Arthritis Advisory Committee Meeting
Briefing Document
for the
20th December 2012 Meeting

AMPLIGEN® (rintatolimod; Poly I : Poly $C_{12}U$)

Treatment of Chronic Fatigue Syndrome

NDA #22-151

AVAILABLE FOR PUBLIC
DISCLOSURE WITHOUT REDACTION

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
ADL	Activities of Daily Living
AE	Adverse Event
AMP	Ampligen
ANA	Anti-Nuclear Antibody
ANCOVA	Analysis of Covariate
AT	Anaerobic Threshold
CDC	Centers for Disease Control and Prevention
CFS	Chronic Fatigue Syndrome
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CPET	Cardiopulmonary Exercise Testing
CRL	Complete Response Letter
DNA	Deoxyribonucleic Acid
DPARP	Division of Pulmonary, Allergy and Rheumatology Products
dsRNA	Double-stranded Ribonucleic Acid
EEG	Electroencephalogram
EKG	Electrocardiogram
ESR	Erythrocyte Sedimentation Rate
ETT	Exercise Tolerance Test
FDA	Food and Drug Administration
GHP	General Health Perception
HHV-6	Human Herpes Virus-6
HPA	Hypothalamic-Pituitary-Adrenal
HU-CFS	Hahnemann University - Chronic Fatigue Syndrome Protocol
INR	International Normalized Ratio
ITT	Intent-to-Treat
IV	Intravenous
KPS	Karnofsky Performance Scale
LFTs	Transitory Liver Function Tests
LQTS	Long QT Syndrome
MDA5	Melanoma differentiation-associated gene-5
MOS	Medical Outcomes Study
MRI	Magnetic Resonance Imaging
MYD88	Myeloid differentiation primary response gene (88)

NDA	New Drug Application
NFKb	Nuclear Factor Kappa B
NHL	Non-Hodgkin's Lymphoma
NIH	National Institutes of Health
NME	New Molecular Entity
NMH	Neurally Mediated Hypotension
NSAIDS	Nonsteroidal Anti-inflammatory Drugs
PLA	Placebo
POTS	Postural Orthostatic Tachycardia Syndrome
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PTYs	Patient Treatment Years
RF	Rheumatoid Factor
SAE	Serious Adverse Event
SCL-90-R	Symptom Checklist-90-R
SEM	Standard Error of Mean
SF-36	Health Survey, Short Form
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SIT	Specific Immunotherapy
SNPs	Single Nucleotide Polymorphisms
SOC	Standard of Care
SEER	Surveillance, Epidemiology, and End Results
SSRI	Selective Serotonin Reuptake Inhibitors
TLR	Toll-like Receptor
TNF	Tumor Necrosis Factor
TRIF	TIR-domain-containing adapter-inducing interferon-β
ULN	Upper Limit of Normal
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WMS	Wechsler Memory Scale

1. EXECUTIVE SUMMARY

Hemispherx Biopharma Inc. is dedicated to developing a treatment for patients with Chronic Fatigue Syndrome (CFS) including a long history with the National Institutes of Health and various academic collaborations. Poly I: Poly $C_{12}U$ (rintatolimod), hereinafter referred to as Ampligen® has been under development for CFS since the late 1980's following its discovery at Johns Hopkins University. Hemispherx has been working in partnership with the FDA to understand this complex disorder for more than two decades. Hemispherx is seeking approval of Ampligen for the treatment of CFS. Ampligen is synthetic immunomodulatory double-stranded RNA compound that activates innate immunity and is a highly selective Toll-like receptor 3 (TLR-3) activator. Ampligen was designed to induce the interferon broad spectrum antiviral/ immunomodulatory "cascades" with a minimal side effect profile.

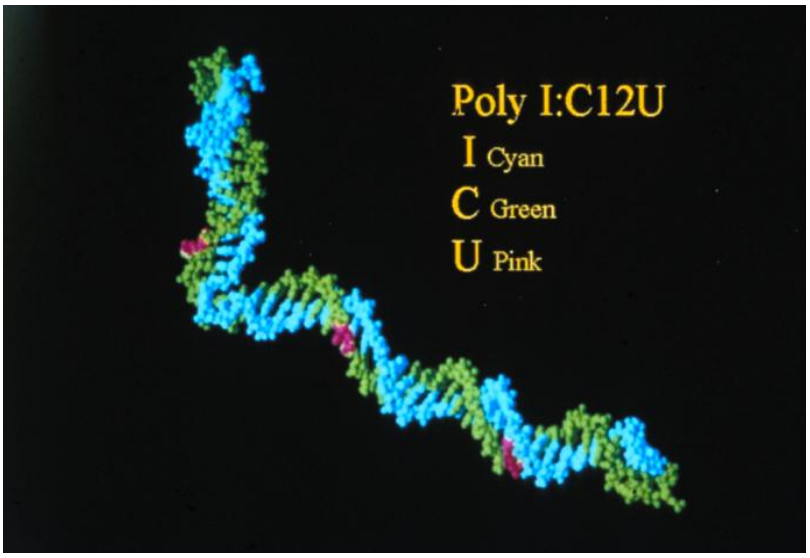


Figure 1.1: Molecular Model of Ampligen

1.1 A SERIOUS AND LIFE-THREATENING DISEASE

CFS is a seriously debilitating and life-threatening disorder. The Centers for Disease Control and Prevention (CDC) characterize it by disabling fatigue and a combination of flu-like symptoms (Fukuda 1994). This fatigue is not improved by bed rest, and may be worsened by physical or mental activity. It involves profound debilitation which dramatically reduces a person's ability to perform normal daily activities and may last for years, even decades (Komaroff 1998). The most severe cases involve 14 to 22 hours of sleep per day. A key symptom is post exertional fatigue. It is typical that one day's worth of normal activity often results in days to weeks of recovery yet never reaching a state of wellness. Other symptoms may include muscle and/or joint pain, sore throat, headache, influenza symptoms and impaired memory or concentration. The inability to physically function is compounded by an inability to have clarity of mind to even watch TV, read a book or work on a computer. The result is substantial reductions in previous levels of occupational, educational, social and/or personal activities. The quality of life is alarmingly low. Current standard of care for CFS is aimed at symptom relief. There is no known cure. No drugs are currently FDA approved for the treatment of CFS or its symptoms.

CFS is characterized by a pattern of relapse and remission over long periods of time. The disease is progressive in many patients and can lead to life-threatening events. CFS affects multiple systems, among them the nervous system which includes the hypothalamic-pituitary-adrenal (HPA) axis, often with low levels of cortisol. Researchers have found abnormalities in the autonomic nervous system that affect heart rate and blood pressure. In the immune system, there is a defect in the natural killer T-cells. Chronic fatigue syndrome causes dysfunction of virtually every organ system in the body, and can lead to premature mortality.

New epidemiologic data on life-threatening sequelae of untreated CFS highlighting specifically the higher incidence of catastrophic cardiac events and suicides and untreatable cancers, especially lymphomas were analyzed in a study of mortality of 144 CFS sufferers (Jason 2006).

Table 1.1: Main Causes of Death in Patients with Chronic Fatigue Syndrome

Cause of Death	Number (%) of Subjects n=144	Percent	
		Male	Female
Heart failure[1]	29 (20.1%)	34.5	65.5
Suicide[1]	29 (20.1%)	17.2	82.8
Cancer	28 (19.4%)	17.9	82.1

[1] Statistically significant at $p<0.01$
Data source: Jason 2006

In that study the median age of death for those with CFS was compared with overall medians in the US (Table 1.2).

Table 1.2: Median Age of Death in Overall U.S. Population vs. CFS Population

Cancer is age 72 overall in the US vs. 47.8 for those with CFS
Suicide is age 48 vs. 39.3 for those with CFS
Heart failure is age 83.1 vs. 58.7 for those with CFS

1.2 UNMET MEDICAL NEED

It is estimated by the CDC that CFS affects approximately 1,000,000 people in the US and that the disease results in over $14 billion in healthcare costs and $9 billion in lost productivity each year. Between 14% to 15% of the total population, approximately 150,000, are severely debilitated. **This severely debilitated population has been the target of the Hemispherx Ampligen clinical development program for more than a decade.**

CFS occurs in all ethnic groups and races, and in countries around the world. It most often occurs in people age 40 to 59 and women are three times more likely than men to develop it (NIAID *NIH Publication No. 04-4697*). **CDC studies have shown that CFS can be as debilitating as multiple sclerosis, lupus, rheumatoid arthritis, heart disease, end-stage renal disease, chronic obstructive pulmonary disease (COPD) and similar chronic conditions.**

The etiology of CFS is still unknown (Komaroff 2006). Earlier theories suggest CFS may have a viral basis such as Epstein-Barr virus or human herpes virus-6 (HHV-6) and it has been suggested that infectious mononucleosis may be a risk factor for CFS in adolescents (Katz 2009), although no specific virus has been firmly identified as the cause. Others speculation has looked to inflammation in the nervous system resulting from a faulty immune system response. Symptomatically, the disease can resemble other disorders, including mononucleosis, Fibromyalgia, Lyme disease, lupus, multiple sclerosis, primary sleep disorders such as narcolepsy or sleep apnea, and hypothyroidism. The patient typically begins with the family practitioner or internist then patients are referred to rheumatologists or other specialists. There are a number of expert research and clinical CFS practices in the US. Health professionals utilize as specific criteria to exclude other illnesses in order to ensure proper diagnosis.

Current standard of care for CFS commonly involves pain relievers as well as antidepressants and medications that affect the central nervous system. The focus is largely on a combination of medications for the relief of specific symptoms experienced by the individual patient. It is not uncommon for patients to develop adverse reactions to these palliative (concomitant) medications and often must discontinue their use. Ampligen is the first drug specifically targeted for the treatment for CFS to provide enhanced capacity for physical activity, cognitive improvement, and increased activities of daily living in severely debilitated CFS patients.

1.3 BASIS FOR LICENSURE

It is the goal of Hemispherx to provide the first drug for the specific treatment of CFS. Hemispherx has completed two well-controlled studies AMP-516 and AMP-502 that support efficacy and demonstrate a wide margin of safety. For the most severely ill patients, improvements in functionality have a significant effect on their lives, such as being able to perform basic tasks such as bathing and dressing. Ampligen is a twice weekly infusion and is generally well-tolerated. The infusion is approximately 30 minutes in duration, and provides an additional level of clinical observation regarding tolerance and safety. Clinical studies have shown that Ampligen reduces the symptom burden of the disorder. Additional observations indicate that Ampligen reduces hospitalizations and could favorably reduce the number deaths in this population.

1.3.1 Brief Clinical History

Hemispherx developed Ampligen for the treatment of CFS and it has been studied in over 1,200 subjects. Of these, 326 participated in two multi-center, double-blind, randomized, placebo-controlled studies, AMP-502 and AMP-516.

Table 1.3: Well-Controlled Studies AMP-502 and AMP-516 are Designed to Provide Complementary Clinical Insights

	AMP-502	AMP-516
Prospective Design	x	x
Multi-Center	x	x
Conducted only in USA	x	x
Randomized-Double-Blind	x	x

Table 1.3: Well-Controlled Studies AMP-502 and AMP-516 are Designed to Provide Complementary Clinical Insights		
Equal Parallel Group Design	x	x
Placebo-Controlled	x	x
Evaluated Efficacy and Safety	x	x
Utilized Ampligen at a Dosage Level of 400 mg given IV Twice Weekly vs. Placebo IV Twice Weekly	x	x
Enrolled Subjects with Severely Debilitating CFS	x	x
Enrolled Subjects between the Ages of 18 - 60	x	x
Enrolled Both Females and Males	x	x
Utilized ETT as a Primary or Secondary Endpoint	x	x
Utilized an Independent Team that Travelled to Each Site to Conduct the ETT Evaluations	x	x
Utilized KPS as a Primary or Secondary Endpoint	x	x
Utilized Concomitant Medication Usage as a Secondary Endpoint	x	x
Utilized ADL as a Secondary Endpoint	x	x
Utilized the Following Four Laboratory Panels to Evaluate Safety: Hematology, Chemistry, Coagulation, and Urinalysis	x	x
Study had Extension Phase to Add Insight into Efficacy/Safety	x	x

Both studies had similar designs, met their endpoints and demonstrated an advanced degree of safety over the current standard of care. In addition to the two pivotal studies, Hemispherx has been conducting a Treatment Protocol (AMP-511) under IND 39,250 since 1997, originally authorized by Dr. Janet Woodcock, now Director of CDER. AMP-511 continues to demonstrate results which are supportive of the findings of studies AMP-502 and AMP-516. Eighty-four percent (84%) of the patients on the treatment protocol remain on study beyond the 24 weeks due to improvement.

1.3.2 Regulatory History

Early pilot programs for Ampligen's use in CFS began in 1988, as a result of a request by the FDA to consider compassionate treatment using Ampligen for a CFS patient in New Mexico. In 1993, Orphan Drug status was granted. Hemispherx currently has five clinical sites conducting the Treatment Protocol. Key clinical trial agreements with FDA divisions include AMP-502 under Biologics, AMP-511 under Anti-Viral and AMP-516 under Special Pathogens. Hemispherx submitted the NDA under Cardio Renal and was moved to Pulmonary, Allergy and Rheumatology in 2011.

Upon the completion of the second placebo-controlled study, AMP-516, Hemispherx submitted the Ampligen NDA in October 2007. It was accepted for review by the FDA in July 2008. In November 2009, Hemispherx received a Complete Response Letter (CRL) which stated that the FDA reviewers could not approve the application in its present form and provided specific

recommendations to address the outstanding issues. The FDA stated that the two primary clinical studies submitted with the NDA did not provide credible evidence of efficacy of Ampligen and recommended at least one additional clinical study which shows convincing effect and confirms safety in the target population.

In June of 2012, Hemispherx met with the current review division to provide an update on progress and review the current state of the science. As a result, the FDA agreed to accept, for review, further analyses of data from Hemispherx's AMP-516 Phase 3 clinical trial and other Ampligen trials (AMP-502 and AMP-516C/E) in lieu of the additional confirmatory Phase 3 study originally called for in the Agency's CRL.

1.3.3 Efficacy

Based on an agreement with the FDA, the AMP-516 protocol utilized Exercise Tolerance Testing (ETT) as the primary endpoint along with Karnofsky Performance Scale (KPS); in AMP-502 KPS was the primary and ETT was secondary. The trend for both studies was congruent and thus the AMP-516 data is substantially supported by AMP-502.

Ampligen has been studied in over 1,200 subjects across various patient populations participating in long-term and short-term studies, including randomized, placebo-controlled clinical studies and open label studies. Ampligen has been administered to 845 subjects, including 589 unique subjects, suffering from severely debilitating CFS. A total of over 90,000 intravenous doses of Ampligen have been administered to CFS subjects. Of these, 326 participated in placebo-controlled studies, AMP-502 and AMP-516. In these studies, patients ranged in age from 20 to age 69 (mean age 44) with a mean age at CFS symptom onset of 35 and had a mean 6 to 9-year history respectively of CFS. Two thirds of the 326 subjects in the placebo-controlled studies AMP-502 and AMP-516 were female. This reflects the social epidemiology of CFS in the general population. Thirty-seven (37) CFS subjects were on Ampligen for more than 104 weeks, and 9 subjects were administered 800 to 1200 mg of Ampligen per week for 1 to 2 years.

The study population consistently resembles that of the CFS population with emphasis on the most severely debilitated. Only those subjects who met the CFS criteria of the CDC (1988 criteria) and had a disease duration of greater than 1 year were allowed to be enrolled. The CDC definition requires just 6 months since onset. No children were studied in the well-controlled CFS studies.

Both AMP-502 and AMP-516 were multi-center, double-blind, randomized, placebo-controlled studies. Both were designed to compare the safety and efficacy of Ampligen versus placebo in the treatment of severely disabled subjects, 92 in AMP-502 and 234 in AMP-516. Adults no older than 60 years who met the case definition for CFS developed by the CDC, but with CFS ongoing for 12 months or more, were eligible if they had a performance reduced quality of life as determined by a KPS between 20 and 60 KPS in AMP-502 and between 40 and 60 in AMP-516.

AMP-502 comprised 24 weeks in total. AMP-516 totaled 76 weeks consisting of Baseline evaluation of up to 12 weeks, followed by Stage 1 for 40 weeks where subjects were stratified by exercise treadmill duration and randomized 1:1 to double-blind treatment and then a 24 week ***crossover study*** (AMP-516C/E) where all subjects compliant in Stage 1 received Ampligen. The double-blind in Stage 1 was maintained during Stage 2. In Stage 2, the previously untreated placebo patients showed dramatic improvement in ETT when placed on Ampligen.

The key endpoints were:

- ETT, looking specifically at exercise duration was the primary endpoint in AMP-516 and a secondary endpoint in AMP-502. Efficacy is established by showing medically and statistically significant improvement in this endpoint. ETT is a well-established indicator and part of Cardio Pulmonary Exercise Testing (CPET) which has been well utilized in chronically disabling diseases.

Table 1.4. Entities which Utilize Exercise Treadmill Testing, a Component of CPET to Evaluate Levels of Physical Disability
The American College of Cardiology
American Heart Association, American Thoracic Society
American College of Chest Physicians
Social Security Administration
American Physical Therapy Association 5.3.1
American Medical Association
American College of Sports Medicine

- KPS is a traditionally used measure that allows patients to be classified as to their functional impairment and was the primary endpoint in AMP-502 and a secondary endpoint in AMP-516. KPS was designed to measure the level of patient activity and medical care requirements.
- Reductions in concomitant medications, a clinical endpoint that has been widely used, was an efficacy endpoint in both studies.

Table 1.5. Summary of Key Results Relating to Efficacy and Clinical Benefit in Subjects Randomized to Ampligen Versus Placebo in the Phase 3 Study AMP-516 (n=234)

Efficacy Endpoints	**Key Secondary Endpoints**
Primary Endpoints: Change from Baseline in Exercise Tolerance Test duration at Week 40: • Ampligen improved placebo-adjusted intra-patient ETT by 21.3% and intra-group ETT by 11.8% (p=0.047) in ITT Subjects • Medically significant improvement over pre-defined intra-group placebo-adjusted benchmark: 11.8% (Ampligen) vs. 6.5% (benchmark) in ITT Subjects • Medically significant improvement over pre-declared intra-group placebo-adjusted benchmark: 14.3% vs. 6.5% in Subjects without significant dose reductions (pre-declared subset) • Proportion of patients with ETT improvement of at least 25%: Ampligen (39.0%) vs. placebo (23.1%) (p=0.013) • Correcting for subjects with reduced dosing compliance increased placebo-adjusted mean intra-patient ETT improvement to 28% (p=0.022)	• Patients with ETT improvement of at least 25% also had a medically significant improvement in KPS of 10 points (p=0.039) • Patients with ETT improvement of at least 25% also had a medically significant improvement in Vitality Scores (SF-36) of 14.6 points (p=0.008) • ITT Population who took concomitant medications: 72% of Ampligen subjects decreased use of concomitant medications vs. 56% who received placebo (p=0.0145)
Clinical Benefits (QT Interval) Placebo subjects had a significantly longer QT interval, compared to Ampligen subjects (p=0.049)	**Cross-over Study (AMP-516C/E)** Placebo subjects crossed-over to receive Ampligen demonstrated an intra-patient improvement in ETT performance at 24 weeks of 39% (p=0.04)

Table 1.6: Summary of Key Results Relating to Efficacy and Clinical Benefit in Subjects Randomized to Ampligen Versus Placebo in the Phase 2 Study AMP-502 (n=92)	
Efficacy Endpoints	**Key Secondary Endpoints**
Primary Endpoint: Increase in KPS at 24 Weeks • Ten (10) point median change in KPS scores in Ampligen-treated patients over placebo (p=0.016) • Increase in median KPS from Baseline of 50 to 60 at Week 24 in Ampligen-treated patients versus placebo with no change (p=0.014)	• Mean ETT duration increased by 95.3 seconds in the Ampligen group versus 57.9 seconds in the placebo group (p=0.010) • In AMP-502, a statistically significant smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use for relief from the symptoms of CFS, compared with the percentage of subjects in the placebo group (44.7%) (p=0.023)
Clinical Benefits Analyses of the distribution of median KPS scores at Week 24 also demonstrated statistically significant improvement at Weeks 16, 20 and 24 (p=0.015, p=0.013 and p=0.015, respectively) in the Ampligen group compared to placebo **Key QT Medication Use** • Placebo subjects increased concomitant medications including those with a known risk of prolonging QT interval compared to Ampligen subjects thereby decreasing their risk for cardiac events	**Clinical Benefits (cont'd)** • Incidence of depression with or without suicide attempt and/or suicide ideation occurred 3 times more often in the placebo group vs. Ampligen (p<0.02) • Suicide attempt/suicide ideation occurred 4 times more often in the placebo group (4) than with Ampligen treatment (1) (difference not statistically significant) • Less hospitalization for depression in the Ampligen group with 0 occurrences vs. 5 in the placebo group (p=0.06) • Percentage of hospitalization for depression out of all hospitalizations was 0% for Ampligen vs. 45% for placebo

Ampligen affords a significant advancement in the treatment of the severely debilitated CFS patient as evidenced by well-controlled trials with historically established therapeutic endpoints.

1.3.4 Exercise Treadmill Testing (ETT)

For AMP-516 the primary efficacy endpoint was intra-group change in duration from Baseline ETT which was met in the study. The ANCOVA analysis of non-transformed ETT data was statistically significant (p=0.047). In the ITT Population, the mean ETT duration in the Ampligen group at Baseline and Week 40 were 576.3 and 672.0, respectively, for a 95.7 second increase over the 40 week treatment period.

Table 1.7: AMP-516 Primary Efficacy Endpoint: Intra-Group Change from Baseline in ETT Duration at Week 40 (ITT Population)

	Ampligen (n=100)	**Placebo (n=108)**	**p-value**
Baseline Seconds Mean	576.3	588.1	
Change from Baseline Mean	95.7	28.2	**0.047**[1]
p-value[2]	<0.001	<0.001	

[1] ANCOVA with Baseline ETT as a covariate
[2] Wilcoxon signed-rank test

In AMP-502, mean ETT duration (seconds) in the Ampligen group at Baseline and at Week 24 were 744.1 and 839.4, respectively, for an increase of 95.3 seconds at Week 24 versus of 57.9 seconds in the placebo group. As shown in Table 1.8, the difference in mean ETT scores for the Ampligen group compared to placebo was statistically significant (p=0.010).

Table 1.8: AMP-502: Intra-Group Change from Baseline in ETT Duration at Week 24 (ITT Population)

	Ampligen	**Placebo**	**p-value**
Baseline Seconds Mean	744.1	602.8	
Change from Baseline Mean	95.3	57.9	**0.010**[1]
p-value[2]	0.050	0.116	

[1] An ANCOVA of log-ratio transformed data with Baseline and sites as covariates.
[2] Wilcoxon signed-rank test.
The ITT Population for ETT analysis will include all subjects who had a Gillespie modified Bruce protocol ETT at Baseline and had a minimum of one post-Baseline Gillespie modified Bruce protocol ETT evaluation.

The Anti-Viral Division asked that for AMP-516 the increase over an ETT benchmark also be evaluated. The benchmark equal to placebo-adjusted mean intra-group increase in ETT duration was based on that for two drugs that had been approved around that time for congestive heart failure (Fosinopril and Captopril) and was set at 6.5%. In AMP-516, the ETT duration increased a mean of 16.6% from Baseline to 40 weeks, compared to 4.8% for the placebo group. **This difference of 11.8% was medically significant as pre-defined in the AMP-516 protocol since it was greater than 6.5%. The table which compares the Ampligen result with that of other drugs that have been approved is found in Table 1.17.**

In addition to the primary endpoint and the absolute difference relative to 6.5%, we have also looked at the proportion of patients who achieved either a "minimum standard for clinical improvement" defined as a ≥25% increase in duration relative to Baseline and a "significant clinical improvement" (≥50% increase in duration, relative to Baseline). **These supplemental analyses are intended to compare the proportion of patients classified as *responders* between the 2 randomized treatment groups**. We believe that these data support our primary endpoint data and can provide a better understanding of the clinical relevance and potential utility that can be derived for the CFS population by such proposed treatment. As shown below there was a statistically significant improvement in the Ampligen arm of the study compared to placebo.

Table 1.9: AMP-516: Frequency Distribution of Percent Change from Mean Baseline Exercise Treadmill Duration at Week 40 (ITT Population)

Improvement from mean Baseline Exercise Treadmill Duration	Ampligen (n=100)	Placebo (n=108)	p-value[1]
Baseline, seconds	576.3	588.1	—
At least 25%, n (%)	39 (39.0)	25 (23.1)	0.013
At least 50%, n (%)	26 (26.0)	15(13.9)	0.028

[1] Probability that a difference between treatment groups exists using the Chi-square test.

The proportions of subjects in the ITT Population with changes from mean Baseline ETT duration at Week 40 of at least 25% and of at least 50% were 1.6 and 1.9-fold greater for subjects randomized to Ampligen than placebo; 39% versus 25%, and 26% versus 15%.

1.3.5 Karnofsky Performance Score (KPS)

In AMP-502, the primary protocol-defined efficacy endpoint was improvement from Baseline to Week 24 in KPS score, which was statistically significantly greater in the Ampligen group than in the placebo group. This endpoint was achieved.

Table 1.10: AMP-502 Primary Efficacy Endpoint: Median and Median Changes in KPS from Baseline to Week 24 (ITT Population)

	Ampligen (n=45)		Placebo (n=47)	
Time	**Median**	**Median Change**	**Median**	**Median Change**
Baseline				
Median	50.0		50.0	
Week 24				
Median	60.0	10.0	50.0	0.0
p-value[1]	**0.014**	**0.016**		

[1] Wilcoxon-Mann-Whitney Test, 2-sided comparing Ampligen vs. placebo groups

Median KPS scores in the Ampligen group from Baseline to Weeks 4, 8, 12, 16, 20, and 24, were 50.0, 50.0, 60.0, 60.0, 60.0, 60.0, and 60.0, respectively, for a 20% increase over time. Differences in median KPS scores from Baseline to completion between Ampligen and placebo became statistically significant (p=0.023) at Week 16 and remained statistically significant through Week 24. Differences in the changes in median KPS scores from Baseline to completion between Ampligen and placebo became statistically significant (p=0.013) at Week 16 and remained statistically significant Week 24.

Analyses of the distribution of median KPS scores at Week 24 also demonstrated statistically significant improvement (p=0.015) in the Ampligen group compared to placebo. Numerous studies in other chronic, severely debilitating diseases have established that the observed differences of this magnitude are medically significant.

Table 1.11: AMP-502: Summary of Distribution of Median KPS from Baseline to Week 24 (ITT Population)

Week	Treatment Group	KPS Score									p-value[1]
		Subjects at Each Level									
		20	30	40	50	60	70	80	90	100	
0	Ampligen	0	2	10	15	18	0	0	0	0	
	Placebo	1	3	8	17	18	0	0	0	0	0.733
24	Ampligen	0	0	4	10	19	7	3	0	2	
	Placebo	0	2	9	13	18	2	2	1	0	**0.015**

[1] Cochran-Armitage trend test, 2-sided.

1.3.6 Concomitant Medications

Both AMP-502 and AMP-516 show credible evidence of Ampligen's clinical effect to decrease the use of concomitant medications to palliate certain symptoms of CFS. The reduction in concomitant medications was a secondary efficacy endpoint.

The number of days of medication use during the first 4 weeks of study and during the last 4 weeks of study were counted to determine the change in use of concomitant medication in AMP-502 and AMP-516. In AMP-502, a statistically significant smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use for relief from the symptoms of CFS, compared with the percentage of subjects in the placebo group (44.7%) (p=0.023). In AMP-516, the proportion of subjects who decreased their use of concomitant medications was significantly greater for subjects who took at least one concomitant medication and received Ampligen (72.3%) versus placebo (55.7%) (p=0.0145).

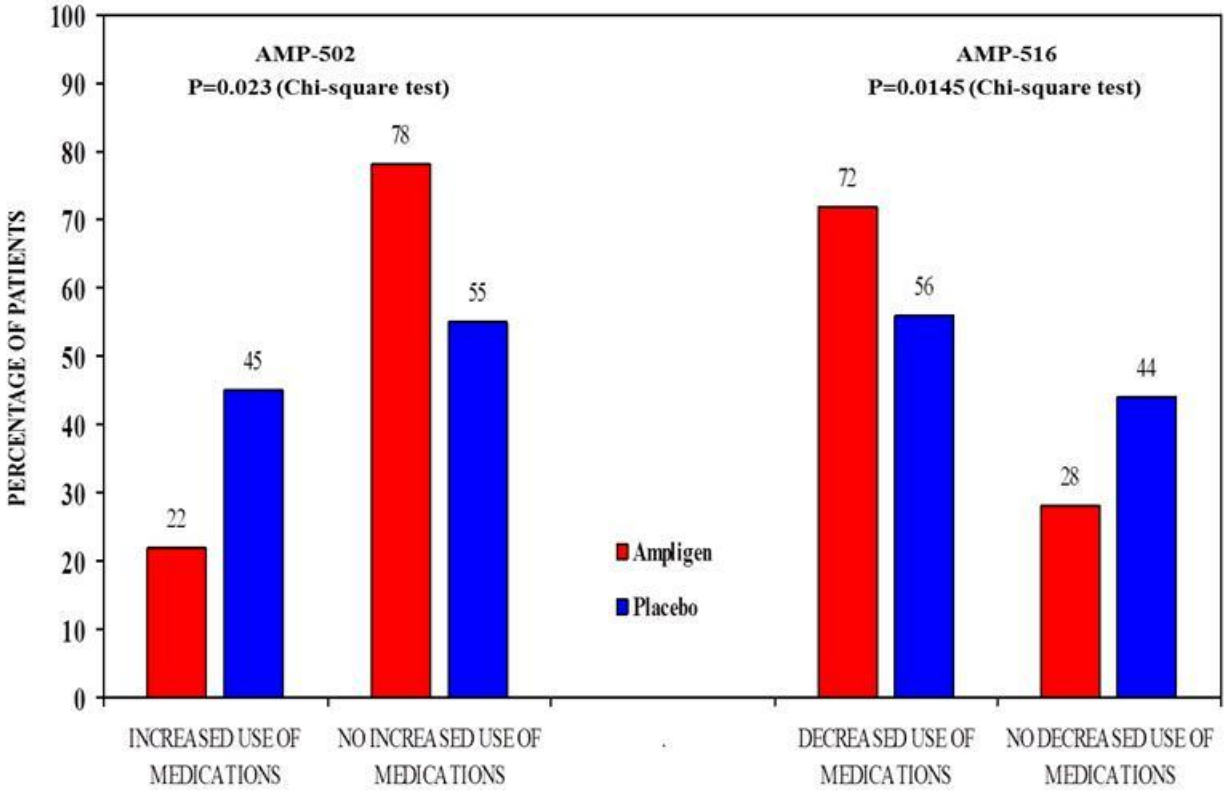


Figure 1.2: Change in Concomitant Medications Used to Palliate Symptoms of CFS

The reduction in the use of concomitant medications used to palliate the symptoms of CFS also includes medications which prolong the QT interval.

1.3.7 Activities of Daily Living, Vitality, and General Health Perceptions

In the dichotomous, proportionate analysis for AMP-516 discussed above, those who increased ETT by 25% or more (39% of the subjects) also saw parallel improvements in their daily life, as measured by Activities of Daily Living (ADL) and vitality and general health perceptions scales.

The vitality and general heath perception scores were analyzed from the SF-36 health survey, specifically the Vitality Index, 4 items in Section 9 of that survey dealing with the amount of time in the past four weeks that the subject felt full of "pep", had a lot of energy, felt worn out, and felt tired; and the General Health Perception, Section 11 of the survey, asking the subject to respond to 4 statements concerning relative health. **The SF-36 was recently independently validated as an appropriate instrument in CFS.**

1.3.8 In AMP-502 Well-Controlled Study, a Reduction in Hospitalizations

The incidence of depression for the CFS subjects was associated with suicide attempt and/or suicide ideation and occurred three times more often in the placebo group (18) vs. the Ampligen cohort (6) ($p<0.02$). Suicide attempt/suicide ideation occurred four times more often in the placebo group (4) than with Ampligen treatment (1), a difference trend, not statistically significant. There was also a trend towards less hospitalization for depression in the Ampligen group with no occurrences in the Ampligen cohort vs. five in the placebo group ($p=0.06$). The percentage of hospitalization for depression out of all hospitalizations was 0% for Ampligen group vs. 22% for the placebo cohort.

Table 1.12: Hospitalization for Depression by Incidence in AMP-502

Parameter Related to Depression and Suicide Attempt/Ideation	Incidence of Event		Fisher's Exact Test p-value (2-tail)
	Ampligen	Placebo	
AE, Depression[1]	6	18	0.02
Hospitalization for Depression[2]	0	5	0.06
AE, Suicide Attempt / Suicide Ideation[3]	1	4	0.37

[1] Depression includes depression with or without suicide attempt/ideation.
[2] Hospitalization includes hospitalization for depression.
[3] Suicide attempt and suicide ideation include with or without depression.

1.3.9 Safety Considerations

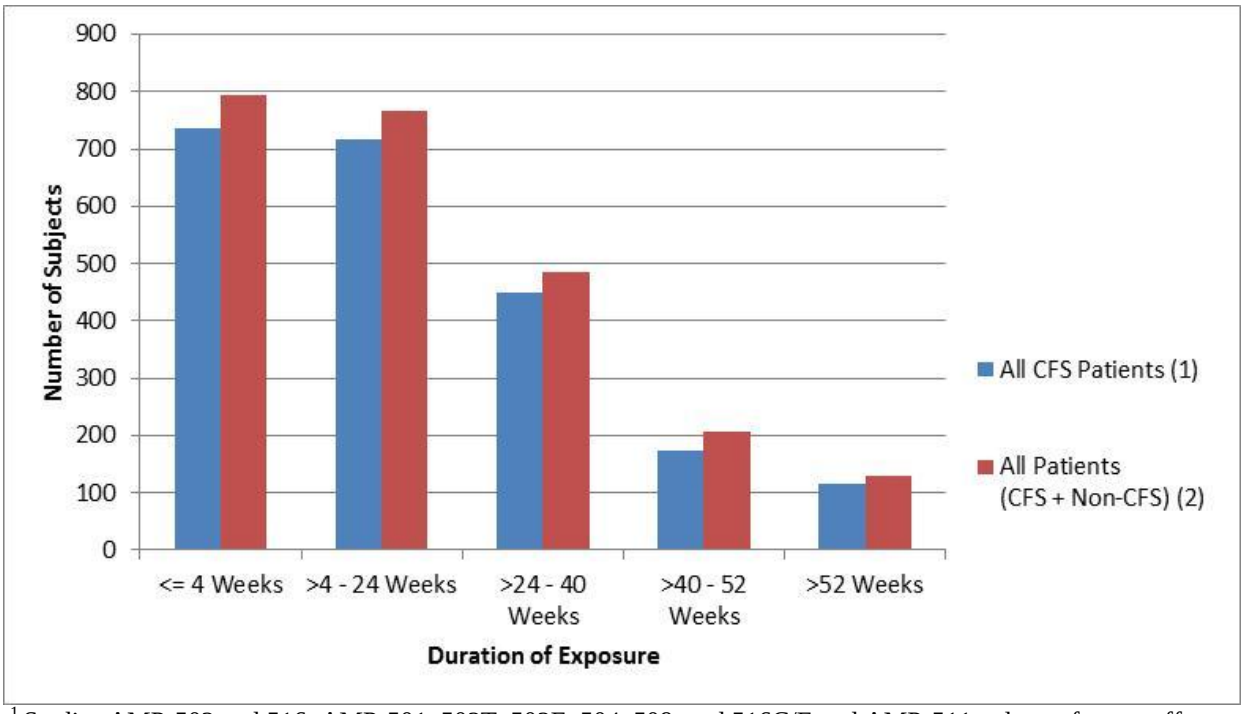
There have been 13 clinical studies conducted by Hemispherx Biopharma, Inc., under IND # 39,250, of Ampligen to assess its safety in subjects with CFS (9 studies), and with supporting safety data from subjects with HIV infection, hepatitis B virus infection, and thermal injury. The data support our conclusion that Ampligen for intravenous infusion is safe in the treatment of adults with CFS.

