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Therapeutic Strategies for Liver Disease

A Report of a Symposium Presented at the
41st Annual Meeting of the European Association
for the Study of the Liver

April 26–30, 2006

Vienna, Austria

Table of Contents

| | |
|--|----|
| Therapeutic Strategies for Liver Disease | 3 |
| Question and Answer Forum | 13 |

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Therapeutic Strategies for Liver Disease

A Report of a Symposium Presented at the 41st Annual Meeting of the European Association for the Study of the Liver
April 26–30, 2006
Vienna, Austria

Abstract

New therapeutic options are needed for chronic hepatitis B and hepatitis C, especially for individuals who do not respond to current therapies. Thymosin alpha-1, or thymalfasin, is an agent with both immunomodulatory and antiviral activity that has been approved for the treatment of chronic hepatitis B in China for a decade, and is being evaluated in Europe and North America. Thymalfasin upregulates expression of major histocompatibility complex class I (MHC-I) through a different mechanism than does interferon. Thymalfasin also stimulates T-cell differentiation, induces TH1-type immune responses, and downregulates T-cell apoptosis. In trials of patients with chronic hepatitis B, combination therapy with thymalfasin and interferon has demonstrated superior virologic, biochemical, and serologic responses compared with interferon monotherapy. When administered with lamivudine, thymalfasin improves response rates and inhibits the development of lamivudine resistance mutations. Thymalfasin has also been evaluated as a treatment for patients with chronic hepatitis C infection. In a pilot study, triple therapy with thymalfasin, peginterferon alfa-2a, and ribavirin was active in patients who had failed to respond to interferon/ribavirin therapy. Multiple trials have shown the agent to be well tolerated and associated with few adverse events. Finally, other studies have explored the potential use of thymalfasin in the management of hepatocellular carcinoma (HCC). In patients receiving transarterial chemoembolization, treatment with thymalfasin was associated with a significantly decreased incidence of serious adverse events. In this small study, investigators noted a trend of longer survival in the thymalfasin arm. In summary, the immunomodulatory agent thymalfasin has demonstrated promising efficacy in a variety of settings in both hepatitis B and hepatitis C infection.

Introduction to Thymosin Alpha-1

Alfred Rudolph, MD, Chief Medical Officer of SciClone Pharmaceuticals in San Mateo, California, presented information on the biochemistry and mechanism of action of thymosin alpha-1, also called thymalfasin or Zadaxin (SciClone Pharmaceuticals). He explained that thymalfasin is a nonglycosylated, N-terminal-acetylated 28-amino acid polypeptide that is produced in the thymus. The peptide was originally identified in laboratory studies as the mediator of immune reconstitution in thymectomized animals. Thymosin forms the N-terminus of the 113-amino acid prothymosin and is present in the circulation at a concentration of approximately 1 ng/mL (0.3 nM). The compound is produced by solid-phase synthesis under current good manufacturing practices and lyophilized in a dose of 1.6 mg per vial. Subcutaneous administration of a 1.6-mg dose yields a peak plasma level

of 50 ng/mL (15 nM), which is 50- to 100-fold greater than the normal circulating level of thymosin.

Peak thymosin alpha-1 levels are reached about 2 hours after injection and the elimination half-life is also approximately 2 hours. Dosing varies according to the indication, with two injections per week for hepatitis, and more frequent injections, up to four or five times per week or higher, for treating certain cancers. The maximum tolerated dose exceeds 20,000 µg/kg.

Thymosin alpha-1 appears to act via two primary mechanisms: immunomodulatory and antiviral (see Figure 1). The compound upregulates expression of MHC-I and glutathione, leading to stimulation of immune responses against infected cells and inhibition of viral replication. The second mechanism of thymosin alpha-1 is its effect on the immune system—thymosin alpha-1 causes stem cells to differentiate into T cells, including CD4+CD8+, and natural killer (NK) cells.

Figure 1. Dual immunomodulatory and antiviral actions of thymosin alpha-1.

IFN = interferon; IL = interleukin; MHC = major histocompatibility complex; NK = natural killer.

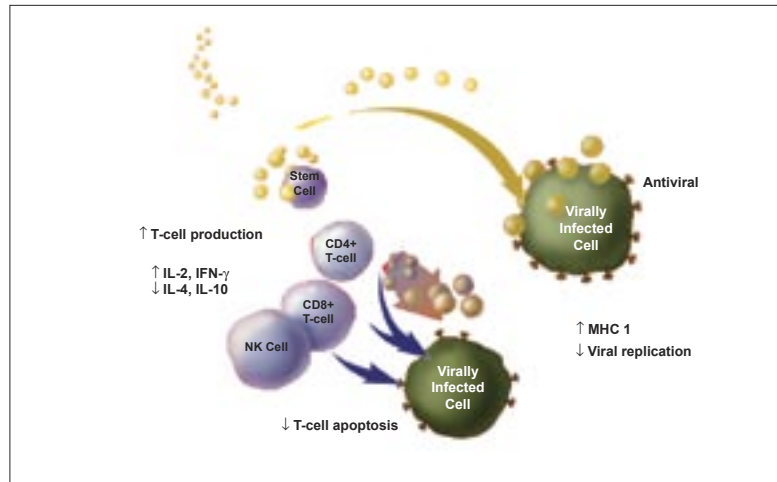
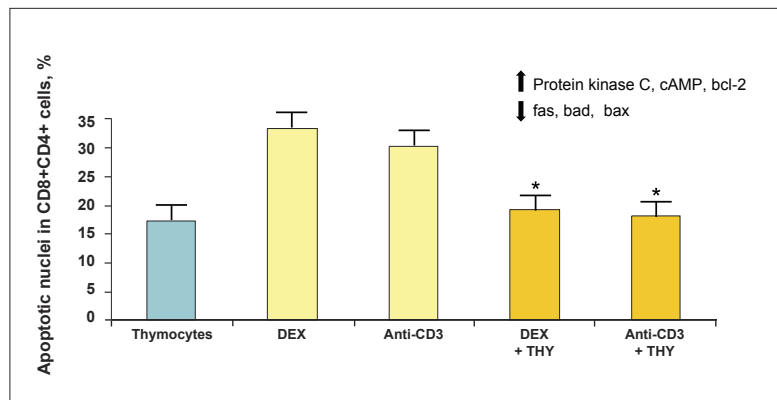


Figure 2. Cellular mouse-model investigating coadministration with dexamethasone (DEX) and anti-CD3 shows that thymosin alpha-1 (THY) blocks apoptosis.

