo controlled trial evaluating the safety and efficacy of IFN  $\alpha$ -3n therapy of WNV meningoencephalitis may provide evidence for a significant therapeutic effect on this, and possibly other flavivirus infections of the central nervous system. We believe that  $\alpha$ -interferon species is the most potentially active alpha interferon product against WNV meningoencephalitis, and that it will be well tolerated at the prescribed dose.

### c. Preliminary Studies

St. Louis (SL) encephalitis virus belongs to the same Japanese encephalitis complex as WNV. The effects of IFN  $\alpha$ -2b on human infection with SL virus was studied by the principal investigator of this proposal in 2001 during an outbreak in West Monroe, Louisiana. Fifteen acutely ill patients with serologically confirmed SL viral meningoencephalitis were treated with IFN  $\alpha$ -2b for 14 days (3 million units IV followed by 3 million units on day 1, then 3 million units s.c. daily to complete 14 days). Patients were examined upon admission and daily thereafter. A neurologic function score was determined at enrollment and after 1, 2, 3 and 4 weeks. The same score had been used to follow the course of 13 untreated patients during a three week period prior to the availability of INF  $\alpha$ -2b. Another 4 untreated patients refused therapy and were followed in the same manner. The neurologic function score was determined as follows:

<u>Neurologic Capacity</u>	<u>Points</u>
Asymptomatic	2, 1 or 0
Walk	2, 1 or 0
Talk	2, 1 or 0
Swallow	2, 1 or 0
Unassisted Respiration	2, 1 or 0

Asymptomatic patients received a score of 10. Those with quadriplegia and a requirement for ventilatory assistance received a score of 0.

Quadriplegia was defined as absent muscle strength in all extremities. Quadriparesis was defined as muscle strength 1-3/5 in all extremities and respiratory insufficiency was

defined as a requirement for any type of respiratory assistance beyond intranasal oxygen.

Quadriparesis or quadriplegia occurred in 8 untreated patients and in 4 treated patients. Respiratory insufficiency requiring ventilatory assistance occurred in 7 untreated patients and 3 treated patients. Persistence of these complications beyond the first week occurred in 11 untreated patients and 2 treated patients; and beyond the second week in 5 untreated and 1 treated patient (Table 5).

**Table 5** Proportion of treated and untreated patients with severe neurologic impairment or respiratory insufficiency at hospital admission and after 1, 2, 3 and 4 weeks of hospitalization.

	<u>15 Treated</u>					<u>17 Untreated</u>				
	<u>*0</u>	1	2	3	4	<u>*0</u>	1	2	3	4
Clinical Complication	<b>** Number of patients</b>									
Quadriplegia	0	0	0	0	0	0	0	2	0	1
Quadriparesis	0	2	1	1	0	0	0	2	2	1
Respiratory Insufficiency	1	2	0	0	0	0	4	2	0	1
One or more of the above										
a) Beyond Week 2					1					5
b) Beyond Week 1					2					11

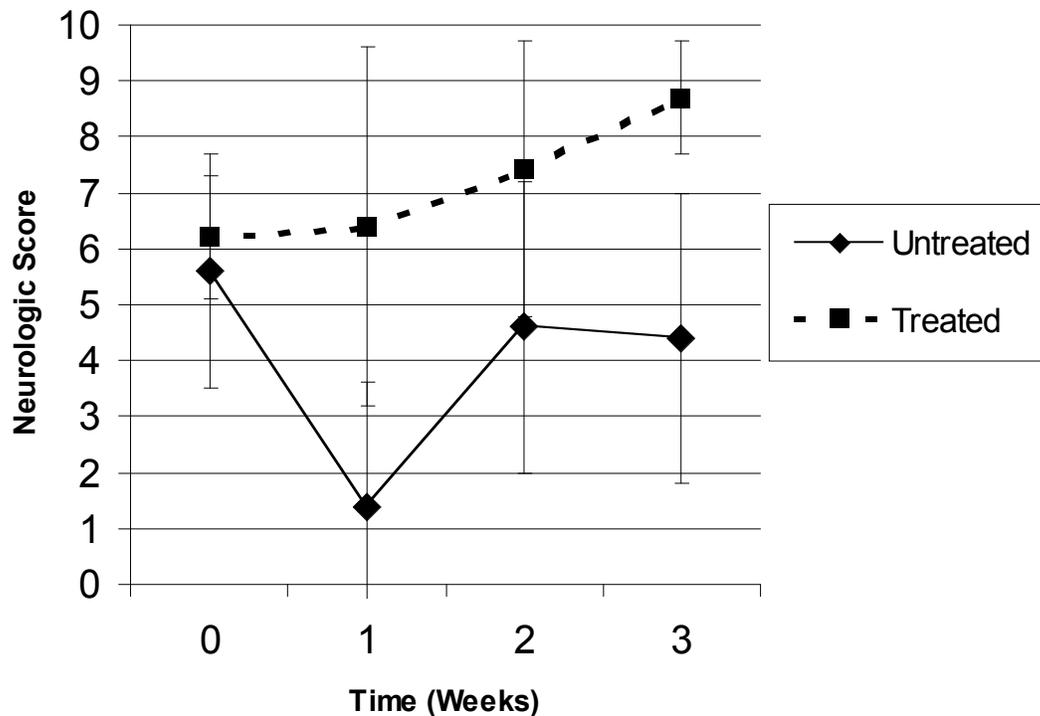
\*0 = Patient status at hospital admission

\*\*Number of patients with one or more of each complication at each week – not cumulative

The mean neurologic score of the untreated patients at hospital admission (week 0) was 5.6 (S.D.  $\pm$  2.1) and of treated patients 6.2 (S.D.  $\pm$  1.1) based upon a possible

range of 0-10. The mean neurologic scores of treated and untreated patients from admission to week 3 are shown in the figure below. Treated patients showed improved neurologic scores during the entire evaluation period as compared to the untreated patients admitted to the same hospital during the prior two weeks (Figure 2). This suggested benefit appears to have occurred primarily during the first seven days of therapy.