Table 1.13: Patients on All Studies of Ampligen				
Protocol	**Phase**	**Number of Patients**	**Number of New Unique Patients**	**Number of New Unique Patients on Ampligen**
CFS Studies				
AMP-516	Phase 3	234	234	117
AMP-502	Phase 2	92	92	45
AMP-516C/E	Phase 3	190	0	100
AMP-502E	Phase 2	34	0	17
AMP-502T	Phase 2	19	19	9
AMP-511	Phase 3	164	125	125
AMP-509	Phase 2	152	152	152
AMP-504	Phase 2	12	8	8
AMP-501	Phase 1/2	16	16	16
Total CFS		**913**	**646**	**589**
Total Non-CFS		**306**	**285**	**256**
Total		**1,219**	**931**	**845**

Ampligen has been in development since 1988 and has been generally well-tolerated in clinical trials that enrolled over 1,200 patients with over 90,000 intravenous doses of Ampligen administered over 600 patient years of Ampligen dosing. Over 200 patients have received Ampligen for at least one year or longer. There has been no evidence of any cumulative toxicities, as no adverse event (AE) became progressively manifest as a function of duration of therapy.

In the CFS studies, 589 unique subjects received treatment with Ampligen. Twenty-five subjects received Ampligen in 2 or more consecutive studies. The average length of Ampligen treatment was 43.6 weeks, while the median was 26.6 weeks. One hundred seventy subjects (30%) received treatment with Ampligen for greater than 1 year, and 37 subjects (6.5%) received treatment for between 2 and 4 years.

The breakdown by duration of exposure for CFS patients and for all patients is set forth below.



[1] Studies AMP-502 and 516; AMP-501, 502T, 502E, 504, 509, and 516C/E and AMP-511 at last safety cutoff
[2] Studies AMP-502 and 516; AMP-501, 502T, 502E, 504, 509, and 516C/E; AMP-511 at last safety cutoff and Studies 700 (HIV), 700E (HIV), 800 (Hepatitis B), and 900(Burn patients)

Figure 1.3: Number of Subjects Who Received IV Ampligen by the Duration of Exposure (CFS and Non-CFS patients)

Overall, infusions of Ampligen were well-tolerated by subjects in the CFS and Non-CFS studies. In the early studies, a number of events (e.g., injection site reactions, flu symptoms) were observed to occur shortly after starting Ampligen infusions. To allow subjects to develop tolerance and minimize these reactions, (**as well as to maintain the double-blind**) subjects in later studies received smaller doses (200 mg) during the first 4 infusions, then the dose was increased to the targeted therapeutic dose (400 mg). Only the dose was changed for these initial infusions; the frequency of drug administration was unaltered.

In the controlled studies, approximately 1% of subjects receiving Ampligen and 14% of placebo subjects discontinued prematurely because of lack of response; a comparable rate of withdrawal for adverse events occurred in both groups (more than 1% and less than 2%).

A high percentage of subjects in the CFS studies reported AEs whether receiving Ampligen or placebo, a reflection of the symptoms associated with CFS. The most frequently reported events included flu syndrome, headache, pain, nausea, asthenia, rash, and myalgia. Adverse reactions observed in controlled clinical studies that occurred in 5% or more subjects and at a rate twice that of placebo were chills, palpitations, and sweating.

There was no significant difference in the number of Serious Adverse Events (SAEs) in AMP-502 and AMP-516 (p>0.4).

Depression considered related to the study medication occurred in 8% of placebo subjects in the controlled studies and 0 subjects on Ampligen. In all the controlled clinical studies with Ampligen, none revealed any increased risk for suicide over placebo.

Table 1.14 below summarizes SAE's in AMP-502 and AMP-516 and their relationship to study drug (i.e., none, remote, possible, probable, or definite) and compares the number of those events to placebo.

Table 1.14: AMP 502 & AMP 516: No Significant Difference in Number of Serious Adverse Events (SAEs)

Relationship to Study Drug	Number of Serious Adverse Events	
	Ampligen	Placebo
No*	16	16
Remote*	4	3
Possible*	2	2
Probable*	0	1
Definite*	0	0
Total	22	22

Concomitant Medications, Including Those that Prolong QT Interval

The reduction in the use of concomitant medications used to palliate the symptoms of CFS (Section 6.3) also includes medications which prolong the QT interval (Stouch 2010). By reducing the need for these QT prolonging medications, Ampligen may help to decrease a parameter that is known to be related to catastrophic cardiac events, especially in middle-age females with CFS (Jason 2006) who otherwise would not be expected to be at risk for catastrophic cardiac events.

In AMP-516, 72% of the patients randomized to receive Ampligen who were taking concomitant medications experienced a reduction in exposure to concomitant medications including those with a known risk of prolonging QT, compared to 44% of the patients randomized to receive placebo. Comparing the proportion of patients between randomized treatment assignment and decreased exposure revealed a significant difference in favor of Ampligen (p=0.0145). **Patients randomized to receive Ampligen were one and three-quarters times more likely to have reduced exposure to medications which include those known to prolong QT, compared to placebo** (Odds ratio: 1.76 [95% CI: 1.00 to 3.11]).

Hepatic Function in Well-Controlled Trials of Ampligen

Transitory liver function tests (LFTs) were slightly elevated in a greater percentage of Ampligen treated patients however, these were clinically and medically insignificant and did not cause CFS subjects termination in the study. Furthermore, at higher levels of LFT abnormalities, placebo and Ampligen patients have similar percentages of abnormality.

No Evidence of Autoimmunity Related to Ampligen on Long Term Exposure

Unlike poly IC, the prototypic "TLR-3 agonist", Ampligen has not been shown to engage cytoplasmic dsRNA sensors and is thus differentiated from the activity of poly IC which acts both through TLR-3 and the intracytoplasmic receptor MDA5. This may account for the differential toxicity, and reflect the more rapid intracellular metabolism of Ampligen. **The highly specific TLR-3 activation of Ampligen was extensively studied by Professor Ralph Steinman, Rockefeller University, who received the Novel Prize in Medicine in 2011.** Ampligen has relatively short half-life of approximately 30 to 40 minutes compared with the >4 hour half-life of poly IC. Avril (2009) evaluated cytokine profiles elicited by various TLR agonists, and found that in his system Ampligen did not induce TNF-α in contrast to poly IC and other compounds. TNF-α has been found to be associated with induction of autoimmune toxicity as well as carcinogenicity in animals, so this finding is consistent with the observed **lack of observed autoimmune toxicity and carcinogenicity associated with the clinical use of Ampligen**.

It has been hypothesized that some TLR agonists may induce undesirable immunological signals. Generally, these TLRs operate by other pathways which trigger more robust cytokines including TNF-α. In contrast, there have been no reports of new onset autoimmune disease reported in any of the CFS clinical trials conducted with Ampligen. Ampligen is quickly metabolized into naturally occurring nucleotides and has a brief half-life of 37 minutes. All metabolic decay of Ampligen is into naturally occurring constituents of RNA, normally found throughout the human body. Further, ten (10) preexisting auto antibodies were found to progressively fall in Ampligen treated subjects and only two (2) new auto antibodies appeared. In particular, we have determined autoantibody levels in 25 AMP-511 subjects who had received Ampligen for one year or longer, including 14 of the subjects who have been on study for 4 years or longer. It showed that a greater number of patients' autoantibody tests converted from an initial antibody positive test result to a negative test (n=10) as compared to an initial antibody negative test result, which thereafter converted to a positive test (n=2) and **indicate no evidence that Ampligen is inducing autoimmune disease or nascent signals of autoimmunity**.

Table 1.15: Number of Subjects by Total Ampligen Exposure Time in the Autoimmune Signal Analysis Program

	>12 Months	**>24 Months**	**>36 Months**	**>48 Months**
No. of Subjects (AMP-511)	25	20	17	14

Ampligen is currently being tested as a safe, efficacious adjuvant with anti-cancer vaccines in FDA-approved clinical studies at the University of Washington, University of Pennsylvania and University of Pittsburgh for breast, ovarian and colorectal cancer.

Ampligen has a strongly positive benefit-risk profile for the treatment of CFS. In Ampligen-treated patients there is a meaningful benefit in terms of physical and cognitive function and decrease in the symptom burden associated with CFS, a decrease in the use of concomitant medications and improvement in the QT interval. In patients who do not achieve ETT improvement, the risks of Ampligen therapy are low since the majority of adverse events are mild, moderate and transient. Eighty five percent (85%) of patients enrolled on the Treatment Protocol, AMP-511 choose to continue on Ampligen after the initial 24 week course of treatment. In fact, for the 138 subjects who have been on the protocol who completed 24 weeks of treatment, 131 (94.9%) elected to continued treatment beyond 24 weeks. Ninety-seven (97) subjects (59.1%) received infusions for at least 48 weeks, and 71 subjects (43.3%) received infusions for at least 72 weeks.

1.3.10 Conclusion

CFS is a serious, life threatening, and unmet medical need. At present there are no other satisfactory treatment options for patients with CFS with the exception of Ampligen. Palliative therapy with other drugs is all that is available and benefits are transitory and present adverse risks including those that are potentially life threatening due to QT interval prolonging attributes. Ampligen, which shortens the QT interval in this high-risk group, reduces the concomitant medicine use. It is the first definitive treatment for CFS. We believe that the preponderance of data clearly provides substantial evidence of efficacy and reasonable safety for this product, for the target population of CFS patients. The efficacy of Ampligen has been demonstrated in numerous clinical studies, including two randomized placebo-controlled studies, AMP-502 and AMP-516, both of which had extension phases.

Summary of combined results of the two well-controlled studies (AMP-516 and AMP-502):

- Clinically meaningful increases in ETT were observed following Ampligen treatment in two multi-centered, randomized, double-blind, placebo-controlled trials which were extensively audited by the FDA.

- A reduction in exposure to concomitant medications typically used to palliate the symptoms associated with CFS, many of which are known to have a detrimental impact on QT interval prolongation, thus creating a potential life-threatening cardiac toxicity.

- The Karnofsky Performance Scale improved significantly relative to Baseline and relative to the placebo group and there was a parallel improvement in activities of daily living.

- Quality of Life measures (SF-36) improved in parallel with ETT outcomes especially in the subgroups of patients which lead a $\geq$25% improvement in ETT.

- "Cognition" improved in patients in the AMP-502 and were also seen in the European-based AMP-509 study, including improvement in their ability to remember things, reduced temper outbursts, doing things more quickly, reduction in "double checking" things, less frequent episodes of one's mind "going blank", and other improvements.

- A greater number of patients significantly improved medically on Ampligen and fewer patients became worse, which may be the natural course of the chronic disease. **Thus,**

clinical deterioration is the natural course of the disease for many CFS patients who are not afforded Ampligen treatment.

- The risks associated with Ampligen are minimal and well-documented.
- Patients on Ampligen showed a reduction in hospitalizations including hospitalizations associated with suicide attempts and suicidal ideation when compared to the placebo group.
- Hospitalizations, emergency room admission and/or inpatient admissions were reduced in patients with CFS receiving Ampligen compared to placebo.
- **Prolonged Ampligen treatment showed no propensity to auto-immunity and indeed patients on Ampligen show a trend to decrease in preexisting auto-antibodies, a potential early step in forming auto immune disease.**

Table 1.16: Benefit of Ampligen against Major Causes of Death in CFS	
Major Causes of Death in CFS	**Established/Potential Ampligen Mitigation Mechanisms**
Heart Failure[1]	Ampligen treatment (in a well-controlled trial) a) reduces use of concomitant drugs including medications which prolong the QT interval, as well as, b) reducing the QT interval as a prognosticator of increased risk for cardiac arrhythmia (AMP 516 and AMP-502) (Stouch 2010), veracity of clinical data has been confirmed by FDA field audits.
Suicide	Ampligen treatment (in a well-controlled trial) reduced incidence of major depression, including suicidal ideation (AMP-502) in which clinical results were also verified by FDA field audits. Derivative macroeconomic benefits to the U.S. health care system were also confirmed by audit of independent experts.
Cancer	Ampligen has broad anti-tumor/immunomodulatory activity against various human tumors (Strayer 1987, Strayer 1986, Mitchell 2007). This is a potential ancillary benefit of Ampligen in immune surveillance of the high risk CFS population. Ampligen treatment decreased the incidence of cancer by approximately 50% in the well-controlled studies. These activities are part of ongoing clinical research at several U.S. based comprehensive cancer centers.

1 Heart failure is associated with a prolonged QT interval

Patients treated with Ampligen often become more productive members of our society. The basis for this therapeutic advantage over the current standard of care is summarized in the following table.

Table 1.17: Comparison of Intra-Group Mean ETT Improvement: Ampligen for CFS vs. Drugs Approved for Non-CFS Severe Exertional Fatigue

Chronic Disease Indication	Drug (Placebo-Controlled Clinical Trial)	Group Analyzed	% Improvement Over Placebo†
Chronic Fatigue Syndrome	**Ampligen (AMP-516)**	**Excluding Significant Dose Reductions**	**14.3***
		Completer	**14.0***
		Intent-to-Treat	**11.8***
		Baseline ETT > 9 minutes	**11.5***
Chronic Congestive Heart Failure	Fosinopril[3]	20 mg dosage group	6.7
	Captopril[4]	150 mg dosage group	6.2
Chronic Angina	Ranolazine[5] (MARISA)	500/1,000 mg Pooled Data 1	6.5
	Ranolazine[6] (CARISA)	750/1,000 mg Pooled Data 1	5.9
Pulmonary Arterial Hypertension[1]	Tracleer (Breathe - 1)	125 mg BID dosage group	10.6
	Remodulin (PO1:04)	1.25-22.5 mg/kg/min	8.0
	Remodulin (PO1:05)	1.25-22.5 mg/kg/min	4.1
	Remodulin (PO1:04-05)	Pooled Data[2]	6.1

* Declared endpoint in pre-specified subsets, not post-hoc analysis (dates agreed upon with FDA) (Reference: AMP 516 Protocol dated 1/24/97 submitted to IND 39,250 on 1/24/97, Serial #056, and response to FDA Comment #1 filed to IND 39,250 on 6/8/01, Serial #136.

† Intra-Group Mean ETT Improvement Over Placebo

1 Data from the FDA summary basis of approval (6 minute walk)

2 Pooled data means results from both study groups were pooled

3 Brown 1995

4 The Captopril-Digoxin Multicenter Research Group 1988

5 Chaitman 2004a

6 Chaitman 2004b

Table 1.18: Relative Therapeutic Advantage: Ampligen vs. Current Standard of Care in Distinct Therapeutic Categories		
	Ampligen Advantage	**Standard of Care Advantage**
Core Fatigue Abatement	Ampligen	None
Improve Activity Performance (≥25% ETT)	Ampligen	None
Global QOL Improvement	Ampligen	None
Polypharmacy abatement	Ampligen	None
EKG Selected Risk(s) Abatement	Ampligen	None
Prevent CFS Disease Progression	Ampligen	None
Lower Incidence of Suicide	Ampligen	None
Lower Incidence of Hospitalizations	Ampligen	None
Macroeconomic (e.g., Hospitalizations, Polypharmacy, Emergency Room Visits) Cost Reduction	Ampligen	None
Probability of Reducing Physician-Based Iatrogenic Error	Ampligen	None

Ampligen helps patients improve their condition enabling them to participate in their lives, while reducing symptom burden, adding quality of life and reducing risks of current treatment. For this group of the most severely ill patients, improvements can have a significant positive impact on their lives as well as having favorable impact on the overall health care and macroeconomic environment. The available evidence supports a positive benefit-risk analysis. Ampligen offers a significant advancement in treatment over the current standard of care. By decreasing the utilization of various palliative, off-label, medications (which include many that prolong QT interval) the availability of Ampligen will contribute to fewer physician based iatrogenic errors in CFS treatment. It can be stated that the burden of the symptoms associated with CFS, is greater than any risks associated with Ampligen; a patient's life is at risk for years of debilitation often followed by premature death.

2. AMPLIGEN

2.1 STRUCTURE

Ampligen (Poly I: Poly $C_{12}U$) is a highly selective Toll-like receptor 3 (TLR-3) activator that activates innate immunity and effectively alters the balance of energy metabolism in patients with CFS. The rationale for the initial trials with Ampligen in CFS was based on its recognized antiviral and immunomodulatory properties and as an optimized endogenous inducer of interferon.

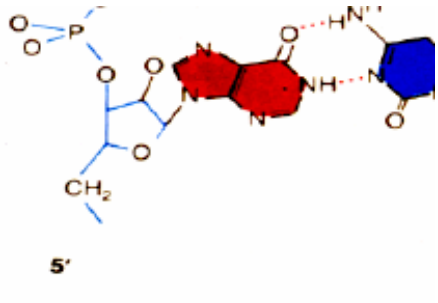


Figure 2.1: Structural Formula of Poly I : Poly $C_{12}U$

Poly I: Poly $C_{12}U$ is a structural analogue of polyribonucleotide, polyriboinosinic acid hydrogen-bonded with poly-ribocytidylic acid (poly I: poly C) containing regularly occurring regions of unmatching (Figure 2.1). In the rC_n strand of Ampligen, uridylic acid substitutions occur on average every 12 to 13 nucleotides (Figure 2.2).

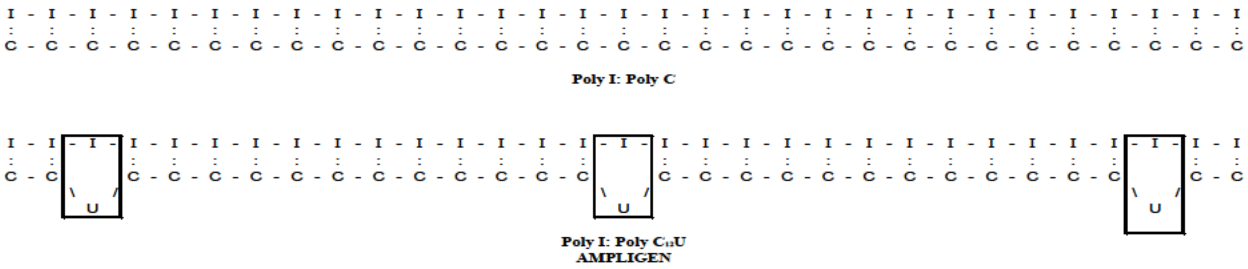


Figure 2.2: Structure of Poly I: Poly $C_{12}U$ (Ampligen)

2.2 MECHANISM OF ACTION

The mechanism of action of Ampligen in CFS is unknown. However, certain genes may be influenced by Ampligen including those for metabolic enzymes and antiviral response elements, and immune modulators. The process is initiated by Toll-like receptors located in the endosomal compartment of various cells involved in antigen processing, notably including dendritic cells. Toll-like receptors account, at least in part, for the specificity of the host defense response. **As primordial transmembrane, pattern recognition receptors, Toll-like receptors trigger alarm signals against invading pathogens by modulating cytokine cascades**. Each subtype of Toll-like receptor has unique ligands for products of pathogens and for infectious microorganisms (Akira 2004). The ligand for TLR-3 is double-stranded RNA (dsRNA), which comprises virus components, e.g., nucleic acids produced by many viruses in the cells they infect. TLR-3 is not a receptor for proteins from host, bacteria, or synthetic compounds (Akira 2004, Kawai 2006). **Local TLR-3 bearing cells respond to the activation of the receptor by triggering multiple innate immune responses (e.g., interferon type 1, a narrow spectrum of cytokines (not including TNF-α), induction of dendritic cell maturation).**

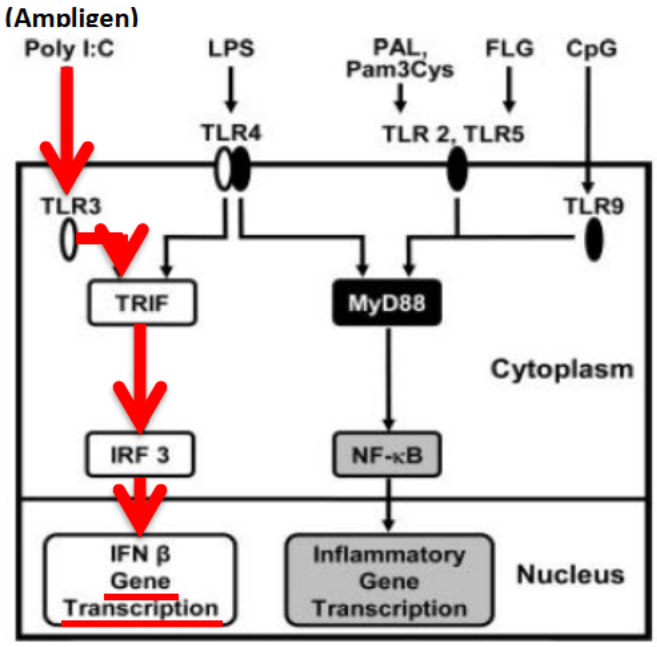


Figure 2.3: Mechanism of Action of Ampligen as a TLR-3 Agonist is Shared in Part with TLR-4 Agonists

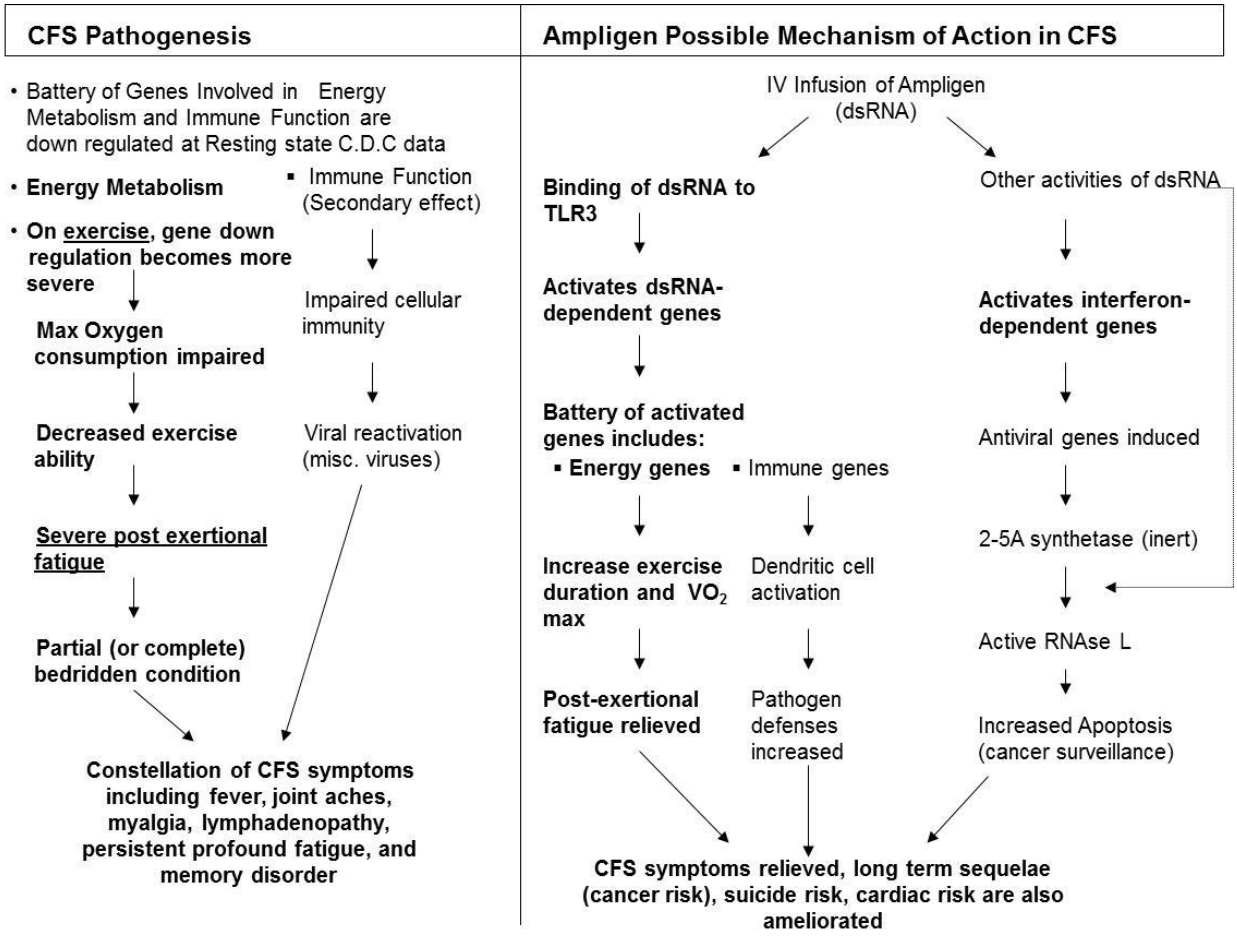


Figure 2.4: Proposed Mechanism of Action Diagram in CFS Treatment (arrows indicate the sequence of events in pathogenesis and treatment)

As noted above, the precise relationship to CFS pathology remains to be elucidated; however the intermittent activation of TLR-3 from twice weekly administration of Ampligen apparently alters the balance of energy metabolism as measured by a reproducible increase in exercise capacity on an exercise treadmill. The fatigue of CFS mimics the fatigue associated with acute and chronic viral infection (Komaroff 2011), and it has been hypothesized that intermittent activation of the pathogen activated metabolic pathway TLR-3 through Ampligen exposure modulates this component of the innate immune response in a manner that favorably impacts patients with this disease.

3. CHRONIC FATIGUE SYNDROME

3.1 A SERIOUS AND LIFE THREATENING DISEASE

Chronic fatigue syndrome is a complex physical illness characterized by debilitating fatigue, post-exertional malaise, pain, cognitive problems, sleep dysfunction and an array of other immune, neurological and autonomic symptoms. The key feature of the syndrome, post-exertional malaise, is the exacerbation of symptoms following minimal physical or mental activity, which can persist for hours, days or even weeks. Rest and sleep produce only modest relief of fatigue and the other symptoms.

Studies performed by the CDC have shown that CFS can be as debilitating as rheumatoid arthritis, multiple sclerosis, lupus, heart disease, end-stage renal disease, COPD, and similar chronic conditions. Table 3.1 looks at the causes of death amongst 144 CFS sufferers who were analyzed by Jason et al. in a 2006 publication (Jason 2006).

Table 3.1: Main Causes of Death in Patients with Chronic Fatigue Syndrome

Cause of Death	Number (%) of Subjects n=144	Percent	
		Male	Female
Heart failure[1]	29 (20.1%)	34.5	65.5
Suicide[1]	29 (20.1%)	17.2	82.8
Cancer	28 (19.4%)	17.9	82.1

[1] Statistically significant at $p<0.01$
Data source: Jason 2006

In that study the median age of death for those with CFS was compared with overall medians in the US (Table 3.2).

Table 3.2: Median Age of Death in CFS Population vs. Overall U.S. Population

- Cancer is age 72 overall in the US vs. 47.8 for those with CFS
- Suicide is age 48 vs. 39.3 for those with CFS
- Heart failure is age 83.1 vs. 58.7 for those with CFS

Another very recent study (Chang 2012) looked at the increased risk of non-Hodgkin's lymphoma (NHL) that is associated with CFS. Using linked Surveillance, Epidemiology, and End Results (SEER)-Medicare registry data, the National Cancer Institute established that, after adjusting for various factors including age and sex, NHL is increased in incidence in people with CFS. In summary, CFS was strongly associated with NHL overall and NHL subtypes.

Importantly, Ampligen may be able to provide benefit in reducing these causes of mortality in CFS patients. Various articles have been published that look at the use of Ampligen and the reduction in causes of mortality in CFS patients, including reductions in concomitant drug use and the relationship with cardiac arrhythmia as well as reduced incidence of major depression and potential antitumor activity (Table 3.3).

Table 3.3: Benefit of Ampligen against Major Causes of Death in CFS	
Major Causes of Death in CFS	**Established/Potential Ampligen Mitigation Mechanisms**
Heart Failure[1] (Jason 2006)	Ampligen treatment (in a well-controlled trial): a) reduces use of concomitant drugs including medications which prolong the QT interval, as well as, b) reducing the QT interval as a prognosticator of increased risk for cardiac arrhythmia (AMP-516 and AMP-502) (Stouch 2010).
Suicide (Jason 2006)	Ampligen treatment (in a well-controlled trial) reduced incidence of major depression, including suicidal ideation (AMP-502). Derivative macroeconomic benefits to the U.S. health care system were also confirmed by audit of independent experts.
Cancer (Jason 2006, Levine 1998, Chang 2012)	Ampligen has broad anti-tumor/immunomodulatory activity against various human tumors (Strayer 1987, Strayer 1986, Mitchell 2007). This is a potential ancillary benefit of Ampligen in immune surveillance of the high risk CFS population. Ampligen treatment decreased the incidence of cancer by approximately 50% in the well-controlled studies. These activities are part of ongoing clinical research at several U.S. based comprehensive cancer centers.

[1] Heart failure is associated with a prolonged QT interval

The illness is also characterized by substantially reduced physical and/or cognitive functioning.

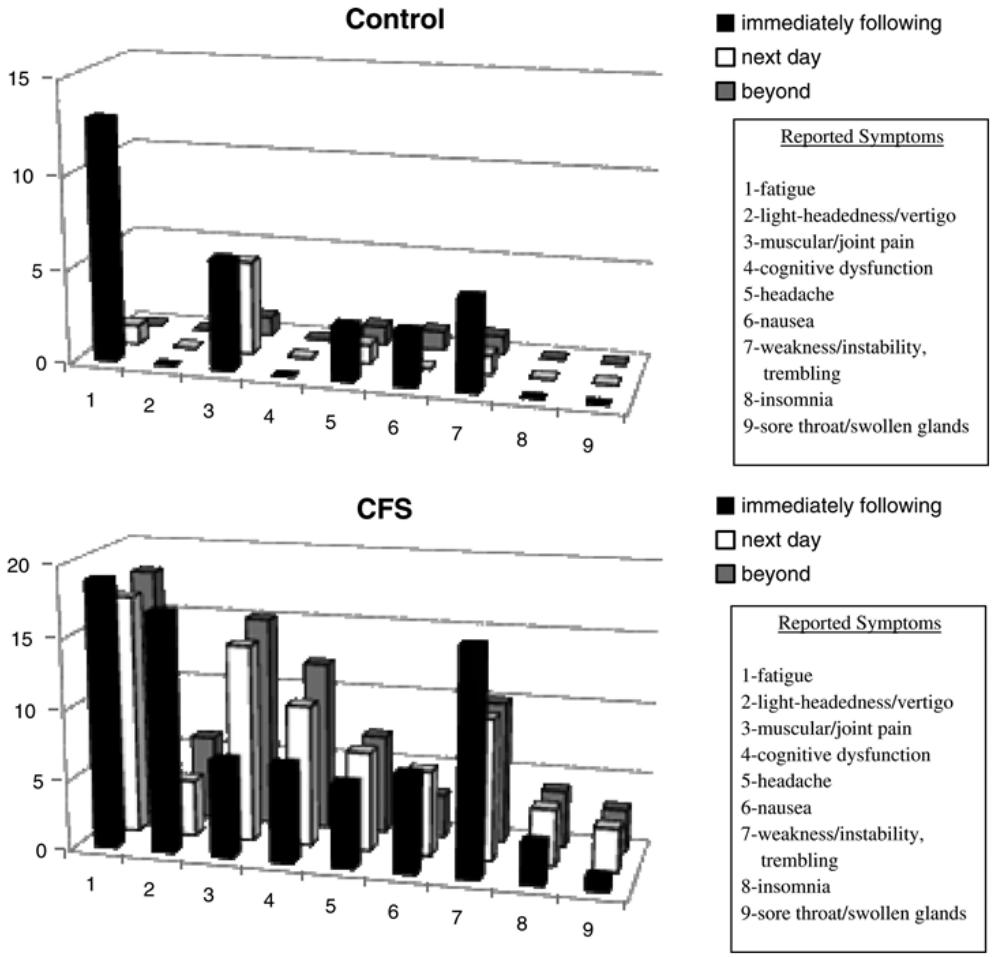


Figure 3.1: Effect of Exercise on Increasing CFS symptomatology as Compared to Matched Control Groups

As shown in Figure 3.1, CFS subjects and control groups were challenged under exercise stress conditions and symptomatolgy was measured thereafter over several days (VanNess 2010). The CFS subjects had much more pronounced symptoms and were much slower to recover across all symptom categories.

Chronic fatigue syndrome causes dysfunction of virtually every organ system in the body and, as noted above, can lead to premature mortality. Researchers have found abnormalities in heart rate and blood pressure (autonomic nervous system), defects in the natural killers T-cells (immune system), reduced cortisol levels (endocrine system), and abnormal metabolism of fatty acids (metabolic system).

The pathophysiological consequences of CFS are multi-systemic and may include: immune and neuroendocrine abnormalities; brain dysfunction and neurocognitive defects; cardiovascular and autonomic disturbances; abnormalities in energy production including mitochondrial dysfunction; and changes in the expression of certain genes. Figure 3.2 presents one possible model of CFS as a multi-system disorder (Chronic Fatigue Syndrome Myalgic

Encephalomyelitis, a Primer for Clinical Practitioners, International Association for CFS/ME 2012 Edition). **The evidence for abnormalities is consistent with recent studies that assess the effects of exertional challenges utilizing physical (exercise or orthostatic) or cognitive (mental) tasks. Importantly, these provocation studies, especially exercise, may be more likely to generate the core symptom of post-exertional malaise.**

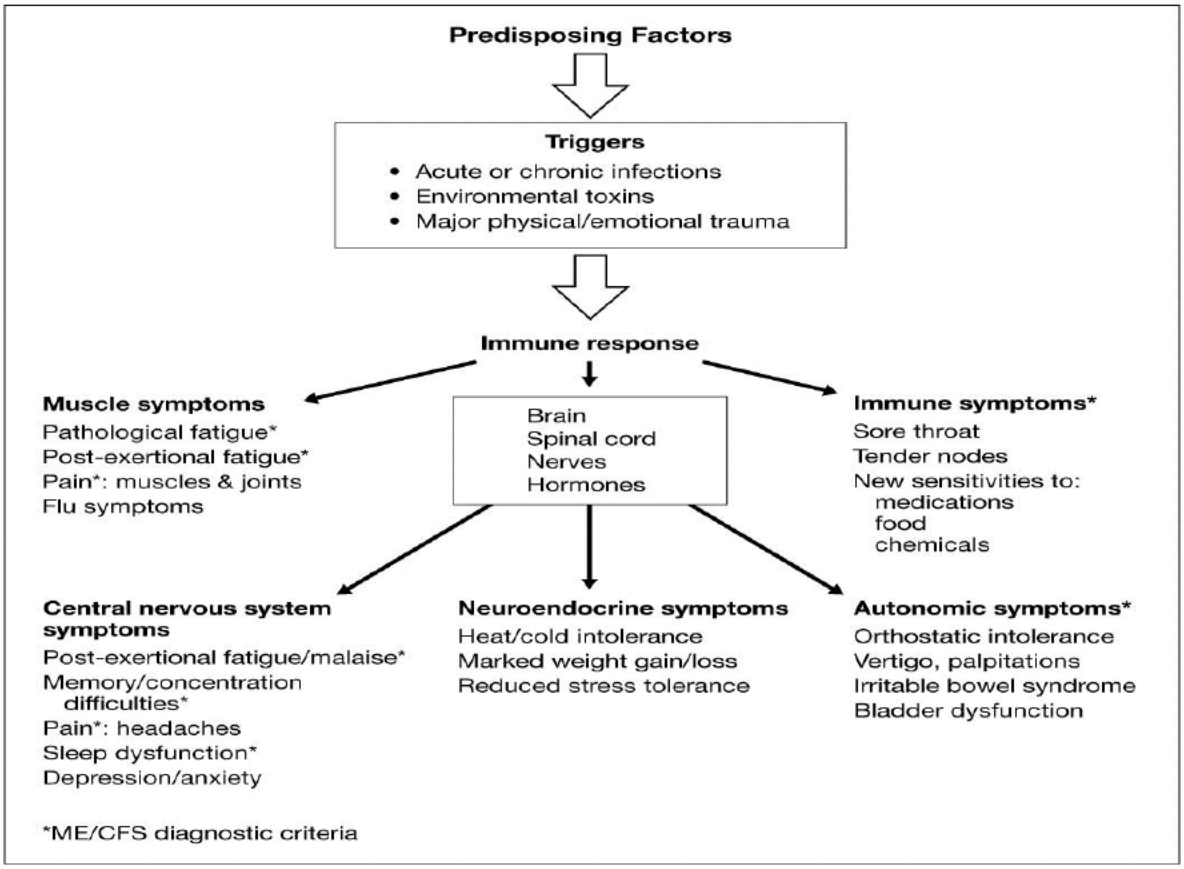


Figure 3.2: Multisystem Dysregulation in CFS

Immune System Abnormalities

The immune system abnormalities in patients with CFS may be associated with symptom severity.

