A phase III trial of interferon α/thymosin α_1 combination therapy for hepatitis C in the United States

Interim results

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Introduction

Chronic hepatitis C virus (HCV) infection has emerged as a major health epidemic in the United States. It is estimated that more than 3.5 million people are infected in this country and that most are unaware of the infection (1). The natural history of the virus remains to be fully elucidated, but evidence suggests that a significant percentage of cases progress to cirrhosis and subsequent liver failure (2). Hepatitis C has become the leading indication for liver transplantation in the United States and the donor pool appears relatively stagnant in terms of growth (3). For these reasons, the treatment of chronic HCV infection has become critically important.

Interferon was first used to treat patients with chronic non-A, non-B hepatitis in the mid-1980s (4). The development of tests for the specific viral entity responsible for the majority of non-A, non-B hepatitis permitted analysis of even early trials in terms of HCV treatment efficacy (5). Early success in an uncontrolled trial led to large randomized placebo controlled and dose ranging trials over subsequent years. These trials demonstrated complete normalization of serum alanine aminotransferase levels in between 30% to 60% of treated patients (6, 7, 8). Unfortunately, most patients relapsed at completion of interferon therapy. Therefore, interferon appeared to be a viable, but suboptimal agent for the treatment of chronic hepatitis C infection. Interferon was approved for sale by the United States Food and Drug Administration in 1991 despite its relatively low efficacy rate.

The concept of combination therapies has been quite common in the field of oncology, but only recently, with the advent of multiply antibiotic resistant organisms has it been widely embraced in the area of infectious diseases. When it became clear that interferon alone was not adequate as a single agent therapy for the majority of chronic HCV infections, many investigators began considering combinations of therapeutic agents to improve response. Thymosin alpha-1 (TA-1) is a 28 amino acid peptide that appears to function as a biological response modifier (9). It is an inducer of native alfa and gamma interferons, increases IL-2 receptor level and affinity and improves activity of natural killer cells (10, 11). While interferon is thought to act mainly by a direct antiviral mechanism, the action of TA-1 is thought to be primarily immuno-

stimulatory. Therefore, it was postulated that the combination of these agents might prove beneficial in the treatment of chronic HCV infection.

Design of the U.S. multicenter trial

The evaluation of the combination of TA-1 and IFN for the treatment of chronic hepatitis C mandated a trial to be designed in a manner that would meet current regulatory requirement and concerns in the United States. Classically, the next step in evaluation of this combination therapy would be the performance of a dose ranging trial. This is designated as a Phase II trial and is often unblinded. Limited availability of drug led to the conclusion that a blinded, randomized, placebo-controlled Phase III trial using a TA-1 dose regimen previously utilized in cancer trials would be appropriate. Like a Phase II trial, considerable attention would be paid to drug safety as well as efficacy.

The patient population was limited to those subjects who had HCV ELISA positive, RIBA confirmed chronic hepatitis C infection characterized by greater than six months of alanine aminotransferase (ALT) abnormality. In addition, all patients underwent liver biopsy. Entry criteria required that the biopsy demonstrate a level of activity that permitted classification as Chronic Active Hepatitis (CAH). Patients with milder liver biopsy findings were excluded from entry into the study. Other exclusion criteria included active hepatitis B, Wilson's disease, alpha-1 antitrypsin deficiency, hemochromatosis, alcoholic hepatitis and autoimmune hepatitis. Patients with decompensated liver disease manifest by the presence of ascites, encephalopathy, severe thrombocytopenia, and significant coagulapathy were also excluded. Cirrhosis with well compensated disease was not an exclusion feature. Finally, patients with severe life threatening illness (including HIV infection), pregnant women, and major psychiatric disorders were excluded from randomization and treatment.

A power analysis, based on estimates of interferon efficacy indicated that 100–120 patients were necessary to determine a clinically meaningful difference between the treatment arms. This cohort was randomized to one of three treatment arms, as shown in table I. The randomization was site specific and performed centrally. Placebo for both active agents was identical to active drug and was administered in the same schedule.

Three treatment sites including Fitzsimons Army Medical Center in Colorado, Walter Reed Army Medical Center in Washington, D.C., and the University of Cincinnati in Cincinnati, Ohio, were utilized. Data presented herein is derived from the first two sites only, since they enrolled patients prior to the opening of the Cincinnati site.

Patients were treated for a total of 26 weeks in the blinded, primary treatment phase of the study. During this phase patients were seen at two to four week intervals and were given symptom questionnaires, directed physical exams, and had serum specimens collected. Routine chemistries were performed at

Table I. Treatment arms.

