



SciClone Pharmaceuticals



SciClone Pharmaceuticals is a revenue-generating, profitable China-centric specialty pharmaceutical company with a substantial business and a product portfolio of novel therapies for oncology, infectious diseases and cardiovascular, urological, respiratory, and central nervous system disorders.

SciClone's ZADAXIN® (thymalfasin) is approved in over 30 countries and may be used for the treatment of hepatitis B (HBV), hepatitis C (HCV), as a vaccine adjuvant, and certain cancers according to the approvals we have in these countries. SciClone markets nearly 20, mostly partnered products in China besides ZADAXIN, including Depakine®, the most widely prescribed broad-spectrum anti-convulsant in China; Tritace®, an ACE inhibitor for the treatment of hypertension; Stilnox®, a fast-acting hypnotic for the short-term treatment of insomnia (marketed as Ambien® in the US); and Aggrastat®, a recently-launched intervention cardiology product. On the development side, SciClone is evaluating SCV-07 in a phase 2b trial for the delay to onset of oral mucositis in patients with head and neck cancer. SciClone is also pursuing the registration of several other therapeutic products in China.

ZADAXIN has not been approved for sale in the United States or Europe.

This product monograph may contain forward-looking statements regarding expected results and expectations. Readers are urged to consider statements that include the words "may," "will," "would," "could," "should," "might," "believes," "estimates," "projects," "potential," "expects," "plans," "anticipates," "intends," "continues," "forecast," "designed," "goal," "unaudited," "approximately" or the negative of those words or other comparable words to be uncertain and forward-looking. These statements are subject to risks and uncertainties that are difficult to predict and actual outcomes may differ materially. Please also refer to other risks and uncertainties described in SciClone's filings with the SEC. All forward-looking statements are based on information currently available to SciClone and SciClone assumes no obligation to update any such forward-looking statements.



Zadaxin Product Monograph

By SciClone Pharmaceuticals

Table of Contents

- List of Tables and Figures
- List of Abbreviations

- Introduction 10

- Chemical Properties of Zadaxin (thymalfasin) 11
- Mechanism of Action of Zadaxin 12
- Use of Zadaxin in Disease States 15

- Animal Models 16
 - Woodchuck Hepatitis
 - Other Animal Models of Infection
 - Animal Models of Cancer

- Hepatitis B 22
 - Hepatitis B Treatment Options
 - Zadaxin Monotherapy in Hepatitis B
 - Zadaxin Plus IFN α Combination Therapy for Chronic Hepatitis B
 - Zadaxin Plus Nucleoside Analogues Combination Treatment for Chronic Hepatitis B
 - Key Takeaways

- Hepatitis C 35
 - Clinical Trials
 - Zadaxin Plus IFN α Combination Therapy for Chronic Hepatitis C
 - US Hepatitis C Trial
 - Italian Hepatitis C Trial & Thailand Hepatitis C Trial, Interim Results
 - Summary and Key Takeaways

- Human Immunodeficiency Virus 44

- Vaccine Enhancer in Immunocompromised Patients 48
 - Introduction
 - Cornell Medical Center (Ithaca, New York) & University of Wisconsin Trials
 - George Washington University Trial
 - Hemodialysis Patients
 - Key Takeaways

Table of Contents (continued)

- Cancer 55
 - Introduction
 - Hepatocellular Carcinoma
 - Non–Small-Cell Lung Cancer
 - Malignant Melanoma
 - Key Takeaways

- Toxicity and Safety 63
 - Toxicity in Animals and In Vitro
 - Safety in Humans

- References 64

List of Tables

- **Table 1**
Effect of Combination Therapy With Zadaxin (ZDX), Amantadine (AMN), and IFN α/β in Mice Infected With Influenza A Virus
- **Table 2**
Hepatitis B Clinical Outcomes (Intent to Treat)

List of Figures

- **Figure 1**
Primary structure of thymalfasin.
- **Figure 2**
Proposed mechanism of action of Zadaxin.
- **Figure 3**
Serum woodchuck hepatitis virus (WHV) DNA in Zadaxin-treated and placebo treated animals.
- **Figure 4**
ZDX in combination with AMN and IFN α/β increases survival of mice infected with influenza A virus.
- **Figure 5**
Relapse time of B16 melanoma bearing mice treated with CY, IFN, and ZDX
- **Figure 6**
Effect of increasing doses of Zadaxin on tumor reduction in murine B16 melanoma.
- **Figure 7**
Comparative adverse-event incidence.
- **Figure 8**
Results at Chang Gung Memorial Hospital, Taiwan hepatitis B trial at 18 months.
- **Figure 9**
Time course of complete response in the Chang Gung Memorial Hospital, Taiwan.

List of Figures (continued)

- **Figure 10**
Results from the Italian hepatitis B trial at 12 months.
- **Figure 11**
Pooled intent-to-treat analysis of end-of-treatment biochemical response.
- **Figure 12**
Results in the US hepatitis C trial at 6 months.
- **Figure 13**
Time to first ALT normalization in the US hepatitis C trial.
- **Figure 14**
Results from the HIV trial in Italy at 12 months.
- **Figure 15**
Clinical response to influenza vaccination.
- **Figure 16**
Results from US influenza trial.
- **Figure 17**
Antibody response to influenza vaccine with Zadaxin enhancer in hemodialysis patients.
- **Figure 18**
Zadaxin+TACE: HCC survival.
- **Figure 19**
Effect of Zadaxin on metastatic melanoma
- **Figure 20**
Serum Zadaxin concentrations after a single subcutaneous dose (1.6 mg) of Zadaxin.

List of Abbreviations

5-FU = 5-flurouracil

AIDS = acquired immunodeficiency syndrome

ALT = alanine aminotransferase

AZT = zidovudine (3'-azido-3'-deoxythymidine)

BIW = twice weekly

CAH = chronic active hepatitis

CD = clusters of differentiation

CHB = chronic hepatitis B

CHC = chronic hepatitis C

CSF = colony stimulating factor

CY = cyclophosphamide

DTH = delayed type hypersensitivity

DTIC = dacarbazine

ELISA = enzyme-linked immunosorbent assay

ETR = end-of-treatment responder

HAI = hepatic activity index

HBeAg = hepatitis B e antigen

HBsAb = antibody to hepatitis B surface antigen

HBsAg = hepatitis B surface antigen

HBV = hepatitis B virus

HCC = hepatocellular carcinoma

HCV = hepatitis C virus

HIV = human immunodeficiency virus

IFN = interferon

IFN α =interferon alpha

IFN α/β = interferon alpha beta (from mouse, as used in this report)

IFN α -2b = interferon alpha-2b (recombinant, as used in this report)

IFN γ = gamma interferon

IL-2 =interleukin-2

IL-2R =interleukin-2 receptor

IM = intramuscular

IV = intravenous

L-IFN = natural human lymphoblastoid interferon

µg = microgram
mg = milligram
mIU = milli international units
MIU =million international units

ng = nanogram
NK =natural killer
NSCLC = non–small-cell lung cancer

PBMC = peripheral blood mononuclear cell
PCR = polymerase chain reaction
pg = picogram
PHA = phytohemagglutinin
ProTα1 = prothymosin alpha 1

QD = per day

SC = subcutaneous
SD = standard deviation

Tα1 = thymalfasin
TF5 = thymic fraction five
TIW = three times per week

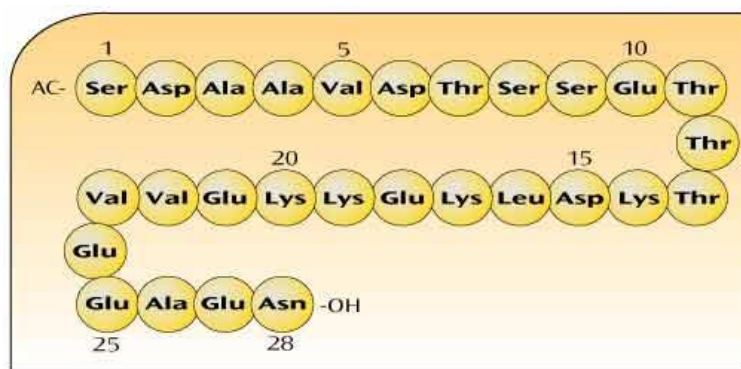
WHV = woodchuck hepatitis virus

ZDX = Zadaxin

Chemical Properties of Zadaxin

Zadaxin (thymalfasin), originally isolated as a natural substance from thymus tissue, is a pure, synthetic amino-terminal acylated peptide of 28 amino acids (molecular weight 3108; Figure 1). Some early studies utilized a partially purified thymic preparation (thymic fraction 5 or TF5) that contained about 1% thymalfasin; (1,2) however, most studies have utilized synthetic preparations of Zadaxin made by solid phase peptide synthesis. (3)

Figure 1. Primary structure of thymalfasin



Endogenous thymalfasin can be detected in serum, where levels measured in healthy adults by immunoassays are in the 0.1 to 1 ng/mL range. (4-7) The circulating concentration of thymalfasin tends to be lower in diseased individuals and higher during pregnancy. (8-11) The source and mechanisms of release and regulation of circulating thymalfasin are unknown. Thymalfasin is contained in the sequence of prothymosin, a 126-amino-acid peptide that is found in the cell nucleus (12-16), and that has been examined in terms of potential effects on cell proliferation. (17-19) Thymalfasin, found in highest concentrations in the thymus, has also been found in spleen, lung, kidney, brain, blood, and a number of other tissues.

Thymalfasin has amino-acid sequence homology with interferon alpha ($IFN\alpha$) and members of the glucagon-vasoactive intestinal peptide (VIP)-secretin family of peptides. Although binding of high concentrations of thymalfasin to VIP receptors has been reported (20,21) and thymalfasin has been found to weakly stimulate adenylate cyclase activity, the receptors for thymalfasin are not known. It is possible that thymalfasin has intracellular receptors, as it can fold into a structured helix in organic solvents and thus may cross the membrane unassisted. (22)

ZADAXIN — Dual Mechanism of Action

Zadaxin has a number of immunomodulatory activities, as well as direct influences on virally infected or cancerous cells. This dual mechanism of action is summarized in [Figure 2](#) and discussed below.

Immunomodulatory Action

Zadaxin stimulates stem cells and increases production of NK, CD4, and CD8 cells. Zadaxin stimulates stem cells to produce increased numbers of mature T cells. The addition of Zadaxin to human CD34 stem cells in culture increased thymopoiesis, resulting in an increase in the number of total CD3 T cells and synthesis of interleukin-7 (IL-7), a cytokine critical for maturation of thymocytes. The predominant subpopulation increased by Zadaxin was helper T cells (CD4). (23)

Zadaxin can enhance production of CD3, CD4, and CD8 cells in patients with chronic hepatitis B24 and cancer, (25) in mice infected with influenza A virus, (26) and in mice immunodepressed by hydrocortisone treatment. (27)

Zadaxin increases NK-cell activity in multiple animal models, (28-31) normal human subjects, (32) and HIV-infected patients. (33) This effect may be important for combating viral infection, as hepatitis C infection has been shown to decrease NK activity. (34)

Zadaxin increases production of Th1 cytokines. Zadaxin can increase production of IFN γ , IL-2, IL-3, and expression of the IL-2 receptor following activation by mitogens or antigens. (24,32,35-39) This pattern of enhanced cytokine production, i.e., IFN γ and IL-2, demonstrates that Zadaxin promotes a Th1 type of immune response. This is important in light of the fact that a Th1 response is associated with a vigorous antiviral response, while a primarily Th2 response is associated with persistence of these infections. (40) In fact, release of Th2 cytokines may be a viral mechanism for avoiding immune surveillance. (41)

In peripheral blood mononuclear cells (PBMCs) from patients with chronic hepatitis C (HCV) infection, Zadaxin induced a significant increase in the production of IL-2. (42) An increase in IL-2 was also seen after treatment of these cells with IFN α , but the increase due to Zadaxin was significantly greater. In fact, incubation of the PBMCs with a combination of IFN α and Zadaxin lead to an additive or even synergistic effect on synthesis of IL-2. Importantly, this study demonstrated that treatment with Zadaxin also leads to a decrease in the Th2 cytokines IL-4 and IL-10, whereas IFN α increased production of these cytokines. Thus, in combination treatment with IFN α , Zadaxin can benefit virally infected patients in two ways: first, by increasing T-cell subsets fundamental for sustained clearance of HCV and, second, by blocking the IFN α -induced Th2 response.