Immune system findings in patients with CFS include:

a) A shift towards a Th2 dominant immune response, with a preponderance of humoral over cell-mediated immunity

b) Immune activation with increased numbers of activated T lymphocytes, including cytotoxic T cells and elevated circulating cytokines

c) Poor cellular function with low natural killer cell cytotoxicity

d) Fatigue and flu-like symptoms may be linked to elevated levels of various cytokines, including interferons and interleukins. The dysregulation of the RNase L pathway supports the hypothesis that viral infection may play a role in the pathogenesis of the illness.

Neuroendocrine Dysregulation

A number of neuroendocrine abnormalities have been found in studies of patients with CFS including:

a) Mild hypocortisolism and attenuated diurnal variation of cortisol

b) Reduced function of the HPA axis, which can affect adrenal, gonad, and thyroid function

Brain Abnormalities

a) Static and dynamic functional brain imaging techniques, EEG studies, and examination of the cerebrospinal fluid have revealed structural, functional, metabolic and behaviorally linked brain abnormalities in patients with CFS. These abnormalities are not unique to the illness nor consistently found. However they can provide clues to illness pathophysiology.

b) Global reductions in gray matter and punctuated areas of high signal intensity (white spots) in the white matter

c) Decreased brain perfusion and glucose metabolism

d) Slower cerebral activity in response to motor and visual imagery tasks than in controls

e) Increased ventricular lactate levels

f) Reduced slow wave sleep and prolonged sleep latency

g) Unique proteins found in cerebrospinal fluid

Cognitive Impairment

Cognitive deficits, when present, are often a principal disabling feature of CFS. Such deficits restrict the patient's ability to function, plan, and complete tasks in real world settings. Documented deficits include impaired working memory, slowed processing speed, poor learning of new information, decreased concentration and attention span, difficulty with word retrieval, and increased distractibility.

Autonomic/Cardiovascular Disturbances

Autonomic dysfunction, when present, is manifested by an inability to maintain an upright posture or feeling faint or weak upon standing (orthostatic intolerance). In such cases, tilt table testing may show neurally mediated hypotension (NMH) or postural orthostatic tachycardia syndrome (POTS).

Mitochondrial /Energy Production Abnormalities

Recent studies suggest that mitochondrial dysfunction may be an important cause of the underlying energy deficit in patients with CFS. One line of evidence indicates that aerobic energy production is impaired. As a result of this impairment, the patient's exertions may exceed aerobic capacity and activate anaerobic metabolic pathways which are far less efficient at producing energy. This process results in the production of lactic acid and a disturbance of ATP/ADP metabolic cycling.

Gene Studies

Gene studies in patients with CFS suggest that the expression of batteries of certain genes may be altered. These include altered expression of genes controlling immune modulation, oxidative stress and apoptosis. Several distinct genomic subtypes have been reported and these may correlate with symptom severity.

In a recent controlled study, two subgroups of patients with CFS were identified with gene expression changes following exercise. The larger subgroup showed increases in mRNA for sensory and adrenergic receptors and cytokines. The smaller subgroup contained primarily patients with orthostatic intolerance, and showed a post-exercise decrease in adrenergic α-2A receptor gene expression.

3.2 DIAGNOSIS

Chronic fatigue syndrome is characterized by a pattern of relapse and remission over long periods of time. A very low percentage of patients recover after 2 to 4 years, whereas the majority remain ill for many years and **the disease is often progressive**. The longer a person is ill before diagnosis, the more complicated the course of the illness appears to be.

A Three-Step Evaluation Process has been proposed that uses the CDC criteria from their definition of CFS (Figure 3.3).

To be classified as CFS, four or more of the following symptoms must be present for greater than or equal to six months:

1. **Impaired memory or concentration**
2. **Sore throat**
3. **Tender cervical or axillary lymph nodes**
4. **Muscle pain**
5. **Multi-joint pain**
6. **New headaches**
7. **Unrefreshing sleep**
8. **Post-exertion malaise**

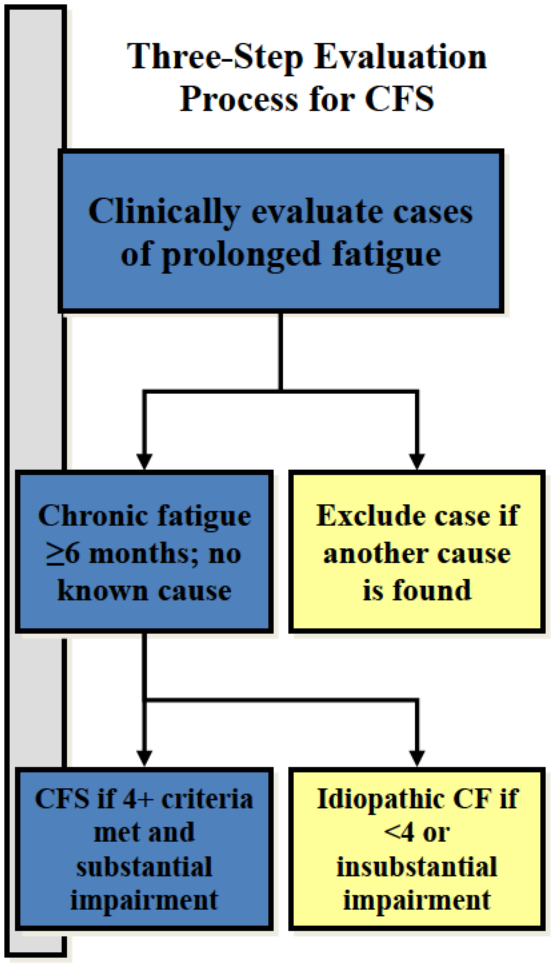


Figure 3.3: CFS is the Unexplained Occurrence of Chronic Fatigue Greater Than or Equal to Six Months

Sources: Recognition and Management of Chronic Fatigue Syndrome. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention. Atlanta. Available at: http://www.cdc.gov/cfs/pdf/HCPManaging.pdf. Accessed February 12, 2008; Fukuda K, Straus S, Hickie I, Sharpe M, Dobbins J, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med*. 1994;121:953-959.

Moreover, subjects who meet the CDC criteria, share common clinical features and common genetically based abnormalities, especially in energy metabolism. These criteria rely on both specific clinical symptoms and rigorous exclusion of other disorders, which may simulate CFS. These other disorders can generally be reliably excluded by validated laboratory tests (such as those specific for chronic infectious diseases, occult neoplasia, and autoimmune diseases, such as systemic lupus erythematosus).

Patients who enrolled in the Ampligen clinical development program after 1988 and before 1994 met the original definition of CFS published in 1988 (Holmes). Patients who enrolled after 1994 met both the original and revised (Fukuda 1994) definitions of CFS. These diagnostic characteristics, described in the CDC case definitions, formed the basis of subject selection and certain endpoints by which the efficacy of Ampligen was established. The table below compares inclusion criteria for the controlled Ampligen CFS studies with the CDC case definitions of 1988 and 1994.

Table 3.4: Criteria for Inclusion in Ampligen Studies of CFS Compared to CDC Case Definitions			
Parameter	**Ampligen Controlled Trials**	**1988 CDC Criteria**	**1994 CDC Criteria**
Karnofsky Performance Score	20-60[1]	<70	<70
Duration of CFS symptoms (months)	12	6	6
No. of CFS Symptoms Required (without physical findings)	8	8	4

[1] AMP-516: 40-60, AMP-502: 20-60

3.3 CFS: AN UNMET MEDICAL NEED

Ampligen would be the first FDA-approved treatment for patients with CFS; currently there are no medications approved. Current treatment of CFS is aimed at symptom relief and improved ambulatory function; there is no known cure (Komaroff 2003).

Presently, a patient with CFS is typically referred from the family practitioner or internist to a rheumatologist or other specialist. The patient may be one of the few who can receive treatment at expert CFS clinics that have arisen over the years. Since the first outbreak of CFS, numerous drugs, both over-the-counter and off-label, have been tried. Treatment commonly involves concomitant use of pain relievers, antidepressants, sleep and orthostatic intolerance medication as well as medications that affect the central nervous system. The focus is largely on the relief of specific symptoms experienced by the individual patient. While some drugs do provide initial benefit, side effects of those drugs frequently exacerbate the symptoms of the disease to the point where the CFS sufferers discontinue their use. At least 8 drugs, other than Ampligen, have been in clinical studies to treat patients for this condition including selective serotonin reuptake inhibitors (SSRI), Acyclovir, Rituxan, Mineralcorticords, adrenocorticords, attention deficit disorder medications and naturopathics (oil of evening primrose), but none have progressed to an advanced clinical status as they failed to reach their pre-declared primary and secondary endpoints.

Ampligen is the first drug specifically targeted for the treatment for CFS to provide enhanced capacity for physical activity, cognitive abilities, and improvement in the quality of life.

Ampligen's transitory side effects such as mild flu-like symptoms and malaise, when seen, usually occur during the initial weeks of treatment and tend to subside on repeated administration. There is no indication of a specific adverse event that appears after long term Ampligen administration (i.e., greater than 1 to 2 years) which would limit a CFS patient's ability to continue treatment.

3.4 ETIOLOGY AND PREVALENCE

The first recorded outbreak of what was eventually characterized as CFS was at Incline Village, NV in 1984. It was the start of a yearlong epidemic involving over 160 cases. Around the same time, clusters of outbreak were occurring in other places as well, including elsewhere in Nevada and in California, but also in the small town of Lyndonville, NY, which reported an outbreak of 60 children and 150 adults in 1985.

The etiology of CFS is still unknown. Earlier theories suggest CFS may have a viral basis such as Epstein-Barr virus or HHV-6 and it is been suggested that infectious mononucleosis may be a risk factor for chronic fatigue syndrome in adolescents (Katz 2009), although no specific virus has been firmly identified as the cause. Other speculation has looked to inflammation in the nervous system resulting from a faulty immune system response. Symptomatically, the disease can resemble many other disorders, including rheumatoid arthritis, fibromyalgia, Lyme disease, lupus, multiple sclerosis and hypothyroidism.

CFS occurs in all ethnic groups and races, and in countries around the world. It most often occurs in people aged 40 to 59 and women are three times more likely than men to experience it (NIAID NIH Publication No. 04-4697). Government estimates place the prevalence of CFS in the US at approximately 1,000,000 people. Other published data have estimated that between 14 to 15%, approximately 150,000 of CFS sufferers have a severe form of the disease (Garcia-Fructuoso 2008). Such patients are house-ridden, bed-ridden and when ambulatory may need to be in a wheelchair. At least 10% of the CFS population is younger than 15, including children under the age of 4.

4. REGULATORY

Hemispherx's collaboration with the FDA on the Ampligen development program began in the late 1980s. Early pilot programs for its use in CFS treatment began in 1988, which were the result of requests by the FDA to consider compassionate treatment using Ampligen for a CFS patient in New Mexico. Ampligen was granted Orphan Drug Status in 1993 and has been in an actively conducted Treatment Protocol since 1997. Throughout this course, one fundamental factor has emerged time and time again: Ampligen patients showed objective improvement and the drug was generally well-tolerated. Table 4.1 below provides a summary of the regulatory history.

Table 4.1: NDA 22-151 Multiple Different FDA Review Divisions During Clinical Development Plan

FDA Division	**BIOLOGICS**	→	**ANTI-VIRAL**	→	**SPECIAL PATHOGENS**	→	**CARDIO-RENAL**	→	**DPARP**
Intra-Division Dates	**3/90 - 5/92**		**5/92 - 10/97**		**10/97 - 6/06**		**6/06 - 4/11**		**4/11 - present**
IND #s and Date Filed	BB-IND #3462 3/90	→	IND # changed to 39,250	→	39,250	→	39,250	→	39,250
Drug Development Milestones and Agreements with FDA (Event and Time)	First randomized, placebo-controlled study (AMP-502) completed July 1991; Second randomized, placebo-controlled study (AMP-502T) competed September 1991	→	Twelve volume Toxicology Amendment submitted to IND #39,250 June 1992; Agreement reached with FDA/FDA Antiviral Drug Advisory Committee, 2/18/93: 1) AMP-502 = first well-controlled (pivotal) study 2) ETT Endpoint for the Phase III study (AMP-516) based on proportion of subjects improving in ETT; End of Phase II meeting 12 November 1996; Treatment protocol (AMP-511) initiated March 1997;	→	Agreement reached with FDA, 1/16/98, concerning Phase III AMP-516: 1) Efficacy will be established by showing a medically significant increase (6.5%) in mean ETT duration (Baseline compared to Week 40) 2) A 10 point increase in KPS is considered a medically significant change; Third randomized, placebo-controlled study (AMP-516) completed February 2004;	→	Agreement reached with FDA at Pre-NDA Meeting, 8/2/06: 1) Analysis of primary endpoint ETT in AMP-516 is at last assessment at 40 weeks compared to Baseline NDA #22-151 submitted 10/10/07; NDA #22-151 resubmitted 4/25/08; NDA #22-151 accepted 7/7/08; NDA #22-151 CRL received 11/25/09;	→	NDA #22-151 request for an extension of time to resubmit NDA 11/16/11; NDA #22-151 request for an extension of time to resubmit NDA deferred 1/6/12; Complete Response to CRL accepted for Review 8/10/12
Phase of Clinical Research	Phase I and Phase II Studies	→	Phase I and Phase II Studies, and Treatment Protocol	→	Phase II and Phase III Studies, and Treatment Protocol	→	Phase II and Phase III Studies, and Treatment Protocol	→	Treatment Protocol

5. CLINICAL DEVELOPMENT PROGRAM

5.1 OVERVIEW

Ampligen has been studied in over 1,200 subjects across various patient populations and indications participating in 19 studies. Nine studies have been conducted in patients with CFS: one Phase 3 and one Phase 2 pivotal study that form the basis of approval (AMP-516 and AMP-502) and seven supportive studies (Table 5.1). Other supportive studies were conducted in patients with HIV infection, hepatitis B virus infection, cancer, and thermal injury provided supportive safety data and well-controlled evidence of positive effects on immunomodulation. No studies have been conducted in healthy volunteers. Ampligen has been administered to 845 subjects (589 unique subjects suffering from severely debilitating CFS. Three hundred and twenty-six (326) subjects in the CFS program underwent 500 patient treatment years (PTYs). Thirty-seven (37) CFS subjects were on Ampligen for more than 104 weeks, and 9 subjects were administered 800 to 1200 mg of Ampligen per week for 1 to 2 years.

The population of adult patients studied in the clinical development program resembled the general CFS population, but with an emphasis on the most severely debilitated. Patients with severely debilitating CFS in the Ampligen development program exhibited at least six CFS symptoms plus two physical findings (Fukuda 1994 criteria), or eight CFS symptoms (Holmes 1988). Adults no older than 60 years who met the case definition for CFS developed by the CDC, but with CFS ongoing for 12 months or more (versus six months in the CDC definition), were eligible if they had a performance reduced quality of life as determined by a Karnofsky Performance Score (KPS) between 20 and 60 KPS in AMP-502, between 40 and 60 in AMP-516.

Table 5.1: Nine Studies of Ampligen in Patients with Chronic Fatigue Syndrome			
Study	**Study Phase and Design**	**Key Efficacy Objective(s) of the Study**	**Patient Population and Number**
Basis for Approval Studies			
AMP-516 (1998-2004) USA 12 Centers	Phase 3 Double-blind, stratified, random assignment, placebo- controlled	Improvements in ETT, KPS; change in use of concomitant medications for palliation of symptoms of CFS; other performance measures	234 Subjects diagnosed with CFS
AMP-502 (1990-1991) USA 4 Centers	Phase 2 Double-blind, stratified, random assignment, placebo-controlled	Improvements in KPS, ADL, ETT; change in use of concomitant medications for palliation of symptoms of CFS; other measures of function	92 Subjects diagnosed with CFS
Follow-on Studies			
AMP-516C/E USA 12 Centers	Phase 3 Open-label extension of 516, both arms received AMP, double-blind maintained	Evaluate ETT changes with crossover of placebo patients to Ampligen and durability of ETT effect in patients that received 24 additional weeks of Ampligen	190 Subjects who completed AMP-516
AMP-502T (1991) USA 3 Centers	Phase 2 Double-blind, random assignment, placebo-controlled; run concurrently with 502	Improvements in KPS, ADL, ETT; change in use of concomitant medications for palliation of symptoms of CFS; other measures of function and other patient reported outcomes	19 Subjects diagnosed with CFS
AMP-502E (1991-1993) USA 4 Centers	Phase 2 Open-label subset of subjects from AMP-502 and AMP-502T	Improvements in KPS, ADL, ETT; change in use of concomitant medications for palliation of symptoms of CFS; other measures of function and other patient reported outcomes	17 Responders and 17 Non-Responders
Other Supportive Studies in CFS Patients			
AMP-511 (1997 to present) USA	Phase 3 Open-label	Evaluate safety and efficacy (KPS, ADL)	164 subjects diagnosed with CFS as of last safety update

Table 5.1: Nine Studies of Ampligen in Patients with Chronic Fatigue Syndrome			
Study	**Study Phase and Design**	**Key Efficacy Objective(s) of the Study**	**Patient Population and Number**
20+ Centers			
AMP-509 Belgium (1993)	Phase 2 Open-label	Improvements in KPS, ETT (bicycle); other measures of function; long term (≥3 years) benefit established)	152 Subjects diagnosed with CFS
AMP-504 (1990-1993) USA 3 Centers	Phase 2 Open-label and compassionate use	Evaluate safety and although not an efficacy study, symptomatic tests were used	10 Subjects diagnosed with CFS
AMP-501 (1989-1990) USA 2 Centers	Phase 1/2 Open-label, dose escalation groups	Evaluate safety and effect on performance	16 Subjects diagnosed with CFS

Key Results for Studies AMP-516 and AMP-502 Are Presented in Tables 5.2 and Table 5.3.

Table 5.2. Summary of Key Results Relating to Efficacy and Clinical Benefit in Subjects Randomized to Ampligen Versus Placebo in the Phase 3 Study AMP-516 (n=234)

Efficacy Endpoints	**Key Secondary Endpoints**
Primary Endpoints: Change from Baseline in Exercise Tolerance Test duration at Week 40: • Ampligen improved placebo-adjusted intra-patient ETT by 21.3% and intra-group ETT by 11.8% (p=0.047) in ITT Subjects • Medically significant improvement over pre-defined intra-group placebo-adjusted benchmark: 11.8% (Ampligen) vs. 6.5% (benchmark) in ITT Subjects • Medically significant improvement over pre-declared intra-group placebo-adjusted benchmark: 14.3% vs. 6.5% in Subjects without significant dose reductions (pre-declared subset) • Proportion of patients with ETT improvement of at least 25%: Ampligen (39.0%) vs. placebo (23.1%) (p=0.013) • Correction for subjects with reduced dosing compliance increased placebo-adjusted mean intra-patient ETT improvement to 28% (p=0.022)	• Patients with ETT improvement of at least 25% also had a medically significant improvement in KPS of 10 points (p=0.039) • Patients with ETT improvement of at least 25% also had a medically significant improvement in Vitality Scores (SF-36) of 14.6 points (p=0.008) • ITT Population who took concomitant medications: 72% of Ampligen subjects decreased use of concomitant medications vs. 56% who received placebo (p=0.0145)
Clinical Benefits (QT Interval) Placebo subjects had a significantly longer QT interval, compared to Ampligen subjects (p=0.049)	**Cross-over Study (AMP-516C/E)** Placebo subjects crossed-over to receive Ampligen demonstrated an intra-patient improvement in ET performance at 24 weeks of 39% (p=0.04)

Table 5.3: Summary of Key Results Relating to Efficacy and Clinical Benefit in Subjects Randomized to Ampligen Versus Placebo in the Phase 2 Study AMP-502 (n=92)	
Efficacy Endpoints	**Key Secondary Endpoints**
Primary Endpoint: Increase in KPS at 24 Weeks • Ten (10) point median change in KPS scores in Ampligen-treated patients over placebo (p=0.016) • Increase in median KPS from Baseline of 50 to 60 at Week 24 in Ampligen-treated patients versus placebo with no change (p=0.014)	• Mean ETT duration increased by 95.3 seconds in the Ampligen group versus 57.9 seconds in the placebo group (p=0.010) • In AMP-502, a statistically significant smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use for relief from the symptoms of CFS, compared with the percentage of subjects in the placebo group (44.7%) (p=0.023)
Clinical Benefits Analyses of the distribution of median KPS scores at Week 24 also demonstrated statistically significant improvement at Weeks 16, 20 and 24 (p=0.015, p=0.013 and p=0.015, respectively) in the Ampligen group compared to placebo **Key QT Medication Use** • Placebo subjects increased concomitant medications including those with a known risk of prolonging QT interval compared to Ampligen subjects thereby decreasing their risk for cardiac events	**Clinical Benefits (cont'd)** • Incidence of depression with or without suicide attempt and/or suicide ideation occurred 3 times more often in the placebo group vs. Ampligen (p<0.02) • Suicide attempt/suicide ideation occurred 4 times more often in the placebo group (4) than with Ampligen treatment (1) (difference not statistically significant) • Less hospitalization for depression in the Ampligen group with 0 occurrences vs. 5 in the placebo group (p=0.06) • Percentage of hospitalization for depression out of all hospitalizations was 0% for Ampligen vs. 45% for placebo

5.2 SUMMARY OF CLINICAL STUDIES

5.2.1 AMP-516

AMP-516 was a prospective, multi-center, double-blind, randomized, placebo-controlled, equal parallel-groups study performed in two stages designed to compare the safety and efficacy of Ampligen versus placebo in the treatment of approximately 240 subjects severely disabled with CFS. The study was conducted at 12 centers in the United States. In Stage 1, subjects were stratified by exercise treadmill duration (≤9 minutes versus >9 minutes) and randomized 1:1 to double-blind treatment for 40 weeks. Treatment consisted of twice weekly IV infusions of study drug, either placebo or Ampligen 200 mg per infusion, for 2 weeks followed by twice weekly infusions of 400 mg per infusion through Week 40. In Stage 2, all subjects compliant in Stage 1 received twice weekly IV infusions of open-label Ampligen 200 mg per infusion for 2 weeks

followed by twice weekly infusions of 400 mg per infusion through Week 24. The double-blind in Stage 1 was maintained during Stage 2. Subjects were enrolled in the study for up to 76 weeks, which included a Baseline evaluation period lasting no longer than 12 weeks.

Eligible subjects:

- were 18 to 60 years of age
- diagnosed with CFS for ≥12 months according to the 1988 and 1994 CDC case definitions
- able to walk a minimum of 20 seconds on an exercise treadmill on 2 occasions during the 12 weeks before study entry
- had a KPS score of 40 to 60
- had laboratory documentation of erythrocyte sedimentation rate (ESR), negative anti-nuclear antibody (ANA) or negative anti-dsDNA, a negative rheumatoid factor, and normal thyroid function
- were either not of child-bearing potential or used acceptable contraception

The primary efficacy endpoint was the comparison of ETT duration in subjects with CFS treated with Ampligen versus placebo. KPS, concomitant medication usage, ADL, Vitality Index and GHP Index of the SF-36, were secondary efficacy endpoints used.

Two hundred thirty-four (234) eligible subjects were enrolled; 117 subjects were assigned to each treatment group and received at least 1 dose of Ampligen or placebo (Safety Population). Two hundred eight (208) subjects received at least one infusion of study drug and were evaluated at least once post Baseline for tolerance to exercise (ITT Population). One hundred ninety-four (194) subjects completed 40 weeks of treatment (Completer Population); the numbers of Ampligen treated subjects (24) and placebo subjects (16) who discontinued early were not significantly different (p=0.220).

Ampligen produced an objective improvement in ETT and a reduction in CFS related concomitant medications, including those that prolong the QT interval, as well as, secondary outcomes. The study demonstrated improvements in the activities of daily living and quality of life measures in the population exhibiting a ≥25% improvement in ETT. The results of this study were evaluated by an anonymous peer-reviewed panel of experts and published by PLoS One (Strayer 2012).

5.2.1.1 Follow-on Study AMP-516C/E

AMP-516C/E was a 6-month open-label crossover study (24 weeks) that followed the 40-week double-blind phase of AMP-516 (Stage 1) to evaluate the long-term safety and efficacy of Ampligen. AMP-516C/E (Stage 2) provided an opportunity to assess if efficacy results achieved in Stage 1 were maintained for Ampligen-treated subjects, and to evaluate the degree to which subjects treated with placebo in Stage 1 improved their efficacy parameters when switched to active treatment. The study was conducted at the same 12 centers in the United States participating in the parent study. All subjects compliant in Stage 1 were eligible to receive twice weekly IV infusions of open-label Ampligen 200 mg per infusion for 2 weeks followed by twice weekly infusions of 400 mg per infusion through Week 24. The double-blind in Stage 1 was maintained during Stage 2. Subjects were enrolled in both Stages for up to 76 weeks, which included a Baseline evaluation lasting no longer than 12 weeks.

Of 194 subjects that completed Stage 1 (AMP-516), 190 were enrolled in Stage 2 of the study. One-hundred sixty three (163) subjects completed Stage 2; 73 in the Ampligen group and 90 in the placebo group. Subjects ranged in age from 19 to 60 years, with a mean age of 43.5 years. The majority of these subjects were Caucasian (95%) and female (73%).

The crossover study established that (under blinded conditions) the subjects previously on placebo in the parent study responded dramatically to Ampligen in terms of ETT improvement.

5.2.2 AMP-502

Protocol AMP-502 was a randomized, double-blind, placebo-controlled study of Ampligen 400 mg IV or placebo given twice weekly. The study was conducted at 4 centers in the United States.

Eligible subjects:

- had CFS for ≥12 months
- were 18 to 60 years of age
- had KPS score of 20 to 60
- could walk on a treadmill for at least 10 seconds
- were either not of child-bearing potential or used acceptable contraception

To ensure a balance between the two study groups in terms of initial performance status, subjects were randomized according to 2 KPS strata: Low (20 to 40) and High (>40 to 60). Each subject assigned to the Ampligen group received 4 doses of 200 mg (twice weekly for two weeks) and then 400 mg twice weekly for the duration of the 6-month study; subjects assigned to the placebo group received an equivalent volume of saline.

The study had originally been designed as a 48 week study. After the first protocol amendment of March 23, 1991, it was shortened to 24 weeks (Stage 1). A second stage was scheduled for subjects who had responded to the study drug. Under the amended protocol, subjects were to be treated until one of the following occurred: 1) 24 weeks had passed, 2) dose-limiting toxicity occurred, or 3) the study was discontinued because of safety concerns.

The primary efficacy endpoints were physical performance as demonstrated by KPS score. Secondary efficacy endpoints included quality of life, which was evaluated by the ADL index score and SF-36, ETT duration, use of concomitant medications for relief of CFS symptoms (which included QT interval prolonging drugs).

Ninety-two (92) subjects were randomized and received at least 1 dose of Ampligen or placebo. Of the 92 subjects randomized, 45 (49%) received study drug and 47 (51%) received placebo. Four (4) subjects in each group withdrew; 3 of the placebo subjects withdrew as a result of intensification of CFS symptoms and 1 subject withdrew for nonmedical reasons, while all 4 subjects receiving study drug withdrew for nonmedical reasons. A total of 84 subjects (91%) completed 24 weeks of treatment. Twenty (20) placebo subjects and 13 subjects receiving study drug missed one or more doses. No subject missed more than 3 of 48 doses. Comparisons of Baseline characteristics demonstrated that the study drug and placebo groups were not different

for age at diagnosis of CFS, age at study entry, overall degree of physical debilitation, and perceived cognitive deficit. Gender distribution, however, was unequal for the study drug and placebo groups; 29 of 45 subjects (64%) in the Ampligen group were female, compared with 40 of 47 subjects (85%) in the placebo group.

Beyond meeting the primary and secondary endpoints, AMP-502 also demonstrated improvements in cognitive functions, decreased hospitalizations including hospitalizations for suicide attempts and suicide ideation. Some of the patients in AMP-502 were significantly more severe than in AMP-516. AMP-502 supports the results of AMP-516 including ETT, KPS, and reduction in concomitant medications including those that prolong QT. Patients improved both physically and cognitively and the drug was well-tolerated.

5.2.2.1 Follow-on Study AMP-502T

This was a Phase 2, double-blind, randomized, placebo-controlled, multicenter safety and efficacy study of Ampligen in CFS, conducted concurrently with Study AMP-502 at 4 centers in the United States. Subjects were randomized to either Ampligen or saline placebo and, following Baseline evaluations, were stratified by Baseline average KPS scores: low (20 to 40) and high (>40 to 60). Subjects were given IV Ampligen 200 mg (or placebo) twice weekly for 2 weeks, then Ampligen 400 mg or placebo twice weekly for 2 or 3 weeks; then Ampligen 400 mg or placebo 3 times weekly through to the end of the 24-week study period. Inclusion required a diagnosis of CFS as defined by the CDC for ≥12 months, KPS score ≥20 and ≤60, and the ability to walk on a moving treadmill for ≥10 seconds. The primary efficacy endpoint was to assess the change in physical performance over time as determined by KPS scores, comparing subjects administered Ampligen with those administered placebo. Secondary endpoints included the change over time in exercise tolerance measured by ETT and the ability to accomplish ADL as measured by the ADL Index.

This study was composed of 19 subjects: 47.4% randomly assigned to Ampligen treatment and 52.6% were randomly assigned to placebo. The majority of subjects were female (74%) and caucasian (95%). Subjects' mean age was 39.1 years, mean weight was 64.5 kg, and mean height was 171.3 cm. The majority of subjects (89%) had a KPS score between >40 and 60. There were no statistically significant differences in demographic and Baseline characteristics between the active treatment group and the placebo group. Overall, the mean age at the onset of CFS was approximately 36 years. The difference in mean time from the diagnosis of CFS to entrance into this study was statistically significantly ($p=0.0491$) greater for the active treatment group (3.41 years) than for the placebo group (1.98 years).

This dose-ranging study established that, while 3x per week dosing maintained efficacy, the offsetting incremental toxicity did not improve on the already acceptable risk-benefit analysis of 2x per week dosing.

5.2.2.2 Follow-on AMP-502E

AMP-502E was initially planned to assess the safety and activity of Ampligen over an additional period of at least 6 months in a subset of subjects who responded to treatment in AMP-502. Seventeen (17) subjects were included who met the initial criteria. Responders were subjects whose KPS scores increased at least 20 points during the 24-week treatment. Non-responders,

whether from the treatment or placebo group, were to be discontinued from study participation; however, some non-responders requested that they be allowed to continue in the open-label extension. As a result, AMP-502E includes responders and non-responders. Thirty-four subjects were included in AMP-502E. All subjects were started or re-started on 200 mg Ampligen for at least 4 doses, then escalated to 400 mg Ampligen twice weekly. AEs and clinical laboratory measurements were the primary safety evaluations. The open-label extension provided an average exposure time nearly 3x as long as the initial 24-week study for 17 subjects who received active drug in Stage 1. This study established longer term durability of the efficacy effect and maintenance of an acceptable safety profile.

5.2.3 Comparison of Studies AMP-502 and AMP-516

Table 5.4 below summarizes the design characteristics that are identical between studies AMP-502 and AMP-516.

Table 5.4: Well-Controlled Studies AMP-502 and AMP-516 are Designed to Provide Complementary Clinical Insights

	AMP-502	**AMP-516**
Prospective Design	x	x
Multi-Center	x	x
Conducted only in USA	x	x
Randomized-Double-Blind	x	x
Equal Parallel Group Design	x	x
Placebo-Controlled	x	x
Evaluated Efficacy and Safety	x	x
Utilized Ampligen at a Dosage Level of 400 mg given IV Twice Weekly vs. Placebo IV Twice Weekly	x	x
Enrolled Subjects with Severely Debilitating CFS	x	x
Enrolled Subjects between the Ages of 18 - 60	x	x
Enrolled Both Females and Males	x	x
Utilized ETT as a Primary or Secondary Endpoint	x	x
Utilized an Independent Team that Travelled to Each Site to Conduct the ETT Evaluations	x	x
Utilized KPS as a Primary or Secondary Endpoint	x	x
Utilized Concomitant Medication Usage as a Secondary Endpoint	x	x
Utilized ADL as a Secondary Endpoint	x	x
Utilized the Following Four Laboratory Panels to Evaluate Safety: Hematology, Chemistry, Coagulation, and Urinalysis	x	x
Study had Extension Phase to Add Insight into Efficacy/Safety	x	x

It is important to note that AMP-516 utilized ETT as the primary endpoint, KPS was a secondary endpoint; in AMP-502 KPS was the primary and ETT was secondary. The findings for both studies corroborated one another.

5.2.4 Other Supportive Studies in CFS Patients

5.2.4.1 AMP-511

AMP-511 is a prospective, open-label, multi-center, expanded-access Treatment Protocol enrolling subjects with severely debilitating CFS. One hundred and forty-one (141) subjects were enrolled at the time of the original submission of the Ampligen NDA. One hundred and sixty-four (164) were enrolled at the time of the last safety update as of September 30, 2011.

This study is ongoing and the portion of the study that was captured in the NDA was conducted at 21 centers in the United States. Two groups of subjects participated: treatment naive subjects (ITT population) and Transfer subjects who had completed the placebo-controlled (AMP-516) and open-label extension stages of AMP-516C/E.