* $P < .05$.

Data from Baumann et al.,² Roy et al.,³ and Gupta et al.⁴



It increases expression of the T_H1 cytokines interleukin (IL)-2 and interferon gamma and downregulates expression of the T_H2 cytokines IL-4 and IL-10. Dr. Rudolph also noted that giving thymosin alpha-1 in conjunction with interferon blunts the T_H2 -type response that is seen with interferon monotherapy. Thymosin alpha-1 also downregulates T-cell apoptosis.

Early studies of the antiviral activity of thymosin alpha-1 were conducted using the woodchuck hepatitis virus (WHV) animal model for hepatitis B. In 1992, Gerin and associates¹ found that treatment with thymosin alpha-1 significantly reduced levels of WHV in the serum compared with control animals.

In the study, WHV DNA remained significantly lower while treatment was maintained, but rebounded when treatment was stopped. Thymosin alpha-1 decreased the progression to HCC in this model as well.

Some of the mechanisms of action of thymosin alpha-1 have been investigated at the molecular level using *in vitro* models of cell death. For example, treatment of CD4+CD8+ thymocytes with dexamethasone or anti-

CD3 is known to induce apoptosis; however, coadministration of thymosin alpha-1 with either stimulus partially inhibits this apoptosis (Figure 2).²⁻⁴ Investigations into its mechanism of action has revealed that thymosin alpha-1 induces upregulation of cyclic adenosine monophosphate, protein kinase C, and Bcl-2, and downregulation of the proapoptotic molecules fas, bad, and bax.

To investigate the effects of thymosin alpha-1 on MHC expression, Giuliani and associates⁵ transfected cells with a plasmid-containing MHC-I and a chloramphenicol acetyltransferase (CAT) reporter. This allowed them to more effectively analyze MHC-I expression. In their studies, MHC-I-transfected cells treated with thymosin alpha-1 showed significantly enhanced CAT activity compared with untreated transfected cells. Deletion of the nuclear factor-kappa B (NF- κ B) site within the MHC promoter region abrogated the activity of thymosin, indicating that this region is involved in the effect of thymosin alpha-1 on MHC-I expression at the transcription level. Conversely, deletion of the interferon response element site, where interferon acts to increase MHC-I expression,

had no effect on the activity of thymosin alpha-1. This indicates that thymosin alpha-1 and interferon interact with different promoter sites and suggests the potential for using both compounds to augment MHC-I expression via different mechanisms.

In summary, the pleiotropic effects of thymosin alpha-1 include immunomodulatory effects on the production of cytokines and their receptors, stimulation of T-cell proliferation and differentiation, and direct inhibition of viral replication or growth of cancer cells. Ongoing research is investigating the subcellular pathways involved in these actions. One mechanism currently being evaluated is the toll-like receptor (TLR) pathway. The link between thymosin alpha-1 and TLRs has been demonstrated in *in vitro* studies. For example, in cells transfected with TLR9, thymosin alpha-1 treatment alone stimulates significant IL-8 production.⁶ However, in cells transfected with TLR2, treatment with thymosin alpha-1 only induces IL-8 production when the ligand for TLR2 is also added to the mixture. These studies indicate that thymosin alpha-1 can activate TLR9 directly and can activate TLR2 by potentiating the ligand-receptor interaction of TLR2. Dr. Rudolph suggested that thymosin alpha-1 likewise interacts with infected hepatocytes through activation of TLRs.

Thymalfasin in China

Gui-Qiang Wang, MD, Professor of Medicine and Director of the Department of Infectious Diseases at Peking University First Hospital in Beijing discussed the experience with thymalfasin in China, where it has been approved for the treatment of hepatitis B for a decade. Hepatitis B virus (HBV) infection is a major health issue in China, with approximately 9% of the population of mainland China testing positive for the hepatitis B surface antigen (HBsAg). Between 25% and 50% of these individuals have chronic hepatitis, with the risk that carries of progression to liver fibrosis, cirrhosis, and HCC. Indeed, effective treatments are greatly needed to treat these individuals.

For more than two decades, thymic extracts from calves have been used in China to treat patients with chronic hepatitis B. In 1996, the synthetic polypeptide thymic hormone thymosin alpha-1, or thymalfasin, was approved for use in China. Since then, the compound has been used in monotherapy and in combination with interferons or nucleoside analogs.

The preliminary clinical trial of thymalfasin in China was published in 1997.⁷ This open-label, single-arm study involved 64 patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B treated at eight centers in

China. All patients had detectable HBV DNA, and 93.8% of patients had elevated alanine aminotransferase (ALT) levels. In the study, patients received 1.6 mg of thymalfasin twice weekly for 6 months. The trial endpoints included undetectable serum HBV DNA and HBeAg loss out to a 6-month follow-up period. At the end of treatment, 39% of patients had responded with HBeAg loss and undetectable HBV DNA. Six months later, at the end of follow-up, 8 patients had reactivated HBV DNA but 9 additional patients responded, for an overall response rate of 40.6%. The investigators observed no significant side effects.