**Figure 2**



Eleven of the 15 treated patients experienced neutropenia and 3 demonstrated an absolute neutrophil count of 900-1000/mm<sup>3</sup> which reversed after withdrawal of therapy. No secondary bacterial infections occurred. Transient mild hepatitis occurred in 11 of the 15 treated patients.

Because the study was initiated as an emergency intervention in an attempt to ameliorate the neurologic effects of an ongoing outbreak of SL meningoencephalitis, it was not a randomized, blinded study and is in press as a pilot study (26). The possible favorable outcome among treated patients and the relative safety of IFN  $\alpha$ -2b supported further study of alpha interferon against previously untreatable flavivirus meningoencephalitis. Thus, in 2002, a study aimed at decreasing the severity and duration of neurologic dysfunction due to WNV meningoencephalitis by treatment with IFN  $\alpha$ -2b for 14 days was initiated by the principal investigator of this proposal under F.D.A. I.N.D.# 10027. The study is entitled "Protocol for Therapy of West Nile Virus Meningoencephalitis with Interferon Alpha-2b: A Randomized Unblinded Clinical Trial". The trial has enrolled patients throughout the United States during 2002 and 2003.

Eligible patients are those 50 years or older with any evidence of central nervous system (CNS) infection due to WNV, or patients 18-49 years old with signs of encephalitis due to WNV. Cerebral spinal fluid (CSF) pleocytosis and/or elevated protein concentration is required. A positive serum and/or CSF test for WNV IgM antibody is waived for entry in an epidemic situation in the same geographic area. Forty patients with WNV infection will be randomized into two groups; 20 treated and 20 untreated. The primary outcome will be the change in neurologic status determined by the N.I.H. Stroke Scale (NIHSS) (see Appendix 1) between that present at randomization to IFN  $\alpha$ -2b therapy or standard supportive care, and that at the third week following randomization (27). Patients are randomized to treatment with IFN  $\alpha$ -2b or standard supportive care after IRB approval, informed consent, and authorization to release individual health information for research are obtained. In an effort to treat patients at the earliest possible stage before brain damage occurs, randomization is required within 4 days of hospitalization. Treatment is 6 million units of  $\alpha$ -IFN on the first day (first dose of 3 million units IV followed by 3 million units s.c.) then 3 million units s.c. daily to complete 14 days. Complete blood count as well as liver and renal function are measured daily during therapy, and subsequently as indicated clinically.

Preliminary efficacy analysis:

To date, 38 patients have been randomized, and evaluable outcome data are available for 23 (15 treated and 8 untreated). The N.I.H. stroke score range is 0-42 (from best to worst) (27). The mean neurologic score improvement for the treated group was 9.6 as compared with 3.0 in the untreated group. Applying the 2-tailed Fisher's randomization test for small sample sizes, the 2-tailed p value is 0.008. This trend is similar to that noted in the study of SL meningoencephalitis.

Preliminary toxicity analysis:

Evaluable toxicity data are available for nine patients treated in this study with IFN  $\alpha$ -2b. Among these, 5 developed grade 2-4 neutropenia, all resolving spontaneously except one which resolved after use of granulocyte colony stimulating factor. The absolute neutrophil count of another 2 patients approached the lower limits of normal (1500 and 1600  $\text{mm}^3$ ). In addition, 7 of 9 treated patients developed transient grade 1-3 hepatitis (maximum serum transaminase level – 229 units/ml), and all resolved spontaneously after discontinuation of therapy (Table 6).

Table 6 – Nine Patients randomized to treatment of West Nile CNS infection with interferon alpha-2b: adverse effects