Eligible treatment-naive subjects:

- were 18 to 65 years of age
- diagnosed with CFS according to the CDC case definition for ≥12 months
- had KPS score of 20 to 60
- had documentation of ESR, negative rheumatoid factor, normal thyroid function (or other laboratory evidence that the subject was euthyroid), negative ANA or negative anti-dsDNA
- and were either not of child-bearing potential or used acceptable contraception

Transfer subjects were not required to meet the inclusion criteria of AMP-511 regarding age, KPS score, or clinical laboratory measurements; however, they were required to begin treatment in the AMP-511 study within 6 weeks after their last treatment in AMP-516. During the treatment period, subjects received IV infusions of 200 mg of Ampligen twice weekly for 2 weeks (Doses 1 to 4) and then received infusions of 400 mg of study drug twice weekly for at least 22 more weeks. A dosage reduction to 25% (100 mg), 50% (200 mg), or 75% (300 mg) of the full 400 mg dose was to be permitted if infusion-related side effects were observed repeatedly with infusions administered over 60±5 minutes. Efficacy evaluations were: KPS score, SCL-90 CD subscale, Vitality Index and GHP Index of the SF-36, and modified Barthel's ADL index. Baseline efficacy measurements were obtained during the pre-study period; on-treatment measurements were performed every 8 weeks after the first dose of study drug and at study termination.

A total of 141 subjects were enrolled at the time of the NDA submission and received at least one dose of Ampligen; there were 103 ITT subjects and 38 transfer subjects. Approximately two-thirds of the ITT population, 66/103, were female (64.1%), and almost three-quarters of the Transfer population, 28/38, were female (73.7%). One hundred twenty-one (121) subjects (86%) completed at least 24 weeks of treatment, which was considered one complete cycle, and 82 subjects (58%) completed 48 weeks. Twenty (20) of 141 subjects (14.2%) discontinued before 24 weeks.

To date, 189 patients have received Ampligen since the study was initiated; 144 patients (80%) have entered the extension phase of the study due to their improvements on the drug. There are currently 5 clinical sites. Hundreds of requests have been received to be a part of the study, however, because the drug is experimental, insurance in many cases did not cover the costs of the treatment as well as the logistical matter of moving near a clinic conducting the trial. This study enlarged the long-term safety data base and allowed for special studies of the immune system, i.e., no evidence of nascent auto-antibody formation.

5.2.4.2 AMP-509

AMP-509 was a Phase 2, prospective, open-label, single-center, expanded-access study to evaluate the efficacy and safety of IV Ampligen in subjects with severely debilitating CFS who had a diagnosis of CFS for at least 12 months; were 17 to 60 years of age; had negative test results for ANA, anti-dsDNA, rheumatoid factor, and thyroid panel; were either not of child-bearing potential or used acceptable contraception; and had a KPS score of 20 to 60. The study was conducted at 1 center in Belgium. Baseline evaluations for safety and efficacy were obtained up to 12 weeks before first dose of Ampligen. The treatment cycle was 24 weeks, and subjects who demonstrated improvement in KPS score at 24 weeks but continued to have KPS<90 were allowed to continue for 2 additional 12-week treatment periods. The primary efficacy evaluation was KPS score. Subjects who entered this study had a KPS score ≥20 and ≤60, assuring that they were severely debilitated with CFS. Secondary efficacy measurements were: ETT (bicycle), SCL-90-R CD subscale, Vitality Index and GHP Index of the SF-36, and modified Barthel's ADL index. Baseline efficacy measurements were obtained during the Baseline period, except for ETT, which was to be performed at Baseline if the subject was able or at Week 12 for severely debilitated subjects. All subjects were to be evaluated for ETT at Week 24 and at study termination if treatment extended beyond 24 weeks.

A total of 152 subjects, 114 (75%) female and 38 (25%) male were enrolled and received at least 1 dose of Ampligen. One hundred and fifty-one (151) subjects had Baseline efficacy measurements and post-Baseline efficacy measurements at one or more time points. Of the 151 subjects, 142 (94%) received study medication for at least 24 weeks.

It should be noted, in AMP-509, the clinical activity of Ampligen was sustained over the course of a long-term follow-up period of nearly 3 years from Week 24. For the 20 subjects who received study drug and for whom follow-up data were available, the median KPS score at follow-up was statistically significantly improved over the score at Baseline and was unchanged from the score at Week 24. Table 5.5 shows the change from Baseline in KPS scores at 24 weeks and 3 years. The therapeutic effect of Ampligen may be durable for up to 3 years (or longer) following initial 24 week treatment.

Table 5.5: Mean and Median KPS Scores at Baseline, Week 24, and 3-Year Follow-Up and Changes From Baseline and Week 24 at Follow-Up			
KPS Score	**Mean**	**Median**	**p-value**
Baseline	53.33	58.33	
Week 24	78.25	80.00	
3-year Follow-up[4]	78.50	77.50	
Change from Baseline at Follow-up	25.17	30.00	<0.0001[1,2,3]
Change from Week 24 at Follow-up	0.25	0.00	0.9259,[1] 0.9131,[2] 1.0000[3]

n=20
[1] Student t-test
[2] Wilcoxon signed rank test
[3] Sign test
[4] Average 2.74 years follow up

The AMP-509 study conducted in Belgium demonstrated the long term benefit of Ampligen. Patients were given Ampligen treatment for the 24 weeks and monitored for up to three years as to their level of improvement. The study also established similar efficacy and safety results regardless of geographic location (i.e., comparison of North American CFS subjects vs. subjects in Europe).

5.2.4.3 AMP-504

Study AMP-504 was a prospective, open-label, multi-center, compassionate use study of subjects with severely debilitating CFS who could not participate in the placebo-controlled studies. The study was conducted at 3 centers in the United States.

Eligible subjects:

- had a diagnosis of CFS for at least 12 months
- were 18 to 60 years of age
- had cognitive dysfunction not due to malignant brain tumors (confirmed by MRI)
- were either not of child-bearing potential or used acceptable contraception
- had a KPS score of 20 to 60

Each subject was to be treated for 12 months, or as long as the subject was benefiting from the treatment and did not experience dose-limiting toxicity. The objective of the study was to give these subjects compassionate access to Ampligen to alleviate their symptoms and disability and to gather additional safety information on Ampligen administered on a long-term basis (≥12 months). Although this was not an efficacy study, symptomatic tests were devised to show the extent of physical and cognitive impairment; good results over time, however, could be interpreted to indicate clinical symptomatic improvement. Evaluations included KPS, ADL index, and SCL-90-R CD subscale.

Ten subjects were enrolled in AMP-504. The KPS, ADL, and SCL-90-R CD subscale were evaluated at Baseline and at intervals during the study. One subject withdrew soon after the first dose of study drug, 9 enrolled subjects were treated for at least 24 weeks, and 8 subjects were treated for 1 to 3 years.

AMP-504 corroborated results from well-controlled studies in the US (AMP-502 and AMP-516) as well as matched cohort studies done in Europe. In collaboration with National Institutes of Health scientists, certain patients who displayed pre-existing titers of HHV-6, a known opportunistic virus in immunosuppressed patients, were monitored over time. Ampligen therapy significantly reduced the circulating blood level of HHV-6.

5.2.4.4 AMP-501

AMP-501 was planned as a pilot study of Ampligen treatment in 10 subjects with CFS, who were to be randomly assigned to 1 of 3 dose regimens. Two open-label protocols initiated at approximately the same time (HU-CFS and Compassionate Use) enrolled 6 subjects, and these 3 studies are collectively reported as AMP-501. The study was conducted at 2 centers in the United States. Amendments to the AMP-501 and HU-CFS protocols extended the treatment period from 16 weeks to indefinite treatment; the Compassionate study was designed to have indefinite participation. In total, 16 subjects who met the CDC criteria for CFS and were also unable to work for 6 months before study enrollment, had abnormal brain MRI, and had at least 1 of 10 additional objectively determined abnormalities (cognitive, physical examination, laboratory test) were administered IV infusions of Ampligen for at least 16 weeks. The 3 dosing regimens to which subjects were randomly assigned included: 300 mg twice weekly, 400 mg twice weekly, or 400 mg three times weekly. All subjects were started on 200 mg twice weekly for varying amounts of time, and then received either 300 mg twice weekly or 400 mg 3 times weekly. A double-blind design with regard to dose regimen was used. In addition, 8 subjects enrolled in the AMP-501 protocol received a placebo infusion (normal saline in same volume as planned treatment) for 4 randomly assigned doses during the first 8 weeks of treatment. Subjects who responded to study treatment were allowed to continue in an indefinite extension of the study as long as no dose limiting toxicities had occurred. The primary efficacy endpoint in this study was improvement in KPS score. The secondary endpoints included ETT, cognitive function (WAIS-R, Halstead-Reitan, WMS), and signs and symptoms of CFS.

Sixteen (16) subjects were enrolled in the study; 16 (100%) were caucasian, 12 (75%) were female, mean age was 41 years, and the average length of illness before enrollment was 25 months. Average KPS score was 46 at Baseline, a level at which the subject would be significantly disabled and require special care and assistance to perform normal activities of daily living. All 15 subjects who underwent ETT at Baseline displayed reduced performance as measured by oxygen consumption. Fifteen of 16 subjects (94%) showed evidence of cognitive dysfunction as evidenced by lower than normal scores on the WMS and the WAIS-R. Fifteen subjects (94%) also had anatomical brain abnormalities on MRI. Giant cell co-culture assays were conducted on blood samples of 14 of the 16 subjects, and HHV-6 was identified in all 14 assays. Ampligen treatment significantly reduced the levels of HHV-6.

Fourteen (94%) of 16 subjects completed at least 16 weeks of treatment; 2 subjects withdrew for reasons not related to treatment (they were re-staged and re-enrolled into the open-label, indefinite-treatment extension study). One (1) subject who completed 16 weeks of treatment did

not continue into the extended phase. The remaining 13 subjects continued and, during the time period covered by the study report, those 13 subjects received uninterrupted treatment for at least 24 weeks. No subjects discontinued treatment for drug-related reasons, and no subjects required a dose reduction. A number of subjects experienced abnormal clinical laboratory values during treatment; however, none required dose reduction or discontinuation. A total of 660 weeks of treatment were administered between August 1988 and June 1990, of which 374 weeks (57%) involved the 400 mg twice weekly dose. Eight subjects were still receiving treatment on extended protocols as of June 1, 1990, the last data collection point for the study report.

This antiviral exploratory study evaluated outcome parameters thereafter used in the well-controlled AMP-502 study (multicenter, placebo-controlled, double-blind randomized).

5.3 ENDPOINTS DESCRIPTION AND CLINICAL RELEVANCE

For the clinical development program, and specifically the two pivotal studies, AMP-502 and AMP-516, the key endpoints indicative of efficacy and clinical benefit in the CFS population are shown below in Table 5.6.

Table 5.6: Key Endpoints in Pivotal Studies

Statistic	Study AMP-516 (Phase 3) n=234	Study AMP-502 (Phase 2) n=92
Primary Endpoint	Change from Baseline in ETT duration at Week 40	Change from Baseline in KPS at Week 24
Secondary Endpoints	Change in use of concomitant medications for palliation of symptoms of CFS, including those that prolong the QT interval Change from Baseline in KPS, SF-36 (general health perceptions and vitality), and ADL at Week 40 in ETT responders (≥25%) Adverse effects and safety	Change in use of concomitant medications for palliation of symptoms of CFS, including those that prolong the QT interval Change from Baseline in ETT and ADL at Week 24 Adverse effects and safety

5.3.1 Exercise Tolerance Test

Exercise Tolerance Testing is a validated clinical endpoint and is consider a standard for the cardio renal group. The Karnofsky Performance Score (KPS) is a traditionally used measurement that allows the physician to classify patients as to their functional impairment and is used for many chronic diseases. KPS was used to help guide inclusion into both studies. KPS was the primary efficacy endpoint in AMP-502, and a secondary efficacy endpoint in AMP-516. At the time of the design of the second pivotal study, AMP-516, CFS was becoming better understood and ETT, looking specifically at exercise duration, offered a clinically measured, objective endpoint related to the primary functional defect (grossly deficient physical activity and post-exertional malaise). In agreement with the FDA and the FDA Antiviral Advisory Committee, ETT was chosen as the primary endpoint for AMP-516.

Over the last two decades, the FDA has utilized exercise testing in various indications including to approve numerous drugs which affect cardio-pulmonary function and ambulation. ETT is a well-established clinical endpoint which has been effectively utilized in a number of chronically disabling diseases. In CFS, ETT is the accepted standard measurement of clinical and functional improvement.

Exercise science is concerned with the capacity of the cardiovascular system to supply oxygen to active muscles and the pulmonary system's ability to clear carbon dioxide from the blood via the lungs. The CDC, using DNA micro-array analysis, has shown that CFS patients have specific deficiencies at rest (or Baseline) in the expression of multiple genes which are involved in energy metabolism, oxygen consumption and immunologic surveillance and that the gene expression deficit becomes dramatically accentuated when CFS patients perform on treadmills (Whistler 2005). Appropriate control groups for these studies included demographically matched females without CFS.

ETT was used as a primary endpoint in the Ampligen clinical development program because it has the ability to establish efficacy by demonstrating medically and statistically significant improvement in exercise duration. **Also, ETT is the superior challenger to bring out the underlying, multiple gene malfunction signatures of CFS subjects**.

5.3.2 Karnofsky Performance Scale

KPS is a measurement tool used in a variety of chronic or debilitating illnesses. KPS allows patients to be classified as to their functional impairment. Conducted by the physician, it can be used to compare the effectiveness of different therapies and to assess individual patient's prognosis. The lower the Karnofsky score, the lower the likelihood of quality of life or even survival for the most serious illnesses.

KPS was designed to measure the level of patient activity and medical care requirements. It is a general measure of patient independence and has been widely used as a general assessment of patients with cancer.

Table 5.7: Clinical Significance of Karnofsky Performance Status Scale Scores		
Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead.

As noted above, KPS score was an entry criteria in both pivotal Ampligen studies. In AMP-516 the entry criterion was KPS of 40 to 60 and in AMP-502 it was an even more debilitated patient population with KPS between 20 and 60. Within the range of 40 to 80, changes in KPS of 10 are considered clinically meaningful (Capewell 1990, McClellan 1991, Wu 1990). Any improvements will greatly impact favorably the lives of CFS sufferers.

5.3.3 Concomitant Medications

Since there is no approved CFS-specific treatment, patients with CFS consume large amounts of medications in order to try to gain some control of their symptoms. It is not uncommon for patients to go on and off a variety of medications, many of which are terminated due to adverse events. Reduction in concomitant medications as a clinical endpoint has been widely used in clinical medicine as a primary or secondary efficacy endpoint. **For example, in 2007, the World Allergy Organization defined the severity of symptoms and the need for concomitant medication as primary parameters in clinical outcome measures of specific immunotherapy (SIT).** Furthermore, it was stated that the symptoms score should always be combined with the rescue medication score. The 'quality of life' is usually used as a secondary outcome measure in clinical trials on SIT (Pfaar 2009).

Many of the concomitant medications used by patients with CFS have the potential to prolong the QT interval. QT interval prolongation and resultant life threatening arrhythmia are common experiences in the practice of clinical medicine.

In the past decade the single most common cause of the withdrawal or restriction of the use of drugs that have been marketed has been the prolongation of the QT interval associated with polymorphic ventricular tachycardia, or torsade de pointes which can be fatal. Clinicians (often non-cardiologists) are increasingly faced with both older and newly approved drugs with labeling that mentions the potential to prolong the QT interval and thus to cause torsade de pointes (Roden 2004).

The classification of medications used to treat symptoms of CFS is listed below.

Table 5.8: Categories of Concomitant Medications Used to Treat Symptoms Associated With Chronic Fatigue Syndrome as Defined by the Centers for Disease Control and Prevention

Symptoms Contained in CDC Case Definition	Main Categories Utilized to Treat Symptoms
Fever	Antipyretics
Sore Throat	Analgesics, Antibiotics
Myalgia	Analgesics, NSAIDS, Narcotics, Muscle Relaxants
Fatigue	CNS Stimulants, Antidepressants, Dietary Supplements, Thyroid Preparations
Headaches	Analgesics, NSAIDS, Narcotics, Anticonvulsants, Migraine Preparations
Arthralgia	Analgesics, NSAIDS, Narcotics, Muscle Relaxants
Neuropsychological Complaints	Thyroid Preparations, CNS Stimulants, Antidepressants, Anticonvulsants, Antianxiety Agents, Anti-panic Agents
Sleep Disturbances	Sedatives, Hypnotics, Analgesics, Narcotics

Many of these medications are known to prolong the QT interval, and therefore may lead to fatal arrhythmias, especially when combinations of these medications are utilized as is seen in CFS. Medications that do prolong the QT interval are known to be related to catastrophic cardiac events, especially in middle-age females with CFS (Jason 2006) who otherwise would not be expected to be at risk for such events.

Concomitant medications taken by patients in AMP-516 and AMP-502 which are associated with prolonging the QT interval are set forth in Table 5.9.

Table 5.9: Concomitant Medications Utilized by CFS Patients with Known Association in Prolonging the QT Interval		
ADDERALL	EPINEPHRINE	PROPULSID
ADIPEX	FASTIN	PROVENTIL INHALER
ALBUTEROL	IMIPRAMINE	PROZAC
ALUPENT INHALER	IMITREX	PSEUDOEPHEDRINE
AMANTADINE	LEVAQUIN	RISPERDAL
AMITRIPTYLINE	LITHOBID	RITALIN
ARALEN	MERIDIA	SEPTRA
AZITHROMYCIN	METHADONE	SEREVENT
BACTRIM	METHYLPHENIDATE	SEROQUEL
BIAXIN	MIDODRINE	SERTRALINE
CELEXA	NEO-SYNEPHRINE 1%	SINEQUAN
CHLOROHYDRATE	NIZORAL	SPORANOX
CIPRO	NORTRIPTYLINE	TECQUIN
DETROL	OXYTOCIN	THIORIDAZINE
DEXEDRINE	PAMELOR	THORAZINE
DIFLUCAN	PAXIL	ZANAFLEX
DROPERIDOL	PHENTERMINE	ZITHROMAX
EFFEXOR	PITOCIN/OXYTOCIN	ZOFRAN
ELAVIL	PROAMITINE	ZOLOFT
		ZOMIG

By reducing the need for these concomitant medications in the treatment regimens for CFS, Ampligen will inevitably reduce the incidence of iatrogenic (physician) induced errors.

The number of days of medication use during the first 4 weeks of study and during the last four weeks of study were counted to determine the change in use of concomitant medication in AMP-502 and AMP-516.

5.3.4 Additional Endpoints

5.3.4.1 Activities of Daily Living

Ability to perform ADLs was assessed with the modified Barthel's ADL Index. This scale includes representative activities (e.g., "brush teeth") in 13 areas of function, such as grooming, bathing, meal preparation, and others. For each item, the subject was to check the response that most accurately reflected his/her ability to perform the task in the previous week. The response choices are:

- No symptoms; no help needed
- Some symptoms; no help needed
- Need some help sometimes
- Need help most of the time
- Unable to do

Scores range from 20 to 100, and a higher score indicates better ADL capacity. ADL was assessed at Baseline, at specified intervals during treatment, and at study termination. The ADL modules assessed from the modified Barthel's ADL index are listed in Table 5.10 below (Collin 1988, Mahoney and Barthel 1965).

Table 5.10: Modules of Activities of Daily Living

Number	Module	Number of Activities
1	Bathing	5
2	Housecleaning	13
3	Communication	6
4	Dressing	6
5	Grooming	3
6	Home management	7
7	Laundry	6
8	Meal preparation	9
9	Mobility/activity	9
10	Physical manipulation	5
11	Transportation (vehicular)	4
12	Toilet	3
13	Yard work/maintenance	7

Activities of daily living was self-assessed as the mean score of 13 modules multiplied by 20. 1=unable to do; 2=need help most of the time; 3=need help some time; 4=no help needed, symptoms present; 5=no help needed, no symptoms.

Scores were calculated by first determining the mean score for each of the 13 modules, then the mean of the 13 modular scores were determined, then the mean of the 13 modular scores was multiplied by 20. The maximum possible ADL score is 100, which would describe a subject who is able to perform all 83 tasks assessed with no help and who had no symptoms.

5.3.4.2 Health Survey, Short Form 36

Short Form 36, Health Survey (SF-36) was developed from work done by the RAND Corporation and the Medical Outcomes Study (MOS) based on the approach used in the RAND Health Insurance Study. Considered a validated patient reported outcome measure, SF-36 is a multi-purpose, short-form health survey. It yields functional health and well-being scores of both physical and mental health measures. The SF-36 has been used in surveys of general and specific populations for comparing the relative burden of diseases across different sub-groups and in differentiating the health benefits produced by different treatments (Turner-Bowker 2008). SF-36 is a recently, independently validated instrument in CFS.

The SF-36 is a self-administered instrument that assesses subjective well-being related to 9 health concepts. The two subscales used in these studies were the Vitality Index and General

Health Perception (GHP) Index. The Vitality Index uses 4 items in Section 9 of the survey, which asks subjects to indicate, How much of the time during the past 4 weeks:

9a. Did you feel full of pep?

9e. Did you have a lot of energy?

9g. Did you feel worn out?

9i. Did you feel tired?

The subject was to pick one of the following responses for each question:

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. None of the time

As a result, scores assigned to responses to items 9a and 9e were reverse coded so that a response of "all of the time" was given 6 points rather than 1 point, "most of the time" was given a score of 5 points, and so on.

The GHP Index uses the responses to Items 1, 11a, 11b, 11c, and 11d. Item 1 asks, "In general, would you say your health is" and the responses are: excellent=1, very good=2, good=3, fair=4, poor=5. Because a higher score indicates perception of better health, scores assigned to the responses are reverse coded so that: excellent=5; very good=4.4; good=3.4; fair=2; poor=1.

The 4 items in Section 11 are:

11a. I seem to get sick a little easier than other people

11b. I am as healthy as anybody I know

11c. I expect my health to get worse

11d. My health is excellent

Responses are:

1. Definitely true
2. Mostly true
3. Not sure
4. Mostly false
5. Definitely false

Responses to items 11b and 11d are reverse coded so that a response of definitely false=1; mostly false=2; mostly true=4; and definitely true=5. Not sure = 3 remains the same.

For each index, the 4 or 5 responses were summed to yield the index score. The SF-36 was administered at Baseline at specific intervals during treatment and at study termination.

6. EFFICACY

6.1 EXERCISE TOLERANCE TEST (ETT)

6.1.1 AMP-516

6.1.1.1 Change from Baseline ETT

The change from Baseline ETT was the primary endpoint in the pivotal Phase 3 AMP-516 study. In AMP-516, patients who received Ampligen extended the duration of their exercise on a treadmill over the 40 week treatment period by a greater amount than patients who received placebo. The ANCOVA analysis of non-transformed ETT data was statistically significant (p=0.047) and therefore the primary efficacy endpoint of AMP-516 was met.

Table 6.1: AMP-516 Primary Efficacy Endpoint: Change from Baseline in ETT Duration at Week 40 (Intention-to-Treat Population)

Week	Exercise Duration - Mean (seconds)		Increase from Baseline - seconds % increase		Improvement Over Placebo -seconds % increase	p-value
	Ampligen	Placebo	Ampligen	Placebo		
Baseline	576.3 n=100	588.1 n=108	-	-	-	-
40	672.0 n=100	616.3 n=108	95.7 16.61[3] 36.5%[4]	28.2 4.80[3] 15.2%[4]	67.5 11.81%[3] 21.3%[4]	0.047[1] 0.107[2]

[1] Analysis of Covariance (Baseline as Covariate) non-transformed ETT data
[2] Analysis of Covariance (Baseline as Covariate) $\log_{10}$ transformed data
[3] Intra-Group Mean ETT Improvement
[4] Intra-Patient Mean ETT Improvement

In order to potentially improve the normality of the data, an ANCOVA based on a $\log_{10}$ transformation was proposed in the final AMP-516 protocol as a variance stabilization procedure. Examination of the ETT values recorded at Baseline (Week 0) and at Week 40 revealed a distribution that failed the test for normality because of the breath of the distribution. However, a comparison of the distribution following the $\log_{10}$ transformation shows an increase in skewness and kurtosis, not an improvement in the distribution as expected. The increase in ETT duration using the ANCOVA is statistically significant using the non-transformed data (p=0.047), but only suggestive of significance if the ANCOVA analysis is performed on $\log_{10}$ transformed data (p=0.107). The preferred statistical approach based on the assumptions for an ANCOVA analysis is using the non-transformed raw data.

6.1.1.2 Intra-Group Mean ETT Improvement over Placebo

In the years between the completion of AMP-502 and the initiation of AMP-516, the FDA and the medical community became more focused on benchmarking cardio-pulmonary function and ambulation as measured by ETT improvement as a quantitative measurement. As a result it was agreed between the Agency and Hemispherx and reflected in the AMP-516 protocol to also measure the percent increase in mean intra-group ETT duration so it could be compared with ETT improvement seen in other severely debilitating chronic diseases. The benchmark that would be used to determine medical significance was based on two drugs that had been approved around that time for congestive heart failure (Fosinopril and Captopril) and medically significant efficacy was defined in the AMP-516 protocol as a placebo-adjusted mean intra-group increase in ETT duration of greater than or equal to 6.5%. In AMP-516, as shown in Table 6.1, the ETT duration increased a mean of 16.6% from Baseline to 40 weeks in the Ampligen group, compared to 4.8% for the placebo group. This difference of 11.8% was medically significant as pre-defined in the AMP-516 protocol since it was greater than 6.5%. Table 6.2 below compares the Ampligen results with that of other drugs that have been approved by the FDA.

Table 6.2: Comparison of Intra-Group Mean ETT Improvement: Ampligen for CFS vs. Drugs Approved for Non-CFS Severe Exertional Fatigue

Chronic Disease Indication	Drug (Placebo-Controlled Clinical Trial)	Group Analyzed	% Improvement Over Placebo[†]
Chronic Fatigue Syndrome	**Ampligen (AMP-516)**	**Excluding Significant Dose Reductions**	**14.3***
		Completer	**14.0***
		Intent-to-Treat	**11.8***
		Baseline ETT > 9 minutes	**11.5***
Chronic Congestive Heart Failure	Fosinopril[3]	20 mg dosage group	6.7
	Captopril[4]	150 mg dosage group	6.2
Chronic Angina	Ranolazine[5] (MARISA)	500/1,000 mg Pooled Data 1	6.5
	Ranolazine[6] (CARISA)	750/1,000 mg Pooled Data 1	5.9
Pulmonary Arterial Hypertension[1]	Tracleer (Breathe - 1)	125 mg BID dosage group	10.6
	Remodulin (PO1:04)	1.25-22.5 mg/kg/min	8.0
	Remodulin (PO1:05)	1.25-22.5 mg/kg/min	4.1
	Remodulin (PO1:04-05)	Pooled Data[2]	6.1

* Declared endpoint in pre-specified subsets, not post-hoc analysis (Reference: AMP 516 Protocol dated 1/24/97 submitted to IND 39,250 on 1/24/97, Serial #056, and response to FDA Comment #1 filed to IND 39,250 on 6/8/01, Serial #136.
† Intra-Group Mean ETT Improvement Over Placebo
1 Data from the FDA summary basis of approval (6 minute walk)
2 Pooled data means results from both study groups were pooled
3 Brown1995
4 The Captopril-Digoxin Multicenter Research Group 1988
5 Chaitman 2004a
6 Chaitman 2004b

6.1.1.3 Pre-Specified Subsets

In addition to the ITT population for AMP-516, an ANCOVA analysis was performed on three **pre-declared subsets** within the ITT population of 208 patients: 1) excluding those patients who had significant reductions in their dosing during the study (n=181) (Table 6.3); 2) patients who completed all 40 weeks of the study (Completers) (n=194) (Table 6.4); and 3) those with Baseline ETT duration >9 minutes (n=126) (Table 6.5). The significant dose reduction population is defined as a combined total of 20 missed doses (out of the 80 overall) or dose reductions of at least 50%. Each of the subsets showed a medically significant improvement relative to the benchmark of greater than or equal to 6.5%.

Table 6.3: Increase in Exercise Treadmill Duration with Ampligen (Pre-declared Subset within ITT Population: Patients without Significant Dose Reductions)

Week	Exercise Duration - mean (seconds)		Increase from Baseline - seconds % increase		Improvement Over Placebo -seconds % increase*	p-value
	Ampligen	Placebo	Ampligen	Placebo		
Baseline	581 n=83	590 n=98	-	-	-	-
40	690 n=83	616 n=98	109 18.73%[3] 43.0%[4]	26 4.47%[3] 15.0%[4]	83 14.26%[3] 28.0%[4]	0.022[1] 0.059[2]

[1] Analysis of Covariance (Baseline as Covariate) non-transformed ETT data
[2] Analysis of Covariance (Baseline as Covariate) $\log_{10}$ transformed data
[3] Intra-Group Mean ETT Improvement
[4] Intra-Patient Mean ETT Improvement

Table 6.4: Increase in Exercise Treadmill Duration with Ampligen (Pre-declared Subset Within ITT Population: Completer)						
Week	**Exercise Duration - mean (seconds)**		**Increase from Baseline - seconds % increase**		**Improvement Over Placebo -seconds % increase***	**p-value**
	Ampligen	**Placebo**	**Ampligen**	**Placebo**		
Baseline	583 n=60	587 n=66	-	-	-	-
40	691 n=60	614 n=66	108 18.56%[3] 40.2%[4]	27 4.60%[3] 15.6%[4]	81 13.96%[3] 24.6%[4]	0.019[1] 0.062[2]

[1] Analysis of Covariance (Baseline as Covariate) non-transformed ETT data
[2] Analysis of Covariance (Baseline as Covariate) $\log_{10}$ transformed data
[3] Intra-Group Mean ETT Improvement
[4] Intra-Patient Mean ETT Improvement

Table 6.5: Increase in Exercise Treadmill Duration with Ampligen (Pre-declared Subset within ITT Population: Baseline ETT Duration > 9 minutes)						
Week	**Exercise Duration - mean (seconds)**		**Increase from Baseline - seconds % increase**		**Improvement Over Placebo -seconds % increase***	**p-value**
	Ampligen	**Placebo**	**Ampligen**	**Placebo**		
Baseline	747 n=60	738 n=66	-	-	-	-
40	820 n=60	725 n=66	+73 +9.77%[3]	-13 -1.76%[3]	83 11.53%[3]	0.0264[1] 0.0289[2]

[1] Analysis of Covariance (Baseline as Covariate)
[2] Analysis of Covariance (Baseline as Covariate) $\log_{10}$ transformed data
[3] Intra-Group Mean ETT Improvement

Importantly, ANCOVA analyses performed on the Baseline ETT duration >9 minutes population using both $\log_{10}$ transformed data as well as non-transformed data are both statistically significant ($p<0.029$) as shown in Table 6.5. Thus, both medically and statistically significant results were seen for this pre-declared ITT subset: Baseline ETT >9 minutes. This subset contains 126 patients and represents 61% of the ITT population.

6.1.1.4 Proportionate Analyses

The original protocol for what became AMP-516 had as its primary efficacy endpoint and reflected in the Statistical Analysis Plan an analysis of the proportion of patients who achieved a

stated percentage increase in ETT duration over Baseline. This dichotomous endpoint, the proportion of subjects improving in ETT, was presented to the Antiviral Drug, FDA Advisory Committee and approved in 1993. The use of that endpoint was subsequently modified at the FDA's request to the change from Baseline ETT endpoint discussed above. Nonetheless, since that time such dichotomous, proportionate analyses have become widely accepted. Therefore, for AMP-516 in addition to the primary analysis of the primary endpoint and the intra-group difference relative to 6.5%, the proportion of patients were analyzed who achieved either a "minimum standard for a significant clinical improvement" defined as a ≥25% increase in intra-patient ETT duration relative to Baseline and a "major clinical improvement" (≥50% increase in duration, relative to Baseline). These supplemental analyses are intended to compare the proportion of patients classified as *responders* between the two randomized treatment groups. As discussed in a recently published paper on AMP-516, we believe that these data can provide a better understanding of the clinical relevance and potential utility that can be derived for this CFS population by such treatment (Strayer 2012). As shown in Table 6.6, there was a statistically significant improvement in the Ampligen arm of the study compared to placebo for the ITT Population.

Table 6.6: AMP-516: Frequency Distribution of Intra-Patient Percent Change from Mean Baseline Exercise Treadmill Duration at Week 40 (Intention-to-Treat Population)

Improvement from mean Baseline Exercise Treadmill Duration	Ampligen (n=100)	Placebo (n=108)	p-value[1]
Baseline, seconds	576.3	588.1	—
At least 25%, n (%)	39 (39.0)	25 (23.1)	0.013
At least 50%, n (%)	26 (26.0)	15 (13.9)	0.028

[1] Probability that a difference between treatment groups exists using the Chi-square test

Similarly, there was a statistically significant improvement in the pre-declared ITT subset with Baseline ETT >9 minutes (Table 6.7).

Table 6.7: AMP-516: Frequency Distribution of Intra-Patient Percent Change from Mean Baseline Exercise Treadmill Duration at Week 40 (Subset of ITT Population with Baseline ETT>9 Minutes)

Improvement from mean Baseline Exercise Treadmill Duration	Ampligen (n=60)	Placebo (n=66)	p-value[1]
Baseline, seconds	747	738	—
At least 25%, n (%)	20 (33.3%)	8 (12.1%)	0.0042
At least 50%, n (%)	14 (23.3%)	3 (4.5%)	0.0031

[1] Probability that a difference between treatment groups exists using the Chi-square test

A frequency distribution analysis comparing ETT improvement by ≥ 25%, no change and worsening of ETT by ≥ 25% shows that Ampligen improved more CFS patients in the ITT Population by at least 25% and fewer Ampligen patients deteriorated by 25% compared to placebo (Table 6.8).

Table 6.8: Frequency Distribution of ≥25% Improvement, <25% Change and ≥25% Worsening in ETT from Baseline at Week 40 (ITT Population, Study AMP-516)

Treatment	Worse n (%)	No Change n (%)	Improved n (%)	p-value[1]
Ampligen (n=100)	17 (17.0%)	44 (44.0%)	39 (39.0%)	0.044
Placebo (n=108)	21(19.4%)	62 (57.4%)	25 (23.1%)	

[1]Probability values derived from the Chi-square test

In those patients with a Baseline ETT duration >9 minutes (n=126), the same frequency distribution analysis (Table 6.9) shows that in the Ampligen group 20 patients (33.3%) improved by ≥ 25%, and 9 patients (15.0%) were worse by ≥ 25% for a net of 11 patients (18.3%) improving by ≥25%. In the placebo group 8 patients (12.1%) improved by ≥25%, but 11 patients (16.7%) were worse by ≥25% for net of -3 patient (-4.6%).

Table 6.9: Frequency Distribution of ≥25% Improvement, <25% Change and ≥25% Worsening in ETT from Baseline at Week 40 (Subset of ITT Population with Baseline ETT >9 Minutes)

Change from Baseline	Treatment	Worse n (%)	No Change n (%)	Improved n (%)	p-value
≥ 25%	Ampligen (n=60)	9 (15.0%)	31 (51.7%)	20 (33.3%)	0.0153[1] 0.0143[2]
	Placebo (n=66)	11 (16.7%)	47 (71.2%)	8 (12.1%)	

[1] Probability values derived from the Chi-square test
[2] 2-tailed Fisher's exact test (Appropriate if any cell has less than five observations)

Table 6.10 shows the frequency distribution around a ≥50% change in the Baseline ETT >9 minutes population. A net improvement of 14 patients (23.3% improved ≥50%) with no patient deteriorating ≥50% was seen in the Ampligen group, while only 3 patients (4.5%) improved ≥50% vs. 6 patients (9.1%) deteriorated ≥50% for a net of -3 patient (-4.6%) in the placebo group.