More recently, You Jing and colleagues⁸ conducted a randomized controlled trial comparing thymalfasin versus interferon alfa in 86 Chinese patients with HBeAg-negative chronic hepatitis B and detectable HBV DNA. Patients received 1.6 mg thymalfasin twice weekly for 6 months (n=26), 3–5 MU interferon alfa for 6 months (n=30), or no treatment (n=30). At the end of treatment, 30.8% of patients treated with thymalfasin achieved a complete response, defined as undetectable HBV DNA and ALT normalization, compared with 46.7% of patients receiving interferon. However, as was observed in the preliminary trial, thymalfasin provided delayed responses in some patients. Six months after stopping treatment, complete response rates were higher among those who had received thymalfasin than those given interferon (42.3% vs 23.3%, respectively). Among untreated patients, 3.3% achieved a complete response.

Chien and colleagues⁹ randomized 98 patients with chronic hepatitis B to receive thymalfasin (1.6 mg twice weekly for either 26 or 52 weeks) or no treatment. At the end of a 12-month follow-up period, the complete response rate, defined as undetectable HBV DNA and HBeAg loss, was highest in the group treated for 26 weeks. Among these patients, 40.6% had complete responses, compared with 38.2% in the group treated for 52 weeks and 9.4% in the control group. The high proportion of responses attained after treatment was stopped again suggests that thymalfasin induces delayed anti-HBV responses (Figure 3).

Combination Therapy With Thymalfasin in HBV

Multiple clinical trials in China have evaluated combination therapy of thymalfasin with other agents. Several studies have investigated the benefit of adding thymalfasin to an interferon regimen. In a multicenter open-label study published in 1997, 188 patients with HBeAg-positive chronic hepatitis B received a 6-month regimen of thymalfasin (n=94), a 3-month regimen of interferon alfa-2b (n=29), or a combination of thymalfasin and interferon (n=64).¹⁰ At the end of the 6-month follow-up period,

Figure 3. Long-term results of the Taiwan study of hepatitis B patients show a delayed response to thymalfasin therapy and best results with a 26-week course of therapy.

Data from Chien et al.⁹

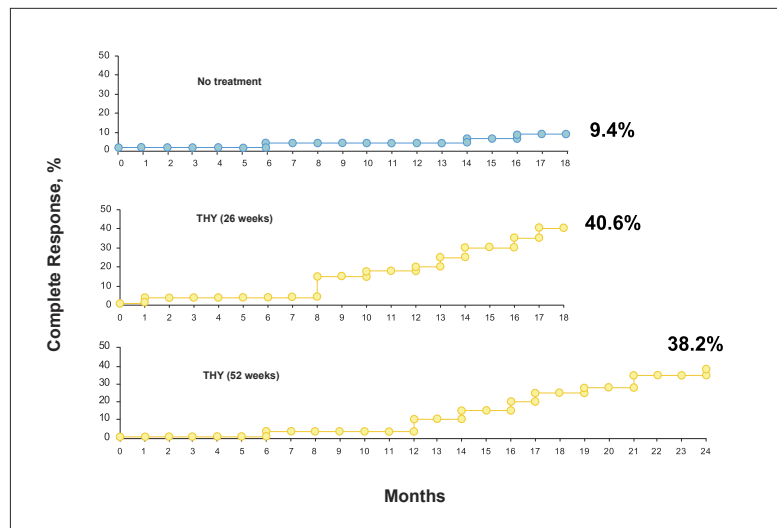


Table 1. Virologic, Biochemical, Serologic Responses With Interferon With or Without Thymalfasin in HBeAg-Positive Patients

| Outcome | Proportion of Patients in Each Arm Achieving Outcome at Each Follow-up Visit, % | |
|-----------------------------|---|-------------------|
| | Thymalfasin + Interferon (n=54) | Interferon (n=49) |
| <i>Undetectable HBV DNA</i> | | |
| End of treatment | 76* | 53 |
| 6 months | 65* | 41 |
| 18 months | 54* | 31 |
| 30 months | 48* | 26 |
| 42 months | 40 | 24 |
| <i>ALT normalization</i> | | |
| End of treatment | 90* | 73 |
| 6 months | 83* | 63 |
| 18 months | 76* | 55 |
| 30 months | 68 | 49 |
| 42 months | 57 | 41 |
| <i>HBeAg loss</i> | | |
| End of treatment | 78* | 55 |
| 6 months | 68* | 47 |
| 18 months | 57* | 37 |
| 30 months | 50 | 35 |
| 42 months | 41 | 28 |

* Denotes statistically significant differences between treatments.

Data from Hu.¹¹

ALT = alanine aminotransferase; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus.

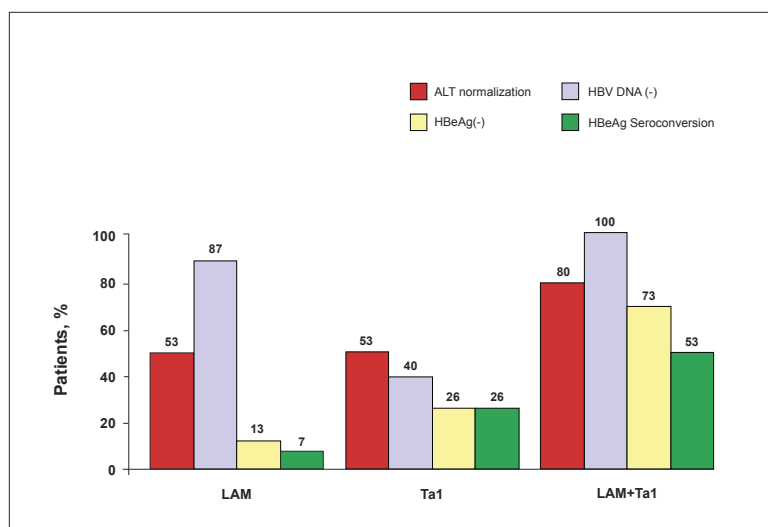


Figure 4. Response after 6 months treatment with thymalfasin monotherapy (Ta1), lamivudine (LAM) monotherapy, and thymalfasin/lamivudine combination therapy in hepatitis B surface antigen–positive patients.