Zadaxin decreases T-cell apoptosis. Zadaxin can antagonize dexamethasone-induced apoptosis in thymocytes in vitro in a dose-dependent fashion. (43, 44) The effects were most pronounced on CD4 and CD8 double positive immature T cells, and treatment with Zadaxin stimulated production of cAMP and activated protein kinase C, suggesting an involvement of these second messenger pathways.

Apoptosis of thymocytes stimulated by serum from tumor-bearing mice was also decreased by treatment with Zadaxin, (45) and in this model system, there was a concomitant decrease in expression of the proapoptotic genes fas, bad, and bax, and an increase in the antiapoptotic gene bcl-2.

Zadaxin has also been used successfully to treat a child with DiGeorge anomaly, (46) a rare congenital disorder characterized by the absence or hypoplasia of the thymus resulting in varying degrees of T-cell immunodeficiency. Prior to treatment with Zadaxin, the patient showed increased lymphocyte apoptosis (increased fas and fas ligand and decreased bcl-2 in both CD4 and CD8 cells) compared to a control subject. After 3 months of treatment with Zadaxin, the proportion of lymphocytes undergoing apoptosis decreased. T-cell responses and B-cell function also improved after treatment, and there was a marked clinical improvement.

Direct Antiviral Actions

Zadaxin increases MHC class 1 expression. Zadaxin has been recently shown to increase expression of MHC class 1 in cultured cells. (47) As both virus infection and cancer are associated with a decrease in expression of the MHC class 1 antigen, (48, 49) and hence a decreased ability of the immune system to recognize those infections or cancers, this represents another mechanism by which Zadaxin can clear these defective cells.

Although IFN α can also lead to an increase in MHC class 1 expression, it was demonstrated in this study that the effect of Zadaxin occurred by a separate pathway from that of IFN α , hence supporting that these two immunomodulatory molecules, Zadaxin and IFN α , act through different mechanisms.

The increase in MHC class 1 expression was found to be due to an effect of Zadaxin on expression of the transcriptional activator NF κ B. Interestingly, this effect on expression of NF κ B could explain the pleiotropic effects of Zadaxin, as this molecule is involved in many pathways of Zadaxin action, including stimulation of Th1 cytokines, inhibition of apoptosis, and inhibition of viral replication. The increase in intracellular NF κ B could thus be an important basis for the mechanism of action of Zadaxin.

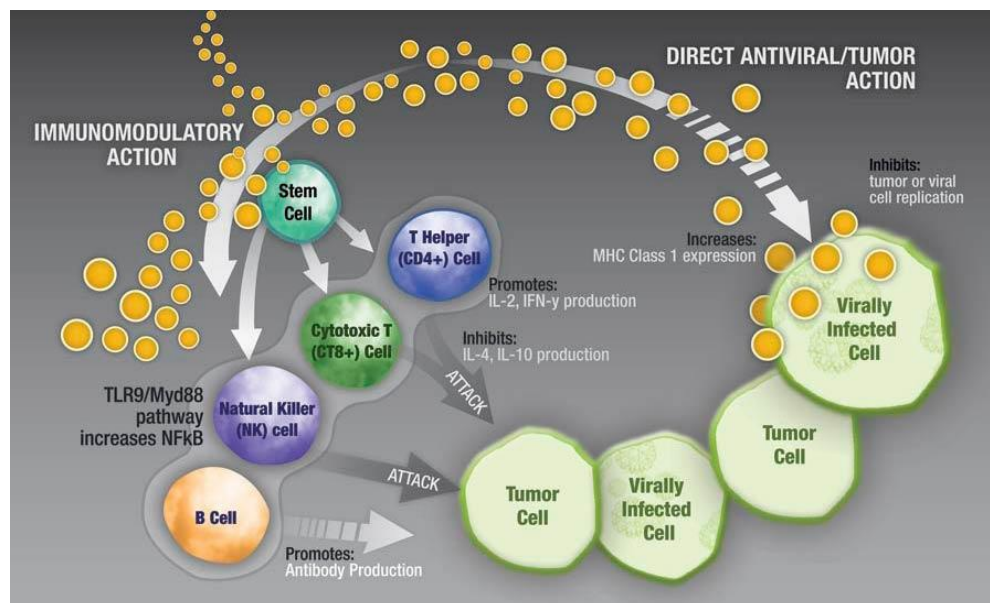
Zadaxin inhibits viral replication. In duck hepatocytes infected with duck hepatitis B virus, Zadaxin treatment significantly reduced viral replication, especially at the level of expression of intrahepatic viral proteins. (50) This demonstrates that Zadaxin acts to inhibit viral replication at a posttranslational step, which is distinct from the antiviral effects of IFN α and nucleoside analogs. Zadaxin treatment leads to a significant dose-responsive decrease in viral growth in various model systems including the HIV-1 virus (data on file) and parainfluenza (Sendai) viruses. (51)

In addition to these direct antiviral effects, Zadaxin has also been reported to inhibit in vitro growth of various non-small-cell lung cancer cell lines. Zadaxin has also been shown to inhibit growth of cancerous cells in vivo, preventing lung adenoma formation in mice (52-54) and breast cancer in rats. (55)

Zadaxin decreases oxidative stress. Zadaxin treatment can lead to increased intracellular glutathione levels under conditions of oxidative stress, both in virally infected cells (51) and in lymphocytes stimulated to undergo apoptosis. (43) These effects are important direct actions of Zadaxin, as oxidative stress and a decrease in glutathione concentrations are hallmarks of viral infection both in vitro and in vivo. (56,57) In fact, restoration of glutathione levels has been shown to dramatically decrease viral replication (58,59) and improve the clinical condition. (60,61)

Given its immunoregulatory properties, Zadaxin has been considered for use in treatment of many of the same disorders for which other immunomodulators are employed. Zadaxin has been investigated in humans for treatment of infectious diseases (hepatitis B, hepatitis C, acquired immune deficiency syndrome), as a vaccine enhancement agent, and for several cancers. Major preclinical and clinical studies are briefly summarized in the subsequent sections, followed by a summary of the safety and pharmacokinetic profile of Zadaxin.

Figure 2. Proposed mechanism of action of Zadaxin. Zadaxin is able to fight disease by stimulating the immune system. Its effects include stimulation of natural killer (NK) cells and cytotoxic lymphocytes (CD8), which directly kill virally infected or cancerous cells. Zadaxin also increases the production of cytokines such as IFN γ and IL-2, and increases the Th1 subset of CD4 cells. Recent evidence has shown that Zadaxin also has direct effect on cells that lead to a decrease in viral replication or cancer-cell growth. Zadaxin increases the expression of surface-marker proteins (MHC-1) on infected cells, providing the immune system with a target for the detection and destruction of diseased cells.



Use of Zadaxin in Disease States

Given its immunoregulatory properties, Zadaxin has been considered for use in treatment of many of the same disorders for which other immunomodulators are employed. Zadaxin has been investigated in humans for treatment of infectious diseases (hepatitis B, hepatitis C, acquired immune deficiency syndrome), as a vaccine enhancement agent, and for several cancers. Major preclinical and clinical studies are briefly summarized in the subsequent sections, followed by a summary of the safety and pharmacokinetic profile of Zadaxin.

- Animal Models
- Hepatitis B
- Hepatitis C
- Human Immunodeficiency Virus
- Vaccine Enhancer in Immunocimpromised Patients
- Cancer

Animal Models

Woodchuck Hepatitis

Woodchuck hepatitis virus (WHV) is closely related to the human hepatitis B virus (HBV), and WHV infection in woodchucks is an animal model for chronic HBV infection in humans. Data is available on effects of Zadaxin from three completed studies with this experimental model.

Study 1 (62)

- 6 WHV chronically infected animals received Zadaxin (10 µg/kg) BIW for 30 weeks
- 6 age- and sex-matched infected animals were used as untreated controls

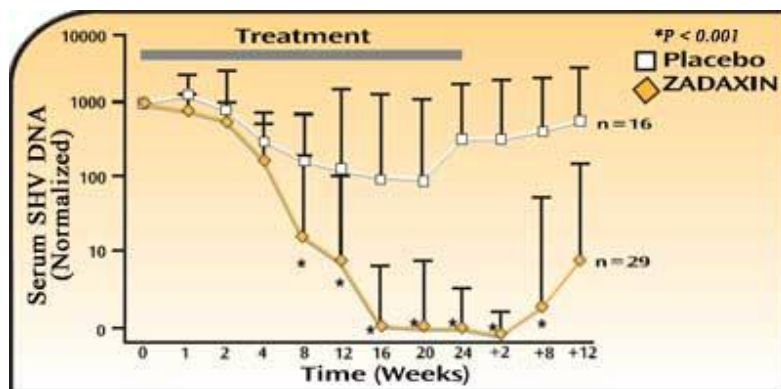
End-of-treatment results:

- Serum WHV DNA levels fell by 99% in all Zadaxin-treated animals
- No changes were observed in serum WHV DNA in the 6 untreated controls

The effect was transient, however, and serum WHV DNA returned to pretreatment levels in the follow-up period.

Figure 3 shows, by logarithmic scale, mean serum levels of WHV DNA for the Zadaxin (10 µg/kg) and placebo-treated animals. These results are similar to those obtained with IFN α . (64)

Figure 3. Serum woodchuck hepatitis virus (WHV) DNA in Zadaxin-treated and placebo-treated animals. Zadaxin was given biweekly for 24 weeks, with 12 weeks follow-up. Zadaxin treatment period is indicated by a solid line at the top of the figure. The figure shows that 6 months of treatment with Zadaxin significantly decreases serum WHV DNA compared with placebo-treated animals. (63)



Study 2 (63)

- 24 WHV chronically infected animals received Zadaxin (900 µg/m², 23 µg/kg, or 10 µg/kg) BIW for 30 weeks
- 12 age- and sex-matched infected animals were used as controls

End-of-treatment results:

- Serum WHV DNA levels fell by 99% to 99.9% in all Zadaxin-treated groups
- No changes were observed in serum WHV DNA in control animals

Study 3 (65)

- 20 WHV chronically infected animals received Zadaxin (900 µg/m²) BIW for 3 years
- 20 age- and sex-matched infected animals were used as placebo controls

End-of-treatment results:

- Serum WHV DNA in treated animals was significantly less than in placebo-treated animals 1 month after beginning treatment ($P < 0.001$)
- Serum WHV DNA level remained significantly lower than placebo controls throughout the remainder of the 152-week treatment
- After 48 weeks, 2 placebo animals died of hepatocellular carcinoma (HCC), while there were no deaths in the Zadaxin-treated animals

Animal Models

Other Animal Models of Infection

Table 1: Effect of Combination Therapy with Zadaxin (ZDX), Amantadine (AMN), and IFN α/β in Mice

Group	Survivors (%)	Viral titer (% Inhibition)	NK-cell activity (Lytic units)	CTL (% Specific lysis)	CD4*	CD8*
Infected Control	12 (20%)	---	2.1 \pm 0.7	6.1 \pm 0.8	14.9 \pm 1.7	9.9 \pm 1.8
AMN**	18 (30%)	57	2.2 \pm 0.2	9.3 \pm 2.1§	18.2 \pm 1.5	11.3 \pm 2.0
ZDX	12 (20%)	---	2.0 \pm 0.6		19.4 \pm 1.8	11.5 \pm 2.1
IFN	15 (25%)	---	2.6 \pm 0.7		17.1 \pm 0.9	11.8 \pm 1.6
AMN+IFN	18 (30%)	50	2.4 \pm 0.4		18.3 \pm 1.4	10.9 \pm 1.2
ZDX+AMN	12 (20%)	47	2.6 \pm 0.3		18.5 \pm 1.6	11.4 \pm 1.9
ZDX+IFN	15 (25%)	31	3.0 \pm 0.7	8.2 \pm 1.3	21.3 \pm 1.8	12.1 \pm 2.0
ZDX+AMN+IFN	36 (60%)†	98‡	9.4 \pm 2.7†	18.2 \pm 1.7†	28.3 \pm 2.4§	15.2 \pm 1.7§

Infected with Influenza A Virus. N = 60 mice. (26)

* Mean number of positive cells \pm S.E. per million spleen lymphocytes

** Amantadine

† P < 0.001 against all other groups

‡ P < 0.01 against all other groups

§ P < 0.05 against all other groups

- After 96 weeks, there were 6 deaths in the placebo group due to HCC and 3 in the Zadaxin treated animals
- There was no evidence of clinical, biochemical, or hematological toxicity in the entire 152-week treatment period

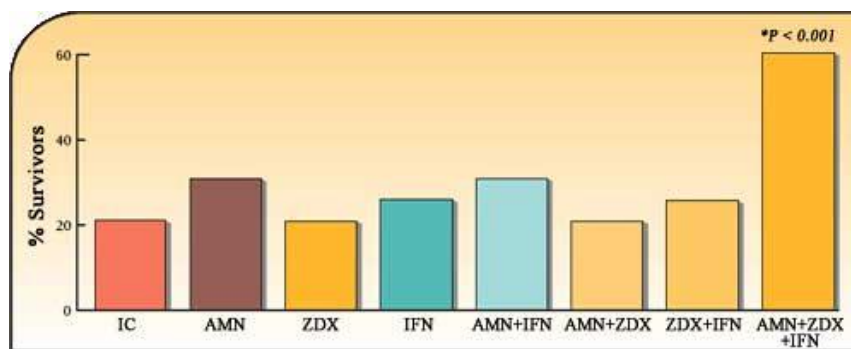
Several studies investigating the effect of Zadaxin on mice infected with various pathogenic organisms have shown beneficial effects of Zadaxin.