Group	Therapeutic agent 1	Therapeutic agent 2
1	interferon alpha-2b, 3MU tiw	thymosin alpha-1, 1.6 mg biw
2	interferon alpha-2b, 3 MU tiw	placebo thymosin alpha-1
3	placebo interferon alpha-2b	placebo thymosin alpha-1

the individual study sites. Storage serums were collected and removed from the clot within three hours and then aliquoted and frozen at –70 °C. At the end of therapy, and following liver biopsy, patients were unblinded.
Follow-up after initial therapy was dependent on response. Response was classified in terms of ALT change. Normalization of ALT at the end of therapy was considered a complete response, while a 50% decrease in ALT was classified as partial response in this interim analysis. Secondary criteria for response were based on the histologic findings as scored by the Histologic Activity Index (HAI) (12). After study initiation, tests of viral load became available and have been incorporated as secondary virologic response criteria (13). Patients classified as responders were followed for an additional 26 weeks, or, if they relapsed, were returned to the drug combination to which they had initially responded. Non-responders to IFN were offered open label IFN/TA-1 combination therapy for a 26 week treatment cycle. Non-responders to combination therapy were considered to be off protocol, and no further follow-up was officially performed. This interim report discusses initial treatment outcome only.

Results

The study protocol called for an interim analysis to be performed when approximately 60 patients had been randomized and completed the primary therapy phase. These data, plus the current status of interferon failures who were retreated with interferon is described. At the time of the interim analysis, 65 patients had been randomized and 61 had completed 26 weeks of therapy. The drop-out rate secondary to non-compliance (2) and side-effects (2) was 6.15% which was less than predicted prior to study initiation. The demographic characteristics of the study population are shown in table II.
Continuous variables were compared by analysis of variance (ANOVA) and were not found to be significantly different from each other. The Histologic Activity Index was scored in a blinded manner by two experienced hepatopathologists who reviewed blinded samples and arrived at an agreement in terms of scoring. Cirrhosis, which has been described as a negative predictor of interferon response was present in less than 5% in all groups and did not differ significantly between groups.

Table II. Comparison of treatment groups.

	Group 1	Group 2	Group 3	Probability
Number of subjects	20	24	21	
% Male	80	79	81	n.s.
Age	44	36.2	42.6	n.s.
Mean ALT (IU/L)	141	164	195	n.s.
HCV RNA	100%	100%	100%	n.s.
HAI	10	9	8.6	n.s.

Mean ALT level by group was compared throughout the course of therapy. Mean ALT level was lowest at all timepoints after week 4 of therapy for patients treated with combination therapy. This group demonstrated statistically significant lowering of ALT compared to both the placebo and interferon arm during the last weeks of therapy. A Kaplan-Meier life-table plot (figure 1) of complete ALT response demonstrates the improvement attributable to combination therapy versus the other treatment groups. Of note is the normalization of ALT beyond the sixth week of therapy that accounts for the end treatment difference between IFN alone and the combination treatment. Complete plus partial response was also more frequent in patients undergoing combination therapy.

The histological activity was evaluated by blinded pair comparison of pre- and post-treatment liver biopsy specimens. Post-biopsy specimens were obtained at or shortly after completion of the primary therapy phase. The greatest degree of histological improvement was observed in patients receiving combination therapy. The mean HAI score improvement in this group was approximately 2.5 points. Patients receiving IFN alone demonstrated a 2 point improvement. While there was a trend towards statistical significance, a clear difference cannot be reliably established at this time. Both therapeutic arms did show a statistically significant improvement over the placebo treated patients, who demonstrated a 1.5 point worsening compared to baseline HAI scores.

Virologic response was evaluated by serial comparison of mean HCV RNA levels as determined by the branched-chain DNA methodology. Serial specimens from weeks 0, 8, 16 and 24 were analyzed. These data are shown in figure 2. Analysis of variance did not reveal a statistically significant difference between treatment groups at baseline, despite the fact that the mean titer for patients in the IFN alone arm was almost half that of the other two groups. This reflects the variability of results seen. The patients taking placebo for IFN and TA-1 had little change in viral titer over the course of treatment. In contrast, patients getting combination therapy demonstrated a significant aggregate decrease in viral titer associated with treatment by the week 8 timepoint. This effect was generally maintained throughout therapy. In contrast,

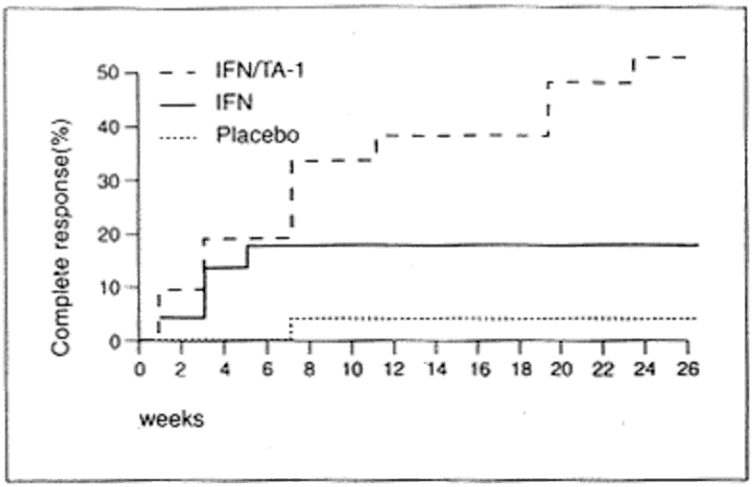

Figure 1. Kaplan-Meier life table analysis of complete biochemical (ALT) response, defined as normalization of ALT at end of treatment cycle. Key is shown.