Patient #	Dates of Therapy	No. of Days	Admission (Adm.) & Weekly Trough WBC (ANC)	Admission (Adm.) & Weekly Peak SGOT/SGPT	*Adverse Effect	Related to Therapy	Conclusion
2	8/30/02-9/02/02 9/09/02-9/18/02 Total	4 <u>10</u> 14	Adm. 13.1 (10.7) Wk 1 7.3 (4.7) Wk 2 7.3 (4.2) Wk 3 8.8 (5.4)	61/21 153/229 96/195 26/40	Hepatitis, grade 3	Yes	Transient, drug-related hepatitis requiring temporary suspension of therapy.
4	9/04/02-9/15/02	12	Adm. 10.2 (7.8) Wk 1 3.2 (1.5) Wk 2 2.6 (1.2) Wk 3 3.0 (1.7) Wk 4 5.5 (3.6)	78/146 49/119 38/141 38/90 20/41	Neutropenia, grade 2 Hepatitis, grade 2	Yes Yes	Transient, drug-related neutropenia and hepatitis. Both resolved after discontinuation of therapy.
5	9/09/02-9/18/02	10	Adm. 10.4 (8.1) Wk 1 3.4 (2.0) Wk 2 2.9 (1.8) Wk 3 3.2 (1.6)	45/57 -- 32/47	None	--	Neutrophils approached lower limit of normal, possibly drug related.
7	9/18/02-9/29/02	12	Adm. 14.1 (11.9) Wk 1 4.3 (2.4) Wk 2 6.3 -- Wk 3 3.2 -- Wk 8 -- --	45/22 -- 79/164 48/105 35/17	Hepatitis, grade 2	Yes	Transient, drug-related hepatitis which resolved after discontinuation of therapy.
9	9/18/02-10/01/02	14	Adm. 8/0 (6.7) Wk 1 2.4 (.27) Wk 2 4.0 (2.6) Wk 3 3.6 (2.0)	20/16 41/57 137/211	Neutropenia, grade 4 Hepatitis, grade 3	Yes Yes	Transient, drug-related neutropenia and hepatitis. Both resolved after discontinuation of therapy.

Table 6 Continued – Nine Patients randomized to treatment of West Nile CNS infection with interferon alpha-2b: adverse effects

Patient	Admission (Adm.)	Admission	*Adverse	Related	Conclusion
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#	Dates of Therapy	No. of Days	& Weekly Trough WBC (ANC)	(Adm.) & Weekly Peak SGOT/SGPT	Effect	to Therapy	
10	9/18/02-10/01/02	14	Adm. 14.5 (11.1) Wk 1 4.7 (2.3) Wk 2 2.9 (1.4) Wk 3 2.9 (.72) GSCF 24.8 (21.6)	16/14 97/92 65/88 27/36	Neutropenia, grade 3 Hepatitis, grade 1	Yes Yes	Transient, drug-related neutropenia and hepatitis. Neutropenia resolved after use of G-CSF, and hepatitis resolved spontaneously.
13	9/26/02-10/09/02	14	Adm. 5.4 (4.5) Wk 1 1.5 (.87) Wk 2 1.9 (.86) Wk 3 4.4 (3.4) Wk 4 --- ---	40/37 37/90 67/55 22/26 30/44	Hepatitis grade 1	Yes	Transient hepatitis which resolved spontaneously after therapy. Neutrophils approached lower limits of normal, possibly drug related.
14	9/27/02-9/19/02	10	Adm. 8.8 (6.8) Wk 1 2.7 (.80) Wk 2 3.3 (1.5) Wk 3 7.0 (4.6) Wk 8 --- ---	29/16 68/37 170/127 65/78 28/38	Neutropenia, grade 3 Hepatitis, grade 2	Yes	Transient, drug-related neutropenia and hepatitis which resolved after discontinuation of therapy.
15	9/07/02-9/19/02 (at home 9/13-9/19)	13	Adm. --- --- Wk 1 --- --- Wk 2 3.5 (1.3) Wk 3 3.6 (1.2) Wk 4 12.6 (7.2)	--- --- 26/40 --- ---	Neutropenia, grade 2	Yes	Transient, drug-related neutropenia which resolved after discontinuation of therapy.

ANC = Absolute Neutrophile Count x 10<sup>3</sup>/mm<sup>3</sup>

\*Grades derived from Cancer Therapy Evaluation Program (CTEP), Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0, - DCTD, NCI, NIH, DHHS – June 10, 2003.

We now propose to replace this study with a more definitive randomized and double-blinded protocol, as described below, in order to obviate bias related to subjective unblinded neurologic evaluations. Comparative studies, cited above, suggest that the use of IFN  $\alpha$ -n3, a mixture of natural interferons, will provide greater specific activity than that with IFN  $\alpha$ -2b alone, and with less toxicity. This proposed placebo controlled study will add endpoints at 3, 6 and 12 months to supplement the primary 3-week endpoint. Our prior experience suggests that a seven-day course of therapy will be sufficient to demonstrate a possible benefit while minimizing potential adverse reactions.

Despite the above preliminary data supporting a beneficial effect of interferon alpha on neurologic status after three weeks among patients with SL or WNV meningoencephalitis, randomization of future patients into a double-blinded placebo controlled trial for WNV meningoencephalitis is essential, feasible, and ethical. No current therapy exists to ameliorate the prolonged neurologic disability of many infected patients, or the mortality rate among the elderly which may reach 35%. The highly variable natural course of viral encephalitis does not allow valid conclusions from unblinded preliminary trials with subjective endpoints such as neurologic function. Further, a favorable relationship between the potential toxicity of interferon alpha and its possible benefit cannot be established from existing data. Thus, future patients, and their physicians, will possess inadequate information to support scientifically rational therapy with any interferon alpha product in the absence of completed studies such as that proposed by this application.

d. Research Design and Methods

i. Design

The design of this study is a prospective, placebo controlled, double blinded trial of IFN  $\alpha$ -n3 therapy for WNV meningoencephalitis to determine its safety and efficacy in improving neurologic outcome.

Patients will be enrolled from June to November, since the incidence of WNV infection is very low during the rest of the year.

Outcome of the treated group at 3 weeks, 3 months, 6 months, and one year after randomization will be compared to the outcome of patients receiving placebo. The primary outcome is defined as the difference between the NIH Stroke Score (27) at randomization and after 3 months.