Data from Lin et al.¹²

ALT = alanine aminotransferase; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus.

responses were significantly greater with thymalfasin plus interferon, which provided an HBeAg seroconversion rate of 70.3%, compared with 46.3% with thymalfasin alone and 32.3% with interferon alone.

In 2001, Hu and colleagues¹¹ published results of a study with a longer follow-up period. The study enrolled 103 patients with HBeAg-positive chronic hepatitis B to receive interferon alfa-2b plus thymalfasin (n=54) or interferon monotherapy (n=49). Patients were treated for 6 months then periodically evaluated during a 4-year follow-up. The combination regimen was significantly superior to interferon monotherapy at the end of treatment through the 18-month follow-up period according to ALT normalization and HBeAg loss, and was superior through the 30-month follow-up according to HBV DNA negativity. The results are summarized in Table 1.

Lin and colleagues¹² investigated the short-term efficacy of a thymalfasin plus lamivudine combination. They enrolled 60 patients who had been HBsAg-positive for at least 12 months and who had ALT levels 2–10 times the upper limit of normal. Patients were randomized to receive 6 months of treatment with one of three regimens: 100 mg lamivudine daily, 1.6 mg thymalfasin twice weekly, or a combination of thymalfasin and lamivudine.

By the end of treatment, 100% of patients receiving the combination regimen achieved undetectable HBV DNA, with an average decline in HBV DNA levels of 4–5 logs. Moreover, 80% of these patients achieved ALT

normalization, 73% achieved HBeAg loss, and 50% had HBeAg seroconversion. These response rates were higher than those observed with either agent alone (Figure 4). No severe side effects were observed in any group, and no mutations were identified in this short study.

A subsequent study by Lin and colleagues¹³ evaluated the combination during a longer treatment and follow-up period. A total of 35 patients received thymalfasin plus lamivudine for 1 year while 37 patients received lamivudine alone. Patients were followed for 1 year after treatment. At both the end of treatment and at the 1-year follow-up, the thymalfasin/lamivudine combination was significantly superior to lamivudine alone in terms of rates of HBeAg loss and HBeAg seroconversion (Table 2).

In addition to providing superior response rates, the combination of thymalfasin and lamivudine was associated with significantly fewer resistance mutations. In fact, no patients treated with the combination developed YMDD mutations, compared with 27% of patients receiving lamivudine ($P=.006$). One patient receiving lamivudine (2.9%) developed pre-C mutations, compared with no patients receiving the combination.

In summary, thymalfasin is used extensively in China. This use is supported by studies with thymalfasin, both as monotherapy and as a component of combination therapy, showing that thymalfasin has promising efficacy in the treatment of chronic hepatitis B. Adding thymalfasin to lamivudine also appears to reduce the incidence

Table 2. Responses to Combination Therapy With Thymalfasin Plus Lamivudine in Patients With Chronic HBV

| Outcome | Thymalfasin + Lamivudine, % (n=35) | Lamivudine, % (n=37) |
|--|------------------------------------|----------------------|
| HBeAg loss at week 52 | 69 | 14 |
| HBeAg loss at 1-year follow-up | 57 | 22 |
| HBeAg seroconversion at week 52 | 51 | 5 |
| HBeAg seroconversion at 1-year follow-up | 43 | 8 |

Data from Lin.¹²

HBeAg = hepatitis B e antigen; HBV = hepatitis B virus.

of lamivudine-associated resistance mutations. Finally, thymalfasin appears to have a favorable safety profile with few side effects.

Use of Thymalfasin for Treating Hepatitis C

Kenneth E. Sherman, MD, PhD, Gould Professor of Medicine and Director of the Division of Digestive Diseases at the University of Cincinnati College of Medicine in Cincinnati, Ohio, spoke about clinical trials of thymalfasin for treating patients with chronic hepatitis C virus (HCV) infection. Hepatitis C is a significant global health problem, affecting an estimated 160 to 200 million persons worldwide. Prevalence varies significantly by geographic region, from infection rates of 1.0% in Europe up to 5.3% in Africa. HCV infection is a leading contributor to the development of both end-stage liver disease and liver cancer.

The past decade has seen a rapid and progressive evolution in treatments for HCV, first by extending the duration of treatment with standard interferon, then with the addition of ribavirin in 1998. Most recently, the introduction of pegylated interferons in the early 2000s increased the response rates from just below 40% to approximately 55%. With the currently available treatments, over half of patients are achieving sustained virologic responses (SVRs), which Dr. Sherman noted are practically considered a cure for this infection.

Despite these recent advances in the treatment of HCV, significant challenges remain. A large proportion of patients do not respond to current treatments and the available agents are all associated with significant side effect profiles. Additionally, some patients, such as those

with decompensated liver disease, are often not eligible for therapy, while other patients, including those with immunosuppression, are not likely to achieve SVRs with current therapies. There is clearly a need for new therapies for individuals not responding to the available treatments for chronic HCV.