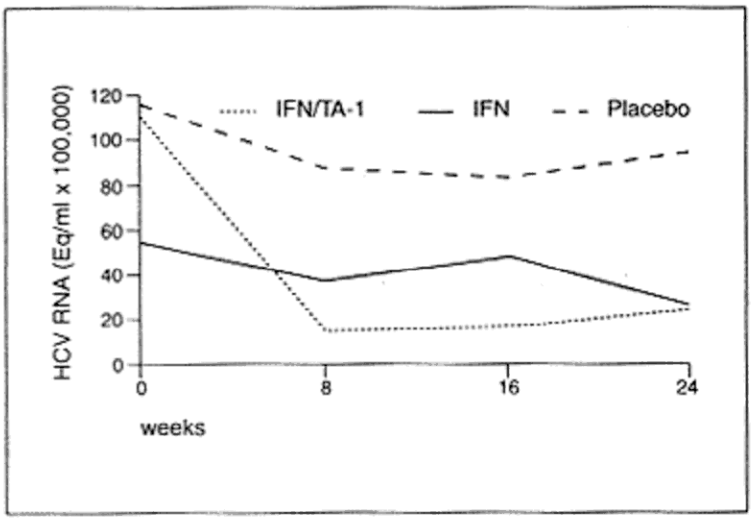

Figure 2. HCV RNA level during blinded treatment. Key is shown.

patients receiving IFN alone had little change from baseline, though a mild decrease in titer at week 24 was enough to make the titer significantly lower than of the placebo treated patients. The side effect profile as reported by patients is shown in table III. Fever, rash and injection site erythema were significantly more common in patients getting active drug(s) than in the placebo treated arm. There was a tendency for somewhat more local erythema in patients receiving combination therapy than in those taking IFN alone. It is interesting to note the high proportion of patients reporting difficulty with concentration and depression in the placebo arm, since this may represent the baseline of these patients with chronic liver disease.

Among key laboratory parameters, the white blood count, absolute neutrophil count and platelet count were all lowered in the active treatment arms versus the placebo controls. There was not a statistically significant difference between INF/TA-1 combination therapy and IFN alone in this regard. Combination therapy was associated with an increase in mean thyroid stimu-

Table III. Side-effect comparison by group.

	Group 1 (%)	Group 2 (%)	Group 3 (%)	Significance (p)
Fever	55.5	45.9	9.5	<0.05
Rash	44.4	31.8	9.5	<0.05
Injection site erythema	77.7	59	23	<0.05
Injection site pain	55.5	50	38	n.s.
Difficulty concentrating	44.4	59	52	n.s.
Depression	60	59	33	n.s.

lating hormone levels during the course of treatment compared with placebo or interferon therapy. However, the proportion of patients developing high or low TSH levels was the same in all groups.
Predictors of response were evaluated to determine if specific markers could be identified. The pretreatment viral titer was found to be important, though in a negative manner. High titers ($>9 \times 10^6$ HCV RNA Eq/ml) were found to be a good predictor of non-response by ALT criteria. Improvement in the HAI was best predicted by week 8 virologic response, defined as the HCV RNA titer less than the detectable titer limit of the assay (3.5×10^5 HCV RNA Eq/ml).

Discussion

This paper describes the interim results of a U.S. multicenter trial of IFN/TA-1 combination therapy for the treatment of chronic hepatitis C infection. The interim analysis was designed to focus on end treatment response (ETR) efficacy by several parameters (biochemical, histological, virological) and to evaluate safety and adverse event profiles for patients on therapy. Recently, much interest in hepatitis C treatment has shifted from ETR to long term response rates, but that aspect was not analyzed in this interim analysis.
Clearly, the combination of IFN/TA-1 appears more efficacious than IFN alone or placebo in terms of biochemical ETR rates. While the benefit was statistically significant, it was not sufficient to stop the trial based on pre-established study criteria which required a $p < 0.001$ for study discontinuation. Similarly there was a trend towards greater efficacy based on histological response for combination therapy than for single agent treatment. Finally, the virological response was much more dramatic in the combination therapy arm. This is particularly pertinent, since lower titers are thought to be a predictor of biochemical response (14). Therefore, the lower titers seen in the interferon group should have conferred a relative treatment advantage.
In terms of side effects, IFN/TA-1 combination therapy does not seem to pose significant disadvantage over IFN alone. There is a mild increase in local

skin reaction reported. Laboratory parameters are also relatively similar between combination and single IFN therapy. While there is a tendency for increases in TSH this was not shown to manifest in an increased rate of clinically relevant thyroid disease.
The current U.S. multicenter trial closed enrollment at the end of 1995. These data will help to establish the efficacy of combination therapy for the treatment of chronic hepatitis C infection.

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