- Zadaxin treatment provided statistically significant protection against lethal infections with *Listeria monocytogenes*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Serratia marcescens* in mice immunosuppressed with 5-fluorouracil (5-FU). (66)
- Zadaxin increased the survival of aged or immunosuppressed mice infected with herpes simplex virus (67) or influenza. (68)
- Zadaxin prolonged survival of mice infected with *C. albicans* and prevented the increased susceptibility to infection by cyclophosphamide treatment. (69)
- Zadaxin in combination with fluconazole increased survival of mice infected with *C. albicans* and immune suppressed by morphine treatment. (70)

The beneficial effects of Zadaxin have been augmented in some animal models by adding other cytokines.

- Combination therapy of hydrocortisone-treated, aged mice with Zadaxin and interleukins augmented the *in vitro* lymphocyte response to interleukins and mitogens as compared with Zadaxin or interleukin treatment alone. (27,71)
- In mice inoculated with influenza A virus and treated with Zadaxin in combination with IFN α/β and amantadine (an antiviral agent), statistically significant increases in survival, NK-cell activity, CD4 and CD8 counts, and cytotoxic activity in the lung (CTL response) were observed, while viral titers in the lung were reduced (Table 1, Figure 4). (26)

Figure 4. ZDX in combination with AMN and IFN α/β increases survival mice infected with influenza A virus. AMN = amantadine, IC = infected control. *P < 0.001 against other groups. (26)



Animal Models

Animal Models of Cancer

Zadaxin has been shown to have beneficial effects in several experimental models of cancer. Recently, Zadaxin treatment has been shown to prevent lung carcinogenesis in mice injected with a chemical carcinogen (52-54) and breast cancer in rats. (55)

Zadaxin, given in combination with chemotherapy and IL-2 or interferon:

- Increased the cytotoxic response of T cells and NK-cell activity
- Reduced tumor size
- Increased survival in many animal models of cancer
 - DHD/K12 colon carcinoma (72,77)
 - B-16 melanoma (29,78)
 - Non-small-cell lung cancer (53)
 - Lewis lung carcinoma (73,98)
 - Friend erythroleukemia (74)
 - P388 or L1210 leukemia (75)
 - Methylcholanthrene-induced fibrosarcoma (76)

Zadaxin treatment shows synergy with IL-2 or IFN α/β :

- Syngeneic BDIX rats with liver metastases from colorectal cancers induced by splenic injection of DHD/K12 cells (1,2-dimethylhydrazine-induced colon carcinoma) (72)
 - Greatly reduced growth of liver metastases
 - Reduced liver invasion (20% vs 62% in controls)
 - Reduced extrahepatic spread
 - Improved median survival time (70.0 ± 8.2 days vs 48.5 ± 8.5 days in controls)
 - Effect not seen with 5-FU alone or in combination with IL-2
 - $P < 0.000$

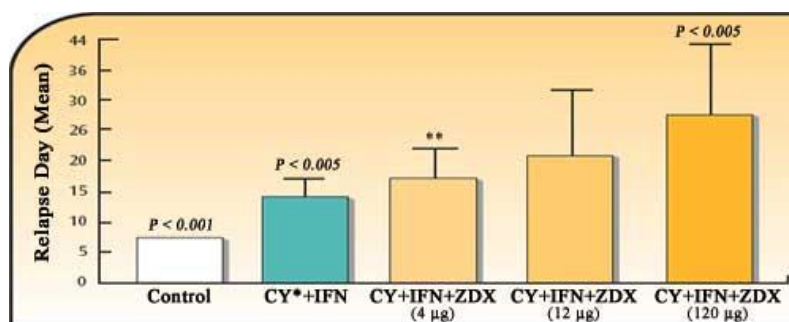
The improvement of survival time in these rats from the use of triple combination therapy allowed for a second cycle of treatment to be provided. (77) This led to:

- Further significant increase in survival time (81% survival at 100 days versus 39% with 5-FU alone or 44% with 5-FU plus IL-2)
- Long-term survival in 2 of the 21 rats treated for two cycles of triple therapy
 - No evidence of disease when sacrificed 5 months after therapy for histologic evaluation
- Significant improvements to parameters of the immune system — compared with controls, increased absolute numbers of peripheral T cells expressing
 - IL-2 receptors
 - CD4
 - CD8

More recent studies with a mouse model of melanoma showed that combination of increasing doses of Zadaxin with IFN α/β and chemotherapy significantly increased time to relapse (Figure 5), decreased the tumor growth rate (Figure 6), and improved survival in a dose dependent fashion. (78) Significantly, the addition of a single cycle of Zadaxin treatment led to a cure in 24% of the mice (5 of 21 animals were alive and disease free 1 year after treatment).

As in other studies investigating the effects of Zadaxin treatment in animal models of cancer, immune parameters were improved. Splenocytes from treated mice showed markedly increased cytotoxic activities against both YAC-1 and autologous B16 tumor cells, and the tumor-induced reduction in percentages of CD3 and CD4 cells was reversed to nontumor levels. (78)

Figure 5. Relapse time of B16 melanoma bearing mice treated with CY, IFN, and ZDX. The figure shows that ZDX increases relapse time of mice with B16 melanoma. Increasing doses of ZDX (up to 120 μg) show the greatest effect on relapse in this model. (78)

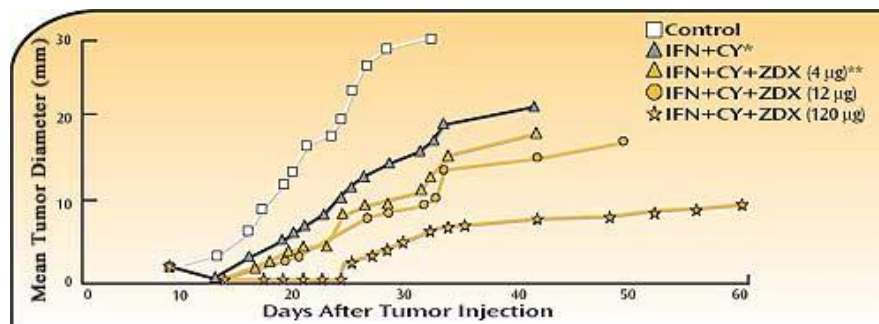


Values are mean \pm S.D.; n = 10

* CY = cyclophosphamide

** Equivalent to human dose

Figure 6. Effect of increasing doses of Zadaxin on tumor reduction in murine B16 melanoma. The figure shows that Zadaxin decreases tumor diameter in mice with B16 melanoma. Increasing doses. Zadaxin (up to 120 μg) increases the effect on tumor shrinkage in this model. (78 of)



* CY = cyclophosphamide

** Equivalent to human dose

Hepatitis B

Chronic hepatitis B (CHB) is a widespread disease associated with significant morbidity and mortality. According to the World Health Organization, more than 350 million people worldwide are chronically infected with the hepatitis B virus (HBV), with carrier rates as high as 20% in some populations. CHB is associated with increased risk for developing cirrhosis, liver failure, and hepatocellular carcinoma. Impaired effectiveness of the host cellular immune mechanisms in clearing HBV-infected hepatocytes has been proposed to explain development of chronic HBV infection.

Hepatitis B

Hepatitis B Treatment Options

Interferon alpha (IFN α) was the first therapy approved for treatment of CHB in the United States and Europe. Initial enthusiasm for this therapy has waned with the realization that response rates are low and relapses are common. Interferon therapy is also associated with significant side effects that can lead to reduction in dose and discontinuation of treatment. In phase 3 trials using IFN α -2b for CHB, 98% of the patients treated at 5 MIU QD, and 90% of the patients treated at 10 MIU QD experienced adverse reactions, with 21% to 44% severe. (79) Side-effect management consists of reducing or discontinuing interferon therapy — in these trials, 25% to 38% of patients interrupted treatment due to side effects. (79) The continued postmarket monitoring of IFN α therapy has revealed new toxicities. (80) The most common adverse events associated with IFN α therapy include the following:

- Flu-like symptoms
- Fatigue
- Anorexia
- Central nervous system reactions
- Psychiatric reactions

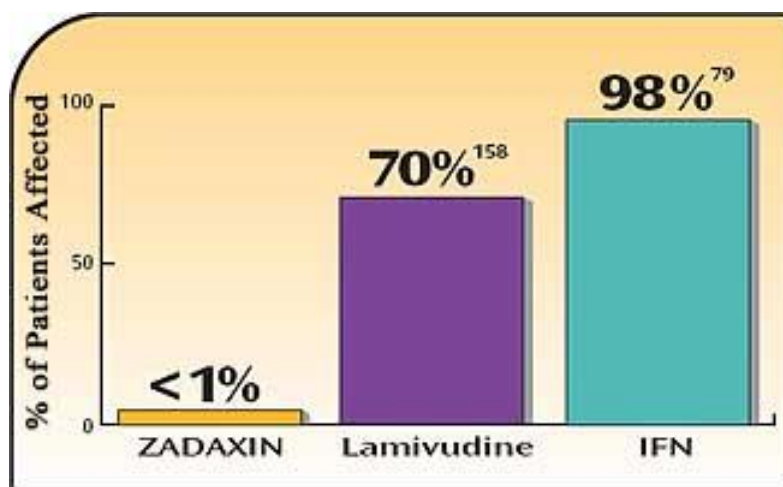
The incidence of depression and suicidal behavior has only recently been fully appreciated, with a significant increase in depression during the sixth month of interferon therapy. (81)

Nucleoside Analogs

Lamivudine has recently been approved in the United States and Europe as a treatment for CHB; famciclovir, adefovir, and entecavir are still under investigation as treatment for CHB. Nucleoside analogs effectively reduce HBV DNA and ALT while the CHB patient is on therapy. However, when therapy with these nucleoside analogs is discontinued, HBV DNA and ALT usually return to pretreatment levels.

In an attempt to produce sustained responses with these drugs, therapy has been continued for 12 months or longer. Alarming, such prolonged therapy is leading increasingly to the selection of HBV mutants that are resistant to these drugs. Lamivudine-treated patients develop lamivudine-resistant mutants at a cumulative rate of approximately 15% to 20% per treatment year. (82) In rare patients, discontinuation of nucleoside analog therapy has also resulted in a flare of the HBV hepatitis with fulminant hepatitis and death. (83) Zadaxin use has not been associated with these problems (Figure 7).

Figure 7. Comparative adverse-event incidence.



Zadaxin

Interest in using Zadaxin for treatment of human hepatitis B was based on the fact that it is another type of immunomodulator, which can trigger maturational events in lymphocytes, augment T-cell function, and promote reconstitution of immune defects. Zadaxin, like interferon, was also effective in the woodchuck hepatitis model for CHB. In clinical studies for CHB, Zadaxin has been primarily investigated as monotherapy, but promising results have also been obtained when Zadaxin is used as an element in combination therapy.

In addition, Zadaxin has an excellent safety record. In treatment of more than 3,000 patients with a range of diseases including hepatitis B and hepatitis C, Zadaxin has been well tolerated and is not associated with any significant side effects. Zadaxin has been administered without adverse incident to elderly subjects (up to 101), children (as young as 13 months), and immunodeficient patients. (84) Patients with decompensated liver disease and renal disease requiring hemodialysis show good tolerance to Zadaxin administration. (85)

Hepatitis B

Zadaxin Monotherapy in Hepatitis B

Several randomized controlled studies have investigated the safety and efficacy of Zadaxin monotherapy for the treatment of CHB. [Table 2](#) presents a summary of results from selected studies.