Thymalfasin Combination Therapy in HCV

Thymalfasin has been evaluated in multiple clinical trials for the treatment of patients with chronic hepatitis C. Early studies investigated the benefit of adding thymalfasin to standard interferon. In 1996, Rasi and associates¹⁴ conducted an open-label trial evaluating a 1-year regimen of thymalfasin plus interferon in 15 patients with chronic hepatitis C, of whom 13 had HCV genotype 1b. At the end of treatment, 11 patients (73%) had undetectable HCV RNA, including 9 patients with HCV 1b and 2 patients who had previously failed interferon treatment. At the end of follow-up 6 months later, 6 patients (40%) had undetectable HCV RNA, including 5 patients (39%) with HCV 1b, which is often more difficult to treat. Dr. Sherman suggested that this was a fairly positive result compared with standard therapy at that time, which did not include ribavirin.

Several years later, a group led by Dr. Sherman conducted a randomized, double-blind, placebo-controlled trial in which patients with chronic HCV infection and biopsy-confirmed hepatitis received either standard interferon plus placebo (n=37), interferon plus thymalfasin (n=35), or placebo (n=37).¹⁵ Dr. Sherman noted that such double-placebo trials are no longer conducted. After a 6-month treatment period, 37% of patients receiving interferon and thymalfasin achieved polymerase chain reaction (PCR)-undetectable HCV RNA level, compared with 19% of patients receiving interferon alone. These results indicated a significant synergistic effect.

More recently, several studies have investigated the feasibility of combining thymalfasin with pegylated interferons. An initial dose-ranging study carried out by Iftikar and associates¹⁶ investigated three doses of thymalfasin administered in combination with peginterferon alfa-2a in patients with chronic HCV (N=31) who had previously failed to respond to interferon with or without ribavirin. All patients had HCV type 1, a high HCV RNA level of at least 2×10^6 copies/mL, elevated ALT, and compensated liver disease. The investigators evaluated HCV RNA levels and peripheral blood T-cell profiles at week 12 in this difficult-to-treat population. They measured HCV RNA using Amplicor (Roche) PCR-based assay and quantified lymphocyte subsets using standard dual color flow cytometry.

For the 12-week treatment period, patients in each arm received peginterferon alfa-2a at 180 µg/week plus

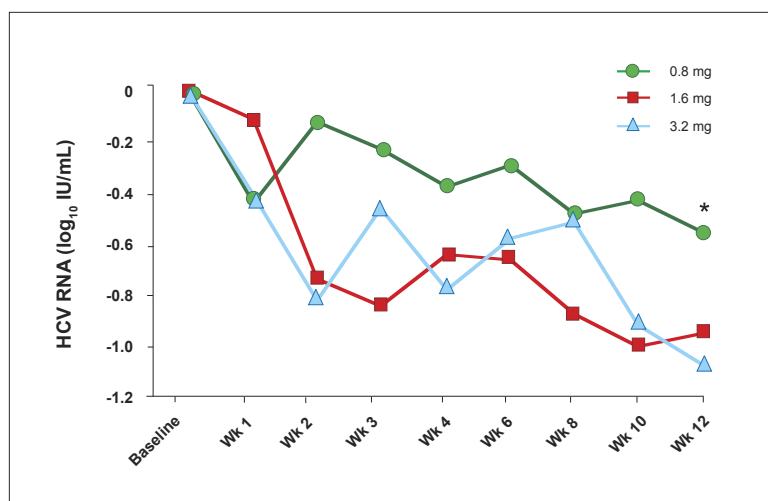


Figure 5. Combination therapy with peginterferon alfa-2a plus three different doses of thymalfasin. The two higher doses (1.6 and 3.2 mg twice weekly) showed a significant dose-response effect in lowering hepatitis C virus (HCV) RNA levels.

* $P < .05$ vs 1.6 mg or 3.2 mg.

Data from Iftikar et al.¹⁶

thymalfasin at varying doses (0.8 mg, 1.6 mg, or 3.2 mg) twice per week. The three arms were well matched, with the majority of patients in each arm having failed an interferon/ribavirin regimen.

At week 12, the median change in HCV RNA level was significantly greater with the two higher doses compared with the lower dose ($P < .05$), suggesting that thymalfasin has a dose-response effect (Figure 5). Early virologic response (EVR), defined as achieving at least a 2-log reduction in HCV RNA or having undetectable HCV RNA at week 12, can be an indication of future responses to treatment. Thymalfasin also showed a dose-effect on EVR, with rates increasing from 20% to 30% to 36% with increasing doses of thymalfasin. T-cell responses to thymalfasin sharply increased from the lowest to the intermediate dose. Although these differences did not reach statistical significance, they do suggest that a threshold is required to observe changes in T-cell counts (Table 3).

In this study, thymalfasin was well tolerated, and no significant adverse events were reported other than the side effects typically observed with peginterferon treatment. Based on these efficacy and safety findings, the 1.6 mg twice-weekly dose of thymalfasin appeared to be the preferred thymalfasin dose.

Phase III Studies of Thymalfasin in HCV Nonresponders

Results from these preliminary studies led to the design of two major clinical trials in the United States to evaluate thymalfasin plus peginterferon in patients not responding to peginterferon alfa with or without ribavirin. The first study, Study 803, enrolled 500 patients with disease ranging from no cirrhosis to early cirrhosis (Metavir score of < 3). The second study enrolled 500 patients with bridging fibrosis (Metavir 3) to those with established cirrhosis but

Table 3. Changes in T-Cell Levels From Baseline to Week 12 With Varying Doses of Thymalfasin in a Thymalfasin/Peginterferon Alfa-2a Regimen

| T Cell Population | Change in T-Cell Count at Week 12 According to Thymalfasin Dose, % | | |
|-------------------|--|--------|--------|
| | 0.8 mg | 1.6 mg | 3.2 mg |
| CD3+ cells | 0 | 8.3 | 7.5 |
| CD4+ cells | -0.2 | 14.8 | 8.8 |

compensated disease (Metavir 3–4). The 1,000 patients in these complementary studies received thymalfasin plus peginterferon alfa-2a or peginterferon alfa-2a plus placebo for 12 months, followed by a 6-month follow-up. The primary endpoints were HCV RNA levels and histology at the end of follow-up (18 months). Patients in Study 803 were well-matched in terms of demographics and treatment history; the majority of patients had failed treatment with interferon plus ribavirin.