Table 6.10: Frequency Distribution of ≥50% Improvement, <50% Change and ≥50% Worsening in ETT from Baseline at Week 40 (Subset of ITT Population with Baseline ETT >9 Minutes)					
Change from Baseline	**Treatment**	**Worse n (%)**	**No Change n (%)**	**Improved n (%)**	**p-value**
≥ 50%	Ampligen (n=60)	0 (0%)	46 (67.7%)	14 (23.3%)	0.0009[1] 0.0004[2]
	Placebo (n=66)	6 (9.1%)	57 (86.4%)	3 (4.5%)	

[1] Chi-square test
[2] 2-tailed Fisher's exact test (appropriate if any cell has less than five observations)

Defining what constitutes *clinically meaningful intra-patient improvement* in ETT was based on intra-patient variability with regard to two ETT examinations performed during Baseline in AMP-516. Accordingly, the variability of treadmill testing of 208 CFS patients taking 2 tests approximately two weeks apart before treatment with Ampligen showed that a 25% minimum level exceeds intra-patient variability in over 90% of the patients.

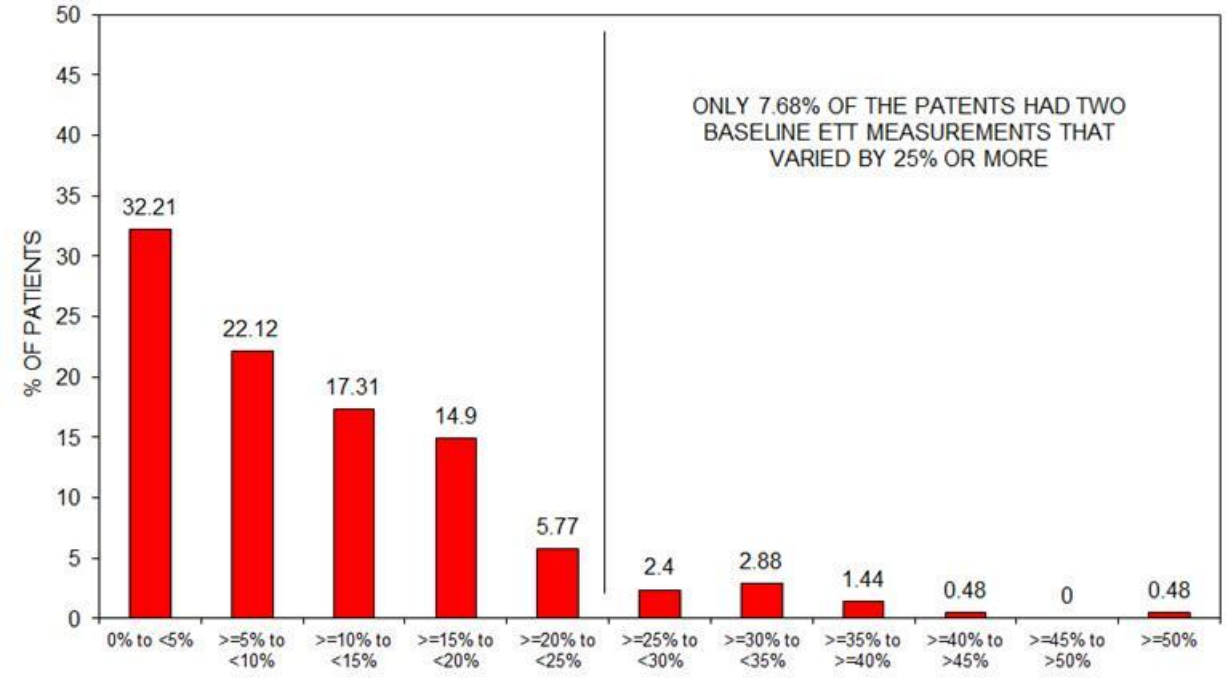


Figure 6.1: AMP-516 ITT Population – Patient Tabulation of the Intra-Patient ETT Differences at Baseline

A ≥25% improvement or deterioration is also supported by the medical literature (Bersin 2012, Chi 2012, Chong 2012, Chu 2011, Demierre 2009, Moppett 2008, Rawlings 2011, and van der Brand 2012).

6.1.1.5 Continuous Responder Analyses

A continuous responder analysis shown below in Table 6.11, calculated using 5% increments from ≥25% improvement to ≥50% improvement demonstrates a benefit in favor of the Ampligen cohort compared to the placebo group at Week 40 for each 5% increment showing the robustness of the Ampligen ETT response. Although, the interrelations of each 5% increment are acknowledged, no correction of the p-values was performed because the point of this table is to show the uncorrected p-value for each of the "Change from Baseline Thresholds". This illustrates that any one of these intervals would be statistically significant.

Table 6.11: Results Based on the AMP-516 ITT Population (n=208) Week 40

Change from Baseline Threshold	Treatment	Failed to Improve n (%)	Improved n (%)	p-value[1]
≥25%	Ampligen	61 (61.0%)	39 (39.0%)	0.013
	Placebo	83 (76.9%)	25 (23.1%)	
≥30%	Ampligen	65 65.0%)	35 (35.0%)	0.018
	Placebo	86 (79.6%)	22 (20.4%)	
≥35%	Ampligen	69 (69.0%)	31 (31.0%)	0.037
	Placebo	88 (81.5%)	20 (18.5%)	
≥40%	Ampligen	70 (70.0%)	30 (30.0%)	0.023
	Placebo	90 (83.3%)	18 (16.7%)	
≥45%	Ampligen	72 (72.0%)	28 (28.0%)	0.032
	Placebo	91 (84.3%)	17 (15.7%)	
≥50%	Ampligen	74 (74.0%)	26 (26.0%)	0.028
	Placebo	93 (86.1%)	15 (13.9%)	

[1] Probability values derived from the Chi-square test

Table 6.12 further demonstrates the robustness of the ETT response for the pre-declared ITT subset (Baseline ETT >9 minutes). The continuous responder analysis shows that the ETT response shows progressive improvement from Week 20 to Week 34. However, the greatest improvement is at Week 40 with each 5% increment having a p-value of <0.01.

Table 6.12: Results at Weeks 20, 34 and 40 for Patients with an ETT >9 Minutes at Baseline (n=126) in AMP-516				
Change from Baseline Threshold	**Treatment**	**Failed to Improve n (%)**	**Improved n (%)**	**p-value[1]**
≥25%				
Week 20	Ampligen	45 (75.0%)	15 (25.0%)	0.2483
	Placebo	55 (83.3%)	11 (16.7%)	
Week 34	Ampligen	42 (70.0%)	18 (30.0%)	0.0254
	Placebo	57 (86.4%)	9 (13.6%)	
Week 40	Ampligen	40 (66.7%)	20 (33.3%)	0.0042
	Placebo	58 (87.9%)	8 (12.1%)	
≥30%				
Week 20	Ampligen	45 (75.0%)	15 (25.0%)	0.0616
	Placebo	58 (87.9%)	8 (12.1%)	
Week 34	Ampligen	45 (75.0%)	15 (25.0%)	0.0335
	Placebo	59 89.4%)	7 (10.6%)	
Week 40	Ampligen	41 (68.3%)	19 (31.7%)	0.0006
	Placebo	61 (92.4%)	5 (7.6%)	
≥35%				
Week 20	Ampligen	45 (75.0%)	15 (25.0%)	0.0132
	Placebo	61 (92.4%)	5 (7.6%)	
Week 34	Ampligen	46 (76.7%)	14 (23.3%)	0.0093
	Placebo	62 (93.9%)	4 (6.1%)	
Week 40	Ampligen	44 (73.3%)	16 (26.7%)	0.0027
	Placebo	62 (93.9%)	4 (6.1%)	
≥40%				
Week 20	Ampligen	48 (80.0%)	12 (20.0%)	0.0114
	Placebo	63 (95.5%)	3 (4.5%)	
Week 34	Ampligen	47 (78.3%)	13 (21.7%)	0.0169
	Placebo	62 (93.9%)	4 (6.1%)	
Week 40	Ampligen	44 (73.3%)	16 (26.7%)	0.0027
	Placebo	62 (93.9%)	4 (6.1%)	
≥45%				
Week 20	Ampligen	50 (83.3%)	10 (16.7%)	0.0381
	Placebo	63 (95.5%)	3 (4.5%)	

Table 6.12: Results at Weeks 20, 34 and 40 for Patients with an ETT >9 Minutes at Baseline (n=126) in AMP-516				
Change from Baseline Threshold	**Treatment**	**Failed to Improve n (%)**	**Improved n (%)**	**p-value[1]**
Week 34	Ampligen	49 (81.7%)	11 (18.3%)	0.0211
	Placebo	63 (95.5%)	3 (4.5%)	
Week 40	Ampligen	45 (75.0%)	15 (25.0%)	0.005
	Placebo	62 (93.9%)	4 (6.1%)	
≥50%				
Week 20	Ampligen	52 (86.7%)	8 (13.3%)	0.1149
	Placebo	63 (95.5%)	3 (4.5%)	
Week 34	Ampligen	49 (81.7%)	11 (18.3%)	0.0211
	Placebo	63 (95.5%)	3 (4.5%)	
Week 40	Ampligen	46 (76.7%)	14 (23.3%)	0.0031
	Placebo	63 (95.5%)	3 (4.5%)	

[1] Chi-square test or if any cell has less than five observations, a 2-tailed Fisher's exact test

6.1.2 AMP-516C/E: Crossover Results

As discussed above, AMP-516C/E was a 6-month open-label crossover study (24 weeks) that followed the 40-weeks of AMP-516 (Stage 1). The AMP-516C/E study provided an opportunity to assess if efficacy results achieved in Stage 1 were maintained for Ampligen-treated subjects, and to evaluate the degree to which subjects treated with placebo in Stage 1 improved their efficacy parameters when switched to active treatment. The double-blind in Stage 1 was maintained during Stage 2, although all subjects received Ampligen during AMP-516C/E.

Table 6.13 below shows the frequency distribution for the ITT Population for subjects with a ≥25% improvement, no change (<25% change), and a ≥25% worsening in ETT from Baseline (Week 40 of Stage 1). Analysis of the ITT Population shows that the PLA→AMP cohort had more improved subjects improving by ≥25%, 27 (30.0%) vs. 8 (11.1%), and less subjects deteriorating by ≥25%, 9 (10.0%) vs. 12 (16.7%), than the AMP→AMP cohort ($p<0.006$). The AMP→AMP cohort continued to show a mean 22.9% intra-patient improvement during AMP-516C/E (Table 6.14).

Table 6.13: Greater Improvement Seen After Switching from Placebo to Ampligen Compared to Patients Who Received 24 Additional Weeks of Ampligen Treatment after an Initial 40 Week Treatment Course AMP-516C/E					
Treatment Stage 1→Stage 2	**n**	**Worse**	**No Change**	**Improved**	**p-value[1]**
AMP→AMP	72	12 (16.7%)	52 (72.2%)	8 (11.1%)	0.006
PLA→AMP	90	9 (10.0%)	54 (60.0%)	27 (30.0%)	

[1] Mantel-Haenszel Chi-square

The mean Baseline Week 40 and Post-Baseline Week 64 ETT values and changes from Week 40 to Week 64 for the ITT Population in AMP-516C/E were also evaluated (Table 6.14). While the AMP→AMP cohorts show increases in mean intra-patient percent improvement (22.9% for ITT), the p value was not <0.05. In contrast, the increase in mean intra-patient percent improvements from Week 40 to Week 64 was 39.0% for the PLA→AMP cohort (p=0.04).

Table 6.14: Greater Improvement in Mean Intra-Patient ETT Seen After Switching from Placebo to Ampligen Compared to Patients Who Received 24 Additional Weeks of Ampligen Treatment after an Initial 40 Week Treatment Course AMP-516C/E			
Treatment Stage 1→Stage 2	**n**	**% Intra-patient Mean Improvement**	**p-value[1]**
AMP→AMP	72	22.9%	0.58
PLA→AMP	90	39.0%	0.04

[1] p-value is from a paired t-test

6.1.3 AMP-502

In AMP-502, mean ETT duration in the Ampligen group at Baseline and at Week 24 were 744.1 and 839.4, respectively, for an increase of 95.3 seconds at Week 24 versus of 57.9 seconds in the placebo group. As shown in Table 6.15, the difference in mean ETT scores for the Ampligen group compared to placebo was statistically significant (p=0.010).

Table 6.15: AMP-502: Intra-Group Change from Baseline in ETT Duration at Week 24 (Intention-to-Treat Population)			
	Ampligen (n=41)	**Placebo** (n=44)	**p-value**
Baseline Seconds Mean	744.1	602.8	
Change from Baseline Mean	95.3	57.9	**0.010**[1]
p-value[2]	0.050	0.116	

[1] An ANCOVA of log-ratio transformed data with Baseline and sites as covariates
[2] Wilcoxon signed-rank test
ITT Population includes all subjects who had a Gillespie modified Bruce protocol ETT at Baseline and had a minimum of one post-Baseline Gillespie modified Bruce protocol ETT evaluation

Two exercise protocols were employed during the AMP-502 study. The initial exercise protocol was utilized on the first seven subjects enrolled, but because of the severe debilitation seen with those subjects, the independent medical personnel who traveled to the clinical sites to ensure standardization of equipment and exercise tolerance test execution recommended a modification of the exercise protocol. The Bruce protocol initially utilized was too strenuous for this severely debilitated CFS population. Although, the first seven subjects were not considered evaluable for ETT, all of the remaining 85 subjects completed the Gillespie modified Bruce protocol at Baseline and were also evaluated during the treatment phase.

6.2 KARNOFSKY PERFORMANCE SCORE

The change from Baseline KPS was the primary endpoint in the pivotal Phase 2 AMP-502 and a secondary endpoint in the pivotal Phase 3 AMP-516.

6.2.1 AMP-502

Hemispherx believes that the primary analysis of the primary endpoint (KPS at 24 weeks) was conclusive for study AMP-502 based on the pre-specified objective criteria. The primary efficacy analysis was conducted on the ITT Population, and the differences in the median KPS scores from Baseline to Week 24 in the Ampligen group compared with the placebo group showed statistical superiority in favor of patients randomized to receive Ampligen. As shown in Table 6.16, p-values for median scores at Weeks 16, 20 and 24 were 0.023, 0.011 and 0.014, respectively. P-values for median change in KPS at Weeks 16, 20 and 24 were 0.013, 0.015 and 0.016, respectively. The improvement in the Ampligen group met the criterion for a *medically significant improvement* in physical performance defined as a ≥10 point increase in the median KPS scores at Week 24. Median KPS scores increased 20% from Baseline to completion of the study in the Ampligen group.

Table 6.16: Median and Median Changes in KPS from Baseline to Weeks 4, 8, 16, 20, and 24 (ITT Population) in AMP-502				
	Ampligen (n=45)		**Placebo (n=47)**	
Time	**Median**	**Median Change**	**Median**	**Median Change**
Baseline				
Median	50.0		50.0	
Week 4				
Median	50.0	0.0	50.0	0.0
p-value[1]	0.74	0.557		
Week 8				
Median	60.0	0.0	50.0	0.0
p-value[1]	0.278	0.199		
Week 12				
Median	60.0	0.0	50.0	0.0
p-value[1]	0.139	0.325		
Week 16				
Median	60.0	10.0	50.0	0.0
p-value[1]	0.023	0.013		
Week 20				
Median	60.0	10.0	50.0	0.0
p-value[1]	0.011	0.015		
Week 24				
Median	**60.0**	**10.0**	50.0	0.0
p-value[1]	**0.014**	**0.016**		

[1] Wilcoxon-Mann-Whitney Test, 2-sided comparing Ampligen vs. placebo groups

As shown in Table 6.17, analyses of the distribution of median KPS scores at Week 24 also demonstrated statistically significant improvement at Weeks 16, 20 and 24 (p=0.015, p=0.013 and p=0.015, respectively) in the Ampligen group compared to placebo. **Numerous studies in other chronic, severely debilitating diseases have established that differences of this magnitude are medically significant.**

Table 6.17: Distribution of Median KPS from Baseline to Week 24 in AMP-502 (ITT Population)

Week	Treatment Group	KPS Score									p-value[1]
		Subjects at Each Level									
		20	30	40	50	60	70	80	90	100	
0	Ampligen	0	2	10	15	18	0	0	0	0	0.733
	Placebo	1	3	8	17	18	0	0	0	0	
4	Ampligen	0	0	13	12	18	2	0	0	0	0.501
	Placebo	1	3	8	15	20	0	0	0	0	
8	Ampligen	0	0	7	15	19	4	0	0	0	0.272
	Placebo	1	2	7	17	18	1	0	1	0	
12	Ampligen	0	0	6	13	19	6	1	0	0	0.188
	Placebo	0	0	12	14	16	4	0	1	0	
16	Ampligen	0	0	5	13	18	7	1	0	1	0.015
	Placebo	0	1	13	12	18	2	1	0	0	
20	Ampligen	0	0	5	8	20	10	0	0	2	0.013
	Placebo	0	1	11	12	18	4	0	1	0	
24	Ampligen	**0**	**0**	**4**	**10**	**19**	**7**	**3**	**0**	**2**	**0.015**
	Placebo	**0**	**2**	**9**	**13**	**18**	**2**	**2**	**1**	**0**	

[1] Cochran-Armitage trend test, 2-sided

As shown in Table 6.18 below, analyses of the distribution of the median changes in KPS at Weeks 16, 20 and 24 relative to Baseline also demonstrated statistically significant improvement (p=0.009, p=0.010, and p=0.013, respectively) in the Ampligen group compared with placebo. Statistical significance occurred at parallel time points for the distribution of both the medians and the median changes in the active treatment group.

Table 6.18: Distribution of Median Changes in KPS from Baseline to Week 24 (ITT Population) in AMP-502

Week	Treatment Group	Change in KPS Relative to Baseline								p-value[1]
		Number of Subjects With Change at Each Level								
		-30	-20	-10	0	10	20	30	40	
4	Ampligen	0	1	2	34	7	1	0	0	0.540
	Placebo	0	1	1	40	5	0	0	0	
8	Ampligen	0	1	1	27	13	3	0	0	0.305
	Placebo	0	1	4	30	10	1	1	0	
12	Ampligen	0	0	2	25	10	8	0	0	0.247
	Placebo	0	1	6	22	14	3	1	0	
16	Ampligen	0	0	1	21	16	6	0	1	0.009
	Placebo	1	0	5	27	11	3	0	0	
20	Ampligen	0	0	2	15	17	9	0	2	0.010
	Placebo	1	0	6	20	16	3	1	0	
24	Ampligen	**0**	**0**	**1**	**17**	**14**	**10**	**1**	**2**	**0.013**
	Placebo	**1**	**1**	**4**	**23**	**11**	**5**	**2**	**0**	

[1] Cochran-Armitage trend test, 2-sided

6.2.2 AMP-516: KPS and Correlation Between KPS and ETT

Table 6.19 shows Baseline and change from Baseline KPS at Week 40 for study AMP-516.

Table 6.19: Baseline and change from Baseline Karnofsky Performance Scores at Week 40 (ITT Population) in AMP-516			
Time	**Ampligen (n=100)**	**Placebo (n=108)**	**p-value**
Baseline Median	50.0	50.0	0.5338[1]
Change from Baseline at Week 40 Median	10.0	0.0	0.5781[2]
p-value[3]	<0.0001	<0.0001	

[1] p-value is from a Wilcoxon rank sum test comparing the mean Baseline values between treatment groups
[2] p-value is from Wilcoxon rank sum test comparing the mean change from Baseline within each treatment group
[3] p-value is from a Wilcoxon signed rank test comparing whether the change from Baseline is equal to zero within each treatment group

Median Baseline KPS scores were 50 in each treatment group. At Week 40, median scores improved by 10 points among subjects randomized to Ampligen, with no change in median score among subjects randomized to placebo. Fifty-five (55%) of subjects randomized to Ampligen had a change from Baseline KPS of at least 10 at Week 40 versus 49 (45%) of subjects randomized to placebo. There was no statistically significant difference between treatment groups in the proportions of subjects with improved KPS of at least 10 points.

The decreased frequency of capturing KPS data in AMP-516 (every 4 weeks) vs. AMP-502 (every week) may explain why the first study, AMP-502, showed a statistically significant improvement in KPS in the Ampligen group with a 10 point median increase compared to placebo, while the later study, AMP-516, also showed a median positive change of 10 points for the Ampligen cohort vs. a 0 (zero) point change for the placebo group, but the p-value was not statistically significant. It was also determined that there was a difference in Cronbach's alpha reliability coefficient which was used to examine the internal consistency of the serial measurements in both AMP-502 and AMP-516. Cronbach's alpha reliability coefficient was calculated from the correlation among the individual values that comprised the Baseline and monthly scores. In AMP-516 the coefficient ranged from 0.76 at Baseline to 0.90 at 40 weeks. These values were lower than the values obtained in AMP-502 in which Cronbach's alpha reliability coefficient ranged from 0.96 at Baseline to 0.99 at the end of the study.

6.2.3 Validation of a ≥25% Increase in ETT as Clinically Significant

In order to determine if subjects who increased ETT ≥25% also showed significant improvements in quality of life assessments, the entire ITT population (AMP-516) was dichotomized based on a ≥25% or <25% improvement in ETT from Baseline to Week 40 and four secondary endpoints which were related to physical performance (KPS and ADL), the subjects' perception of their energy level (vitality) and GHP were analyzed (Table 6.20).

With regard to KPS, subjects who had a significant (≥25%) ETT clinical response (n=64) also had a medically significant and statistically significant improvement in median KPS of 10 points (p=0.017) compared to the subset of subjects (n=144) that had a <25% improvement in ETT with no improvement in KPS (KPS of 50 at both Baseline and Week 40). As shown below, medically significant and statistically significant improvements were also found in Activities of Daily Living (ADL) and the SF-36 Vitality and GHP Scores.

Table 6.20: Dichotomizing ITT Population Based on Significant Clinical Improvement at Week 40 in ETT Duration - Corresponding Improvement in Secondary Endpoints, KPS, ADL, Vitality and GHP				
Secondary Endpoint		**Dichotomized by ETT Improvement**		**p-value**
		<25% (n=144)	**≥25% (n=64)**	
KPS[1]	Baseline	50	50	
	Week 40	50	60	0.021
	Change	0	10	0.017
ADL[2]	Baseline	68.27	69.01	
	Week 40	69.36	74.15	0.022
	Change	1.088	5.143	0.009
Vitality[3]	Baseline	11.18	10.55	
	Week 40	14.55	25.16	0.0001
	Change	3.02	13.99	0.0001
GHP[3]	Baseline	16.67	18.94	
	Week 40	19.04	23.54	0.031
	Change	2.10	5.20	0.031

[1] Median with p-value based on Wilcoxon Two-Sample test (two-sided)
[2] Mean with p-value based on 1-factor ANOVA model
[3] Mean with p-value based on ANCOVA model with Baseline as covariant

Based on the foregoing we believe that a ≥25% increase in ETT duration relative to Baseline represents a significant clinical improvement in ETT and is associated with medically significant improvement in KPS, ADL, vitality and general health perceptions.

6.2.4 AMP-516: Determination that a ≥25% Increase in Intra-Patient ETT Duration is Medically Significant with Regard to Improved KPS and Vitality Scores (SF-36)

As shown in Table 6.21 for the ITT Population treated with Ampligen in AMP-516, a ≥25% increase in ETT duration relative to Baseline represents a significant clinical improvement in ETT.

Also shown in Table 6.21 below, there were also corresponding improvements in KPS and Vitality Scores in the subset of the ITT Population treated with Ampligen that experienced a clinically significant increase in ETT duration (i.e., ≥25% increase in ETT from Baseline to Week 40).

In the AMP-516 study, the Ampligen treated subset of the ITT Population that had a significant ETT clinical response (≥25% increase in exercise duration from Baseline to Week 40) also had a medically significant and statistically significant improvement in median KPS from 50 to 60 (p= 0.005) (Wilcoxon Two-Sample test, two-sided) compared to the subset of subjects that had a <25% improvement in ETT and had a median KPS of 50 at both Baseline and Week 40.

Table 6.21: Dichotomizing the Ampligen Treated ITT Population Based on Significant Clinical Improvement at Week 40 in ETT Duration - There is Corresponding Clinically Significant Improvements in Secondary Endpoints, KPS and Vitality				
Secondary Endpoint		**Dichotomized by ETT Improvement**		**p-value**
		<25% (n=61)	**≥25% (n=39)**	
KPS[1]	Baseline	50	50	
	Week 40	50	60	0.005
	Change	0	10	0.039
Vitality[2]	Baseline	9.84	9.49	
	Week 40	14.34	24.10	0.008
	Change	4.51	14.62	0.008

[1] Median with p-value based on Wilcoxon Two-Sample test (two-sided)
[2] Mean with p-value based on 1-factor ANOVA model

Thus, a ≥25% improvement in ETT duration from Baseline to Week 40 resulted in significant improvements in KPS and vitality in the significant ETT response subset of the Ampligen treated ITT Population compared to the subset that had <25% improvement in ETT duration.

The improvement seen in vitality scores from 9.49 to 24.10 in the ≥25% ETT responder subset represents a major clinical response compared to the <25% ETT improvement cohort.

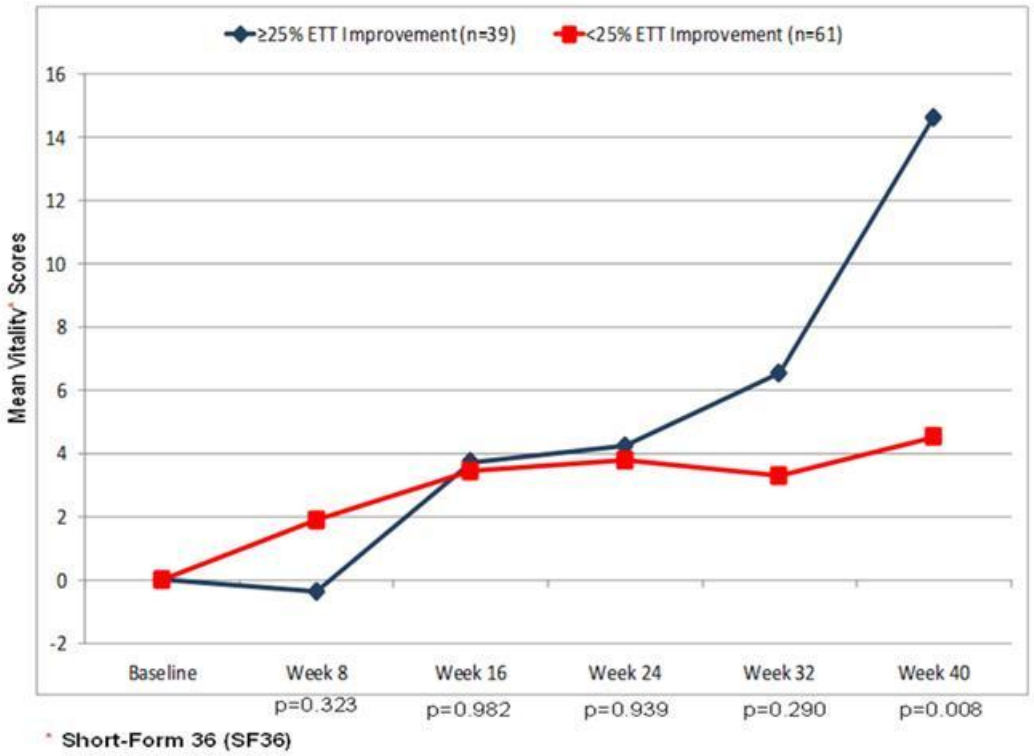


Figure 6.2: Change from Baseline for Vitality Scores for Patients With and Without a ≥25% Improvement in ETT at Week 40 (Ampligen Treated Patients from ITT Population)

6.2.5 AMP-509: KPS Scores at Baseline, Week 24, and 3-Year Follow-Up

In AMP-509, the clinical activity of Ampligen was sustained over the course of a long-term follow-up period of approximately 3 years from Week 24. For the 20 subjects who received study drug and for whom follow-up data were available, the median KPS score at follow-up was statistically significantly improved over the score at Baseline and was unchanged from the score at Week 24. Table 6.22 shows the change from Baseline in KPS scores at 24 weeks and at mean duration of follow-up equal to 2.74 years.

Table 6.22: Mean and Median KPS Scores at Baseline, Week 24, and 3-Year Follow-Up and Changes From Baseline and Week 24 at Follow-Up

KPS Score	Mean	Median	p-value
Baseline	53.33	58.33	
Week 24	78.25	80.00	
3-year Follow-up[4]	78.50	77.50	
Change from Baseline at Follow-up	25.17	30.00	<0.0001[1,2,3]
Change from Week 24 at Follow-up	0.25	0.00	0.9259,[1] 0.9131,[2] 1.0000[3]

n=20
[1] Student's t-test
[2] Wilcoxon signed rank test
[3] Sign test
[4] Average 2.74 years follow up.
Source: AMP-509 CSR, Table 7-18

6.2.6 AMP-511: KPS

As shown in Table 6.23, the mean scores showed progressive increases from Baseline (48.16) to Week 72 (61.36) and mean changes from Baseline were statistically significant, $p<0.05$, at each time point. At Week 24, the mean score was 57.58, a 20% increase, and at week 48 the mean score was 60.10, a 25% increase compared to Baseline. Median changes from Baseline were statistically significant, $p<0.05$, at each time point and the median value changed from 50 at Baseline to 60 at Week 24 and remained at 60 through Week 72. The median change from Baseline was 10.0 from Week 24 to Week 72, which represents a medically significant improvement.

Table 6.23: Changes in Mean and Median KPS from Baseline to Weeks 8, 16, 24, 32, 40, 48, 56, 64 and 72 in AMP-511 (ITT Population)		
	Ampligen (n=103)	
Time	**Mean***	**Median***
Baseline	48.16	50.0
Week 8		
Change from Baseline	**3.01**	**0.0**
Week 16		
Change from Baseline	**5.63**	**0.0**
Week 24		
Change from Baseline	**9.42**	**10.0**
Week 32		
Change from Baseline	**10.10**	**10.0**
Week 40		
Change from Baseline	**11.07**	**10.0**
Week 48		
Change from Baseline	**11.94**	**10.0**
Week 56		
Change from Baseline	**12.72**	**10.0**
Week 64		
Change from Baseline	**12.43**	**10.0**
Week 72	61.36	60.0
Change from Baseline	**13.20**	**10.0**

*All post-Baseline mean and median differences from Baseline were statistically significant; $p<0.05$ by paired t-test (mean) or sign test (median)

6.3 CONCOMITANT MEDICATIONS

Both AMP-502 and AMP-516 show credible evidence of Ampligen's clinical effect to decrease the use of concomitant medications to palliate symptoms of CFS. The reduction in concomitant medications was a secondary efficacy endpoint.

The number of days of medication use during the first 4 weeks of study and during the last 4 weeks of study were counted to determine the change in use of concomitant medication in both studies. In AMP-502, subjects were encouraged through repeated interviews to discontinue medications before starting study drug. In this study, the use of concomitant medications was reported to have either increased or not increased. In AMP-516, subjects were neither counseled

nor encouraged to discontinue medications before starting study drug. In this study, the use of concomitant medication was reported to have either decreased or not decreased.

In AMP-502, a statistically significantly smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use in an attempt to palliate the symptoms of CFS, compared with the percentage of subjects in the placebo group (44.7%) (p=0.023). In AMP-516, the proportion of subjects who decreased their use of concomitant medications was significantly greater for subjects who took at least one concomitant medication and received Ampligen (72%) versus placebo (56%) (p=0.0145).

6.3.1 AMP-516

Changes from initial use of concomitant medications related to CFS at the end of the study are summarized below in Table 6.24 first for all subjects in the ITT Population and then in Table 6.25 for subjects in the ITT Population who took at least one concomitant medication. The use of concomitant medications decreased from Baseline for most subjects in each treatment group, but a larger proportion of subjects who received Ampligen took fewer concomitant medications than subjects who received placebo, 68% versus 55%, respectively (p=0.048). A similar trend was seen in Table 6.25 for the ITT Population who took concomitant medications where 72% of subjects on Ampligen took fewer concomitant medications vs. 56% who received placebo (p=0.0145).

Table 6.24: Change from Initial Use of Concomitant Medications Related to CFS at End of Study AMP-516 (ITT Population)

Direction of change[1]	Number (%) of subjects		p-value[2]
	Ampligen (n=100)	Placebo (n=108)	
Decrease	68 (68.0%)	59 (54.6%)	0.0482
No decrease	32 (32.0%)	49 (45.4%)	

[1] The change in use of concomitant medications was calculated for each subject by subtracting the number of days each concomitant medication was taken during the last 4 weeks that the subject was in the study from the number of days each concomitant medication was taken during the first 4 weeks of the study

[2] p-value is from a chi-square test

Table 6.25: Change from Initial Use of Concomitant Medications Related to CFS at End of Study AMP-516 (ITT Population Who Took at Least One Concomitant Medication)

Direction of change[1]	Number (%) of subjects		p-value[2]
	Ampligen (n=94)	Placebo (n=106)	
Decrease	68 (72.3%)	59 (55.7%)	0.0145
No decrease	26 (27.7%)	47 (44.3%)	

[1] The change in use of concomitant medication was calculated for each subject by subtracting the number of days each concomitant medication was taken during the last 4 weeks that the subject was in the study from the number of days each concomitant medication was taken during the first 4 weeks of the study

[2] p-value is from a chi-square test

6.3.2 AMP-502

In AMP-502, as shown in Table 6.26, a statistically significantly (p=0.023) smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use compared with the percentage of subjects in the placebo group (44.7%). Notably, the requirement for medication for relief from symptoms of CFS was approximately twice that for subjects given placebo than for subjects given Ampligen. The number and percentage of these medication used during the first 4 weeks and the last 4 weeks of the study are presented below.

Table 6.26: Change from Initial Use of Concomitant Medications in an Attempt to Palliate Symptoms of CFS at End of Study AMP-502 (Subjects in the ITT Population Who Took Concomitant Medications)

Direction of change[1]	Ampligen (n=45) n (%)	Placebo (n=47) n (%)	p-value[2]
Increased	10 (22.2%)	21 (44.7%)	0.023
Not Increased	35 (77.8%)	26 (55.3%)	

[1] The change in use of concomitant medication was calculated for each subject by subtracting the number of days each concomitant medication was taken during the last 4 weeks that the subject was in the study from the number of days each concomitant medication was taken during the first 4 weeks of the study

[2] p-value is from a chi-square test

6.3.3 Activities of Daily Living

In the more severely debilitated AMP-502 patient population, the improvement from Baseline to Week 24 in ADL score was statistically significantly greater in the Ampligen group than in the placebo group. The change in mean ADL scores from Baseline to completion (Week 24) for the Ampligen group was 13.00, a statistically significant (p=0.022) difference compared to placebo. Differences in mean changes in ADL scores between Ampligen and the placebo from Baseline became statistically significant at Week 12 and remained statistically significant at Weeks 16, 20 and 24, respectively. The mean changes from Baseline ADL scores increased monotonically in both treatment groups beginning at Week 4 in Study AMP-502. By Week 12 and continuing to Week 24, increases in mean change from Baseline in ADL scores were higher in the Ampligen treatment group than placebo. These changes indicate meaningful improvements in patient function at Week 24 for all patients who received Ampligen. A summary of the mean change in ADL scores from Baseline to Weeks 4, 8, 12, 16, 20, and 24 for the ITT Population is presented in the Table 6.27 below.