These studies show that Zadaxin promotes disease remission in 26% to 41% of the patients treated.

A recent, independent meta-analysis of 435 patients entered into randomized, controlled trials of Zadaxin monotherapy for CHB demonstrated a statistically significant benefit in favor of Zadaxin therapy, inducing a sustained virological response over placebo (odds ratio, OR = 2.87; 95% CI 1.58 – 5.22; P = 0.0005). (86) The same study also demonstrated a trend in favor of Zadaxin compared with IFN α for sustained virological response (OR = 2.62; 95% CI 0.80 – 8.56).

Taiwan Trial

This randomized, open-label, controlled, 18-month study was conducted at the Chang Gung Memorial Hospital.

Patients and protocol:

- 32 patients randomized to 6 months treatment with Zadaxin 1.6 mg SC TIW; 12 months follow-up
- 34 patients randomized to 12 months treatment with Zadaxin 1.6 mg SC TIW; 6 months follow-up
- 32 patients randomized to 18 months observation without specific treatment
- Baseline HBV DNA (mean)
 - 122 pg/mL (6-month Zadaxin)
 - 138 pg/mL (12-month Zadaxin)
 - 87 pg/mL (control)
- Baseline ALT (mean)
 - 156 (6-month Zadaxin)
 - 138 (12-month Zadaxin)
 - 140 (control)
- Complete response prospectively defined as clearance of both HBV DNA and HBeAg at 18 months

Table 2: Hepatitis B Clinical Outcomes (Intent to Treat) §.

Group	Study Country	# Patients Enrolled	Clinical Outcome*	
Randomized, placebo-controlled double-blind (phase 2)	United States	20	ZDX: Placebo: P < 0.04	83% 25%
Multicenter, randomized, placebo-controlled double-blind (phase 3)	United States	99	ZDX: Placebo: P < 0.11	24% 12%
Multicenter, randomized, controlled open-label (phase 3) IFN	Taiwan 15 (25%)	158	ZDX (6 mo Rx): ZDX (12 mo Rx): Control:	36% 27% 25%
		98†	ZDX (6 mo Rx): ZDX (12 mo Rx): Control: P < 0.004 ZDX (6 mo Rx) vs Control	41% 38% 9%
Multicenter, randomized, controlled (phase 3)	Italy	33	ZDX: IFN α 2b: Control‡: P = 0.025 ZDX vs Control	41% 25% 7%
Meta-analysis §	United States, Taiwan, Italy	223	ZDX (6 mo Rx): Control: P = 0.04	36% 19%

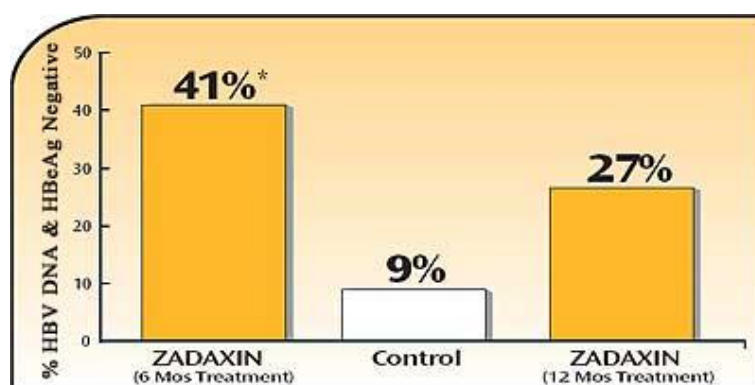
* End of follow-up or extended follow-up

† Data from the Chang Gung Memorial Hospital, the major center for the Taiwan trial. Twelve months treatment plus twelve months follow-up period

‡ Historical control matched for age, sex, biochemical, histological, and serological parameters

§ Meta-analysis performed by MetaWorks, Inc. (Boston, Massachusetts); includes all patients in the four studies

Figure 8. Results at Chang Gung Memorial Hospital, Taiwan hepatitis B trial at 18 months. The figure shows data for the 6-month treatment group, the 12-month treatment group, and the control group at 18 months from the start of the trial for patients treated at the Chang Gung Memorial Hospital. *P = 0.004, Zadaxin monotherapy (6 months treatment) vs untreated control. (87)



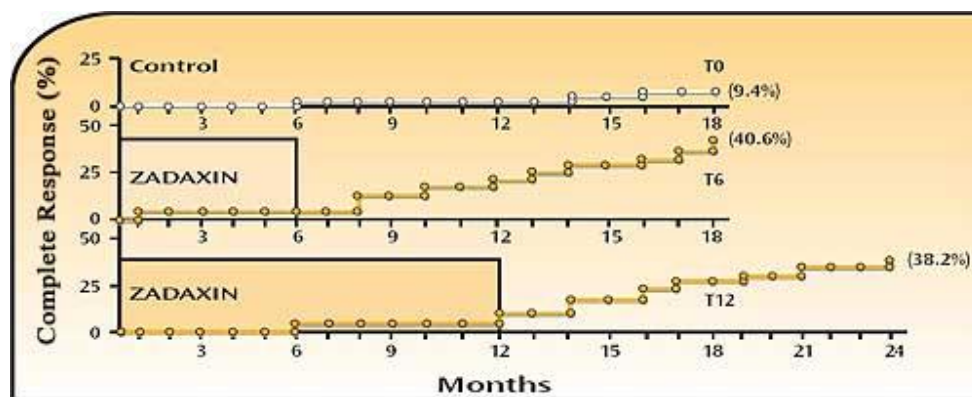
Results:

- 6-month treatment group complete response rate: 41% (13/32) (P = 0.004 compared with control)
- 12-month treatment group complete response rate: 26% (9/34)
- Control group complete response rate: 9%

The 34 patients in the 12-month treatment group were evaluated again at the end of 24 months, ie, 12 months after their Zadaxin treatment had ended. In the 6-month period since the previous follow-up, the number of responders rose from 9 (27%) to 13 (38%).

Figure 9 shows the time course of complete response, including the extended data from the 12-month treatment group. As shown, the percentage of patients reporting both negative HBV DNA and HBeAg in both treatment arms continued to rise after treatment ended, compared with a relatively flat spontaneous remission in the control arm. The increase in virologic response after treatment was significant (P = 0.014). (87)

Figure 9. Time course of complete response in the Chang Gung Memorial Hospital, Taiwan. The percentage of patients reporting both negative HBV DNA and HBeAg in both treatment arms continued to rise after treatment was completed, compared with a relatively flat spontaneous remission in the control arm ($P = 0.014$). This delayed response is typical of CHB patients treated with Zadaxin. The top portion of the figure (T0) shows the control group, the middle portion (T6) shows the 6-month Zadaxin treatment group, and the bottom portion (T12) shows the 12-month Zadaxin treatment group including extended follow-up data for this treatment group. Darkened areas represent the time of Zadaxin administration for each treatment group. (87)



These results reflect an emerging pattern of Zadaxin efficacy that was observed in earlier studies: Response rates increase and are sustained after treatment has ended. The mechanism for this is not fully understood but may be a consequence of immune-system activation during therapy. This pattern is in contrast to that observed with $IFN\alpha$ therapy in CHB, in which treatment responses generally occur during the first 4 months of therapy and relapse over time.

Phase 3 Italian trial (Zadaxin vs $IFN\alpha$ -2b). This multicenter, randomized, controlled trial tested Zadaxin versus $IFN\alpha$ -2b in difficult-to-treat patients infected with the hepatitis B precore mutant virus, who are characterized by being HBeAg negative, HBV DNA positive.

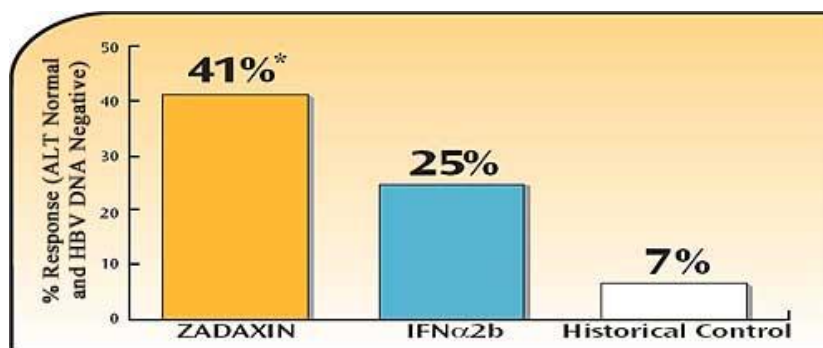
Phase 3 Italian trial (Zadaxin vs $IFN\alpha$ -2b)

This multicenter, randomized, controlled trial tested Zadaxin versus $IFN\alpha$ -2b in difficult-to-treat patients infected with the hepatitis B precore mutant virus, who are characterized by being HBeAg negative, HBV DNA positive.

Patients and protocol:

- 33 patients with CHB (presence of HBsAg in serum for at least 12 months, HBV DNA and HBe antibody positive, and ALT levels 1.5 times the normal upper limit for at least 12 months)
- Both groups comparable with regard to age, gender, serological, biochemical, and histological parameters including Knodell score
- 16 patients received IFN α -2b (5 MIU, TIW)
 - Mean ALT = 182
- 17 patients received Zadaxin (0.9 mg/m², BIW)
 - Mean ALT = 142
- Retrospective comparison group: 15 untreated historical controls followed for 12 months
 - Comparable to the treated groups with respect to age, sex, and biochemical, histological, and serological parameters
- 6-month treatment
- 6-month follow-up
- Complete response defined by ALT normalization and HBV DNA loss after 6-month follow-up period

Figure 10. Results from the Italian hepatitis B trial at 12 months. Patients were treated for 6 months and followed for 6 months. At the end of followup, 25% of patients in the IFN α -2b group and 41% of the patients in the Zadaxin group had cleared HBV DNA and normalized ALT. The Zadaxin results are significant (*P < 0.05, Fisher's Exact Test) when compared with a matched group of untreated patients (historical control; 7%). In contrast to IFN α -2b, Zadaxin was better tolerated and was not associated with adverse side effects. (88)



Results:

- Zadaxin-treated group
 - Complete response in 41% (7/17)
 - Significantly higher than untreated group (P < 0.05)
 - 58% histological improvement after 6-month follow-up
 - 25% were unchanged after 6-month follow-up
 - 17% worsened after 6-month follow-up
 - No associated side effects

- IFN α -2b-treated group
 - Complete response in 25% (4/16)
 - Insignificant relative to untreated controls
 - 36% improvement in liver histology after 6-month follow-up
 - 36% unchanged after 6-month follow-up
 - 27% worsened after 6-month follow-up
- Untreated patients
 - Complete response in 7% (1/15)

The data from this trial illustrate an important apparent difference between Zadaxin and interferon treatment. With interferon, responses are typically seen during the first 4 months of therapy, with frequent relapses in the follow-up period. In this trial, the response rate in the IFN α -2b group declined from 44% at the end of 6-month treatment to 25% at the end of 6-month follow-up. With Zadaxin, responses continued to occur after treatment was stopped (29% at the end of 6-month treatment and 41% at the end of 6-month follow-up). This delayed response to Zadaxin treatment has been seen in other hepatitis B studies. (87,89)

While interferon is known to promote a quick response during treatment, relapses are common. (90-92) Treatment of hepatitis B with IFN α typically results in rapid and significant inhibition of viral replication during the treatment phase, but resistant viral variants or residual viruses rebound after therapy is withdrawn and the patient relapses. (90) Zadaxin, on the other hand, appears to work more slowly than interferon, perhaps by long-term enhancement of the cellular immune system and, thus, provides a greater longterm therapeutic value.

It is likely that Zadaxin is effective in Mediterranean, Asian, and Caucasian patients, since available data include all of these populations and the trend in all studies is similar. Zadaxin also has a good response rate in both HBeAg+ and HBeAg- patients. (87,88) With IFN α treatment, however, Asian patients, HBeAg- or HBeAg+ patients, children, immunodeficient patients, and highly viremic patients are less likely to respond. (93) In a randomized controlled trial of chronic hepatitis B in Chinese adults, 15% of the patients had sustained clearance of HBeAg after a 6-month course of IFN α - 2b.⁹⁴ Another trial in Chinese chronic hepatitis B patients showed a response rate, including partial responses, of 12.5% in IFN-treated patients. (95)

The experience with IFN α in Taiwan in chronic hepatitis B is similar, with up to 24% response to IFN α therapy. (96) Interferon response is better in Chinese patients with elevated ALT and comes closer to the 36% typical response rate seen with Zadaxin. (95) Patients with HBeAb have shown 60% to 80% relapse of viremia and disease during follow-up after treatment with IFN α . (93,97)

Hepatitis B

Zadaxin Plus IFN α Combination Therapy for Chronic Hepatitis B

Although Zadaxin is an effective monotherapy for CHB, a number of studies suggest that Zadaxin can work in synergy with other immune modulators or antiviral agents.