The secondary outcome will be the change in functional score - Barthel Index (BI) (see Appendix 2) [28] at 3 months, 6 months, and 1 year. Additional secondary endpoints will be the length of hospital stay and mortality. Safety will be assessed by comparing complete blood counts, and measurements of liver and renal function in the treatment and placebo groups.

## II. Methods

### A. Randomization

Eligible patients will be randomized by Hemispherx Biopharma, Inc. to receive either IFN  $\alpha$ -n3 or placebo with blinding of patients and investigators.

Randomization will occur after written informed consent form is obtained from the patient or patient's surrogate. Authorization to release individual health information for research will be obtained also. Informed consent will explain the study purpose, procedures, possible risks and benefits, and the current standard of care (supportive measures). Patients will be informed that supportive care may be chosen as an alternative to study enrollment. Patients will be informed that 20 ml of blood and 5 ml cerebrospinal fluid will be drawn upon enrollment to obtain IgM antibody against WNV, WNV nucleic acid and interferon assays. Another 10 ml of blood may be drawn after one or two weeks if measurement of convalescent WNV antibody, or repeat nucleic acid and interferon assays are indicated.

### B. Patient Population

#### Inclusion Criteria:

The population in this study will include any patient 18 years of age or older, male or female, of any race who meet the following criteria:

- Patients age 50 or greater with clinical evidence of CNS infection supported by CSF pleocytosis and/or elevated protein concentration, absence of organisms on gram or fungal stain, and epidemiologic factors consistent with WNV infection.
- Patients 18-49 with evidence of encephalitis due to WNV. Such evidence consists of CSF abnormalities as noted above, and one or more of the following symptoms or signs: decreased level of consciousness, confusion, focal neurologic signs, focal sensory changes, paralysis, seizure, respiratory insufficiency, or new motor weakness.
- Requirement of a positive serum and/or CSF test for flavivirus IgM antibody will be waived in an epidemic situation in which such documentation has occurred in 2 or more prior patients in the same geographic area within a 3 week period. However, specific identification of antibody to WNV should be confirmed subsequently. If not, the patient will be withdrawn from efficacy evaluation.
- Patients must be enrolled within 5 days of hospitalization in order to avoid randomization of potentially irreversible, advanced central nervous system disease.

#### Exclusion Criteria:

Patients will not be eligible for the study if any of the following is present:

- Signs of advanced central nervous system dysfunction, supported by electroencephalographic or CT evidence of severe brain damage; for example, such patients may have poor or no response to deep stimulation, absent pupillary or corneal reflexes, and/or absence of spontaneous respiratory activity.
- An initial peripheral absolute neutrophil count below 1000/mm<sup>3</sup>.
- Absence of signed informed consent by patient or appropriate surrogate.
- Allergy to eggs or mice.
- Age less than 18 years.
- Alternate clinical diagnosis that explains the patient's condition.
- Pregnant or nursing. If female, a negative pregnancy test will be required within 5 days prior to randomization, unless patient is post-menopausal for at least 2 years, or is using an effective method of birth control (birth control pills, implants, intrauterine device, diaphragm or is surgically sterile).

#### Proposed Population Sample Composition

We estimate enrolling a total of 60 patients as follows: 31 females and 29 males; 8 Hispanic or Latino and 52 Not-Hispanic or Not-Latino; 46 Whites, 8 Blacks, 1 Native Hawaiian, 4 Asians and 1 American Indian.

#### C. Study Treatment

Either 3 million units of IFN  $\alpha$ -n3 (0.6 ml) or placebo (0.6 ml) will be administered as in initial intravenous dose immediately after randomization. This will be followed by a subcutaneous dose of 0.6 ml 12 hours later, and then 0.6 ml by subcutaneous injection every 24 hours to complete seven days of therapy. The patient will receive a total of eight doses of either IFN  $\alpha$ -n3 or placebo. Vital signs (temperature, heart rate, respiratory rate, and blood pressure) should be recorded immediately prior to each injection of study drug and 30-60 minutes after.

#### D. Clinical Evaluation

##### 1. Safety data:

Safety and tolerability of the drugs will be assessed by the investigators at each center. Adverse effects of treatment will be scored according to the National Cancer Institute Common Toxicity Criteria – Version 2.0 (see Appendix 3) and recorded on adverse event forms (see Appendix 4). This will be evaluated for any trend towards harm in interim analyses by the Data and Safety Monitoring Board (DSMB).

##### 2. Clinical Parameters:

\* History and physical before randomization and after the end of therapy on Day 7.

- \* CT or MRI of the brain before randomization and thereafter as clinically indicated.
- \* Electroencephalograph (EEG) as indicated clinically.
- \* NIH Stroke Scale (NIHSS), prior to randomization, then at end of weeks 1, 2 and 3, and at 3 months.
- \* Barthel Index (BI) prior to randomization, then at the end of weeks 1, 2, and 3, and at the end of 3 months, 6 months and 12 months.

**Note:** The NIHSS and BI at 3 months, 6 months and 1 year can be done within 7 days ( $\pm$  7 days) from the actual date.