Table 6.27: Mean Change from Baseline Activities of Daily Living Scores (Intent-to-treat Population, Study AMP-502)			
Statistic	**Ampligen n=45**	**Placebo n=47**	**p-value**
Mean Baseline	61.10	57.09	0.084[2]
Change from Baseline at Week 4[1]	3.97	2.35	0.347[3]
Change from Baseline at Week 8[1]	7.37	4.26	0.087[3]
Change from Baseline at Week 12[1]	9.03	5.07	0.042[3]
Change from Baseline at Week 16[1]	9.37	5.08	0.029[3]
Change from Baseline at Week 20[1]	10.98	5.64	0.016[3]
Change from Baseline at Week 24[1]	13.00	6.97	0.022[3]

[1] Mean change adjusted by least square means
[2] Student t-test
[3] An ANCOVA with baseline as covariate

Patients receiving Ampligen demonstrated a general progressive improvement within all activity modules, including those involved with sustained manual activities (e.g., cleaning, laundry, yardwork/maintenance) as shown below in Figure 6.3.

Patients increased activity sufficiently to move to the next ADL ability category in 10 of the 13 modules. In 10 of 11 modules, their average increase in ability was more than 0.5 unit of ability. Thus, the Ampligen effect was distributed generally over all the ADL modules.

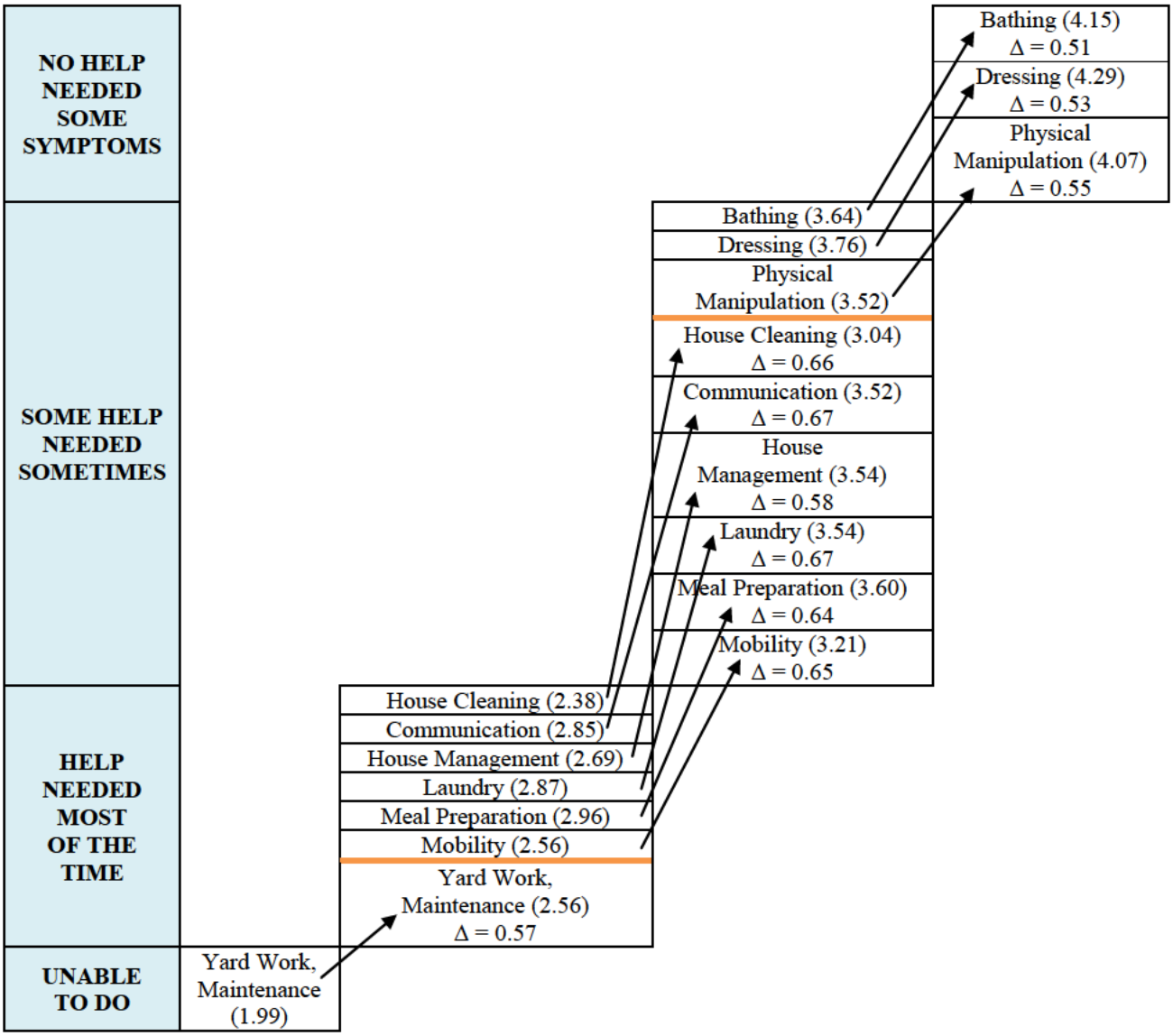


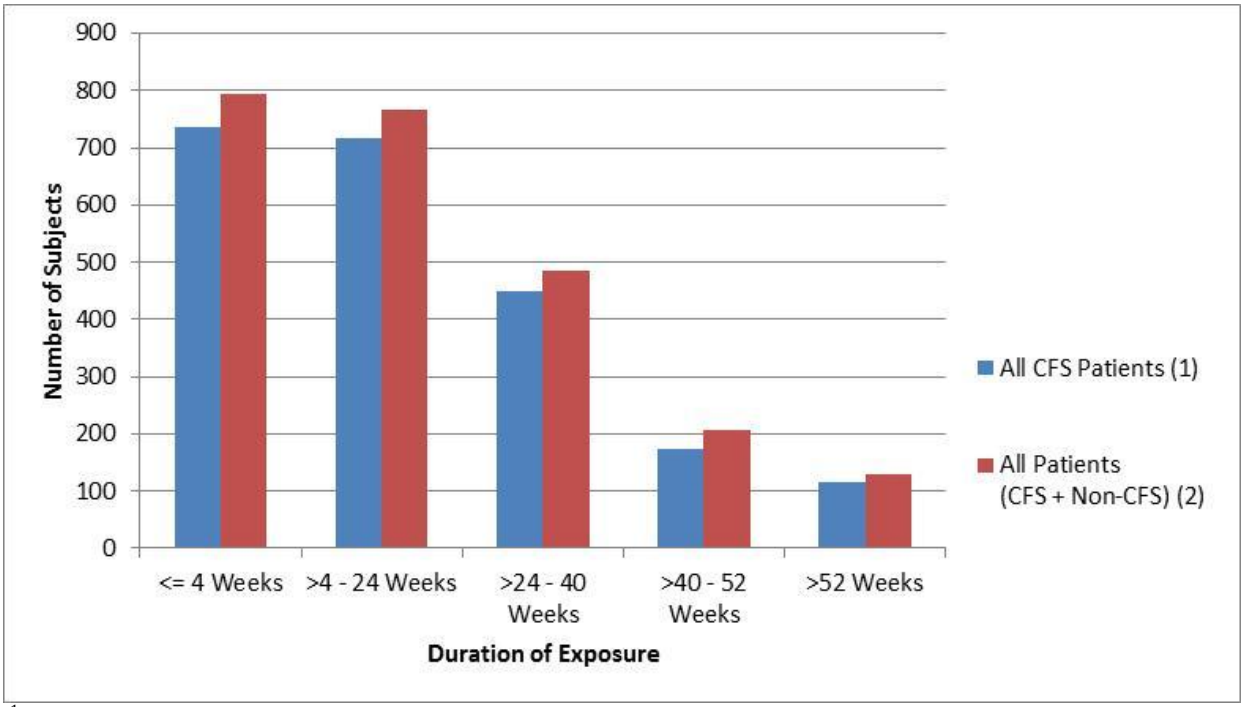
Figure 6.3: Characterization of Improvements in Activities of Daily Living (All Patients Receiving Ampligen in AMP-502)

7. SAFETY

There have been 9 clinical studies conducted to assess Ampligen's safety in subjects with CFS with additional supporting safety data from subjects with HIV infection, hepatitis B virus infection, cancer, and thermal injury. No studies were conducted in healthy volunteers. The data support our conclusion that Ampligen for intravenous infusion is safe for the treatment of adults with CFS. Ampligen for the CFS indication has been in development since 1988 and has been generally well-tolerated in clinical trials that enrolled over 1,200 patients with over 90,000 intravenous doses of Ampligen administered over 600 patient years of Ampligen dosing. Over 200 patients have received Ampligen for at least 40 weeks to one year. There has been no evidence of any cumulative toxicities, as no adverse event (AE) became progressively manifest as a function of duration of therapy.

7.1 EXPOSURE

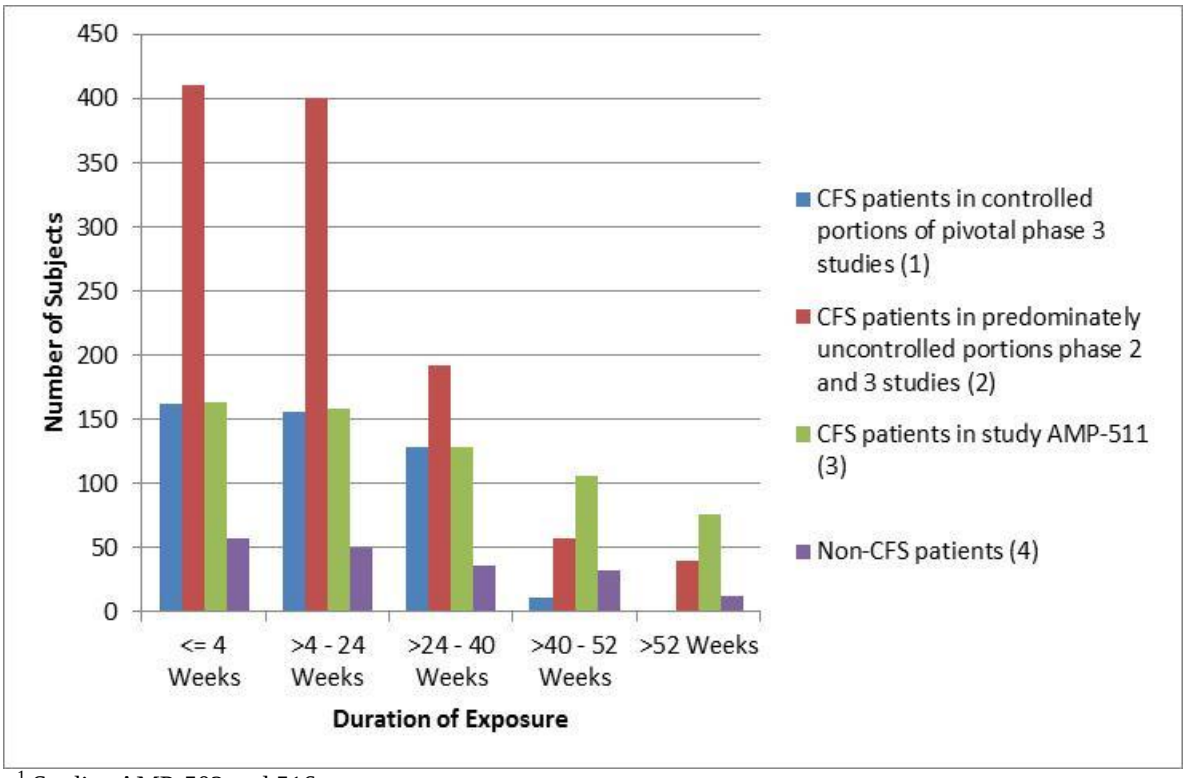
In the CFS studies, 589 unique subjects received treatment with Ampligen with an additional 256 subjects in non-CFS studies. Twenty-five subjects received Ampligen in 2 or more consecutive studies. All other subjects received Ampligen in only 1 study, although some subjects may have received placebo in a previous study for this indication. The average length of Ampligen treatment was 43.6 weeks, while the median was 26.6 weeks. Over 100 subjects have received treatment with Ampligen for greater than 1 year, and over 30 subjects received treatment for between 2 and 4 years. The breakdown by duration of exposure for CFS patients and for all patients is set forth below.



[1] Studies AMP-502 and 516; AMP-501, 502T, 502E, 504, 509, and 516C/E and AMP-511 at last safety cutoff
[2] Studies AMP-502 and 516; AMP-501, 502T, 502E, 504, 509, and 516C/E; AMP-511 at last safety cutoff and Studies 700 (HIV), 700E (HIV), 800 (Hepatitis B), and 900(Burn patients)

Figure 7.1: Number of Subjects Who Received IV Ampligen by the Duration of Exposure (CFS and Non-CFS patients)

The further breakdown of exposure by study type is shown in Figure 7.2.



[1] Studies AMP-502 and 516
[2] Studies AMP-501, 502T, 502E, 504, 509, and 516C/E
[3] Studies AMP-511 at last safety cutoff
[4] Studies 700 (HIV), 700E (HIV), 800 (Hepatitis B), and 900 (Burn patients)

Figure 7.2: Number of Subjects Who Received IV Ampligen by the Duration of Exposure in the Controlled and Uncontrolled Portions of the Clinical Studies

Overall, infusions of Ampligen were well-tolerated by most subjects in the CFS and Non-CFS studies. In the early studies, a number of events (e.g., injection site reactions, flu symptoms) were observed to occur shortly after starting Ampligen infusions. To allow subjects to develop tolerance and minimize these reactions, subjects in later studies received smaller doses (200 mg) during the first 4 infusions, then the dose was increased to the targeted therapeutic dose (400 mg). The dose was changed for these first infusions; the frequency of drug administration was unaltered. This approach also allowed for the double-blinded condition to be maintained in the well-controlled studies.

7.2 SAFETY OVERVIEW

7.2.1 Deaths, Serious Adverse Events and Adverse Events

With regard to the overall incidence of AEs for all CFS studies (controlled and uncontrolled), there was no significant difference in number of AEs per 100 patient treatment years (PTYs) in the Ampligen cohort (2296) compared to placebo (2332) (Table 7.1). There was also no difference in the percentage of patients discontinuing secondary to an AE across all studies for Ampligen (3.0%) versus placebo (3.0%). **With regard to the incidence of SAEs per 100**

PTYs, a slightly higher incidence was seen with the placebo cohort (21.1%) compared to the Ampligen cohort (16.6%). Also, **a slightly higher percent of patients had a SAE in the placebo cohort (8.6%) vs. the Ampligen cohort (7.7%)**. There were 3 deaths which occurred in the Ampligen open-label studies. None of these were related to the Ampligen treatment. Two deaths occurred in study AMP-516C/E (38LC 22(b) (6) had pneumonia and hemoptysis secondary to a coagulopathy from warfarin (Prothrombin Time (PT) was 7.7 times normal (INR=7.7) indicating a high risk for bleeding), and died of respiratory failure, and 54MS 11-(b) (6) with road rage, was cornered by police and committed suicide). 34DB 024-(b) (6) with a history of depression committed suicide after completing study AMP-509. **With regard to the controlled portions of the two pivotal studies (AMP-502 and AMP-516) there were no deaths.** In those studies, the incidence of AEs per PTY was 18% lower in the placebo cohort compared to the Ampligen group. This difference is explained by an increased number of infusion reactions and flu-like symptoms associated with Ampligen treatment, especially during the initiation of therapy (similar to the flu-like symptoms seen with the initiation of interferon therapy). The majority of the flu-like symptoms were mild or moderate (89% for Ampligen vs. 93% for placebo). There was no significant difference between Ampligen and placebo with regard to the overall severity profile for flu-like symptoms. **The total number of SAEs was the same (n=22) in the Ampligen and placebo arms of the controlled studies (AMP-502 and AMP-516) as was the number of patients discontinuing because of AEs (n=5).**

Table 7.1: Incidence and Frequency of Deaths, Serious Adverse Events, and Adverse Events in the Ampligen Program Through the Last Safety Cutoff

Category	Controlled Portions of Pivotal Studies		Controlled and Uncontrolled Studies	
	Studies 502 and 516		CFS Studies[1]	
	Ampligen	Placebo	Ampligen	Placebo
Number of Patients Treated	162	164	737	174
Mean Patient-Years of Follow-Up on Study Drug	0.63	0.66	0.68	0.65
Total Patient-Years of Follow-Up on Study Drug	101.6	109.0	498.6	113.7
Mean Number of IV Infusions	64.1	67.4	59.8	67.0
Total Number of IV Infusions	10389	11051	44054	11656
Deaths				
Patients Who Died, n (%)	0	0	3 (0.41%)[2]	0
Incidence of Death Per 100 Patient-Years on Study Drug	0	0	0.60	0
SAEs				
Number of SAEs	22	22	83	24
Incidence of SAEs per 100 Patient-Years on Study Drug	21.7	20.2	16.6	21.1

Table 7.1: Incidence and Frequency of Deaths, Serious Adverse Events, and Adverse Events in the Ampligen Program Through the Last Safety Cutoff				
Category	**Controlled Portions of Pivotal Studies**		**Controlled and Uncontrolled Studies**	
	Studies 502 and 516		**CFS Studies[1]**	
	Ampligen	**Placebo**	**Ampligen**	**Placebo**
Patients With SAEs, n (%) (Deaths on Study Reported Separately)	17 (10.5%)	13 (7.9%)	57 (7.7%)	15 (8.6%)
Number of AEs	2827	2488	11448	2652
Incidence of AEs per 100 Patient-Years on Study Drug	2782.5	2282.6	2296.0	2332
Patients With AEs, n (%)	161 (99%)	160 (98%)	685 (93%)	170 (98%)
Discontinuations, n (%)	28 (17%)	20 (12%)	98 (13%)	20 (11%)
Discontinuations Secondary to AEs, n (%)	5 (3.1%)	5 (3.0%)	22 (3.0%)	5 (3.0%)

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

[2] These 3 deaths were not related to study drug
Two deaths occurred in AMP-516C/E (38LC 22-(b) (6) with pneumonia and hemoptysis secondary to coagulopathy from warfarin (Prothrombin Time (PT) was 7.7 times normal (INR=7.7) indicating a high risk for bleeding), died of respiratory failure, and 54MS 11-(b) with road rage, was cornered by police and committed suicide). 34DB 024-(b) with a history of depression committed suicide after completing study AMP-509.

As shown in Table 7.2, there were five serious adverse events (SAEs) that were judged by the principal investigators to be possibly or probably related to the study drug, which occurred in the placebo-controlled studies (AMP-502 and AMP-516). Table 7.3 provides a summary of these 5 SAEs.

Table 7.2: Serious Adverse Events (SAEs) in AMP 502 and AMP 516 Pivotal Studies		
Relationship to Study Drug	**Number of Serious Adverse Events**	
	Ampligen	**Placebo**
No*	16	16
Remote*	4	3
Possible*	2	2
Probable*	0	1
Definite*	0	0
Total	22	22

*Relationship based on contemporaneous clinical observations of "blinded" Principal Investigators at study sites

Table 7.3: Subjects Who Experienced at Least One SAE that was Possibly, Probably, or Definitely Related to Study Drug[1]									
Study Treatment	**Center/ Subject Number**	**Sex, M/F**	**Age[2], yr**	**Adverse Event (investigator text)**	**Reason Why the Event was Considered Serious**	**Time from Start of Treatment to Becoming Serious, Days**	**Duration of Event if Resolved, Days**	**Action Taken with Respect to Study Drug**	**Causality as Assessed by the Investigator**
AMP-516 **Ampligen**	53/011	F	28	Suicide attempt (Suicide attempt)	Life-threatening	121	3	None	Possible
AMP-516 **Placebo**	37/019	F	42	Chest pain (Difficulty breathing, chest tightness)	Hospitalization	117	Not resolved	Dose(s) held	Probable
AMP-516 **Placebo**	52/037	F	35	Convulsion (Seizures)	Hospitalization	251	Not resolved	Dose(s) held	Possible
AMP-502 **Ampligen**	13/072	F	39	Abdominal pain (Abdominal pain)	Hospitalization	36	5	None	Possible
AMP-502 **Placebo**	23/031	F	31	Headache (Accelerated headache syndrome)	Hospitalization	155	9	None	Possible

[1] Controlled studies AMP-502 and AMP-516
[2] Age at enrollment

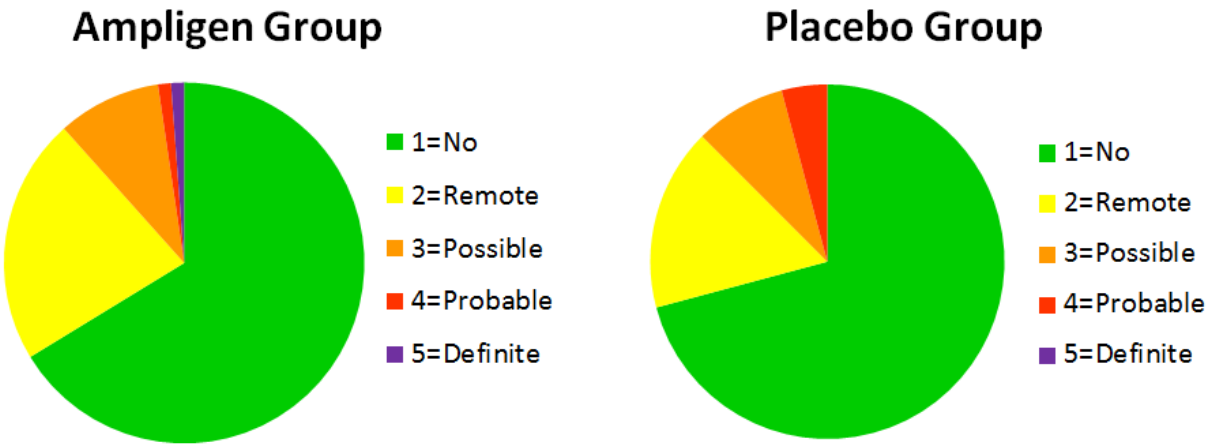
As shown in Table 7.3, the only SAE that was judged to be probably related to study drug occurred in a placebo patient. This patient (AMP-516 37BS 019 (b)) developed chest pain and difficulty breathing after receiving an infusion of study drug (placebo) and was hospitalized. During hospitalization a myocardial infarction was ruled out, but no definite diagnosis was made. A pulmonary specialist was consulted who suggested that the subject discontinue the study and the patient concurred.

Two Ampligen and two placebo patients had SAEs which were judged by the principal investigators to be possibly related to study drug. One placebo patient (AMP-516 52CI 037 (b) (6)) was admitted to the hospital with a seizure, which was not well-controlled on seizure medication. The principal investigator believed the seizures were possibly related to the study drug (placebo). This patient received no further doses of study drug (placebo). The second patient (AMP-502 23CC 031(b) (6)) was admitted to the hospital because of intensification of severe headaches, which failed to respond to headache medication and were associated with progressive sleep deprivation and exhaustion. During hospitalization the patient responded to treatment and continued on the study. The principal investigator considered the headache syndrome to be possibly related the study medication (placebo).

One Ampligen patient (AMP-516 53KH 011 (b)) was admitted to the hospital after attempting suicide by taking 40 alprazolam (0.5 mg), atenolol and 12-16 ounces of alcohol. The patient recovered and was discharged two days later. In the principal investigator's opinion, the attempted suicide was considered to be life-threatening and possibly related to study drug (Ampligen), but more likely related to a hypomanic reaction to parotoxine in conjunction with social difficulties. The Ampligen treatments resumed four days after hospital discharge and the patient completed the AMP-516 study. The second Ampligen patient (AMP-502 13PI 072 (b) (6) developed abdominal pain associated with headache, nausea, malaise and weakness following an infusion of Ampligen and was hospitalized. On hospital admission the patient had elevated liver enzymes with a SGOT of 814 and SGPT of 810 (both 20x ULN). The patient rapidly improved with bed rest alone and the study drug was not discontinued. The infusions were continued at a 50% reduction (200 mg instead of 400 mg) and the patient received 48 additional doses of study drug without further similar symptoms or liver function test elevations. The principal investigator considered the abdominal pain and elevated liver enzymes to be possibly related to study drug (Ampligen).

As shown in Table 7.1, for all CFS studies (Controlled and Uncontrolled) the incidence of SAEs per 100 patient-treatment years (PTYs) on study drug is 16.6 SAEs for Ampligen vs. 21.1 SAEs per 100 PTYs for placebo.

The relationship of SAEs to study drug across all CFS studies is shown in Figure 7.3. There was no significant difference in the relationship profile for SAEs in the Ampligen vs. placebo cohorts ($p>0.8$, Chi-square test). Only one SAE was judged to be definitely related to the study drug in the CFS developmental plan. This patient had pre-existing Chilblain syndrome which was believed by the investigator to have exaggerated the subcutaneous tissue response to study drug (Ampligen) which leaked from their vein during an infusion. The patient recovered completely (see Section 7.2.9 for a more detailed description of this event).



Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

Figure 7.3: SAEs in the Ampligen CFS Program – Relationship to Study Medication in All Studies[1]

7.2.2 Serious Adverse Events by System Organ Class and Preferred Term

Table 7.4 shows that there was a slightly lower overall incidence of SAEs for all CFS studies (controlled and uncontrolled) for the Ampligen cohort (8.0%) vs. placebo cohort (8.6%). As shown in Table 7.4, in 7 of the 11 body systems across all the controlled and uncontrolled studies the difference in the percentage of patients between the Ampligen vs. placebo arms was 0.5% or less. With regard to specific body systems, the percentage of patients with SAEs was >0.5% lower in the Ampligen cohort vs. placebo for two body systems (body as a whole and nervous), while the percentage was >0.5% lower in the placebo cohort vs. Ampligen for 2 other body systems (cardiovascular and respiratory). **As shown in Figure 7.3, there is no difference in the SAE relationship profile for the Ampligen vs. placebo cohorts.**

With regard to the controlled portions of the pivotal studies AMP-502 and AMP-516, the percentage of patients with SAEs was higher by >0.5% in four body systems for the Ampligen cohort vs. three body systems categories for placebo. As shown in Tables 7.4 and Figure 7.3, the SAE relationship profiles were well-balanced between the Ampligen and placebo groups.

Table 7.4: Summary of All Patients with Serious Adverse Events (SAEs) in Any Treatment Group, by System Organ Class and Preferred Term				
	Controlled Portions of Pivotal Studies		**Controlled and Uncontrolled Studies**	
	Studies 502 and 516		**CFS Studies[1]**	
	Ampligen	**Placebo**	**Ampligen**	**Placebo**
	(n=162)	**(n=164)**	**(n=737)**	**(n=174)**
Patients with SAEs	17 (10.5 %)	13 (7.9 %)	59 (8.0%)	15 (8.6%)
Body as a Whole	11 (6.8 %)	8 (4.9 %)	31 (4.2%)	10 (5.7%)
Cardiovascular	3 (1.9 %)	0	9 (1.2%)	0
Digestive	0	2 (1.2 %)	6 (0.8%)	2 (1.1%)
Endocrine	0	0	1 (0.1%)	0
Metabolism and Nutrition	0	1 (0.6 %)	4 (0.5%)	1 (0.6%)
Musculoskeletal	0	0	1 (0.1%)	0
Nervous	3 (1.9 %)	6 (3.7 %)	9 (1.2%)	6 (3.4%)
Respiratory	1 (0.6 %)	0	7 (0.9%)	0
Skin	0	0	1 (0.1%)	0
Special Senses	0	0	1 (0.1%)	0
Urogenital	2 (1.2 %)	0	4 (0.5%)	0

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

Table 7.5 shows a slightly lower overall incidence of AEs in the Ampligen cohort vs. placebo for all CFS studies (controlled and uncontrolled), while the controlled portions of the pivotal studies AMP-502 and AMP-516 is slightly higher. Only one body system (musculoskeletal) has more patients in the Ampligen cohort vs. placebo for both categories of the studies (all CFS studies and controlled studies). The cardiovascular body system was perfectly balanced for the CFS studies overall at 36.8%, while the Ampligen cohort was 6.6% higher than the placebo cohort for the controlled studies.

With regard to the controlled studies, the 6.6% or 10 patient increase in the Ampligen cohort vs. placebo for cardiovascular body system is explained by a flushing reaction (vasodilation) which can occur with Ampligen administration (27 Ampligen patients vs. 15 placebo patients).

With regard to the musculoskeletal system, the 3.6% or 5 patient increase in the Ampligen cohort vs. the placebo cohort is explained by several of the flu-like symptoms including arthralgia (22 Ampligen vs. 19 placebo) and myalgia (37 Ampligen vs. 34 placebo) seen with Ampligen administration.

With regard to special senses, the 3.5% or 5 patient increase in the Ampligen cohort vs. placebo is explained by tinnitus (7 Ampligen patients vs. 1 placebo patient).

With regard to urogenital the 9% or 14 patients in the Ampligen cohort cannot be explained by less than 6 separate adverse event categories and overall the percent of patients with urogenital AEs is not increased relative to placebo when considering all CFS studies.

Table 7.5: Summary of All Patients with Adverse Events in Any Treatment Group, by System Organ Class and Preferred Term

	Controlled Portions of Pivotal Studies		Controlled and Uncontrolled Studies	
	Studies 502 and 516		CFS Studies[1]	
	Ampligen	Placebo	Ampligen	Placebo
	(n=162)	(n=164)	(n=737)	(n=174)
Patients with any Adverse Events	161 (99.4%)	160 (97.6%)	685 (92.9%)	170 (97.7%)
Body as a Whole	155 (95.7%)	153 (93.3%)	646 (87.7%)	161 (92.5)
Cardiovascular	70 (43.2%)	60 (36.6%)	271 (36.8%)	64 (36.8%)
Digestive	103 (63.6%)	103 (62.8%)	427 (57.9%)	111 (63.8%)
Endocrine	3 (1.9%)	3 (1.8%)	13 (1.8%)	3 (1.7%)
Hematology and Lymph	48 (29.6%)	50 (30.5%)	142 (19.3%)	57 (32.8%)
Metabolism and Nutrition	30 (18.5%)	33 (20.1%)	117 (15.9%)	36 (20.7%)
Musculoskeletal	61 (37.7%)	56 (34.1%)	271 (36.8%)	60 (34.5%)
Nervous	119 (73.5%)	111 (67.7%)	424 (57.5%)	119 (68.4%)
Respiratory	94 (58.0%)	94 (57.3%)	345 (46.8%)	102 (58.6%)
Skin	85 (52.5%)	88 (53.7%)	317 (43.0%)	96 (55.2%)
Special Senses	72 (44.4%)	67 (40.9%)	222 (30.1%)	70 (40.2%)
Urogenital	59 (36.4%)	45 (27.4%)	195 (26.5%)	47 (27.0%)

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

7.2.3 Adverse Events of Special Interest

Table 7.6 shows a 7.1% increase in the percent of patients with infusion reactions in the Ampligen cohort vs. placebo for the controlled studies, while the percent difference is only 0.9% overall when considering all CFS studies (see also Section 7.2.9 Suspected Adverse Reaction).

Table 7.6: Patients with AEs of Special Interest (Safety Population)				
	Controlled Portions of Pivotal Studies		**Controlled and Uncontrolled Studies**	
	Studies 502 and 516		**CFS Studies[1]**	
	Ampligen	**Placebo**	**Ampligen**	**Placebo**
Number patients treated	162	164	737	174
AEs of interest				
Number of patients with infusion reaction (%)	50 (30.9%)	39 (23.8%)	172 (23.3%)	39 (22.4%)
Total number of infusion reactions	131	83	434	83
Number of patients with anaphylactic reaction (%)	0	0	0	0
Total number of anaphylactic reaction	0	0	0	0
Number of patients with liver function test abnormalities (%)	2 (1.2%)	0	6 (0.81%)	0
Total number of occurrences of liver function test abnormalities	2	0	7	0
Number of patients with leucopenia abnormalities (%)	3 (1.9%)	0	8 (1.1%)	0
Total number of occurrences of leucopenia	3	0	12	0
Number of patients with flu-like symptoms (%)	139 (85.8%)	120 (73.2%)	572 (77.6%)	128 (73.6%)
Total number of occurrences of flu-like symptoms	546	414	2858	440
Number of patients with neoplasia (%)[2]	1 (0.6%)	2 (1.2%)	9 (1.2%)	2 (1.1%)
Total number of occurrences of neoplasia[3]	1	2	13	2
Total number of patients with cancer (%)	1 (0.6%)	1 (0.6%)	2 (0.3%)	1 (0.6%)
Total number of occurrences of cancer	1	1	2	1
Number of patients with development of autoimmune disease (%)	0	0	2 (0.27%)[4]	0

Table 7.6: Patients with AEs of Special Interest (Safety Population)				
	Controlled Portions of Pivotal Studies		Controlled and Uncontrolled Studies	
	Studies 502 and 516		CFS Studies[1]	
	Ampligen	Placebo	Ampligen	Placebo
Total number of occurrences of development of autoimmune disease	0	0	2	0
Number of patients with AEs of depression, hospitalization for depression, or suicide attempt/ideation	19 (11.7%)	21 (12.8%)	62 (8.4%)	22 (12.6%)
Total number of occurrences of AEs of depression, hospitalization for depression, or suicide attempt/ideation	25	42	87	43

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E
[2] The higher incidence of neoplasia relative to cancer is related to the frequent occurrence of benign lipomas
[3] Excludes non-melanoma skin cancers (see text above)
[4] There were no patients who developed new onset of autoimmune disease (see text above)

A 12.6% increase was seen in the percent of patients reporting flu-like symptoms in the Ampligen cohort compared to placebo in the controlled studies (AMP-502 and AMP-516). These flu-like symptoms are generally mild to moderate and typically lessen in intensity with continued Ampligen administration. The majority of the flu-like symptoms were mild or moderate (89% for Ampligen and 93% for placebo).

There was no evidence for an increase in cancer in the Ampligen cohort vs. placebo (see Table 7.20).

In AMP-516, one patient (52CI 20 (b) (6), a 53 year old female receiving Ampligen was diagnosed with a B-cell non-Hodgkin's lymphoma. The diagnosis was based on a bone marrow core biopsy, which was performed because of long standing lymphocytosis dating back to 1998 prior to the patient's enrollment in the AMP-516 study. Also, a recent publication shows that patients with CFS have an increased risk of developing non-Hodgkin's lymphoma (Chang 2012). In study AMP-502, a 38 year old female receiving placebo (23CC 43 (b) (6)) developed rectal carcinoma (carcinoid tumor) which was resected and the patient continued dosing and completed the study. In study AMP-511, a 61 year old female receiving Ampligen developed a breast carcinoma which was resected and the patient continued on study for an additional 24 weeks.

With regard to autoimmune disease there were no patients who developed an onset of autoimmune disease. One patient (502E 27SH 015 (b) (6), a 50 year old female, entered the AMP-502/502E studies with a history of systemic lupus erythematosus, "Lupus" and "vasculitis". She experienced AEs of "Lupus rash" and vasculitis during AMP-502E which were not related to study medication and continued on Ampligen for an additional 26 weeks

completing Study AMP-502E. A second patient (501 13PI 011 (b) (6), a 26 year old female, experienced mild bloody diarrhea, and mild "inflammatory bowel disease" which resolved with conservative treatment. The verbatim term "inflammatory bowel disease" was coded as ulcerative colitis (preferred term) although there was no evidence for ulceration colitis and the patient continued on Ampligen and received 17 additional weeks of treatment without any recurrence of bloody diarrhea or inflammatory bowel disease. A larger autoimmune Lupus antibody panel examining 16 autoantibodies was completely negative, indicating there was no laboratory evidence of an autoimmune disorder.