- In cell culture, Zadaxin combined with IFN α enhanced NK activity in human peripheral blood lymphocytes and purified large granular lymphocytes to a greater extent than that which would have resulted from an additive effect alone (32)
- In animal studies, Zadaxin in combination with IFN α increased NK activity in immunosuppressed mice (28,29) and had greater antiviral and antitumor activity than that which would have resulted from an additive effect alone (26,74,78,98)

As discussed below, Zadaxin works in synergy with IFN α for treating hepatitis C. Therefore, human trials to improve the effectiveness of other CHB monotherapies by the addition of Zadaxin are being explored. Moreover, because of its excellent safety profile, Zadaxin may be combined with other therapies such as IFN α or nucleoside analogs to enhance their efficacy without increasing their toxicity.

Phase 2 Italy trial (IFN α plus Zadaxin)

This open label study tested the combination of low-dose lympho-blastoid IFN α (L-IFN α) and Zadaxin. (99)

Patients and protocol:

- 15 patients with CHB
 - HBsAg positive
 - HBV DNA positive
 - ALT levels > 1.5 times normal
- 11 treatment failures on standard IFN α -2b therapy
- 4 previously untreated patients
- Zadaxin (1 mg) subcutaneously on 4 consecutive days, L-IFN α (3 MIU) intramuscularly on the fourth day
- Subsequently, Zadaxin and L-IFN α biweekly for 26 weeks
- 12-month follow-up
- Response defined as loss of HBV DNA and normalization of ALT at 18 months.

Results:

- Overall sustained response of 60% (9/15)
- Disease remission in 55% (6/11) of previous IFN α -2b treatment failures
- No reactivation of disease in any sustained responders followed beyond follow-up period

It should be noted that in this trial, the dose of L-IFN α (3 MIU BIW) was a fraction of the total standard dose (15-30 MIU) usually given in 3 to 6 injections per week. These data suggest that responses of patients to the Zadaxin plus IFN α combination may be higher than has been the general experience with either Zadaxin or IFN α monotherapy. The results in patients who were previous interferon failures are particularly striking, in light of the fact that historical retreatment response rate with a second course of IFN α would be expected to be no better than 10%. These results are consistent with studies conducted in vitro and in animals (discussed above) that suggest that Zadaxin acts in synergy with other immune modulators.

Phase 2 Turkish trial (Zadaxin and IFN α -2b)

This trial compared IFN α -2b monotherapy with Zadaxin plus IFN α -2b combination therapy. (100)

Patients and protocol:

- 31 patients with CHB
 - Treatment naïve
 - Anti-HBe positive
 - HBV DNA positive
- Group 1
 - 21 patients
 - Weeks 1 to 26: 1.6 mg Zadaxin SC BIW + 10 MIU IFN α -2b SC TIW
 - Weeks 27 to 52: 10 MIU IFN α -2b SC TIW
 - 26-week follow-up
- Group 2
 - 10 patients
 - Weeks 1 to 52: 10 MIU IFN α -2b SC TIW
 - 26-week follow-up
- Endpoints
 - Normalization of ALT: weeks 52 and 78
 - HBV DNA negative: weeks 52 and 78
 - Improved liver histology: week 78

Results:

- Response at week 52
 - 87.7% HBV DNA negative with normal ALT: Zadaxin + IFN α -2b
 - 70% HBV DNA negative with normal ALT: IFN α -2b monotherapy
 - Response at week 78
- Response at week 78
 - 76.2% sustained response: Zadaxin + IFN α -2b
 - 40% sustained response: IFN α -2b monotherapy
 - P = 0.002

Hepatitis B

Zadaxin Plus Nucleoside Analogues Combination Treatment for Chronic Hepatitis B

In contrast to the disease in the West, the natural history of CHB in Asia is characterized by an initial active viral replicative state with minimal liver damage (immune tolerance phase). Immune-tolerant patients are usually asymptomatic and have normal or near-normal ALT. This phase is followed by an active immune clearance phase with chronic active hepatitis. However, most patients do not clear HBV DNA, which then becomes integrated into the host's genome. Many of these patients will eventually progress to develop cirrhosis and hepatocellular carcinoma (HCC).

The main aim of treatment is to suppress HBV replication before there is any significant irreversible liver disease. As most of the liver damage occurs during the immune clearance phase (when HBV replication is being suppressed spontaneously), it would be optimal to suppress HBV replication in the earlier, immune tolerant phase. Unfortunately, patients in the immune tolerant phase do not respond to current therapies, and an effective therapy remains elusive.

The response rate to interferon in Asian patients is low: Only 15% to 20% will clear HBeAg and HBV DNA, and most of these responders are already in the immune clearance phase and, thus, would have eventually spontaneously cleared their infection without any treatment. However, in immune-tolerant patients, response to interferon is even lower, with less than 5% of patients clearing HBV DNA. One of the factors that impair the antiviral effect of immunomodulatory agents is a high pretreatment HBV DNA level. Recently, second-generation nucleoside analogues such as lamivudine and famciclovir have been shown effective in suppressing HBV replication. It would, therefore, be logical to use a combination of immunomodulatory agents and second-generation nucleoside analogues in the treatment of CHB in the immune-tolerant phase.

Pilot Hong Kong study (Zadaxin plus famciclovir) (101)

This study was populated with patients whose ALT levels made them highly unlikely to undergo spontaneous seroconversion to negative HBV DNA and HBeAg.

Patients and protocol:

- 32 immune-tolerant adult Chinese patients with vertically transmitted CHB
 - ALT <2.5 times upper limit of normal
 - High HBV DNA load: HBV DNA titers > 4000 mEq/mL (Chiron Quantiplex bDNA assay)
- 6-month treatment
 - Zadaxin (1.6 mg SC BIW)
 - Famciclovir (500 mg TID)
- 12-month follow-up
- Complete virological response defined as disappearance of HBV DNA and HBeAg
- Significant improvement in liver histology characterized by improvement =2 points, Knodell hepatitis activity index (HAI)

Results:

- Virological and histological response evaluated
- 3 patients (9.09%) demonstrated a sustained complete virological response after treatment and follow-up
- 27.3% patients had significant improvement in liver histology
- Combination was well tolerated
- No side effects reported

These results are highly promising since immunetolerant patients usually do not respond to any available drug therapy.

Unfortunately, therapy with nucleoside analogues leads to the selection of HBV mutants that are resistant to these drugs. Lamivudine-treated patients develop lamivudine-resistant mutants at a cumulative rate of approximately 20% per treatment year. (82) HBV mutants are also induced by famciclovir therapy. However, famciclovir-induced HBV mutants do not show cross-resistance to lamivudine. Therefore, it may be possible that the use of a combination of famciclovir and lamivudine may decrease the risk of emergence of HBV mutants.

Pilot Hong Kong study (Zadaxin plus lamivudine plus famciclovir) (102)

Patients and protocol:

- 11 Chinese patients with vertically transmitted CHB
 - High levels of HBV DNA
- 12-month treatment period
 - Zadaxin (1.6 mg BIW for 6 months)
 - Lamivudine (100 mg/day for 12 months)
 - Famciclovir (500 mg TID for 12 months)
- 12-month post-treatment follow-up
- Complete virological response defined as disappearance of HBV DNA and HBeAg

Results:

- 64% (7/11) complete virological response
- ALT normalized in all patients
- 91% (10/11) HBV DNA negative
- Drugs were well tolerated
- No side effects noticed

These data support the hypothesis that a combination of Zadaxin and nucleoside analogues may be a safe and effective therapy for CHB, especially in difficult-to-treat cases such as patients with vertically transmitted diseases and nonresponders to previous therapy.

Pilot Turkish study (Zadaxin plus lamivudine in pediatric patients unresponsive to previous treatment). (103)

Patients and protocol:

- 10 pediatric patients with vertically transmitted CHB
- Nonresponders to previous IFN α or IFN α plus lamivudine
- 12-month treatment period
 - Zadaxin (1.6 mg/m² BIW for 6 months)
 - Lamivudine (3 mg/kg/day for 12 months)
- 12-month post treatment follow-up

Results:

- 70% (7/10) HBV DNA negative
- Drugs were well tolerated
- No side effects noticed

Key Takeaways

- Zadaxin monotherapy is at least as effective as IFN α in HBV and has more success in populations with low response rates to IFN α , eg, vertically transmitted HBV patients and previous nonresponders to IFN α or IFN α plus lamivudine.
- Zadaxin-treated patients have fewer relapses.
- Zadaxin has a greater long-term response after treatment is over.
- Patients receiving Zadaxin alone report no serious drug-related toxicities during treatment.
- Zadaxin/IFN combination therapy has a high short- and long-term efficacy rate among previous IFN failures.
- Zadaxin/lamivudine combination therapy has a high short- and long-term efficacy rate among previous IFN failures.
- Zadaxin/IFN combination therapy almost doubles long-term efficacy compared with IFN monotherapy in treatment-naïve patients.
- Zadaxin plus nucleoside analogues are extremely effective in treating HBV, even in immunetolerant patients with high HBV DNA levels.
- Zadaxin/lamivudine combination therapy has a high short- and long-term efficacy rate among previous IFN or IFN plus lamivudine failures.

- Zadaxin can be added to therapies like IFN or nucleoside analogues, enhancing their efficacy but not increasing their toxicity.
- Zadaxin is safe and efficacious in pediatric patients.

Hepatitis C

Clinical Trials

Hepatitis C is recognized as a global health problem, with an estimated worldwide prevalence of more than 170 million and no foreseeable vaccine. As in hepatitis B, Zadaxin is safe and effective for the treatment of chronic hepatitis C when used in combination with IFN α . Although the hepatitis B and C viruses are not structurally related, they are similar in that they are both associated with a high incidence of liver disease, including cirrhosis and hepatocellular carcinoma. They both induce hepatocellular damage, whether through direct cytotoxicity or through induction of immune mechanisms that lead to hepatocellular necrosis. Clearance of viral infection in both viral diseases requires immune involvement, although the exact mechanism for clearance may be different. A much higher percentage of patients (ca. 85%) infected with the hepatitis C virus go on to chronic infection.

Current management of hepatitis C is centered on the use of interferons. Unfortunately response occurs in a minority of patients and sustained response in fewer. IFN α -2b and ribavirin, a new combination, has been approved to treat chronic hepatitis C in naïve patients and in patients who have relapsed following standard interferon treatment. The new combination shows fewer relapses than interferon alone but with additional side effects. In addition to the typical side effects from interferon, ribavirin causes anemia in up to 25% of patients, (104) which can be serious especially in patients with underlying cardiovascular disease. Depression, suicidal intention, and suicides have occurred in patients treated with the combination of ribavirin and IFN α .

More recently, PEG-IFN α , a covalent conjugate of recombinant IFN α with a PEG polyethylene glycol (PEG) moiety, has been approved as monotherapy and in combination with ribavirin for the treatment of naïve patients with chronic hepatitis C. Pegylation involves the attachment of polyethylene glycol to the interferon molecule. Pegylation results in slower clearance of the interferon molecule, allowing it to remain in the bloodstream longer, thereby providing a more convenient, once-weekly dosing schedule for patients and maintaining its ability to consistently suppress the hepatitis C virus over the 1-week dosing period. In vitro and in vivo studies suggest that the biological activity of PEG-IFN α is derived from its interferon alfa moiety. Despite the recent improvements, the sustained response rate remains around 50%. The dissatisfaction with the treatment response rate and the sustained response rate has led to studies of interferon combined with other modalities, such as Zadaxin.