### 3. Laboratory Parameters:

- \* Complete blood count (CBC) with differential and platelet count prior to randomization then on Day #1, Day #3, Day #5, Day #7, Day #9, and Day #14 if patient is still hospitalized.
- \* Creatinine, and Alkaline Phosphatase, total Bilirubin, serum glutamic oxaloacetic transaminase (AST), serum glutamic pyruvic transaminase (ALT) prior to randomization, then on Day #1, Day #3, Day #5, Day #7, Day #9 and Day #14 if patient is still hospitalized.
- \* Routine urine analysis and bacterial cultures prior to randomization and on Day #7 and Day #14 if patient is still hospitalized.
- \* CSF for cell count and differential, protein, glucose concentrations and WNV IgM antibody before randomization.
- \* EKG prior to randomization, then as clinically indicated.
- \* Chest x-ray prior to randomization, then as clinically indicated.
- \* If available, 2c.c. of CSF should be frozen at  $-20^{\circ}\text{C}$ , in 1c.c. aliquots. 1c.c. will be tested for presence of WNV Ribonucleic Acid by polymerase chain reaction technology. The second c.c. will be assayed for  $\alpha$ -interferon levels.
- \* 2c.c. of serum on Day #1, before starting treatment, and 2c.c. of serum on Day #7 [10 hours ( $\pm$  2 hours) after the last dose of interferon] should be frozen in 1c.c. aliquots at  $-20^{\circ}\text{C}$ .

**Note:** Each site will be provided with mailing labels to ship the specimens to Focus Technologies, Inc. The specimens should be shipped over dry ice.

E. Reporting Safety Information

Adverse Event – An adverse event is any problematic occurrence, whether or not considered to be study drug related, during the acute phase of the study or during the follow-up period.

The investigator will monitor each patient for evidence of drug intolerance/toxicity and for the development of clinical and/or laboratory evidence of an adverse effect. The investigator is to assess whether the adverse event has any relationship to the study medication. All adverse events should be followed to resolution. The minimum information required for each adverse event includes; date, time of onset, length of time on study medication, severity, relationship to the study agent, relevant medical history, concomitant medications, outcome of event with date and time of resolution and plans for follow-up, contraindication for receiving additional doses of study medication.

Guidelines for determining severity and relationship to study medications are as follows:

No:	No drug relationship exists
Remote:	Less than two of the four criteria are met
Possible:	At least two of the criteria are met
Probable:	At least three of the criteria are met
Definite:	Drug caused the event

Criteria:

1. A temporal relationship exists between the event and the use of the drug.
2. Re-administration of study medication results in reappearance or worsening of the reaction.
3. Previous experience with the suspected or related compounds resulted in a similar situation.
4. Event is not related to any concomitant disease, pre-existing condition, other drug therapy, or environmental factors.

Severity:

Mild – transient and easily tolerated.

Moderate – causes discomfort and interrupts normal activity.

Severe – considerable interference with normal activities and may be incapacitating.

Serious Adverse Event (SAE):

A serious adverse event or reaction is any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or

prolongation of existing hospitalization, results in persistent or significant disability/incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

The investigator should exercise his/her judgment when deciding if expedited reporting is appropriate in situations not meeting the SAE criteria.

Serious adverse events must be reported during the acute phase of the study, as well as during the follow-up period. For life-threatening or fatal events, the investigator must report the SAE within 24 hours, or sooner, after he/she identifies the SAE. This must be followed by a narrative report by the investigator outlining the details of the adverse event, within 3 working days. If the event is not life-threatening or fatal, the investigator must report the SAE within 48 hours of recognition. Reporting of an SAE is performed by the investigator by completing the "Adverse Event" form and faxing it to 718-661-7899, as well as by phone contact with the Principal Investigator. Hemispherx Biopharma, Inc. will be immediately notified of all SAE. All SAE will be reported to the FDA.

The investigator is responsible for follow-up reports of any information related to the SAE. The result of any additional evaluations conducted by the investigator must be reported to the Principal Investigator.

All SAEs should be reported to the Institutional IRB.

SAEs that are unexpected and serious, and possibly associated with the use of the study agent, will be reported to all participating sites within a reasonable period of time.

F. Break Randomization

The medication identity can be revealed to the investigator in case of a medical emergency. The Principal Investigator approval must be sought for unblinding.

G. Criteria for Discontinuing Study Medication

Adverse effects of  $\alpha$ -IFN on blood/bone marrow, hepatic, pulmonary, or renal/genitourinary function will be scored according to the National Cancer Institute Common Toxicity Criteria – Version 2.0 (refer to Appendix 3). Scoring for each of the above categories will be used to grade adverse effects on the following parameters, and to discontinue therapy.

<u>Laboratory Parameter</u>	<u>Grade Requiring Discontinuation</u>	<u>Attribution for Discontinuation</u>
Hemoglobin	$\geq 2$	Probable or Definite
Neutrophils/Granulocytes	$\geq 3$	Probable or Definite

Platelets	≥ 2	Probable or Definite
*Alkaline Phosphatase	≥ 2	Probable or Definite
*Bilirubin	≥ 2	Probable or Definite
*SGOT (AST)	≥ 2	Probable or Definite
*SGPT (ALT)	≥ 2	Probable or Definite
*Creatinine	≥ 3	Probable or Definite
Proteinuria	≥ 3	Probable or Definite
Pulmonary Infiltrates	≥ 1	Probable or Definite

#### Grades (General Definitions)

0 = No adverse event or within normal limits

1 = Mild adverse event

2 = Moderate adverse event

3 = Severe and undesirable adverse event

4 = Life-threatening or disabling adverse event

5 = Death related to adverse event

\*Grades will refer to multiples of values present at onset of therapy rather than multiples of the upper limits of normal (ULN).