The number of patients with AEs of depression, hospitalization for depression, or suicide attempt/ideation was higher in the placebo cohort than the Ampligen cohort, both in the controlled studies (12.8% vs. 11.7%) and in the CFS studies overall (12.6% vs. 8.4%) (see also Section 7.2.10 Depression, Suicide Ideation and Suicide Attempt and Section 7.2.11 Hospitalization).

7.2.4 Liver Function Test

Table 7.7 shows that 16.0% of Ampligen patients compared to 7.3% of placebo patients had an increase in at least one liver function test (primarily SGPT) in the controlled studies (AMP-502 and AMP-516). It was relatively rare for the SGPT to increase over 3x the upper limit of normal (ULN) (6.2% of Ampligen patients vs. 1.8% of placebo) and only one Ampligen patient (0.6%) increased SGPT 5x the ULN over that seen for placebo.

Overall, there were 11 (1.5%) Ampligen and 2 (1.1%) placebo patients in the controlled and uncontrolled studies with one or two SGPT values >5x ULN. Nine of the Ampligen patients had one (n=7) or two (n=2) SGPT values >5x ULN which normalized with continued Ampligen dosing at 400 mg (n=6) or at a reduced dose of 300 mg (n=2) or 200 mg (n=1). All nine patients completed their respective study. Two patients, one Ampligen with two SGPT values >5x ULN and one placebo patient with a single SGPT value >5x ULN completed their respective studies without SGPT normalization. The last SGPT values were 77 (ULN=40) for the Ampligen patient and 202 (ULN=40) for the placebo patient. One placebo patient with a single SGPT value >5x ULN was dosage reduced 50% (equivalent to 200 mg). The SGPT normalized and patient continued to study completion. The final Ampligen patient had a single SGPT value >5 ULN discontinued the study because of travel being too far (patient lived out-of-state) and did not return for follow-up blood work.

There was no increase in total bilirubin in the Ampligen cohort vs. placebo in the controlled studies (AMP-502 and AMP-516). Also, there were no patients that had an increase in SGPT or SGOT >2x the ULN and also had a total bilirubin >1.5x ULN.

Table 7.7: Assessment of Liver Function Tests in Patients Who Received Ampligen in the CFS Development Program

	Controlled Portions of Pivotal Studies		Controlled and Uncontrolled Studies	
	Studies 502 and 516		CFS studies[1]	
	Ampligen	Placebo	Ampligen	Placebo
Number patients treated	162	164	737	174
Number of patients with ≥ liver function test elevation, n (%)	26 (16.0%)	12 (7.3%)	88 (11.9%)	12 (6.9%)
SGOT (AST) >2X ULN, n (%)	6 (3.7%)	4 (2.4%)	30 (4.1%)	4 (2.3%)
SGOT (AST) >3X ULN, n (%)	0	2 (1.2%)	7 (0.95%)	2 (1.1%)
SGOT (AST) >5X ULN, n (%)	0	1 (0.6%)	4 (0.54%)	1 (0.57%)
SGPT (ALT) >2X ULN, n (%)	20 (12.3%)	9 (5.5%)	56 (7.6%)	9 (5.2%)
SGPT (ALT) >3X ULN, n (%)	10 (6.2%)	3 (1.8%)	26 (3.5%)	3 (1.7%)
SGPT (ALT) >5X ULN, n (%)	3 (1.9%)	2 (1.2%)	11 (1.5%)	2 (1.1%)
Total bilirubin >1.5X ULN, n (%)	2 (1.2%)	3 (1.8%)	19 (2.6%)	3 (1.7%)
Total bilirubin >2X ULN, n (%)	1 (0.6%)	1 (0.6%)	10 (1.36%)	1 (0.57%)
SGPT or SGOT >2X ULN and total bilirubin > 1.5X ULN, n (%)	0	0	0	0
SGPT or SGOT >3X ULN and total bilirubin > 2X ULN, n (%)	0	0	0	0

[1]Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E
[2] No development of autoimmune disease was seen

7.2.5 Hematology and Chemistry Adverse Events

Table 7.8 shows that the incidence of grade 2 plus grade ≤1 hematocrit and hemoglobin decreases were well-balanced for both the controlled studies, 6.2% for Ampligen vs. 4.2% for placebo, and 16.0% for Ampligen vs. 15.8% for placebo, respectively. Grade ≤1 decrease in hemoglobin occurred in 2.0% more Ampligen patients compared to placebo (15.4% vs. 13.4%) in the controlled studies (AMP-502 and AMP-516). With regard to hematocrit, a grade ≤1 decrease occurred in 2.6% more Ampligen patients compared to placebo (5.6% vs. 3.0%). Grade 2 decreases in hematocrit and hemoglobin occurred in 0.6%-1.8% more placebo patients in the controlled studies compared to the Ampligen patients. The overall results from the controlled and uncontrolled CFS studies supported the hematocrit and hemoglobin findings of the controlled studies.

In the controlled studies, grade ≤1 decreases in WBC were seen in 28.4% of Ampligen patients compared to 26.8% of placebo. Grade 2 decreases in WBC were much less frequent occurring in 4.9% of Ampligen patients compared to 4.3% of placebo. Overall, five (5) Ampligen patients had a grade ≥3 decrease in WBC:

1) Patient (502 13PI 041 (b) (6)) had a single low WBC value on 12/6/90 of 1.8K, it was repeated 4 days later on 12/10/90 and found to be normal at 4.7K. Patient continued to be dosed with Ampligen 400 mg and completed the study.

2) Patient (502 27SH 002 (b) (6)) had a single low WBC value on 9/26/90 of 1.8K. Patient continued to receive Ampligen at full dose (400 mg) and WBC increased to normal (8.5K) on 11/20/90. Patient completed study AMP-502.

3) Patient (502E 27SH 063 (b) (6) had a single low WBC value of 1.0K on 6/23/91 secondary to "extensive leukocyte deterioration" in the laboratory specimen. Patient continued to receive Ampligen at full dose (400 mg) and the WBC=3.9K on 7/10/92 and 6.6K on 8/18/92. Patient completed study AMP-502E.

4) Patient (509 34DB 028 (b) (6) had a single low WBC of 1.2K on 6/24/96. Patient continued to receive Ampligen at a dosage of 300 mg and WBC increased into the normal range at 6.5K on 7/26/96. Patient continued to receive Ampligen and completed the study with the WBC in the normal range at 5.4K.

5) Patient (502E 13PI 068 (b)) had a single low WBC value of 1.3K on 2/27/92 secondary to "extensive leukocyte deterioration" in the laboratory specimen. Patient continued to receive Ampligen at a dosage of 300 mg to 400 mg and had a normal WBC value of 6.6K on 4/16/92. WBC value continued in the normal range while patient was receiving a dosage of 400 mg. Patient completed study AMP-502E with a WBC of 5.9K.

With regard to thrombocytopenia, in the controlled studies, grade ≤1 decreases in platelet counts were seen in 3.1% of Ampligen patients vs. 2.4% of placebo patients and overall in the controlled plus uncontrolled studies grade ≤1 decreases were seen in 2.6% of Ampligen patients vs. 2.3% of placebo patients. Three (3) Ampligen patients experienced grade 2 decreases in platelet counts. In two cases the grade 2 decreases in platelet counts were transient and both subjects (502E 27SH 015 (b) (6) and 511 38LC 082 (b) (6)) continued to receive Ampligen with platelet count increasing above 100K. Both patients completed their respective studies receiving the full dosage level of 400 mg twice weekly. In one case (502E 27SH 031 (b) (6)) the grade 2 platelet count was drawn 8 month post study completion. At time of study completion the platelet count was in the normal range (244K).

Grade ≤1 increases in creatinine were seen in 1.9% of Ampligen patients compared to 0.6% of placebo patients in the controlled studies.

Table 7.8: Hematology AEs and Chemistry AEs in the Ampligen Development Program for CFS					
		Controlled Portions of Pivotal Studies		**Controlled and Uncontrolled Studies**	
		Studies 502 and 516		**CFS Studies[1]**	
		Ampligen	**Placebo**	**Ampligen**	**Placebo**
Number Patients Treated	Lab Parameter Range	162	164	737	174
Patients with hematocrit decreased					
Grade ≤ 1, n (%)	28 to <32	9 (5.6%)	5 (3.0%)	37 (5.0%)	5 (2.8%)
Grade 2, n (%)	<28	1 (0.6%)	2 (1.2%)	5 (0.68%)	2 (1.1%)
Patients with hemoglobin decreased					
Grade ≤ 1, n (%)	9.5 to <11.5	25 (15.4%)	22 (13.4%)	109 (14.8%)	22 (12.6%)
Grade 2, n (%),	<9.5	1 (0.6%)	4 (2.4%)	6 (0.81%)	4 (2.3%)
Patients with neutrophil count decreased					
Grade ≤ 1, n (%)	1.5 K to <1.9 K	0	0	1 (0.14%)	0
Grade 2, n (%)	1.0 K to <1.5 K	0	0	0	0
Grade ≥ 3, n (%)	<1.0 K	0	0	0	0
Patients with WBC count decreased					
Grade ≤ 1, n (%)	3.0 K to <4.0 K	46 (28.4%)	44 (26.8%)	191 (25.9%)	46 (26.4%)
Grade 2, n (%)	2.0 K to <3.0 K	8 (4.9%)	7 (4.3%)	34 (4.6%)	7 (4.0%)
Grade ≥ 3, n (%)	<2.0 K	2 (1.2%)	0	5 (0.68%)	0
Patients with thrombocytopenia					
Grade ≤ 1, n (%)	90 K to <130 K	5 (3.1%)	4 (2.4%)	19 (2.6%)	4 (2.3%)
Grade 2, n (%)	50 K to <90 K	0	0	3 (0.41%)	0
Grade ≥ 3, n (%)	<50 K	0	0	0	0
Patients with increased creatinine					
Grade ≤ 1, n (%)	>1.5 to 2.0	3 (1.9%)	1 (0.6%)	10 (1.4%)	1 (0.57%)
Grade 2, n (%)	2.1 to 4.0	0	0	0	0
Grade ≥ 3, n (%)	>4.0	0	0	0	0

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

7.2.6 Vital Sign Abnormalities

With regard to Table 7.9 and adverse events related to hypertension, hypotension and tachycardia, the differences between Ampligen and placebo are less than 1% except for the controlled plus uncontrolled CFS studies where 3.4% of the placebo patients had hypertension compared to 1.6% of Ampligen patients.

With regard to fever, approximately 8 to 9% more Ampligen patients experienced a fever compared to placebo patients. The vast majority of these fevers were mild or moderate (97% for Ampligen vs. 100% for placebo). There was no significant difference in the overall severity profile between the Ampligen and placebo cohorts ($p>0.7$, Chi-square).

Table 7.9: AEs relating to vital sign abnormalities by preferred term in the Ampligen development program for CFS

	Controlled Portions of Pivotal Studies		**Controlled and Uncontrolled Studies**	
	Studies 502 and 516		**CFS Studies[1]**	
	Ampligen	**Placebo**	**Ampligen**	**Placebo**
Number patients treated	162	164	737	174
Patients with hypertension, n (%)	4 (2.5%)	5 (3.0%)	12 (1.6%)	6 (3.4%)
Patients with tachycardia, n (%)	6 (3.7%)	5 (3.0%)	23 (3.1%)	6 (3.4%)
Patients with hypotension, n (%)	3 (1.9%)	3 (1.8%)	16 (2.2%)	3 (1.7%)
Patients with orthostatic hypotension, n (%)	0	0	5 (0.68%)	0
Patients with fever, n (%)	32 (19.8%)	19 (11.6%)	152 (20.6%)	20 (11.5%)

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

7.2.7 Pre- and Post-Infusion Vital Signs

As shown in Table 7.10, when the mean pre-infusion vital signs (heart rate, blood pressure, respiratory rate, and temperature) were compared for the Ampligen cohort vs. placebo cohort, there were no clinically significant changes.

Table 7.10:	**Vital Signs Pre- and Post-Infusion - Safety Population (Controlled CFS Studies AMP-502 and AMP-516)**			
Measure	**Pre- and Post-Infusion**		**Controlled CFS Studies (AMP-502 and AMP-516)**	
			Ampligen (n=162)	**Placebo (n=164)**
Heart rate (bpm)	Pre-Infusion	Mean Standard Deviation Median	**79.2** 10.04 **80.0**	**77.2** 9.28 **76.0**
	Post-Infusion	Mean Standard Deviation Median	**76.2** 10.67 **76.0**	**75.4** 8.95 **76.0**
Systolic Blood Pressure (mm Hg)	Pre-Infusion	Mean Standard Deviation Median	**116.8** 13.74 **114.0**	**115.4** 15.85 **112.0**
	Post-Infusion	Mean Standard Deviation Median	**114.5** 12.2 **114.0**	**114.8** 14.22 **112.0**
Diastolic Blood Pressure (mm Hg)	Pre-Infusion	Mean Standard Deviation Median	**73.2** 9.87 **72.0**	**73.3** 10.26 **73.5**
	Post-Infusion	Mean Standard Deviation Median	**72.9** 8.29 **72.0**	**72.4** 9.76 **70.0**
Respiration (breaths/ minute)	Pre-Infusion	Mean Standard Deviation Median	**17.3** 1.89 **18.0**	**17.7** 2.11 **18.0**
	Post-Infusion	Mean Standard Deviation Median	**17.1** 1.96 **16.0**	**17.4** 2.01 **18.0**
			AMP-516 Only	
			Ampligen (n=117)	**Placebo (n=117)**
Temperature (degrees C)	Pre-Infusion	Mean Standard Deviation Median	**36.5** 0.56 **36.5**	**36.5** 0.41 **36.4**

7.2.8 Adverse Events Leading to Death

In the 9 CFS studies combined, 3 deaths were reported. None of these were related to the Ampligen treatment. Two deaths occurred in study AMP-516C/E (38LC 022 (b) (6) a 59 year old male developed pneumonia and had hemoptysis secondary to an iatrogenic coagulopathy from warfarin (Prothrombin Time (PT) was 7.7 times normal (INR=7.7) indicating a high risk for bleeding), and died of respiratory failure attributed to pre-existing conditions, not to study drug, and 54MS 011 (b) (6) a 38 year old male with road rage, was cornered by police and committed suicide. He was reported to have been shooting at motor vehicles and, when cornered by police officers, turned the gun on himself. 34DB 024 (b) (6) with a history of depression committed suicide after completing study AMP-509.

7.2.9 Suspected Adverse Reaction

A severe and unexpected local skin reaction to extravasation of Ampligen at the infusion site in the dorsum of the left hand was reported in one CFS subject (502E 24LP 012 (b) (6), a 29 year old female, with preexisting chilblain syndrome, which is a rare inflammatory disorder of subcutaneous and perivascular tissue including blood vessels. Over a 5.5 hour period post-infusion, this patient experienced progressive swelling and pain in her hand and arm to the point of inability to move her fingers. A limited hand fasciotomy (a surgical decompression procedure) was performed with subsequent improvement of symptoms and full recovery. The investigator considered the reaction definitely related to study medication, as the subcutaneous volume of liquid leaking from the vein caused the internal pressure and the serious symptoms, necessitating surgery. It was the investigator's opinion that there was a strong possibility the underlying chilblains and accompanying subcutaneous tissue inflammation caused an exaggerated and unique response. The subject withdrew at the investigator's request because of the event. This SAE was the only SAE judged by a principal investigator to be definitely related to study drug in the CFS development program.

7.2.10 Depression, Suicide Ideation and Suicide Attempt

As shown in Table 1.1, CFS increases a person's risk for premature death from suicide as well as heart failure, cancer and other complications of CFS. In all the controlled clinical studies with Ampligen, none revealed any increased risk for suicide over placebo. Of the two pivotal studies, AMP-502 enrolled the more severely debilitated and, as a result, depression, suicide ideation and suicide attempt were more likely to occur. In fact, as shown in Table 7.11, the incidence of depression as an adverse event was associated with suicide attempt and/or suicide ideation and occurred three times more often in the placebo group (18) vs. the Ampligen cohort (6) ($p<0.02$). Suicide attempt/suicide ideation occurred four times more often in the placebo group (4) than with Ampligen treatment (1), but this difference was not statistically significant. There was a trend towards less hospitalization for depression in the Ampligen group with no occurrences in the Ampligen cohort vs. five in the placebo group ($p=0.06$). The percentage of hospitalization for depression out of all hospitalizations was 0% for Ampligen vs. 45% for the placebo cohort.

Table 7.11: Hospitalization for Depression by Incidence in AMP-502			
Parameter Related to Depression and Suicide Attempt/Ideation	**Incidence of Event**		**Fisher's Exact Test p-value (2-tail)**
	Ampligen	**Placebo**	
AE, Depression[1]	6	18	0.02
Hospitalization for Depression[2]	0	5	0.06
AE, Suicide Attempt / Suicide Ideation[3]	1	4	0.37

[1] Depression includes depression with or without suicide attempt/ideation
[2] Hospitalization includes hospitalization for depression
[3] Suicide attempt and suicide ideation include with or without depression

7.2.11 Hospitalizations

Table 7.12 below looks more broadly at visits and stays in the hospital. The greater number of inpatient hospital days for the placebo group 101 vs. 15) was statistically significant ($p<0.05$).

Table 7.12: Health Care Utilization of in AMP-502					
Utilization Parameter	**Ampligen n=45**		**Placebo n=47**		**p-value[1]**
	Total Number	**Mean**	**Total Number**	**Mean**	
Emergency Room Visit	4	0.09	13	0.28	ns
Inpatient Hospital Days	15	0.33	101	2.15	$p<0.05$
Number of Hospitalizations	3	0.07	11	0.23	ns

[1] Independent Sample t-test (natural log)

In addition, as shown in Table 7.13, for those subjects who were hospitalized, the number of hospitalizations per subject and the length of their hospital stay were both significantly statistically longer in the placebo group.

Table 7.13: Relative Incidence of hospitalizations in CFS Patients Receiving Ampligen Versus Placebo in AMP-502				
		Ampligen	**Placebo**	**Mann-Whitney**
Number of Admissions per Hospitalized Patient	Mean	1.0	3.4	p<0.005
	Median	1.0	3.0	
Number of Hospital Days per Patient	Mean	2.7	16.3	p<0.005
	Median	1.0	18.0	

Hospitalization was defined as either an emergency room admission or an admission to the inpatient service.

7.2.12 Discussion of Adverse Events of Special Interest: Autoimmunity

Ampligen is a highly-selective agonist for Toll-like receptor 3 (TLR-3) and as discussed in the section above the development of new onset of autoimmune disease has not been seen with Ampligen. The reason for this may be that TLR-3 is unique amongst the 10 human TLRs in that it utilizes a different pathway that may induce immune responses distinct from the other TLRs. The ligand for TLR-3 is double-stranded RNA (dsRNA). Many studies have been conducted to address the potential toxicities of dsRNAs and have demonstrated fundamental differences in the mechanism of action and safety profile of Ampligen, including work done by Professor Ralph Steinman at Rockefeller University (2011 Nobel laureate in medicine) who used "knock-out" technology to show that the action of Ampligen was tightly restricted to TLR-3 activation. Moreover, Ampligen induces far fewer regulated genes than, poly IC, which may be related to Ampligen's alternative way of sensing dsRNA, which does not rely solely on other types of receptors like MDA5 (Avril 2009).

Nonetheless, there may be a perceived risk associated with Ampligen based on its pharmacology. In particular, it has been hypothesized that TLR agonists may induce undesirable immunological signals. **Ampligen contains a phosphodiester backbone (not phosphorothiolate) and so is quickly metabolized into naturally occurring nucleotides. Also, Ampligen has a brief half-life of 37 minutes which contributes to its lack of toxicity. All metabolic decay of Ampligen is into naturally occurring constituents of RNA, normally found throughout the human body in the nucleotide "pools"**.

Avril (2009) evaluated cytokine profiles elicited by various TLR agonists, and found that in his system Ampligen did not induce TNF-α in contrast to poly IC and other compounds. TNF-α has been found to be associated with induction of autoimmune toxicity, **so this finding is consistent to the observed lack of autoimmune toxicity associated with the clinical use of Ampligen.**

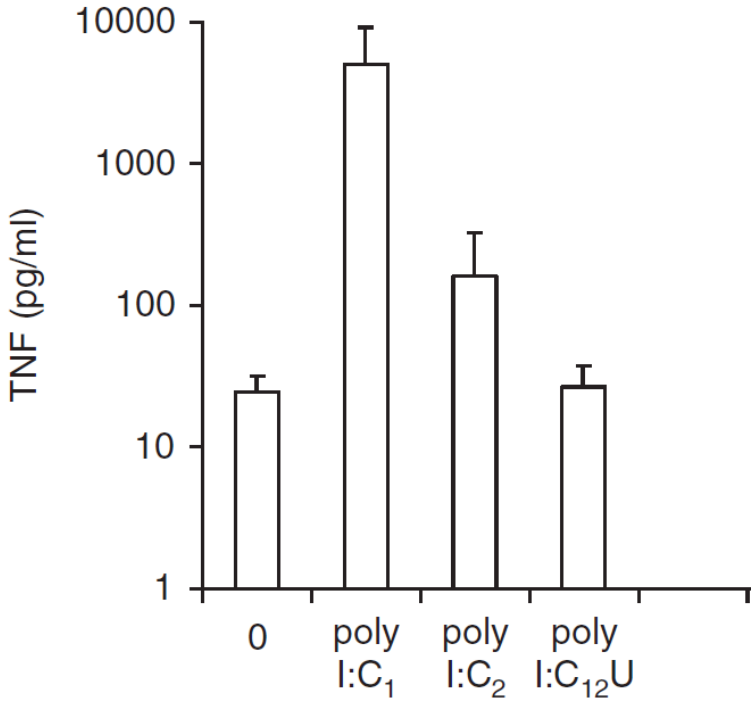


Figure 7.4: Poly IC Induces High Levels of TNF-α; Ampligen (Poly I : Poly $C_{12}U$) Does Not (Avril 2009)

In Figure 7.4, TNF-α expression was measured in the supernatant of DCs cultured overnight with the three TLR agonists. Data represent mean ±SEM from three different healthy donors.

Ampligen comprises a single polypurine (inosine) strand and a single polypyrimidine (cytosine and uridine) strand. These are assembled into a double-stranded RNA structure that is maintained under physiological conditions by typical "Watson-Crick" hydrogen bonding between purine and pyrimidine base pairs. The introduction of the base uridine at a 1:12 ratio (U:C) into the polypyrimidine strand maintains the overall double-stranded structure, but creates sites of thermodynamic instability that allow rapid hydrolysis of Ampligen (T½ ≈ 35-40 minutes) by serum nucleases to simple nucleosides (natural substances that are always found in living cells) and are used by cells to produce normal nucleic acids.

It is a specific TLR-3 agonist, being a double stranded RNA molecule, so it is recognized by the endosomal pathogen-associated molecular pattern receptor (Toll receptor type 3) in the endosomal compartment of antigen processing cells which results in receptor activation and signal transduction via the NFKb pathway. Secondary cytokine release and up and down regulation of host immune response pathways, including type I interferon induction occurs locally and systemically. Ampligen functions via the MYD88 independent pathway utilizing the TRIF adaptor molecule. As such Ampligen is a primary activator of a component of the innate response.

Ampligen has not been shown, however, to engage cytoplasmic dsRNA sensors and is thus differentiated from the activity of poly IC which acts both through TLR-3 and the intracytoplasmic receptor MDA5. This may account for the differential toxicity, and reflect the more rapid intracellular metabolism of Ampligen.

Ampligen entered development as an interferon inducer before the Toll-like receptor family had been elucidated. Type I interferon induction is now established to be the result of TLR-3 activation in the endoplasmic reticulum of TLR-3 expressing antigen presenting cells. Ampligen was developed as a less toxic analog of poly IC.

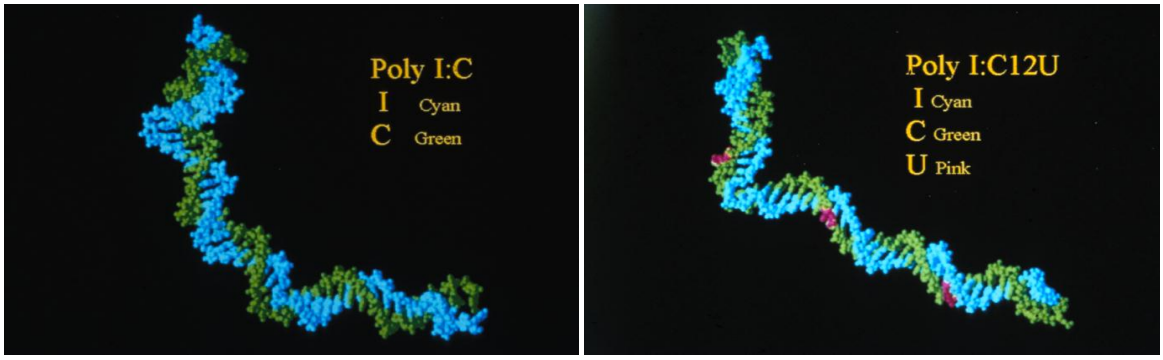


Figure 7.5: Molecular Models of Ampligen and Poly IC

Poly IC has activity mediated not only by TLR-3 but also MDA5, an intracytoplasmic dsRNA detector, however Ampligen does not have activity mediated by MDA5 (Trumpfheller 2008). TLR-3 knock-outs do not respond to Ampligen whereas poly IC maintains activity in the TLR-3 knock out model (Gowen 2007). Finally TLR-3 specific activation is mediated by the TRIF adaptor and not MyD88, thus Ampligen is an MyD88 independent gene activator (Bagchi 2007). Other work suggests that Ampligen lacks direct intracytoplasmic activity unlike other TLR-3 agonists and may thus be unlikely to generate toxicities associated with intracytoplasmic effects, a finding consistent with the acute and chronic safety record established following the administration of 90,000 doses during clinical trials in humans. The structure of Ampligen assures a steady metabolism of the product to inactive short RNA fragments and nucleotide precursors through the action of endogenous ribonucleases. The distribution of bioactive Ampligen dsRNA fragments is continuously diminished upon infusion and distribution of the product, and endosomal TLR signaling elicited with infusion is thus pulsatile in nature, in contrast to other TLR agonists. The serum concentration associated with 200 to 400 mg infusions is consistent with cell culture concentrations that are optimal in various *in vitro* models of biological activity.

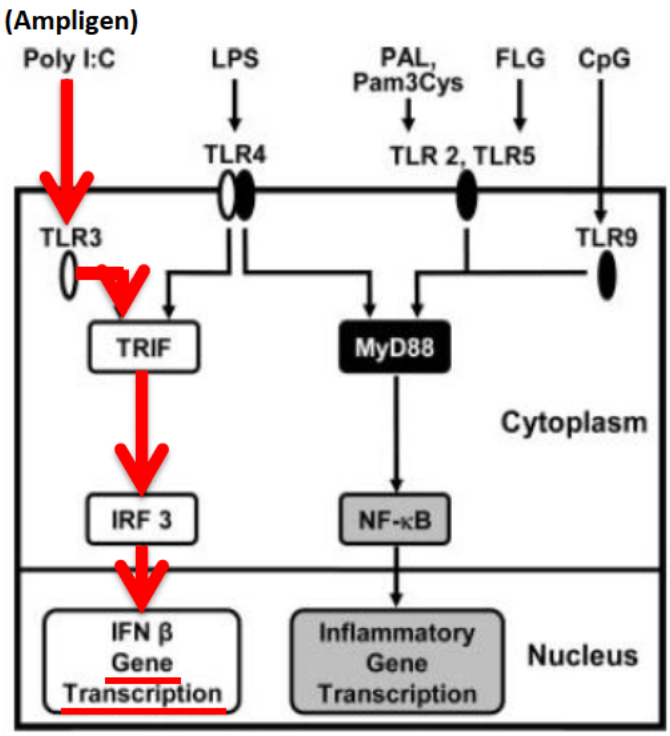


Figure 7.6: Mechanism of Action of Ampligen as a TLR-3 Agonist is Shared in Part with TLR-4 Agonists

Hemispherx instituted a long-term, active program in conjunction with the FDA, of conducting a comprehensive autoimmune profile (anti-actin antibody, ANA, anti-dsDNA antibody, anti-LKM-1 antibody, rheumatoid factor (RF), anti-thyroid peroxidase and anti-thyroglobulin antibody, and anti-islet cell) on subjects enrolled in its ongoing Treatment Protocol (AMP-511).

In particular, we have determined autoantibody levels in 25 AMP-511 subjects who had received Ampligen for one year or longer, including 14 of the subjects who have been on study for 4 years or longer. In fact, the study showed that a greater number of patients' autoantibody tests converted from an initial antibody positive test result to a negative test (n=10) as compared to an initial antibody negative test result, which thereafter converted to a positive test (n=2) and thus indicates no evidence that Ampligen is inducing autoimmune disease.

Table 7.14: Number of Subjects by Total Ampligen Exposure Time in the Autoimmune Signal Analysis Program in AMP-511

	>12 Months	>24 Months	>36 Months	>48 Months
No. of Subjects	25	20	17	14

Long-term data available in these 25 AMP-511 subjects who received Ampligen for one year or longer, including 14 of the subjects who had been on study for 4 years or longer is shown in Table 7.15.

Table 7.15: Longitudinal Changes in Autoantibodies in Subjects who received Ampligen for one year or longer (n=25) in AMP-511					
Autoantibody Test	**Autoantibody Test Results (Number of tests)***				
	Initial Result ↓ Last Result	**Negative ↓ Negative**	**Negative ↓ Positive**	**Positive ↓ Negative**	**Positive ↓ Positive**
Anti-Actin (Smooth Muscle)		22	0	1	2
ANA Screen		22	0	3	0
Anti-dsDNA		23	1	1	0
Anti-Liver/Kidney Microsomal		25	0	0	0
Rheumatoid Factor		23	0	1	1
Anti-Thyroid Peroxidase		18	1	1	5
Anti-Thyroglobulin		19	0	1	5
Islet Cell Antibodies		23	0	2	0
Totals		175	2	10	13

* Comparing initial value to last value. Data collected over a 1 year period during which the 25 subjects were tested 8 times for a total of 200 tests.

Table 7.16 also shows that Ampligen does not cause the development of autoantibodies. In the controlled study AMP-516, no development of anti-dsDNA or Rheumatoid Factor antibodies was seen for the Ampligen cohort vs. 1 placebo patient (3.7%) who became positive for RF.

In patients in AMP-511 who received Ampligen for less than one year, there was one patient who was anti-dsDNA borderline positive once at Day 35 with a reading of 31 where less than 30 was a negative reading. Repeat testing of this patient (125) was negative for anti-dsDNA at Days 171 and 343 despite continued Ampligen dosing.

Table 7.16: Assessment of Autoantibodies in Patients in Controlled Study AMP-516		
	Ampligen	**Placebo**
Number patients treated	162	164
Number of patients with samples for autoantibody assessment	Anti-dsDNA n=35	Anti-dsDNA n=29
	RF n=35	RF n=27[1]
Mean duration of therapy (weeks) at time of antibody assessment	Anti-dsDNA 31.5	Anti-dsDNA 32.0
	RF 31.5	RF 32.3
Number of patients with Baseline negative autoantibodies who developed positive autoantibodies at any point during follow-up, n (%)	Anti-dsDNA 0 / 35 0%	Anti-dsDNA 0 / 29 0%
	RF 0 / 35 0%	RF 1 / 27 3.7%

[1] Two placebo patients had a positive Rheumatoid Factor (RF) at Baseline

As noted above, both AMP-502 and AMP-516 show credible evidence of Ampligen's clinical effect to decrease the use of concomitant medications to treat symptoms of CFS. A statistically significant smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use for relief from the symptoms of CFS, compared with the percentage of subjects in the placebo group (44.7%) (p=0.023). In AMP-516, the proportion of subjects who decreased their use of concomitant medications was significantly greater for subjects who took at least one concomitant medication and received Ampligen (68%) versus placebo (59%) (p=0.0145).

7.2.13 Use of Medications Including Those Which Prolong the QT Interval

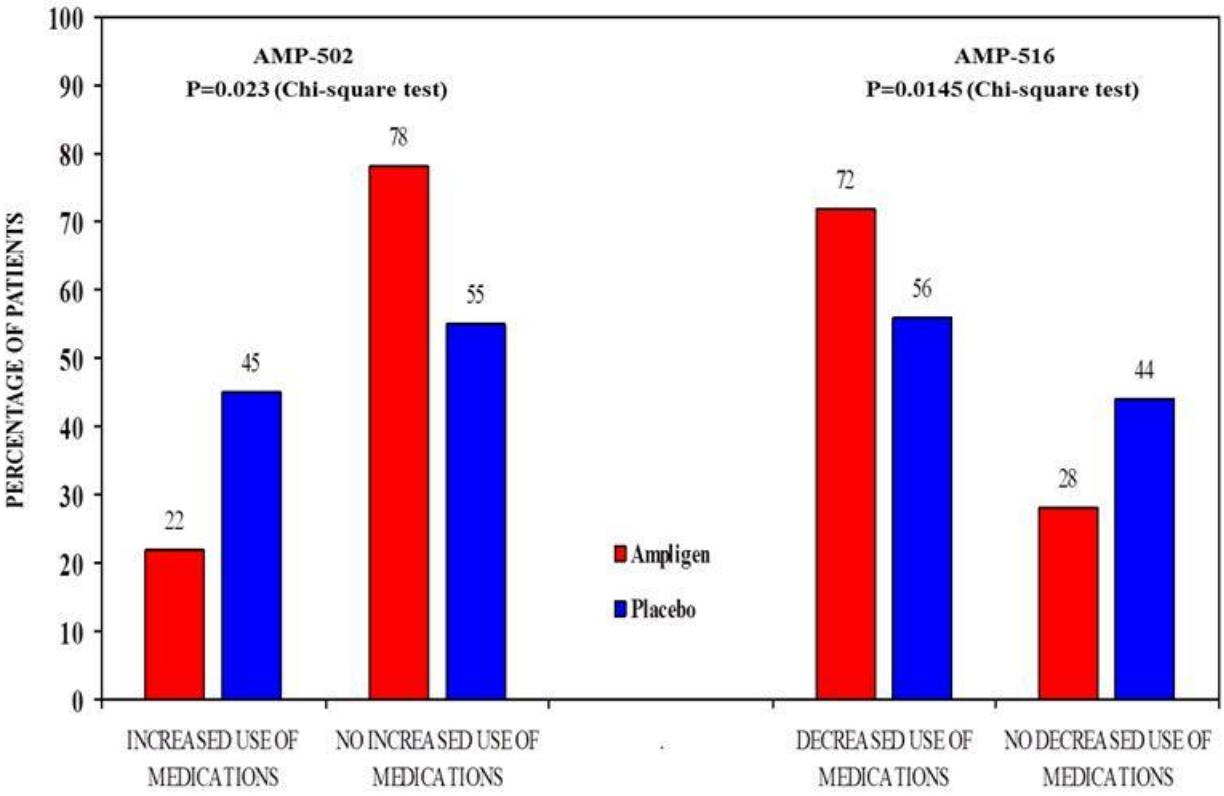


Figure 7.7: Change in Concomitant Medications Used to Palliate Symptoms of CFS

The reduction in the use of concomitant medications used to palliate the symptoms of CFS shown in Figure 7.7 is discussed in Section 6.3. These concomitant medications also include medications which prolong the QT interval (Stouch 2010, and Table 5.11). By reducing the need for these QT prolonging medications, Ampligen may help to decrease a parameter that is known to be related to catastrophic cardiac events, especially in middle-age females with CFS (Jason 2006) who otherwise would not be expected to be at risk for catastrophic cardiac events.