Hepatitis C

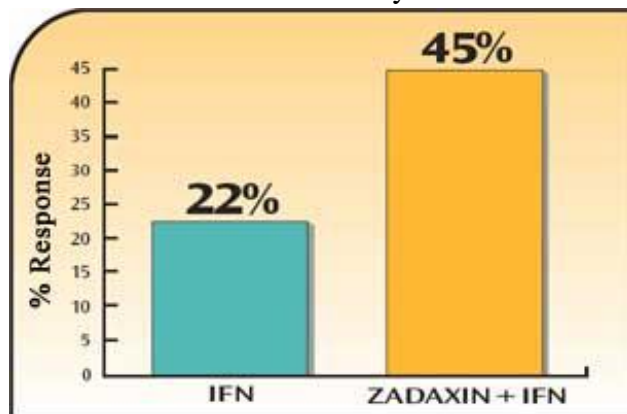
Zadaxin Plus IFN α Combination Therapy for Chronic Hepatitis C

Three studies have investigated the therapeutic effect of Zadaxin in combination with IFN for treatment of chronic hepatitis C. These are a phase 3 study in the United States (105) and two phase 2 studies in Italy. (106,107) These studies have been analyzed independently and using pooled and meta-analysis techniques. The data in combined and individual analyses show that Zadaxin in combination with IFN is safe, effective, and significantly superior to IFN alone at the end of treatment. Using Zadaxin plus IFN combination therapy, both the end-of-treatment and sustained responses are twice those obtained with IFN alone.

Pooled and meta-analysis


The three hepatitis C studies listed were included in the pooled and meta-analyses. (108) An end-of-treatment response was defined as normalization of serum ALT (biochemical response) or negative HCV RNA by PCR (virologic response) at the end of treatment. Sustained response (SR) was also subdivided into biochemical and virologic outcome parameters. A total of 136 patients (67 Zadaxin plus IFN combination therapy, 54 IFN monotherapy, and 15 monotherapy historical controls) were included in the meta-analysis. A total of 121 patients (67 Zadaxin plus IFN combination therapy and 54 IFN monotherapy) were included in the pooled analysis.

Figure 11. Pooled intent-to-treat analysis of end-of-treatment biochemical response. P = 0.0096. (108)



Pooled intent-to-treat analysis results:

- End-of-treatment biochemical response (ALT) (Figure 11)
 - 45% in the Zadaxin plus IFN combination treatment group
 - 22% in the IFN monotherapy group
 - P = 0.0096
- Sustained biochemical response (normal ALT 6 to 12 months after completion of treatment)
 - 22% in the Zadaxin plus IFN combination therapy
 - 9% of patients treated with IFN alone



In both of these pooled analyses, end-of-treatment and sustained biochemical response, the Zadaxin plus IFN combination treatment response more than doubles that to IFN alone.

Meta-analysis results:

- Biochemical response odds ratio > 1
 - 95% confidence interval (CI) > 1
 - Odds ratio of 3:1
 - Zadaxin and IFN combination: > 3 times more likely to normalize ALT at end of treatment
 - Statistically significant result
- Sustained response odds ratio > 1
 - 95% CI slightly overlapping 1
 - Zadaxin plus IFN combination therapy superior to IFN monotherapy for sustained biochemical response
- Virological response: end of treatment and sustained (undetectable HCV RNA by PCR 6 to 12 months after end of treatment)
 - Response odds ratio with 95% CI > 1 for combined studies
 - Combination therapy statistically significantly superior to IFN monotherapy

Hepatitis C

U.S. Hepatitis C Trial

This was a randomized, placebo controlled, double-blind, multicenter trial comparing Zadaxin plus IFN α -2b combination treatment to IFN α -2b monotherapy or placebo.

Patients and protocol:

- 110 patients enrolled and randomized
 - IFN α -2b alone
 - Zadaxin (1.6 mg SC BIW) plus IFN α -2b (3 MIU SC TIW) combination
 - Placebo
 - 107 patients considered evaluable
 - 109 patients used in investigator's intent-to-treat analysis
 - 110 patients used in sponsor's intent-to-treat analysis
 - 103 patients considered evaluable by investigator
 - 107 patients considered evaluable by sponsor
- 6-month treatment period
 - Treatment identities unblinded after treatment
- 6-month follow-up of responders
- Nonresponders to monotherapy and combination therapy offered 6-month combined regimen of Zadaxin plus IFN α -2b
- Primary clinical endpoint: complete biochemical response defined as normal ALT level on last two study visits at the end of the 6-month treatment period

It should be noted that the primary clinical endpoint definition in this study was more stringent than what was reported in the literature with IFN monotherapy at the time, where the single last ALT is used to determine end-of-treatment response.

Results:

- Evaluable patients
 - Combination response of 42% was significantly greater than IFN α -2b response of 19% ($P = 0.04$)
 - Combination response of 42% was significantly greater than placebo response of 5% ($P < 0.001$) (Figure 12)
- Partial responders were considered as nonresponders in efficacy analysis

Figure 12. Results in the US hepatitis C trial at 6 months. The figure shows the complete biochemical response (ALT) at the completion of the 6-month treatment for the three treatment groups. Patients were treated with either Zadoxin plus IFN α -2b, IFN α -2b, or placebo. N = 107 evaluable. Data on file. (159)

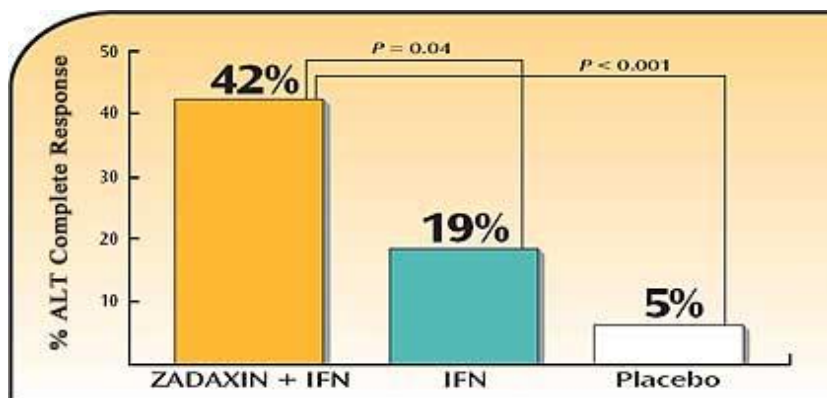
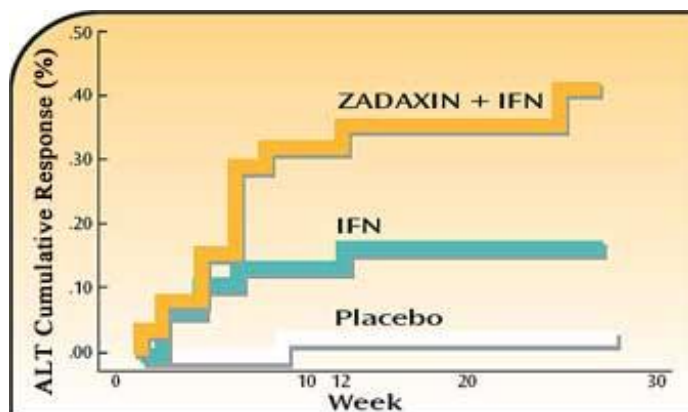


Figure 13. Time to first ALT normalization in the US hepatitis C trial. The figure shows that Zadoxin plus IFN α -2b combination therapy enhances later treatment response for a longer duration than typically seen with IFN alone. This cumulative biochemical response accounts for the observed differences in end-of-treatment response between Zadoxin plus IFN α -2b combination and IFN α -2b alone. (105)



ZDX + IFN vs IFN, P = 0.007
 ZDX + IFN vs PBO, P = 0.0001
 IFN vs PBO, P = 0.009

Intent-to-treat analysis:

- Viral clearance defined as end-of-treatment viral load compared with viral load at baseline
- Significant difference between Zadoxin plus IFN α -2b and placebo groups (P < 0.001)
- Statistical trend in the difference between Zadoxin plus IFN α -2b and IFN α -2b (P = 0.1) in HCV RNA levels

- Patients with histological activity index (HAI) score improvement >2 points
 - Zadaxin plus IFN α -2b group: 47% (16/34)
 - IFN α -2b group: 36% (12/33)
 - Placebo group: 14% (5/36)
 - P = 0.01 among the three treatment groups
 - P = 0.004 between Zadaxin plus IFN α -2b combination and placebo
- Concordance in both active treatment groups between improved HAI and response on the basis of ALT (data on file)

After 6 months of therapy, 11 patients who did not respond to IFN α -2b were placed on a 6-month combined regimen of Zadaxin plus IFN α -2b. Ten of the 11 patients (one patient dropped out after 12 weeks secondary to pre-existing coronary artery disease) completed 6 months of the combined regimen.

Re-treatment results:

- 40% showed normalization of ALT
- 80% demonstrated at least a 50% decrease in viral titer
- 30% classified as complete virological responders (viral titers below assay's detection limit)

Following 6 months of treatment, patients were followed for 6 months to evaluate sustained response. Patients who relapsed were offered an additional 6 months re-treatment with the same treatment: combination IFN α -2b plus Zadaxin or IFN α -2b monotherapy. Re-treated relapsers were then followed for 6 months to evaluate sustained response.

Sustained response results:

- Analysis included patients treated for 6 months and relapsers retreated for a total of 12 months
- Sustained biochemical response
 - 19.2% for Zadaxin plus IFN α -2b
 - 9.4% for IFN α -2b
- Zadaxin plus IFN α -2b combination therapy was superior to single-agent IFN α -2b in treatment of patients with CHC
- Treatment was generally well tolerated

Hepatitis C

Italian Hepatitis C trial and Thailand Hepatitis C trial, Interim Results

Italian hepatitis C trial #1 (106)

This was an open-label, phase 2 study of CHC patients using a combination of Zadaxin and lymphoblastoid IFN α (L-IFN α).

Patients and protocol:

- Enrolled patients had biochemical-, histological-, and serological-confirmed CHC
- 15 patients entered into the study
 - 4 previous IFN α -2b therapy failures
 - 13/15 patients were genotype 1b (least responsive to interferon therapy)
 - 6 patients with active cirrhosis
- Initial treatment of 1-week inductive therapy (1 mg Zadaxin SC, days 1 to 4; 3 MIU L-IFN α IM, day 4)
- Maintenance treatment weeks 2 to 52 (1 mg Zadaxin SC, BIW; 3 MIU L-IFN α , IM, TIW)
- 6-month follow-up after completion of 12-month treatment period (18 months total)
- Response defined as negative serum HCV RNA by PCR at 12 months
- Sustained response defined as negative serum HCV RNA by PCR after a 6-month follow-up

Results:

- After 12-month treatment period
 - 73% (11/15) had loss of serum HCV RNA
 - 2 (of original 4) were IFN α -2b failures
 - 8 also responded with normal ALT
- 69% (9/13) with HCV type 1b responded to therapy
- 6 months post treatment
 - 39% (5/13) responders with HCV type 1b still negative for serum HCV RNA
 - 50% (3/6) with active cirrhosis responded with loss of HCV RNA
 - 33% (2/6) with active cirrhosis still negative
 - 40% (6/15) overall sustained response
 - 5/6 HCV RNA negative patients at 18 months also had normal ALT levels
 - Patients with sustained response to treatment showed significant improvement in HAI after treatment (Knodell HAI; $P < 0.05$)
 - No major toxicity observed
 - No patient reduced IFN α -2b dosage or suspended treatment

In this study, combination treatment for 12 months resulted in a greater sustained response rate over that seen with 6-month treatment.

Italian hepatitis C trial #2 (107)

This was a randomized study to compare the efficacy of Zadaxin plus IFN α -2b combination treatment with IFN α -2b monotherapy.

Patients and protocol:

- 34 patients
- Treatment naïve
- Histologically proven HCV-positive chronic active hepatitis
- Persistent mean ALT = 2 times upper limit of normal
- 17 patients randomized to receive IFN α -2b (3 MIU TIW) and Zadaxin (2 mg BIW) for 6 months
- 17 patients received IFN α -2b alone (3 MIU TIW)
- 12-month post-treatment follow-up
- Complete response defined as normal ALT

Results:

- After 6-month treatment period
 - 71% (12/17) complete response in patients treated with Zadaxin plus IFN α -2b combination
 - 35% (6/17) complete response in patients treated with IFN α -2b alone
 - P = 0.04
- After 12-month follow-up
 - 29% (5/17) in Zadaxin plus IFN α -2b combination group
 - 18% (3/17) in IFN α -2b monotherapy group

Thailand hepatitis C trial, interim results (109)

This is an ongoing, single-arm study to investigate the efficacy of Zadaxin plus IFN α -2a combination treatment in Thai patients with chronic HCV infection.