If a patient's treatment is discontinued due to an adverse event, the patient's course will be followed and the outcome reported.

#### H. Concomitant & Prohibited Medications

The use of antibacterial agents, acyclovir, or anti-HIV agents will be allowed as indicated clinically. Antivirals that are not allowed are ribavirin, or commercially available interferons.

#### I. Withdrawals (of patients)

Patients may be withdrawn from study for the following reasons:

1. Patient or patient surrogate wishes to withdraw
2. Development of serious adverse event
3. Trial termination

#### J. Protocol Violations & Departure From Protocol

Protocol violations that result in an adverse event should be reported to the Principal Investigator within 24 hours. A written report should be faxed to the Principal Investigator as soon as possible addressing the nature of the error, patient's clinical status before and after, steps taken to review the error and to assure it will not happen again.

All such events should be reported to the Investigator's IRB. Protocol violations not resulting in an adverse event error should be reported to the Principal Investigator.

If a circumstance arises which may justify a departure from protocol for an individual patient, the Investigator should contact the Principal Investigator for a joint decision as to whether the protocol may be modified for that patient, or whether the subject should be prematurely discontinued from the study. The reason for such a joint decision will be documented in writing and may require Institutional Review Board approval.

K. Data Management

Case Report Form Development:

Development of CRFs will be performed by the Data Manager in Microsoft Word. The Protocol will be the guide used in designing the CRF. The Medical Director will provide input on the design of the CRF. Data Manager has at least 10 years of experience in designing CRFs.

Database Design and Programming:

The Software that will be used is ClinAccess Powered by SAS 4.0. Double data entry will be performed. Audit trails are performed in ClinAccess. Printouts of the audit trail may be provided whenever necessary. The screens will be developed to look, as much as possible, like the CRF. Testing of the database will be performed before releasing for data entry.

Data Flow and Data Entry Procedures:

The CRFs will be completed by the clinical site. Monitors will visit site to review the completeness of the CRFs before pulling and sending CRFs to Hemispherx. A tracking log will be completed by monitor when sending CRFs to Data Manager at Hemispherx. Data Manager or designee will then review the CRF pages against the tracking log for completeness. Any inconsistencies with data recorded on the CRF pages will be reported. Tracking Log will be entered into the data base. CRFs are then ready for data entry. Document IDs will be created to identify each CRF page. This information will be logged into ClinAccess for data entry.

First and second data entry will be done by two different persons. Data queries may be generated during data entry. ClinAccess will then match first entry with second entry. All discrepancies will be moved to verification. A third person will generate the verification report and store this report. This person will then go through the process of selecting which answer is correct in ClinAccess. Once all discrepancies are resolved, ClinAccess will move these CRFs to the Master database.

Data Quality Assurance – Queries and Audits:

Double data entry is done independently. Verification is done by a third person not associated with first or second entry. When data entry is complete, 20 percent of the completed cases are then reviewed against the database. Any data entry errors will be recorded in ClinAccess. Queries are generated during data entry and when CRFs are being reviewed. Also, an edit check program can

be written to run in ClinAccess to generate queries. ClinAccess allows you to generate a DCFs (Data Clarification Form). Each time a DCFs report is generated, ClinAccess provides the report with a batch number. DCFs is printed, reviewed by the Data Manager. The DCFs is then photocopied onto two part paper (white and yellow) and sent to site for clarification. Site will date and initial each query then send the White pages to Hemispherx and keep the yellow pages for their records. Data Management will then resolve the queries in ClinAccess. Each and every one of these actions is recorded in ClinAccess. The Audit trail is updated constantly.

Confidentiality:

All CRFs are stored in a designated confidential filing area. Only the Medical department and Data department has access to these files. Patients are identified by a code, not patient name. Database can only be accessed by the Data Department. Each user is restricted access to what their job description requires.

Data Security:

System Administrator is the only person that has access to the server. It is password protected. ClinAccess is password protected. You must have a password to enter the ClinAccess. Full daily backups are performed . Testing of the backups is done every two weeks for recovery.

L. Statistical Considerations

Randomization Methods:

Subjects will be randomized using the method of permuted blocks. There will be no stratification variables. A randomization schedule will be computer-generated by the Biostatistics Unit at the North Shore-LIJ Research Institute. In order to maintain blinding, the schedule will be delivered only to a single, designated individual supervising the labeling of medication. None of the investigators will be aware of the randomization schedule.

Endpoint Variables:

The endpoint variable will be the NIH Stroke Scale score (NIHSS) measured at baseline and 3 months. The NIHSS ranges from 0 to 42, with higher scores indicative of greater neurological disability. It is understood that some patients will not be available for long-term follow-up due, primarily, to refusal to continue participation.

The secondary endpoint is the change in the Barthel Index at 3 months, 6 months, and 1 year. Additional secondary endpoints will be effect of this agent on length of hospital stay and mortality.