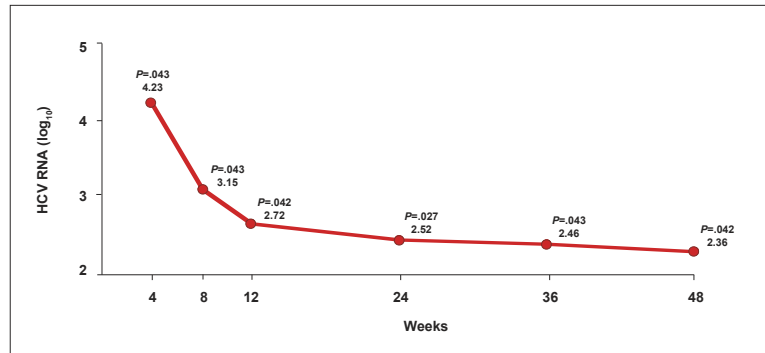
In Study 803, results at the 6-month follow-up did not show a statistically significant benefit with thymalfasin according to SVR rates, improvement in fibrosis, ALT normalization, or changes in CD4+ cell count. However, because the importance of ribavirin was not fully appreciated when this trial was designed, ribavirin was not included in the regimens.

Triple Therapy With Thymalfasin for HCV

Poo and colleagues¹⁷ subsequently undertook a pilot trial to evaluate the efficacy of thymalfasin in combination with peginterferon alfa-2a and ribavirin in patients who did not respond to interferon/ribavirin therapy. Patients in this open-label, single-arm study received a 48-week regimen that included 1.6 mg thymalfasin twice

Figure 6. Reduction of hepatitis C virus (HCV) RNA levels in peginterferon/ribavirin combination nonresponders when treated with combination therapy plus thymalfasin.

Data from Poo et al.¹⁷



weekly, 180 µg peginterferon alfa-2a once weekly, and 800–1,000 mg ribavirin daily. Dr. Sherman mentioned that this ribavirin dose would now be considered suboptimal. The primary endpoint of the study was SVR at week 72, with secondary endpoints including EVR at week 12 and end-of-treatment response at week 48.

The study enrolled 30 patients from Mexico with characteristics different from those typically seen in HCV trials. The majority were female (n=21) and the average age was 57 years. All were HCV RNA-positive, and 19 patients had high viral loads. HCV genotypes represented included 1a (n=11), 1b (n=15), and 2 (n=4).

In this nonresponder population, the triple-therapy regimen provided significant decreases in HCV RNA throughout the treatment period (Figure 6). At week 12, 56.6% of patients had achieved an EVR. Half of patients responded by the end of treatment, and the SVR at week 72 was 21.4%. Among genotype 1 patients, the SVR rate was 25%. Dr. Sherman noted that this result was fairly interesting, given that in standard retreatment trials with peginterferon/ribavirin, SVR rates are typically between 5% and 15%. The investigators reported that thymalfasin was well tolerated, without side effects.

Future Directions of Thymalfasin for HCV

Given the apparent activity of thymalfasin plus peginterferon and ribavirin in the pilot study, a large randomized phase III trial was designed to further evaluate the efficacy and safety of this triple combination. This study is underway in Europe, under the leadership of Mario Rizzetto, MD. The multicenter, international, double-blinded trial is evaluating peginterferon alfa-2a plus ribavirin with either thymalfasin or placebo in 550 patients who failed to respond to prior peginterferon alfa-plus-ribavirin therapy.

Following an 8-week screening period, patients are being randomized to receive peginterferon alfa-2a at a standard dose plus a weight-based ribavirin dose, with

either placebo or 1.6 mg thymalfasin twice a week. Investigators are evaluating virologic responses at week 12 and 24; patients with detectable HCV RNA at week 24 are considered nonresponders. Patients who respond by week 24 continue treatment for another 24 weeks, and are evaluated after a 24-week follow-up period for SVR.

Patients are being stratified by viral load ($\geq 800,000$ IU/mL or $< 800,000$ IU/mL), category of treatment failure (peginterferon alfa-2a vs peginterferon alfa-2b), and the presence or absence of cirrhosis. For European clinicians with patients meeting the eligibility requirements, Dr. Sherman encouraged participation in the trial, as it was still actively enrolling throughout Europe at the time of the symposium.

Applications for Thymalfasin in the Management of Hepatocellular Carcinoma

Robert Gish, MD, Medical Director for the Liver Disease Management and Transplant Program at California Pacific Medical Center in San Francisco, presented several studies evaluating the use of thymalfasin for treating patients with HCC. HCC accounts for more than half a million deaths worldwide each year, making it one of the top three causes of cancer death; however, no medications are currently approved for the treatment of HCC in the United States, the European Union, or globally. Although liver transplantation can cure HCC and the underlying cirrhosis that is commonly present, supply of organs is limited and most patients with advanced disease are not candidates for surgery.

A number of other therapeutic options are available, including ablative techniques, which can provide short-term benefits. Transarterial chemoembolization (TACE), however, is the only therapy that has demonstrated survival benefits in randomized controlled trials. Ongoing studies are evaluating additional modalities, such as thermal radiofrequency ablation and injection therapy.