Patients and protocol:

- 12 patients
- 8 treatment naïve
- 4 nonresponders to previous IFN therapy
- Histologically proven HCV-positive acute chronic active hepatitis
- Patients received IFN α -2a (3 MIU TIW) and Zadaxin (1.6 mg BIW) for 48 weeks

Results:

- At 24 weeks
 - 33.3% virological response (HCV RNA negative)
- At 48 weeks
 - 45.5% overall virological response (HCV RNA negative)
 - 80% virological response in naïve patients

Hepatitis C

Summary & Key Takeaways

Hepatitis C Summary

The United States National Institutes of Health (NIH) Consensus Meeting held in March 1997 reviewed new data in the treatment of CHC. The NIH CHC conference concluded that Zadaxin, when used in combination with IFN α , is “the most promising of the cytokines or immunomodulators tested thus far.” (110)

Key Takeaways

- Zadaxin added to IFN more than doubles the efficacy rate of IFN alone in end-of treatment and sustained response.
- Zadaxin plus IFN is effective in IFN nonresponders and in cases of relapse after IFN treatment.
- Response rates increase during a full 48 weeks of therapy.
- Zadaxin combination therapy has a high response rate even in genotype 1 patients.
- No major toxicity has been observed in patients receiving combination therapy.

Human Immunodeficiency Virus

Clinical Trials

Stimulation of the immune system, especially in combination with antiviral agents has received considerable interest as a potential means to treat acquired immunodeficiency syndrome (AIDS)- and HIV-infected patients. Both preclinical and clinical studies have shown a high degree of immune restoration from the combined administration of Zadaxin and IFN α . Thus, Zadaxin in combination with AZT and IFN α has been investigated for treatment of HIV-infected patients.

Phase 2 Italian HIV trial (111)

This open-label, initial phase 2 study investigated the combination of Zadaxin, IFN α , and AZT for treatment of HIV-infected patients with CD4 counts of 500 or lower.

Patients and protocol:

- 7 patients in each of four treatment groups
 - Zadaxin (1.0 mg BIW) plus IFN α (2 MIU BIW) plus AZT (500 mg/day)
 - Zadaxin (1.0 mg BIW) plus AZT (500 mg/day)
 - IFN α (2 MIU BIW) plus AZT (500 mg/day)
 - AZT (500 mg/day)
 - 12- to 18-month treatment period

Results:

- Zadaxin plus IFN α plus AZT group
 - CD4 cells increased from 309 ± 77 before treatment to 496 ± 230 ($P = 0.029$)
 - The addition of Zadaxin stimulated lymphocyte cytotoxic activity against NK-sensitive target cells compared with other treatment groups

Phase 3 Italian HIV trial (84)

This was a larger, multicenter, randomized, phase 3 study to determine the safety and efficacy of Zadaxin plus IFN α plus AZT combination therapy in HIV-infected patients.

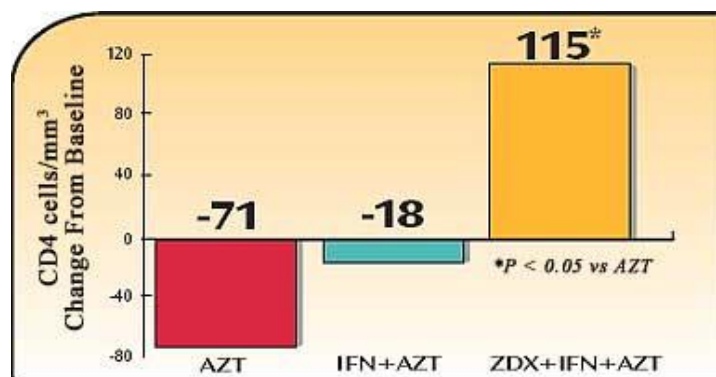
Patients and protocol:

- 92 HIV-infected patients
 - Asymptomatic or with AIDS-related complex
 - CD4 counts 200 to 500/mm³
 - No more than 1 month previous AZT
- 3 treatment groups
 - AZT (500 mg/day)
 - AZT plus IFN α (3 MIU IM BIW)
 - AZT plus IFN α plus Zidaxin (2 mg SC BIW)
- 1-year treatment period

Results:

- Sustained increase in CD4 counts in Zidaxin plus IFN α plus AZT combination group
- Decreased CD4 counts in other treatment groups
- Median CD4 counts at 12 months relative to baseline
 - Zidaxin plus IFN α plus AZT combination group: +69 cells/mm³
 - IFN α plus AZT combination group: -52 cells/mm³
 - AZT group: -65.5 cells/mm³
- Greatest CD4 effect seen in patients with baseline CD4 <350 mm³ (Figure 14)
 - +115 cells/mm³, Zidaxin plus IFN α plus AZT
 - -18 cells/mm³, IFN α plus AZT
 - -71 cells/mm³, AZT

Figure 14. Results from the HIV trial in Italy at 12 months. The figure shows the median change in CD4 cells/mm³ at the end of 12 months of treatment relative to baseline for patients with baseline CD4 =350 mm³. The three treatment groups were Zidaxin plus IFN α plus AZT, IFN α plus AZT, and AZT. (84)



In this trial, plasma HIV RNA and p24 antigenemia data also suggested the superiority of the triple combination therapy.

Plasma HIV RNA changes:

- Mean change from baseline at 5 months
 - Ziadaxin plus IFN α plus AZT: decrease of 16,000 copies/mL
 - AZT monotherapy: decrease of 5,000 copies/mL
- Mean change from baseline at 12 months
 - Ziadaxin plus IFN α plus AZT: decrease of 15,000 copies/mL
 - AZT monotherapy: increase to 7,000 copies/mL above baseline

p24 antigenemia results at 12 months:

- Ziadaxin plus IFN α plus AZT: maximum change from baseline of -232 pg/l
- IFN α plus AZT: increase above baseline of +1.6 pg/l
- AZT monotherapy: increase above baseline of +33 pg/l

Key Takeaways

- Ziadaxin plus IFN α plus AZT treatment leads to sustained increases in CD4 counts.
- Ziadaxin plus IFN α plus AZT combination therapy triples the reduction of HIV RNA copies relative to AZT monotherapy.
- Ziadaxin's superior safety profile makes it ideally suited for special patient populations, including immune-suppressed patients.
- Ziadaxin does not compound the side effects of IFN α or AZT.

Vaccine Adjuvant in Immunocompromised Patients

Introduction

Immune senescence, a normal aging process, has been related to a gradual decline in thymus function and thymic hormone production. The lack of thymic hormones may contribute to the decline in immune function, particularly the T-cell component. (112-114) In the elderly, quantitative and qualitative analysis of a specific antibody response after vaccination has been shown to be compromised when compared with response in young subjects. (115,116)

Decreased antibody response to T-cell dependent antigens may be one factor that accounts for insufficient efficacy of certain vaccination programs (eg, influenza). Diminished antibody responses have also been reported in patients with end-stage renal disease. The evidence for impairment of cell-mediated immunity in hemodialysis patients has been attributed to incompetence in T-cell-mediated immune responses. (117-121) Several studies have reported poor antibody response after hepatitis B vaccination in hemodialysis patients. (122-124)

Since Zadaxin can enhance T-cell-dependent specific antibody production, the addition of Zadaxin to vaccination programs for immunocompromised individuals should be effective. In vaccinated elderly individuals, in vitro influenza antibody synthesis was augmented with the addition of Zadaxin. (115) Enhancement of specific antibody responses to tetanus toxoid and sheep red blood cells also was observed by the in vivo administration of Zadaxin to older mice or mice immunosuppressed with cocaine. (125,126) These experimental data support that Zadaxin, when appropriately administered with a vaccine, can enhance immune function and enhance an antibody response in individuals with a compromised immune system.

Six clinical studies have been completed that evaluated the efficacy of Zadaxin as an adjuvant for influenza and hepatitis B antiviral vaccines in subjects immunocompromised due to age or hemodialysis. When compared with vaccine plus placebo, administration of Zadaxin in conjunction with vaccine increased and sustained the specific antibody response, increased protection against illness, and overcame previous lack of specific antibody response and age-associated decline in specific antibody response. The studies also show that Zadaxin is safe for administration to immunocompromised subjects, and no serious adverse effects were observed in any of the studies.

Vaccine Adjuvant in Immunocompromised Patients

Cornell Medical Center (Ithaca, New York) and University of Wisconsin Trials

The immunoenhancing effect of Zadaxin for influenza vaccination was examined by Gravenstein et al at the University of Wisconsin and Cornell Medical Center. (127)

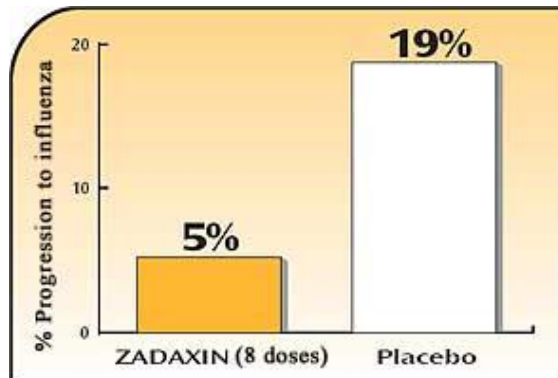
Patients and protocol:

- 9 elderly subjects (age range 65 to 99 years)
- Subjects nonresponsive to influenza vaccination 1 year earlier
- Initial administration of influenza vaccine
- Subsequent biweekly Zadaxin (0.9 mg/m²) injections for 5 weeks

Results:

- 67% (6/9) of Zadaxin subjects responded with high levels of anti-influenza antibodies
- 10% typical response rate to revaccination in elderly subjects

Figure 15. Clinical response to influenza vaccination. There was a lower incidence of influenza in subjects who received 8 doses of Zadaxin compared with placebo (6/100 [5.5%] vs 21/110 [19%]; $P = 0.002$). (129)



This trial was followed by a double-blind, randomized, placebo-controlled study at the Wisconsin Veterans' administration Medical Center, Madison, conducted by the same group of researchers. (128)

Patients and protocol:

- 90 male veterans > 64 years of age
- Mean age 77.3 years
- Age range 65 to 99 years
- 45 subjects randomized to Zadaxin 0.9 mg/m² SC BIW for 4 weeks following vaccination IM with trivalent influenza vaccine
- 45 subjects randomized to placebo injections SC BIW for 4 weeks following vaccination IM with trivalent influenza vaccine
- Groups were similar with respect to age, underlying disease, and medications
- Effective immunization defined as fourfold or greater rise in antibody titer over 3 to 6 weeks as measured by ELISA

Results:

- 69% (31/45) of Zadaxin subjects effectively immunized
- 52% (21/40) of placebo subjects effectively immunized
- P = 0.023
- Differences were greater in subjects older than 77 years
- Relationship between antibody levels and age (P < 0.039)
- Antibody levels declining with age in placebo subjects
- Antibody levels remaining stable with age in Zadaxin-treated subjects
- Antibody levels in Zadaxin-treated older subjects comparable to levels in younger subjects

Vaccine Adjuvant in Immunocompromised Patients

George Washington University Trial (129)

Patients and protocol:

- 330 subjects
- Initial vaccination with trivalent influenza vaccine (B/Ann Arbor, A/H3N2 Leningrad, A/H1N1 Taiwan)
- Subsequent treatment
 - 4 SC injections of Zadaxin (0.9 mg/m²)
 - 8 SC injections of Zadaxin (0.9 mg/m²)
 - Placebo SC injections

Results – All Age Groups

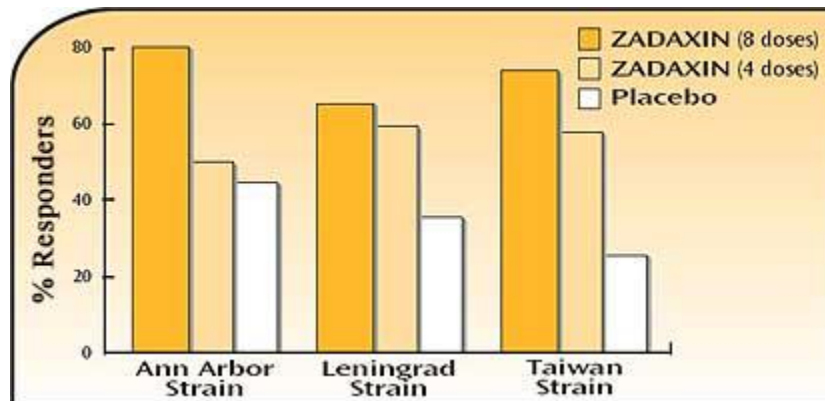
- A/H1N1 Taiwan antigen
 - Greater antibody levels in patients receiving 8 doses of Zadaxin than either other group (P = 0.015)
- Lower incidence of influenza in patients receiving 8 doses of Zadaxin versus placebo (P = 0.002) (Figure 15)
 - Zadaxin: 6% (6/100)
 - Placebo: 19% (21/110)