In AMP-516, 72% of the patients randomized to receive Ampligen who were taking concomitant medications experienced a reduction in exposure to concomitant medications including those with a known risk of prolonging QT, compared to 56% of the patients randomized to receive placebo. Comparing the proportion of patients between randomized treatment assignment and decreased exposure revealed a significant difference in favor of Ampligen (p=0.0145). Similar results were seen in the well-controlled study AMP-502 (Figure 7.6). **Patients randomized to receive Ampligen were one and three-quarters times more likely to have reduced exposure to medications which include those known to prolong QT, compared to placebo** (Odds ratio: 1.76 [95% CI: 1.00 to 3.11]).

Two aspects of the QT prolongation issue have been evaluated. First, is Ampligen associated with prolongation of the QT interval and, secondly, does the use of Ampligen in this patient population potentially benefit those patients with a resultant decrease in the use of those medications and/or an improvement in the QT interval.

In AMP-516, the safety population evaluated for QT prolongation consisted of 190 subjects (91 received Ampligen and 99 placebo) with a Baseline and post-Baseline QT interval determination performed under standardized conditions. The same EKG analysis equipment was utilized for all subjects at all study sites throughout the study by an experienced team headed by an exercise physiologist. This population has been labeled the *Safety Population Evaluable for QT Interval Prolongation* for summarization and analysis. The difference between the 234 enrolled subjects and the ITT Population (n=208) consisted of 26 subjects who discontinued from the study before Week 20 and did not have a post-Baseline ETT or EKG performed and accordingly they were not included in the analysis of the QT data.

With regard to the first aspect - is Ampligen associated with prolongation of the QT interval - the EKG database from AMP-516 consisted of 12-lead surface EKGs obtained during Baseline and at Weeks 20, 34, and 40 during the AMP-516 study. The EKG evaluations obtained at Baseline were averaged and compared to the maximum QT interval recorded at Weeks 20, 34, or 40. Maximum on-study QT and QTc intervals (QTcB and QTcF) were determined for each subject and then categorized as being abnormal using the cutoff points specified in the E14 Guidance Document, QT/QTc intervals > 500, > 480, and > 450 msec, and QT/ QTc increases from Baseline of > 60 and > 30 msec.

A summary of these results are presented below. Table 7.17 shows that there is no difference in number of subjects with abnormal maximum post-Baseline QTc (Bazett) interval parameters in Ampligen vs. placebo. Table 7.18 shows there is no difference in the number of subjects with abnormal maximum post-Baseline QTc (Fridericia) interval parameters in Ampligen cohort vs. placebo.

Table 7.17: AMP-516 Safety Population Evaluable for QT Interval Prolongation: No Difference in Number of Subjects with Abnormal Maximum Post-Baseline QTc (Bazett) Interval Parameters in Ampligen Cohort vs. Placebo

QTc (Bazett) Interval	Treatment	Number of Subjects with Abnormal QT Parameter	Fisher's Exact p-value
>500 msec	Ampligen	0	--
	Placebo	1	
>480 msec	Ampligen	1	1.000
	Placebo	1	
>450 msec	Ampligen	6	0.317
	Placebo	11	
>30 msec increase over Baseline	Ampligen	12	0.327
	Placebo	19	
>60 msec increase over Baseline	Ampligen	1	0.622
	Placebo	3	

Table 7.18: AMP-516 Safety Population Evaluable for QT Interval Prolongation: No Difference in Number of Subjects with Abnormal Maximum Post-Baseline QTc (Fridericia) Interval Parameters in Ampligen cohort vs. Placebo			
QTc (Fridericia) Interval	**Treatment**	**Number of Subjects with Abnormal QT Parameter**	**Fisher's Exact p-value**
>500 msec	Ampligen	0	--
	Placebo	0	
>480 msec	Ampligen	0	--
	Placebo	1	
>450 msec	Ampligen	1	0.371
	Placebo	4	
>30 msec increase over Baseline	Ampligen	10	0.520
	Placebo	15	
>60 msec increase over Baseline	Ampligen	0	--
	Placebo	3	

QT prolongation was also examined using the data from AMP-516. Results were examined based on the post-Baseline values, using the actual recorded QT value, and the corrected values using Bazett's ($QT_c=QT/RR^{1/2}$) and Fridericia's ($QT_c=QT/RR^{1/3}$) formula. Figure 7.8 contains the post-Baseline results for the QT interval, observed and corrected. The patients randomized to receive placebo had a significantly longer QT interval, compared to patients randomized to receive Ampligen (p=0.049). Although trending in the same direction, the corrected QT intervals were not significantly different at the alpha = 0.05 between patients randomized to receive placebo, and patients randomized to receive Ampligen. Based on the foregoing, there is no evidence of an increased risk of proarrhythmic potential in patients randomized to receive Ampligen.

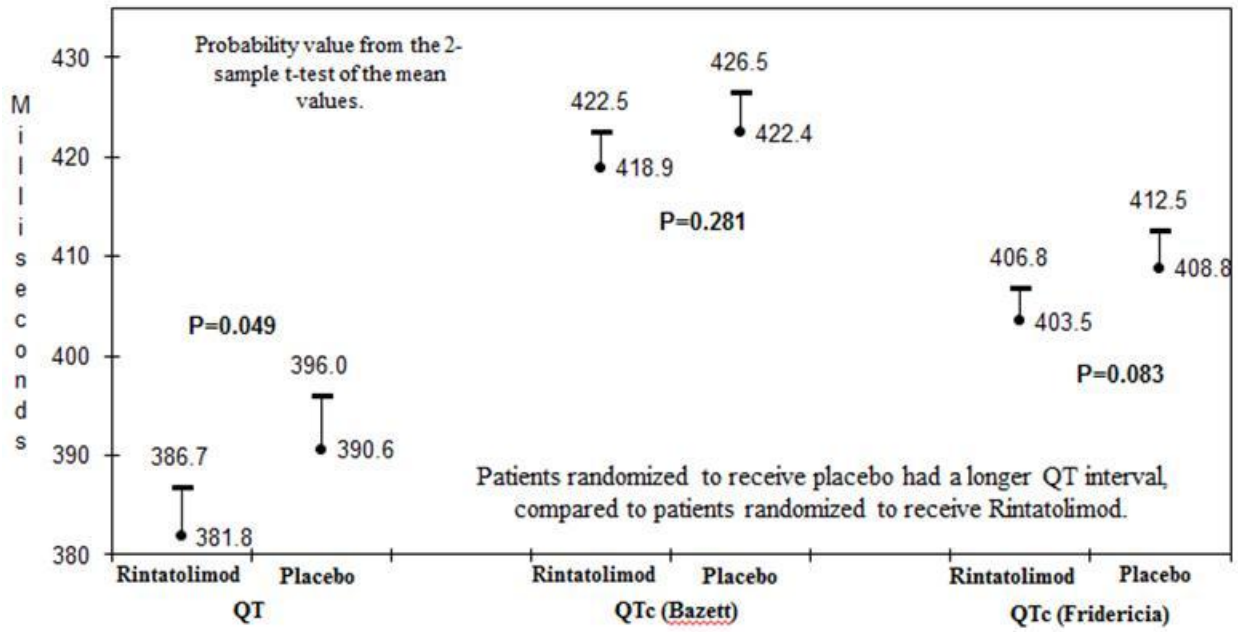


Figure 7.8: No Evidence of QT Prolongation Through Examination of the Upper Sided 95% Confidence Limits Based on the Mean QT (milliseconds) of the Post-Baseline Values by Randomized Treatment Assignment

Concomitant drug use and the potential relationship with cardiac arrhythmia in the female CFS population between the ages of 35 and 50 years is a new hypothesis for early cardiac life threatening events and will obviously require much larger prospective studies in the future. The article by Jason et al. is summarized below in Table 7.19. The QT prolonging interval pattern observed in the placebo CFS group may also be contributed to by concomitant medications not formally classified as such at present, but this presence contributes to QT prolongation in the poly-pharmacy environment that is utilized by CFS patients in an unsuccessful effort to mitigate severe symptomatology.

Table 7.19: Medical Significance of CFS: Principal Causes of Increased Mortality in CFS Patients	
Major Causes of Death in CFS	**Established/Potential Ampligen Mitigation Mechanisms**
Heart Failure[1] (Jason 2006)	Ampligen treatment (in a well-controlled trial), a) reduces use of concomitant drugs including medications which prolong the QT interval, as well as, b) reducing the QT interval as a prognosticator of increased risk for cardiac arrhythmia (AMP-516 and AMP-502) (Stouch 2010).

[1] Heart failure is associated with a prolonged QT interval

7.2.14 Incidence of Cancer are Not Increase by Ampligen Exposure

As shown in Table 7.20, the overall incidence of cancer in the controlled and uncontrolled studies per 100 PTYs on study drug is lower for Ampligen (0.401) compared to placebo (0.880). This is consistent with Ampligen's demonstrated anti-proliferative activity (Hubbell 1985, Hubbell 1987, Hubbell 1990, Strayer 1986).

Table 7.20: Incidence and Frequency of Cancer in the Ampligen Program Through the Last Safety Cutoff

Category	Controlled Portions of Pivotal Studies		Controlled and Uncontrolled Studies	
	Studies 502 and 516		CFS Studies[1]	
	Ampligen	Placebo	Ampligen	Placebo
Number of Patients Treated	162	164	737	174
Mean Patient-Years of Follow-Up on Study Drug	0.63	0.66	0.68	0.65
Total Patient-Years of Follow-Up on Study Drug	101.6	109.0	498.6	113.7
Mean Number of IV Infusions	64.1	67.4	59.8	67.0
Total Number of IV Infusions	10389	11051	44054	11656
Total number of patients with cancer (%)	1 (0.6%)	1 (0.6%)	2 (0.3%)	1 (0.6%)
Total number of occurrences of cancer	1	1	2	1
Incidence of cancer per 100 patient-years on study drug	0.984	0.917	0.401	0.880

[1] Studies 501, 502, 502T, 502E, 504, 509, 511 (at last safety cut-off), 516, and 516C/E

7.2.15 Dose Response

An initial study in 16 patients with CFS (Study AMP-501) did not find significant differences in laboratory parameters among patients treated with Ampligen 200 mg, 300 mg or 400 mg twice weekly. The 400 mg twice-weekly schedule became established as the biologically effective dose based on two studies in patients with CFS (Study AMP-502T and Study AMP-516) and in one study in patients with HIV/AIDS (Study AMP-700). In AMP-700 patients received doses of up to 700 mg twice weekly.

These studies established that a dosage of 400 mg of Ampligen infused twice weekly following four initial doses of 200 mg given twice weekly over 2 weeks consistently triggered biological activity in the relative absence of potential adverse events such as acute flu-like symptoms or flushing reactions.

7.3 PRECLINICAL SAFETY STUDIES

As discussed in other sections, Ampligen operates via a TLR-3 cell surface receptor. This receptor is fundamentally different in lower animals (primary structure, expression, tissue distribution, etc.) thus making transitions from animal toxicology data to man somewhat problematic. In vivo nonclinical toxicity studies were conducted in rat, rabbit, dog, and monkey. The rabbit is most vulnerable to Ampligen-induced toxicity, followed by the dog and rat, with primates exhibiting little toxicity. Most studies were conducted in rat and monkey.

Since the filing of NDA #22-151, various additional preclinical studies have been conducted. These can be grouped as follows and are listed in Table 7.21.

- Two additional studies of repeat dose toxicity of Ampligen in rats and monkeys, including pharmacokinetics and cytokine with dsRNA antibody profiles
- Two additional developmental toxicity studies of Ampligen in the rabbit
- One additional pharmacodynamic study of Ampligen in rats and monkeys
- Two metabolic studies examining cytochrome P450 induction and inhibitory potential in fresh human hepatocytes and human hepatic/microsomal preparations
- One additional study of the inhibitory potential of Ampligen towards P-glycoprotein and BCRP using Caco 2 cell monolayers
- One study of the hemolytic potential of Ampligen and plasma compatibility

More detail on these studies is shown below:

Table 7.21:	**Non-Clinical Studies Evaluating Ampligen Conducted Since Filing NDA #22-151**		
09-5-11	Pharmacodynamics	Rat and Monkey	Ampligen (Poly I : Poly $C_{12}U$): Cytokine Sera Levels Obtained Pre-Infusion and 4, 24 and 72 Hours Post-Infusion in Rats and Non-Human Primates
FY07-062B	Repeat-Dose Toxicity	Monkey	Poly I : Poly $C_{12}U$ (Ampligen) Sixteen Week Repeat Dose Intravenous Pharmacokinetic Study and Cytokine and dsRNA Antibody Profiles in Cynomolgus Macaques
FY07-063B	Repeat-Dose Toxicity	Rat	Poly I : Poly $C_{12}U$ (Ampligen): Eight Week Repeat Intravenous Pharmacokinetic Study and Cytokine and ds RNA Antibody Profiles in Male and Female Sprague-Dawley Rats
ESL00001	Toxicity	Rabbits	Intravenous (Infusion) Dosage-Range Developmental Toxicity Study of Ampligen® (Poly I : Poly $C_{12}U$) in Rabbits
ESL0002	Toxicity	Rabbits	Intravenous (Infusion) Developmental Toxicity Study of Ampligen® (Poly I: Poly $C_{12}U$) in Rabbits
8268185	Hemolytic	Human	Hemolytic Potential and Plasma Compatibility Testing with Ampligen
8268420	Metabolic	Human	Determination of the Inhibitory Potential of Ampligen towards P-glycoprotein and BCRP using Caco 2 Cell Monolayers
8268421	Metabolic	Human	Inhibitory Potential of Ampligen Towards Human Hepatic Microsomal Cytochrome P450Isoenzymes
8268422	Metabolic	Human	Evaluation of Cytochrome P450 Induction Following Exposure of Ampligen to Primary Cultures of Human Heptocytes

8. BENEFIT RISK ASSESSMENT

8.1 BENEFIT

Ampligen has a favorable risk benefit/risk profile for the treatment of CFS, a serious and life-threatening indication with an unmet medical need. Ampligen also displays a set of therapeutic advantages over symptom directed therapy. At present there are no FDA-approved treatments specifically targeting the treatment of CFS.

Patients with severely debilitating CFS suffer for years, both physically and cognitively with little to no quality of life. The impact is felt by their families and our nation as a medical/social consequence. The current standard of care (SOC) is palliative and provides little to no permanent relief. In many instances these SOC drugs create additional safety considerations and health burdens including medications that may prolong QT interval in a population already at risk of pre-mature heart failure. Patients also die prematurely of organ failure, cancer and suicide, a result of the life-threatening complications of current patient management. Ampligen has been the only drug to date to demonstrate that it provides direct improvement to patients with CFS, both physically and cognitively, significantly advancing their quality of life and it has shown to be well-tolerated by a severely sick population.

The proposed indication for Ampligen is for the treatment of chronic fatigue syndrome (CFS) as the disease has been defined by the Centers of Disease Control, the Agency with relevant statutory authority for examining the incidence of the disorder in the US population.

CFS occurs most often in persons 40-59 years of age and is seen 3 times more frequently in women. Productivity among people with CFS is estimated to decline by 37% in the household and by 54% in the labor force. **The CDC has published extensively and identified CFS as an economically and emotionally devastating illness, which can exceed the morbidity associated with multiple sclerosis, heart disease and other chronic illness.** There is no specific standard of care for CFS and patients utilize a large number of drugs ("off label") in an attempt to palliate the debilitating symptoms of the illness. **Ironically, the use of these drugs in various combinations may lead to early death.**

- 1200 patients have been exposed to Ampligen across all indications
- >800 patients with CFS
- >90,000 doses of Ampligen administered
- >600 patient years of drug exposure
- >200 patients have received Ampligen for at least one year; and there is no indication of any AE which appears after long term Ampligen administration (i.e., >1 year), which would limit a CFS patient's ability to continue on Ampligen treatment

Table 8.1: Summary of Key Results Relating to Efficacy and Clinical Benefit in Subjects Randomized to Ampligen Versus Placebo in the Phase 3 Study AMP-516 (n=234)	
Efficacy Endpoints	**Key Secondary Endpoints**
Primary Endpoints: Change from Baseline in Exercise Tolerance Test duration at Week 40: • Ampligen improved placebo-adjusted intra-patient ETT by 21.3% and intra-group ETT by 11.8% (p=0.047) in ITT Subjects • Medically significant improvement over pre-defined intra-group placebo-adjusted benchmark: 11.8% (Ampligen) vs. 6.5% (benchmark) in ITT Subjects • Medically significant improvement over pre-declared intra-group placebo-adjusted benchmark: 14.3% vs. 6.5% in Subjects without significant dose reductions (pre-declared subset) • Proportion of patients with ETT improvement of at least 25%: Ampligen (39.0%) vs. placebo (23.1%) (p=0.013) • Correction for subjects with reduced dosing compliance increased placebo-adjusted mean intra-patient ETT improvement to 28% (p=0.022)	• Patients with ETT improvement of at least 25% also had a medically significant improvement in KPS of 10 points (p=0.039) • Patients with ETT improvement of at least 25% also had a medically significant improvement in Vitality Scores (SF-36) of 14.6 points (p=0.008) • ITT Population who took concomitant medications: 72% of Ampligen subjects decreased use of concomitant medications vs. 56% who received placebo (p=0.0145)
Clinical Benefits (QT Interval) Placebo subjects had a significantly longer QT interval, compared to Ampligen subjects (p=0.049)	**Cross-over Study (AMP-516C/E)** Placebo subjects crossed-over to receive Ampligen demonstrated an intra-patient improvement in ET performance at 24 weeks of 39% (p=0.04)

Table 8.2: Summary of Key Results Relating to Efficacy and Clinical Benefit in Subjects Randomized to Ampligen Versus Placebo in the Phase 2 Study AMP-502 (n=92)	
Efficacy Endpoints	**Key Secondary Endpoints**
Primary Endpoint: Increase in KPS at 24 Weeks • Ten (10) point median change in KPS scores in Ampligen-treated patients over placebo (p=0.016) • Increase in median KPS from Baseline of 50 to 60 at Week 24 in Ampligen-treated patients versus placebo with no change (p=0.014)	• Mean ETT duration increased by 95.3 seconds in the Ampligen group versus 57.9 seconds in the placebo group (p=0.010) • In AMP-502, a statistically significant smaller percentage of subjects in the Ampligen group (22.2%) increased their medication use for relief from the symptoms of CFS, compared with the percentage of subjects in the placebo group (44.7%) (p=0.023)
Clinical Benefits Analyses of the distribution of median KPS scores at Week 24 also demonstrated statistically significant improvement at Weeks 16, 20 and 24 (p=0.015, p=0.013 and p=0.015, respectively) in the Ampligen group compared to placebo **Key QT Medication Use** • Placebo subjects increased concomitant medications including those with a known risk of prolonging QT interval compared to Ampligen subjects thereby decreasing their risk for cardiac events	**Clinical Benefits (cont'd)** • Incidence of depression with or without suicide attempt and/or suicide ideation occurred 3 times more often in the placebo group vs. Ampligen (p<0.02) • Suicide attempt/suicide ideation occurred 4 times more often in the placebo group (4) than with Ampligen treatment (1) (difference not statistically significant) • Less hospitalization for depression in the Ampligen group with 0 occurrences vs. 5 in the placebo group (p=0.06) • Percentage of hospitalization for depression out of all hospitalizations was 0% for Ampligen vs. 45% for placebo

Over the years and throughout the extensive clinical program, Ampligen patients continued to respond and the drug was generally well-tolerated. Ampligen has clearly demonstrated clinical efficacy and a consistent safety profile. A clinically meaningful increases in ETT was observed following Ampligen treatment in 2 multi-centered, randomized, double-blind, placebo-controlled trials. A reduction in exposure to concomitant medications typically used to treat the symptoms associated with CFS, many of which are known to have a detrimental impact on QT interval prolongation and therefore may contribute to sudden death. The Karnofsky Performance Scale improved significantly relative to Baseline and relative to the placebo group. Quality of Life measures (SF-36) improved in parallel with ETT outcomes especially in the subgroups of patients which lead a $\geq$ 25% improvement in ETT. "Cognition" improved in patients in the AMP-502 and AMP-509 study, including improvement in their ability to remember things, less frequent episodes of one's mind "going blank", and other improvements. **A greater number of patients significantly improved medically on Ampligen and fewer patients became worse, which may be the natural course of the chronic disease. Ampligen treatment therefore alters the expected downhill trajectory of untreated debilitating, life threatening CFS.** Patients on Ampligen showed a reduction in hospitalizations including hospitalizations associated with suicide ideation when compared to the placebo group. Hospitalizations, emergency room admission and/ or inpatient admissions were reduced in patients with CFS receiving Ampligen compared to placebo. Prolonged Ampligen treatment showed no propensity to auto-immunity and indeed patients on Ampligen show a trend to decrease in preexisting auto-antibodies, a potential early step in forming autoimmune disease. The risks associated with Ampligen are minimal and well-documented. Ampligen treated patient show no increase in the incidence of cancer.

AMP-516 findings are confirmed by the supportive multi-center, randomized, double-blind, placebo-controlled AMP-502 study where the following observations were made:

1. 57.8% of Ampligen-treated patients experienced similar reduced exposure to concomitant medications compared to only 31.9% in the placebo group.
2. When comparing the proportion of patients between randomized treatment assignment and decreased concomitant medication exposure, a significant difference was revealed in favor of Ampligen ($p=0.013$).
3. Ampligen-treated patients were approximately 3 times more likely to have reduced exposure to concomitant medications including those known to prolong QT, compared to patients randomized to receive placebo (Odds ratio: 2.92 [95% CI: 1.25 to 6.84]).

Increased stamina and tolerance to exercise are important factors in reducing the cardiovascular risk associated with CFS. In both AMP-502 and AMP-516, Ampligen resulted in a statistically significant ($p<0.05$) increase in mobility and stamina (exercise tolerance). Reducing the sedentary influence of CFS through increased stamina coupled with the reduced dependence on medications that can prolong QT, is an obviously important step in the successful management of this chronic disease. **The analysis of the intra-patient QT interval data from AMP-516, where over 1,000 EKGs were recorded during the course of the study revealed evidence of an increased risk of pro-arrhythmic potential in the placebo group, compared to the Ampligen-treated group.**

In the past decade, the single most common cause of the withdrawl or restriction of the use of drugs that have been marketed has been the prolongation of the QT interval associated with polymorphic ventricular tachycardia, or torsade de pointes (TdP) which can be fatal. Clinicians (often non-cardiologists including CFS treatment specialists) will be increasingly faced with both older and newly approved drugs with labeling that mentions the potential to prolong the QT interval and thus to cause *torsade de pointes* (Roden 2004).

Ampligen treatment allowed CFS subjects to reduce their dependence on concomitant medications used to treat the debilitating symptoms of CFS with a corresponding reduction in the exposure to drugs known to prolong the QT interval. Accordingly, with further clinical experience post marketing, this approach may provide a therapeutic strategy to mitigate the incidence of heart failure/sudden death in this patient population by reducing their therapeutic dependency on palliative medications associated with increased risk of TdP associated sudden death.

Ampligen AMP-516 and AMP-502 both met their primary and secondary key endpoints. Ampligen has clearly demonstrated clinical efficacy and a consistent safety profile.

Reduction in Depression, Suicide Ideation and Suicide Attempt by Ampligen Treatment (AMP-502)

CFS increases a person's risk for premature death from suicide as well as heart failure, cancer and other complications (Jason 2006). In all the controlled clinical studies with Ampligen, none revealed any increased risk for suicide over placebo. Of the two pivotal studies, the more severely debilitated were those in AMP-502. Accordingly, suicide ideation and suicide attempt were more likely to occur. In fact, incidence of depression as an adverse event was associated with suicide attempt and/or suicide ideation and occurred three times more often in the placebo group vs. the Ampligen cohort ($p<0.02$). Suicide attempt/suicide ideation occurred four times more often in the placebo group than with Ampligen treatment. There was a trend towards less hospitalization for depression in the Ampligen group with no occurrences in the Ampligen cohort vs. five in the placebo group ($p=0.06$). The percentage of hospitalization for depression out of all hospitalizations was 0% for Ampligen vs. 45% for the placebo cohort.

Table 8.3: Hospitalization for Depression by Incidence in AMP-502

Parameter Related to Depression and Suicide Attempt/Ideation	Incidence of Event		Fisher's Exact Test p-value (2-tail)
	Ampligen	Placebo	
AE, Depression[1]	6	18	0.02
Hospitalization for Depression[2]	0	5	0.06
AE, Suicide Attempt / Suicide Ideation[3]	1	4	0.37

[1] Depression includes depression with or without suicide attempt/ideation.
[2] Hospitalization includes hospitalization for depression.
[3] Suicide attempt and suicide ideation include with or without depression.

Table 8.4: Health Care Utilization in AMP-502					
Utilization Parameter	**Ampligen n=45**		**Placebo n=47**		**p-value[1]**
	Total Number	**Mean**	**Total Number**	**Mean**	
Emergency Room Visit	4	0.09	13	0.28	ns
Inpatient Hospital Days	15	0.33	101	2.15	p<0.05
Number of Hospitalizations	3	0.07	11	0.23	ns

[1] Independent Sample t-test (natural log)

Table 8.5: Relative Incidence of Hospitalizations[1] in CFS Patients Receiving Ampligen Versus Placebo in AMP-502				
		Ampligen	**Placebo**	**Mann-Whitney**
Number of Admissions per Hospitalized Patient	Mean	1.0	3.4	p<0.005
	Median	1.0	3.0	
Number of Hospital Days per Patient	Mean	2.7	16.3	p<0.005
	Median	1.0	18.0	

[1]Hospitalization was defined as either an emergency room admission or an admission to the inpatient service

Ampligen sterile solution for intravenous infusion is indicated for treatment of subjects with CFS. In clinical studies, Ampligen improved functional capacity and other clinical endpoints measuring severity of CFS symptoms.

Ampligen, as a CFS therapeutic, can lessen the patient's exposure to these symptomatic treatments, hence their potential negative side effects. Thus, Ampligen provide a significant safety margin in light of the well-documented life threatening events in this CFS population: (a) cardiac death, b) suicides, c) fatal cancers).

Table 8.6: Relative Therapeutic Advantage: Ampligen vs. Current Standard of Care in Distinct Therapeutic Categories		
	Ampligen Advantage	**Standard of Care Advantage**
Core Fatigue Abatement	Ampligen	None
Improve Activity Performance (≥25% ETT)	Ampligen	None
Global QOL Improvement	Ampligen	None
Polypharmacy abatement	Ampligen	None
EKG Selected Risk(s) Abatement	Ampligen	None
Reduced CFS Disease Progression	Ampligen	None
Lower Incidence of Suicide	Ampligen	None
Lower Incidence of Hospitalizations	Ampligen	None
Macroeconomic (e.g., Hospitalizations, Polypharmacy, Emergency Room Visits) Cost Reduction	Ampligen	None
Probability of Reducing Physician-Based Iatrogenic Error	Ampligen	None

Common Adverse Reactions

Adverse reactions observed in controlled clinical studies that occurred in 5% or more subjects and at a rate twice that of placebo were chills, palpitations, tinnitus, and sweating. These adverse events were transitory, mild/moderate and likely due to endogenous interferon production.

Flu-like reactions

Transient flu-like events (including fatigue, low-grade fever, headache, pharyngitis, arthralgia and myalgia, and nausea) occurred in more Ampligen-treated subjects than in placebo subjects during the initial (first 10) doses in controlled studies most likely were secondary to interferon production. Occasionally, such reactions were accompanied by tightness in the chest and shortness of breath. These reactions were readily controlled by reducing the rate and/or the dose of the initial infusions.

Adverse Reactions Leading to Discontinuation

In controlled studies, approximately 1% of subjects receiving Ampligen and 14% of placebo subjects discontinued prematurely because of lack of response; a roughly comparable rate of withdrawal for adverse events occurred in both groups (more than 1% and less than 2%).

There is no evidence of autoimmunity and/or nascent autoimmune signals related to Ampligen therapy as described in the earlier safety section.

There is no observable difference in the incidence of new medical conditions indicative of potential new onset autoimmune disease. It is important to note that not all TLR's are endogenous inducers of interferon, and some, acting primarily by completely different mechanisms of action than TLR-3, may incite autoimmune disorders in other groups of patients.

Table 8.7: Summary of Key Cardiac Toxicity Results – Studies AMP-516 and AMP-502; Evidencing a Consistent Protective Advantage of Ampligen Over Placebo	
Study	**Result**
AMP-516	A ≥5 ms post-treatment mean increase in QT prolongation was observed in the placebo group, while a <5 ms mean post-treatment increase in QT interval prolongation was observed in Ampligen-treated patients
	Based on the lowest threshold value (>450 msec), over 8% of the patients in the QTc analysis had an average Baseline QTc value of >450 msec (95% CI: 4.9% to 13.3%)[1]
	Patients randomized to receive placebo had a significantly longer QT interval, compared to patients randomized to receive Ampligen (p=0.049)
	Within the placebo group, the change from Baseline in QT interval was significantly different than zero (p<0.05) for placebo subjects who were exposed to 1 or more concomitant medications known to prolong the QT interval
	68.0% of Ampligen-treated patients experienced a reduction in exposure to concomitant medications with a known risk of prolonging QT interval compared to 54.6% in the placebo group. Comparing the proportion of patients between randomized treatment assignment and decreased exposure revealed a significant difference in favor of Ampligen (p=0.048)
	Ampligen-treated patients are 1.76 times more likely to have reduced exposure to medications know to prolong QT compared to placebo (Odds ratio: 1.76 [95% CI: 1.00 to 3.11]
AMP-502	57.8% of Ampligen-treated subjects experienced a reduction in exposure to concomitant medications with a known risk of prolonging QT interval compared to 31.9% of placebo subjects
	Comparing the proportion of patients between randomized treatment assignment and decreased exposure revealed a significant difference in favor of Ampligen (p=0.013)
	Patients randomized to receive Ampligen were approximately 3 times more likely to have reduced exposure to medication known to prolong QT compared to placebo (Odds ratio: 2.92 [95% CI: 1.25 to 6.84])
AMP-516 and AMP-502	Ampligen resulted in statistically significant (p<0.05) increase in mobility and stamina (ETT) [2]

[1] This finding suggests that a recognizable population of CFS patients who participated were at increased risk of cardiovascular disease and cardiac-related events.

[2] Increased stamina and tolerance to exercise are important factors in reducing the cardiovascular risk associated with CFS. Reducing the sedentary influence of CFS through increased stamina, coupled with the reduced dependence on medications that can prolong QT is an important step in disease management.

Ampligen has a favorable risk benefit/risk profile for the treatment of CFS, a serious and life-threatening indication with an unmet medical need. Ampligen also displays a set of therapeutic advantages over the present standard of care.

Table 8.8: Risk Benefit Analysis: Summary of Clinical Benefits Offered with Ampligen Treatment Relative to Standard of Care		
	Ampligen	**Current Approaches**
Targets Core Symptom (Fatigue) of CFS[a]	Yes	No
Targets Treatment of Individual CFS Symptoms[b]	Yes (indirectly)	Yes
Improves Exercise Tolerance (ETT) and Activity Level[c]	Yes	No
Improved Quality of Life[d]	Yes	Sporadic
Reduction in Concomitant Medication Usage[e]	Yes	No (Goes in opposite direction)
Reduction in Exposure to Concomitant Medications that Include QT Interval Prolonging Drugs[f]	Yes	No May actually contribute to increase cardiac death rate
Reduces QT Interval[g]	Yes	No
Evidence of Autoimmunity Potential[h]	None in humans	Unknown
Decreases Suicide Incidence Rate (Jason 2006)[I] and *Torsades de Pointes*	Yes	May contribute to increased suicide rate (Especially SSRIs)
Decreases Heart Failure Incidence (Jason 2006)[j]	Presumptive effect due to EKG improvement	No. May actually contribute to increase cardiac death rate
Cancer Incidence (Jason 2006, Levine 1998, Chang 2012)[k]	Should be expected to be reduced based on clinical observations to date	No evidence
Improved Cognition[l]	Yes	No evidence of any effect
Decreases Incidence of Worsening CFS (Breaks Disease Progression)[m]	Yes	No evidence of any effect
Improved Overall Acceptable Safety Given Life-Threatening Disease and Unmet Medical Need[n]	Yes	Has not been assessed in this patient population

[a] Ampligen treatment is directed towards the core symptom of CFS (fatigue) with ancillary secondary benefits
[b] Each medication used in a polypharmacy treatment approach targets a specific CFS symptom
[c] Subjects receiving Ampligen for 40 weeks improved intra-patient placebo-adjusted ET 21.3% (p=0.047) from Baseline in an ITT analysis (Study AMP-516)
[d] Transitory improvement of quality of life may be seen with current medications; however, treatment with Ampligen has a more profound and sustained impact on patient QOL (SF-36 and KPS)
[e] Ampligen treatment allows CFS subjects to reduce their dependence on concomitant medications used to treat debilitating symptoms of CFS
[f] Ampligen treatment allows CFS subjects to reduce their dependence on concomitant medications used to treat debilitating symptoms of CFS, coincidentally reducing exposure to drugs known to prolong the QT interval
[g] A ≥5 ms post-treatment mean increase in QT prolongation was observed in the placebo group, while a <5 ms mean post-treatment increase in QT interval prolongation was observed in Ampligen-treated patients
[h] There is no evidence to date of autoimmune toxicity associated with Ampligen treatment

[i] Ampligen therapy has shown to help patients avoid therapies that are associated with increasing suicidal behaviour by reducing the incidence of major depression, including suicidal ideation, side effects which are typical of several concomitant therapies for CFS symptoms

[j] Advantage realized in Ampligen treated patients that benefit by avoiding concomitant therapy that is known to prolong QT interval

[k] Ampligen may have broad antitumor and/or immunomodulatory activity against various tumors; this is currently under active clinical investigation in US against breast, ovarian, colorectal and others

[l] Cognition improvement exhibited by improvements in patient's memory; reduction in emotional outbursts; and other improvements (Studies AMP-502 and AMP-509)

[m] Fewer Ampligen-treated patients become worse following initiation of therapy

[n] The risks associated with Ampligen are minimal and well-documented and no SAEs have been directly attributable to the drug in the two well-controlled studies. The other drugs have not been well-studied in any CFS population.

Table 8.9: Truncated Summary of Benefit-Risk Assessment Relative to Current Standard of Care: A Qualitative Framework: Analyzing Ampligen for Severely Debilitating , Life Threatening CFS

Consideration	Favorable Benefit-Risk	Unfavorable Benefit Risk
Severity of Condition	Yes	No
Unmet Medical Need	Yes	No
Clinical Benefit	Yes	No
Risk	Yes	No
Risk Management	Patients would be under continuous physician care with treatment requiring infusions 2 times/week	

Excerpted public presentation by Dr. John Jenkins, CDER

CONCLUSION

In summary, CFS is a serious, life threatening and unmet medical need. There are currently no FDA approved products specifically for the treatment of CFS. Ampligen has the potential to be a positive therapeutic option for patients with CFS who do not have an alternative to directly treat the condition. Given the overwhelming physical and cognitive health issues; the decrease in activities of daily living and overall lack of quality of life associated with CFS, Ampligen clearly represents a clinically meaningful advance for a significant unmet medical need, and the weight of evidence supports FDA goals to advance treatment for chronically ill patients with severe debilitating, life threatening, conditions such as severe CFS.

It can be stated that the burden of the symptoms associated with CFS is greater than any risks associated with Ampligen, since a CFS sufferer's life may be at risk for years of debilitation often followed by premature death.

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