Statistical Analysis:

The t-test will be used to compare NIHSS scores for IFN  $\alpha$ -n3 vs. control at each time point. (Although repeated measures ANOVA would be the method of

choice, it is likely that there will be more and more missing data at later time points. Even though, the generalized linear model can handle missing data in a RMANOVA, it seems more appropriate to follow a simple approach using two-sample t-tests.) Based on previous studies, we expect the t-test assumptions to hold; however, if the use of a t-test cannot be justified, then the Mann-Whitney test will be used.

It should be noted that most of the missing data at later follow-up will be due to the fact that patients are feeling well and do not want to submit to the NIHSS evaluation. We will perform a secondary analysis, whereby a “good” score will be inputted for those subjects. Of course, this analysis may be biased, but it will provide some suggestion as to ultimate effect size for this trial.

As for mortality analysis, this will be purely descriptive since the number of expected death is very small.

#### Sample Size Justification:

We plan to randomize a total of 40 subjects, half to receive IFN and half placebo. There are no previous studies of WNV using the NIHSS that will help plan for power-based sample size calculations. In a reasonable attempt to perform an appropriate calculation, we have used the data that we have previously collected on St. Louis Encephalitis, using a 10-point neurological assessment scale, suitably “mapped” into the NIHSS 42-point scale.

Based on our data, we assume that, at 3 months, the mean NIHSS for the control group will be 22.4 and the mean for the IFN  $\alpha$ -n3 group will be 15.7 with a common standard deviation of 7.0. To detect a difference of this magnitude with 80% power (two-tailed t-test,  $\alpha=0.05$ ), a sample size of 19 patients per group (i.e., total 40) will be required.

In anticipation of patients lost for follow-up, 30 patients per group (i.e., total 60) would be randomized.

#### M. Pitfalls

There are two main concerns with this study. First, the timing and sites where West Nile virus will re-emerge is not predictable. Thus, the pre-selected study sites might not be sufficient to enroll enough patients. During our prior unblinded study we were able to enroll patients with West Nile meningoencephalitis from centers throughout the United States. On occasion we were not able to enroll the first patient from such sites due to pending IRB approval. However, with experience in completing the process, we were able to obtain IRB approvals within a very short period of time so that other patients could be enrolled. Participating centers for the present proposal have been selected in locations where a substantial number of clinical infections with West Nile virus occurred in 2003, or where further spread is anticipated in 2004.

Second,  $\alpha$ -IFN is an FDA approved medication for treatment for hepatitis C and several formulations are widely available. Patients might elect not to participate in this study, choosing to receive a recombinant  $\alpha$ -IFN as an alternate treatment for West Nile virus meningoencephalitis. However, enrollment in our prior pilot studies has not been limited by this factor since definitive data are not available as yet regarding the relative efficacy and toxicity of  $\alpha$ -IFNs for this entity.

e. Human Subjects Research  
Protection of Human Subjects

Institutional Review Board (IRB) approval will be obtained prior to the initiation of any clinical protocols.

1. Risks to the subjects

a. Subject Selection:

This study will aim to enroll hospitalized patients 18 years of age or older with evidence of WNV CNS infection. Older people have been shown to be at an increased risk for severe illness and death. During the New York City area outbreak in 1999-2000, 12 (15%) of 78 hospitalized patients, were < 50 years of age, 28 (36%) were between 50 and 69 years of age, and 38 (49%) were older than 70. Nine of these 78 people died (case fatality rate, 12%); all were >65 years of age (29). Only 28% (13/47) of the patients who survived encephalitis or neuromuscular weakness, or both, during the Ontario outbreak in the summer of 2002, were discharged home without additional support (30). Because of these more severe outcomes in older patients within prior outbreaks, all patients over 50 years of age with evidence of CNS infection (meningitis or encephalitis) will be eligible for enrollment. Patients of age 18-49 will be enrolled if there is evidence of encephalitis manifested by decreased level of consciousness, confusion, focal neurologic signs, focal sensory change, paralysis, seizure or respiratory insufficiency. Those with severe neurologic illness for several days will be excluded. Prior autopsy results have shown degenerating neurons and necrobiosis of ganglion cells, suggesting that reversal of neurologic dysfunction by study agents would be unlikely (31, 32). We will aim to enroll a total of 60 patients.

b. Sources of Material

Serum specimens as well as CSF specimens will be collected from patients after Informed Consent is signed and authorization is obtained to release individual health information for research. At the time of consent, patients may agree or refuse to have their specimens used in future WNV research. Patients will do so by initialing the appropriate box of the Informed Consent Form. These specimens will be stored at participating centers. To protect patient confidentiality, specimens will be labeled with a code and not with the patient's name.

c. Potential Risks

Patients will be randomized to receive IFN  $\alpha$ -n3 or placebo in a double blinded approach. The potential adverse effects of IFN  $\alpha$ -n3 include suppression of bone marrow, hepatic, or renal function; psychiatric disorders; and alteration of thyroid

function. Granulocytopenia and hepatitis are the more likely adverse events. These side effects have been shown to be transient and reversible once treatment is stopped.

At the present time, there is no treatment for West Nile virus. Patients usually receive supportive care (2). Case-fatality rates among patients hospitalized during recent outbreaks have ranged from 4% in Romania (1996), to 12% in New York (1999), 14% in Israel (2000), in Michigan (2002), and 18% in Canada (2002). The mortality rate of patients aged 65 or older was 35% (33).