Thymalfasin and Transarterial Chemoembolization for HCC

Several recent studies have investigated the feasibility of incorporating thymalfasin into treatments for HCC. Dr. Cheng Shuqun at the Eastern Hepatobiliary Surgery Hospital in Shanghai evaluated three different treatment strategies for 57 patients with HCC: hepatectomy plus TACE plus thymalfasin postoperatively, hepatectomy plus TACE alone, or hepatectomy alone. The thymalfasin-treated group had a significant survival advantage ($P=.0039$) compared with the two other arms, which were pooled in this analysis.

The same center and investigators also evaluated the activity of thymalfasin after hepatectomy in HBV-positive patients with HCC and active viral replication. A total of 70 patients were treated with hepatectomy only ($n=35$) or hepatectomy followed by lamivudine plus thymalfasin ($n=35$). The treatment group had a significantly improved median overall survival of 12.5 months versus 6 months in the control group ($P=.023$; Figure 7).¹⁸

In an open-label, phase II randomized pilot study (protocol T α 1-HCC-2K1001), Dr. Gish and associates¹⁹ evaluated the safety and efficacy of TACE plus 1.6 mg thymalfasin 5 times weekly for 6 months versus TACE alone. A total of 28 patients enrolled at five study centers in the

United States. Three subjects withdrew before receiving treatment, leaving a final analysis of 25 patients.

All patients had unresectable HCC and were not candidates for liver transplantation. In order to enroll, patients had to have biopsy-confirmed HCC or other predefined evidence of HCC if no biopsy was available. For this small study, patients were required to have a mild-to-moderate Child-Pugh category (A or B), a Model End-stage Liver Disease (MELD) score less than 20, normal renal function (serum creatinine <1.5 mg/dL), and no main portal vein invasion.

Patients were stratified by tumor stage and randomized to receive TACE or TACE plus thymalfasin. After the 24-week treatment period, patients were monitored through week 72 and received TACE as needed if tumor growth occurred. Survival was evaluated through month 30 after randomization. The investigators evaluated tumor responses using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, overall survival and progression-free survival, reduction in alpha-fetoprotein (AFP) levels, and Eastern Cooperative Oncology Group (ECOG) performance status.

The two treatment groups were comparable according to relevant baseline criteria, including Okuda status and AFP, ALT, aspartate aminotransferase (AST) and alka-

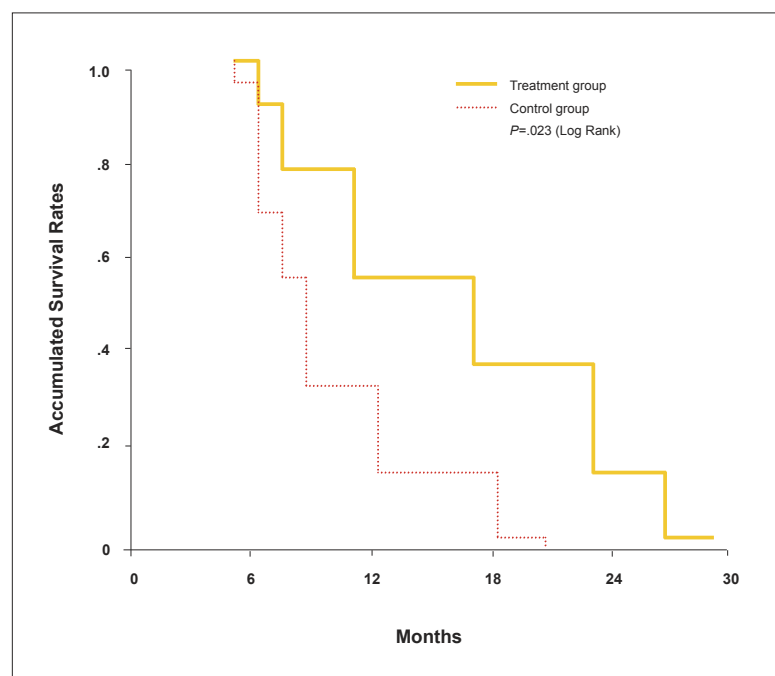


Figure 7. Survival curves for hepatocellular carcinoma patients treated with hepatectomy only (control group) versus hepatectomy followed by lamivudine plus thymalfasin (treatment group), showing survival advantage to thymalfasin-treated patients.

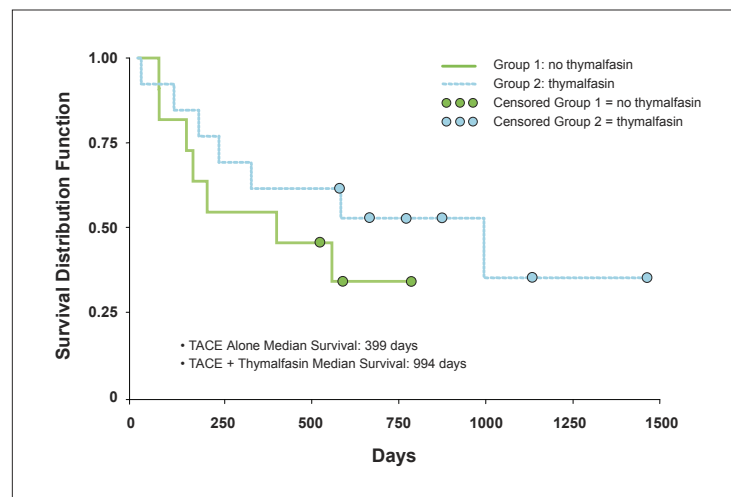
Data from Shuqun et al.¹⁸

Figure 8. Survival analysis of the Response Evaluation Criteria in Solid Tumors trial.

$P=.34$ (log-rank); $P=.36$ (Wilcoxon).

TACE = transarterial chemoembolization.