## 2. Adequacy of Protection Against Risks

### a. Recruitment and Informed Consent:

Hospitalized patients, at centers with IRB approval, who meet the inclusion criteria will be approached for enrollment by the investigator at that site. Patients or their surrogates must sign the informed consent prior to enrollment. This research study's purpose will be introduced to the patients. Patients will understand that the study is blinded and randomized. The required blood tests and CSF tests will be explained to the patients. The nature, route and possible discomforts and risks of therapy will be discussed with each patient. The

possible benefits from being in the study, and alternative treatment options, i.e., supportive care, will be explained to each patient. The person(s) to call in case of any questions or concerns will be provided. The right to leave the study, or study termination, will be explained to each patient. A special section for consent to use specimens in future research will be provided. Each patient or designated family member, a witness, and the investigator will sign the informed consent form prior to enrolling the patient in the study. A copy of the informed consent will be provided to the patient. The original signed consent will be filed in the Investigator Document File Binder and will be available for inspection. Another copy of the signed consent form will be maintained in the patient's permanent medical record.

### b. Protection Against Risk:

Upon satisfactory receipt of all necessary paperwork, including signed FDA Form 1572, study material will be delivered to the study site and a mutually convenient time will be set up for the conduct of an initiation visit. At this meeting, all personnel expected to be involved in the conduct of the study will undergo an orientation to include review of study protocol, instruction for case report form completion and overall responsibilities including those for drug accountability and study file maintenance. Case report forms will be provided for the collection of all study data. A copy is to be retained in the investigator's files. It will be the obligation of the investigator to review each page of the case report form. The investigator will permit a representative of Hemispherx Biopharma or a contract research organization (CRO), or the FDA to inspect all case report forms and the study patient's medical records (including office and hospital) at regular intervals throughout the study. These inspections are for the purpose of verifying adherence to the protocol and the completeness and exactness of the data being entered on the case report forms. The

investigational materials are to be prescribed by only the principal investigator or the sub-investigators named in FDA Form 1572. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. Accurate records must be maintained accounting for the receipt of the investigational materials and for the disposition of the material. All serious adverse events shall be reported immediately to the Medical Director of Hemispherx Biopharma, Inc., and to the Principal Investigator, and will be followed by a written narrative report by the Investigator outlining the details of the adverse event. The report will be sent to Hemispherx Biopharma, Inc., and to the Principal Investigator within three (3) working days. Serious adverse events shall also be documented in the case report form. Daily evaluation of subjective complaints and physical exam while patients are hospitalized and at follow-up clinic visits will be done and addressed accordingly. Patients will receive all needed medication interventions as deemed clinically appropriate by the investigator. At the termination of the study, the investigator must return any unused supplies to Hemispherx Biopharma. Upon completion of termination of the study, the Principal Investigator will submit a final written report as required by Federal Regulations. The report should be submitted to the sponsor and to the IRB within 90 days of completion or termination of the study. All information obtained during the conduct of this study will be regarded as confidential in accordance with HIPPA and IRB regulations.

Routine reports will be sent to an independent Data Safety and Monitoring Board for review as well as all serious adverse event reports to insure safety of the subjects.

3. Potential Benefits of the Proposed Research to the Subjects and Others

WNV is a human neuro-pathogen. Most of the deaths occur due to CNS infection. Case fatality rate ranges between 4% to 18%. At the present time patients receive only supportive care, and only 28% of the patients who survive encephalitis or neuromuscular disease are discharged to their home without additional support (30). During the 2003 summer outbreak, 8,912 patients were diagnosed with WNV infection and 30% were reported as WNV meningitis or encephalitis. The potential benefit of this study is that a relatively safe treatment for WNV meningoencephalitis will reduce the severity and duration of neurologic abnormalities caused by this infection. The duration of hospital stay, death rate, or degree of long term neurologic abnormality may also be reduced.

4. Importance of the Knowledge to be Gained

WNV is a single-stranded RNA virus of the family *Flaviviridae*. Currently there is no treatment for flaviviral infections. Moreover, there is no treatment for RNA viruses infecting the CNS. This proposed research follows upon the suggestion of Merigan in 1982 that the action of alpha interferon be examined against CNS viral infections that are particularly severe and caused by RNA viruses for which we have no other available therapy (34). The proposed research aims to determine whether IFN  $\alpha$ -n3 is beneficial and safe when given to patients with WNV meningoencephalitis. If this potential outcome is validated, it will provide efficacious therapy for a previously untreatable, potentially fatal, infection, and will serve as the basis for testing alpha interferon against other serious infections caused by RNA viruses.

5. Inclusion of Women:  
All hospitalized patients meeting inclusion criteria will be offered enrollment regardless of their gender. Both males and females will be eligible for the proposed clinical trial.
6. Inclusion of Minorities:  
All hospitalized patients meeting inclusion criteria will be offered enrollment regardless of their race. Subjects from minority population will be encouraged to enroll in the clinical trial to insure that members of minority groups, and their subpopulation are included in the study.
7. Inclusion of Children:  
Children younger than 18 will not be enrolled because of the high likelihood of other diagnoses that mimic WNV meningoencephalitis in this group. Moreover, children are at low risk of severe WNV disease.
8. Data and Safety Monitoring Plan  
After the case report forms are reviewed for completeness and quality, they will be entered into the database at Hemispherx Biopharma. Routine reports will be sent to the Data Safety and Monitoring Board where they will be evaluated.

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