Data from Gish et al.¹⁹



line phosphatase levels. In an intention-to-treat analysis, median survival was 994 days with TACE plus thymalfasin versus 399 days for TACE alone (Figure 8). This difference did not reach statistical significance, but Dr. Gish suggested that the trend supports the value of conducting a large phase III trial to further evaluate this regimen. In terms of response rates, no significant differences were noted between treatments. However, for almost half of patients in the study, not enough data are available yet to determine best response by the RECIST standards.

Safety analysis revealed fewer serious adverse events with the addition of thymalfasin (32% vs 68% with TACE alone). Dr. Gish suggested that the immunomodulatory effects of thymalfasin might therefore have some benefit for managing the complications of HCC, which could be an important application of the agent.

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Question and Answer Forum

Drs. Wang, Sherman, Rudolph, and Gish answer further questions on thymalfasin therapy for hepatitis B and C.

In patients with hepatitis B, why do you think the combination of thymalfasin plus lamivudine is active, given that the interferon/lamivudine combination has not demonstrated substantial improvements over interferon alone?

Dr. Gui-Qiang Wang Nucleoside analogs are in fact very good drugs for HBV infection. They work quickly, but have a limited ability to induce sustained responses in terms of HBV DNA suppression and ALT levels. I think this is because lamivudine does not stimulate the immune system. As we know, many doctors believe that a combination of nucleoside analogs and an immune system-enhancing drug is the best way to attain sustained HBV inhibition. Right now, thymalfasin appears to be a good candidate for an immunoenhancer. So the nucleoside inhibitor is active against the virus, and thymalfasin can activate the immune system. This kind of combination should be active, and preliminary studies have demonstrated that it does appear to work well. Moreover, the lack of YMDD mutations with the thymalfasin/lamivudine combination was another important finding.

Dr. Kenneth Sherman Those data on the YMDD are really very dramatic because at this point, worldwide, lamivudine is a very inexpensive drug but it suffers from a very bad mutation profile.

Are there any data or any studies underway that you are aware of using any other agents combined with thymalfasin, such as adefovir or entecavir?

GW There are no large trials. Doctors in China are evaluating adefovir and thymalfasin combination, but no data are available.

Based upon the features of thymalfasin as demonstrated in the basic science studies, can thymalfasin be used in patients with HIV infection or coinfection?

Dr. Alfred Rudolph Coinfection with HIV and HBV or HCV is a larger and fairly difficult problem to address.

We have done studies with thymosin in HIV and shown that as with IL-2 there are T-cell responses, but in the end, outcomes are the same: patients still succumb to the same sorts of opportunistic infections. The new approach is to use highly active antiretroviral therapy (HAART) in addition to low-dose IL-2 or something like thymalfasin, and I would be very interested in a trial combining HAART and thymalfasin in people coinfecting with hepatitis and HIV. It is a logical thing to do, but I know of no large cohort that has been tested yet.

Are there any data on the use of thymalfasin in patients undergoing surgical resection or radiofrequency ablation as an adjunctive therapy?

Dr. Robert Gish There are no studies ongoing that I know of in the United States or European Union with thymalfasin right now. I would propose that those studies should take place. Radiofrequency ablation has been used extensively, not just as a bridge to transplant but as a separate treatment. In this case, I believe that there may be tumor responses. Given the basic science studies, thymalfasin may stimulate an immune response that could help improve outcomes, as we've seen with interferon. Moreover, it might also help manage the cirrhotic complications and the complications of treating the tumor with TACE. Therefore, I think it would be important to look at decompensation, infections, and adverse events, as well as tumor responses, in a larger study.

What might be the feasibility of using thymalfasin at higher doses to replace the interferon component of a treatment regimen?

KS Thymalfasin probably cannot be used as a single agent. Early single-agent studies failed to show a big effect with thymalfasin alone in hepatitis C, and there was a good rationale for combining these agents. However, what I find very intriguing is the potential use of a safe, well-tolerated agent like thymalfasin in combination with some of the new agents, such as the RNA-dependent polymerase inhibitors or the serine protease inhibitors, where an immune modulation response combined with a

very strong direct antiviral effect from those agents might be beneficial.

What are the primary differences between HBV infections in Asian patients versus Western patients, and how might these differences affect some of the results in the Chinese studies of thymalfasin?

GW In the case of interferons, data have shown different responses in Asian patients versus Western patients. In Asian patients, the HBeAg seroconversion rate is about 22%, compared with 33% in Western patients. However, such data are not available regarding nucleoside analogs. An important difference between the populations is in the prevalence of different HBV genotypes. In China, about 70% of HBV-positive persons have genotype C infection, which is associated with poorer responses to interferon.

Regarding the interaction of thymosin alpha-1 and TLRs, does thymalfasin regulate specific TLRs? Also, how does thymalfasin act as a ligand in this setting?

AR Thymalfasin seems to have TLR activity in two separate receptors. In one of them it seems to potentiate

the interaction of the receptor and the ligand, and in the other it directly stimulates the receptor without potentiation of the ligand-receptor interaction. The TLRs that seem important are 7, 9, and 2, but this research is in an early phase, and it may well be that there will be other interactions demonstrated in the future.

Has thymalfasin been evaluated in HCV nonresponders with genotypes 2 and 3?

KS In at least some of the earlier studies, those patients were included. These days, with at least 80% of those patients being cleared with peginterferon plus ribavirin, the pool of patients with genotypes 2 and 3 is much smaller, and for that reason, the studies to date have concentrated on patients with genotype 1 infection. That said, it is important to note that the new generations of drugs being developed are all aimed at the genomic structure of HCV genotype 1, and therefore it is likely that we will see a gap in terms of drugs that might be available for the small but important subset of patients with genotypes 2 and 3 who fail to respond. Use of interferons with drugs like thymalfasin may have a role in that setting.