Results – Subjects 80 years or older

- Fourfold increase in specific influenza antibody levels (considered clinically effective) in patients who received 8 doses of Zadaxin relative to placebo
- P = 0.031 for B/Ann Arbor
- P = 0.044 for A/H3N2 Leningrad
- P = 0.027 for A/H1N1 Taiwan

Greatest effect on augmentation of antibody levels by Zadaxin observed in subjects ≥ 80 years with prevaccination antibody levels $<$ median level for this age group (Figure 16)

Figure 16. Results from US influenza trial. This figure shows the percent of subjects aged 80 to 101 years with baseline antibody levels less than the median who responded with a fourfold rise in antibody levels for each antigen strain at 6 weeks post vaccination. The three treatment groups were Zadaxin (8 doses following vaccination), Zadaxin (4 doses following vaccination), and placebo (8 doses following vaccination). $P < 0.05$ Zadaxin (8 doses) compared with placebo, all influenza strains. (129)



Vaccine Adjuvant in Immunocompromised Patients

Hemodialysis Patients

Two separate randomized, double-blinded, placebocontrolled trials evaluated the adjuvant effect of Zadaxin on two different vaccines in subjects immunocompromised by chronic renal failure and undergoing hemodialysis. (85,130,131)

Study 1 (130,131)

Patients and protocol:

- 23 hemodialysis patients
- Nonresponders to a course of Heptavax B vaccination \geq 3 months earlier
- 3 vaccine injections (Heptavax B) given 1 month apart
- Each followed by 5 biweekly injections of Zadaxin 0.9 mg/m² or placebo

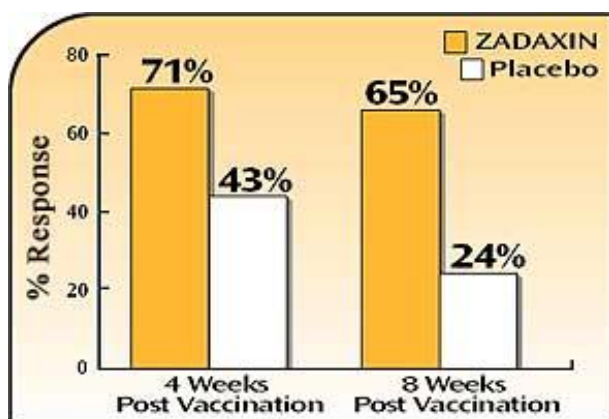
3-month results:

- 375% increase in patients producing clinically significant ($P < 0.002$) HBsAb titers (>10 mIU/mL)
 - 64% (7/11) of Zadaxin-treated patients
 - 17% (2/12) of placebo-treated patients

12-month results:

- 45% of Zadaxin-treated patients achieved sustained, clinically significant HBsAb titers ($P < 0.002$)
- 0% of placebo-treated patients developed sustained, clinically significant HBsAb titers
- Higher antibody levels in Zadaxin-treated patients

Figure 17. Antibody response to influenza vaccine with Zadaxin adjuvant in hemodialysis patients. The figure shows the percent response (defined as fourfold or greater increase in specific anti-influenza antibody) of hemodialysis patients to influenza vaccination treated with Zadaxin adjuvant therapy or placebo. The Zadaxin-treated patients exhibited a statistically significantly greater fourfold antibody response compared with placebo (4 weeks post vaccination $P < 0.002$; 8 weeks post vaccination $P < 0.001$). (85)



Study 2 (Figure 17) (85)

Patients and protocol:

- 97 hemodialysis patients
- Vaccination with monovalent A/Taiwan/1/86 (H1N1) influenza vaccine
- 8 subsequent injections (2 per week) of Zadaxin 0.9 mg/m² or placebo

Results:

- 4 weeks post vaccination
 - 71% (34/48) of Zadaxin-treated patients developed a fourfold or higher titer of specific antibody
 - 43% (21/49) of placebo-treated patients developed a fourfold or higher titer of specific antibody
 - $P < 0.002$
- 8 weeks post vaccination
 - 65% (31/48) response rate in Zadaxintreated patients (fourfold or higher titer of specific antibody)
 - 24% (12/49) response rate in placebo-treated patients (fourfold or higher titer of specific antibody)
 - $P < 0.001$



Vaccine Adjuvant in Immunocompromised Patients

Key Takeaways

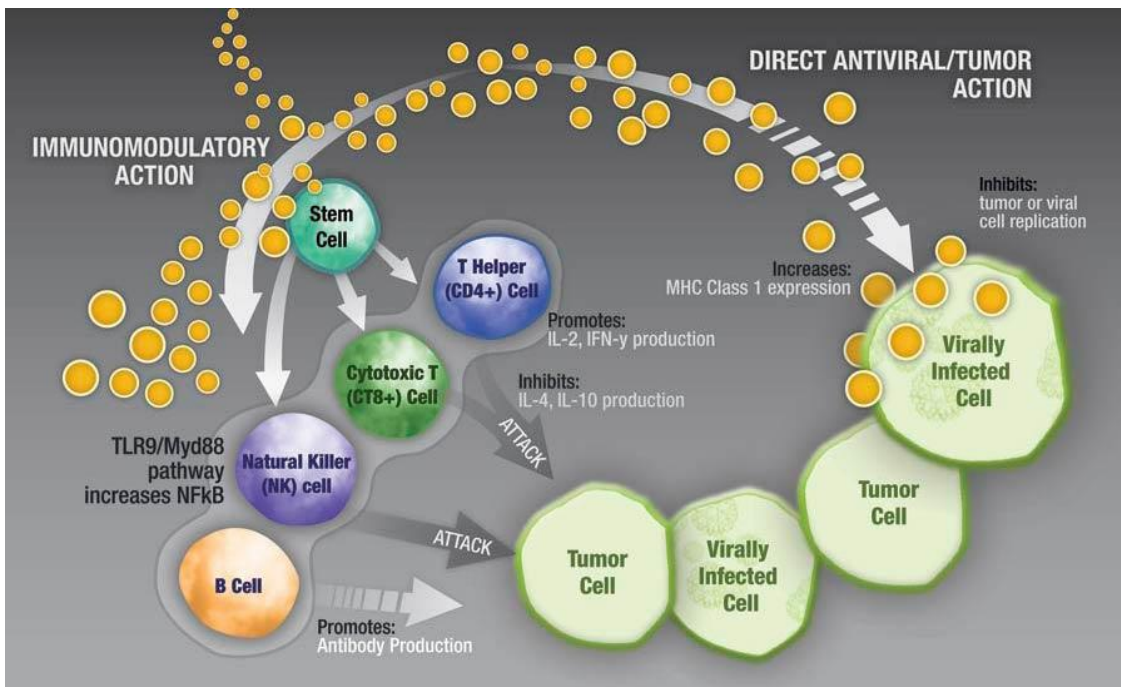
- Zadaxin adds power to hepatitis B and influenza vaccines.
- The exemplary safety profile of Zadaxin allows for use in renally compromised and elderly patients.
- Zadaxin provides more than 6 times the typical response to revaccination in previous nonresponders.
- Zadaxin has shown the greatest effect in patients older than 80 years and has been used safely in patients as old as 101 years.

Cancer

Introduction

The therapeutic usefulness of Zadaxin has been examined in several types of cancers. Zadaxin has efficacy in several animal cancer models and has been shown to improve immune function in these models. Many cancer patients have depressed cellular immunity, and progression of some cancers appears to be related to impaired suppression of the tumors by the immune system. This has been shown to be the case for melanoma, hepatocellular carcinoma (HCC), and renal-cell carcinoma. (132)

Immune modulation (eg, with IL-2) has shown promising results for treatment of human cancers, (133-135) and the results summarized below suggest that Zadaxin may have usefulness in treating certain forms of cancer.



Cancer

Hepatocellular Carcinoma

Hepatocellular carcinoma is the most prevalent malignant disease in the world, killing up to 1.25 million people per year. HCC accounts for more than 80% of all primary liver tumors and has a worldwide annual incidence of approximately 1 million new cases, (136) with a male to female ratio of about 3-8:1. (137) HCC is a common malignancy in Africa and Asia, and it accounts for approximately 4000 to 6000 cases per year in the USA. (138)

Eighty to ninety percent of patients with HCC have underlying cirrhosis; alcoholic cirrhosis is the predominant type in Western countries, whereas in southeast Asia, posthepatitis cirrhosis is more common. There is a strong correlation between HCC and chronic hepatitis B. High incidence rates in Africa and Asia have been associated with high endemic HBsAg carrier rates. (139) In these areas highly endemic for hepatitis B, an association between HCC rates and mycotoxin contamination of food has been detected. (138) One of the identified mycotoxins is one of the most potent natural chemical carcinogens known: aflatoxin B1. This toxin, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, is usually associated with grains and food products such as peanuts and rice. (140)

More recently, an association between HCC and chronic hepatitis C has been determined. Antibodies to hepatitis C virus are found in as many as 80% of patients with hepatocellular carcinoma in countries including Japan, Spain, and Italy. (141) HCC carcinogenesis has also been associated with radiation, thorotrast, smoking, alpha-1 antitrypsin deficiency, hemochromatosis, Budd-Chiari syndrome, porphyria, oral contraceptives, and anabolic androgenic steroids.

When identified in its early stages, HCC can be treated with surgical resection or liver transplantation, and some patients may be cured. However, the disease is often not amenable to surgical treatment, either because of tumor size or because of poor liver function. In these situations, the prognosis is dire.

Other treatment approaches have been tried when surgery or liver transplantation are not feasible. Systemic chemotherapy results are at best dismal. Conversely, a large number of reports have provided encouraging perspectives for regional chemotherapy. (138) Transcatheter arterial chemoembolization (TACE) is a combination of regional chemotherapy and some form of hepatic artery occlusion. Consistently higher response rates have been reported for TACE when compared with systemic chemotherapy. Combining immunomodulatory therapy with TACE was expected to increase the efficacy level even higher.

Italian trial of Zadaxin in HCC (142)

This phase 2 trial examined the efficacy and safety of Zadaxin for treatment of HCC.

Patients and protocol:

- 12 patients
- 11 patients with Child class A or B cirrhosis, Okuda stage I or II tumors
- 1 patient with Child class C cirrhosis, Okuda stage III tumor
- Diagnosis based on ultrasonography and histology
- 6 months treatment with 0.9 mg/m² Zadaxin (SC, BIW)
- TACE (40 to 60 mg of doxorubicin)
- Historical control group matched for gender, age, Okuda staging, Child score, a-fetoprotein serum levels, and viral infection, treated with TACE alone

Results:

- Longer survival times in Zadaxin-treated patients
 - Statistical significance achieved 7 months after end of treatment
 - 82% versus 41% survival
 - $P < 0.05$
- Significant increase in cytotoxic T cells (CD8) in Zadaxin-treated patients at 3 months after end of treatment
- Significant increase in NK cells (CD16 and CD56) in Zadaxin-treated patients at 1 month after end of treatment

The delayed after-treatment effect of Zadaxin observed in this study is concordant with studies in hepatitis B where it is common to see patients respond to Zadaxin during the follow-up period.

Chinese Trial of Zadaxin in HCC

This open-label, historically controlled trial evaluated the efficacy and safety of adding Zadaxin therapy to TACE. (143)

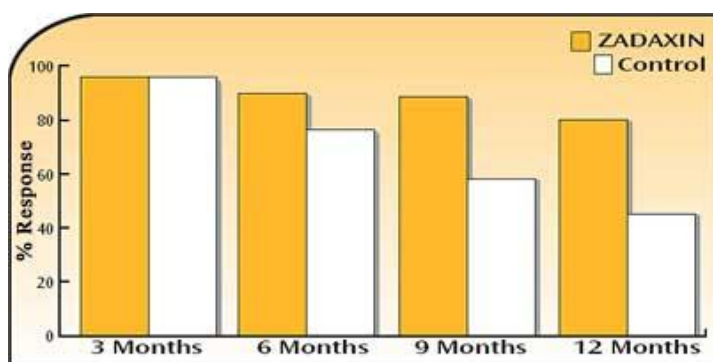
Patients and protocol:

- 32 patients
- TACE procedure
 - 5 to 15 mL iodized oil
 - 40 to 60 mg doxorubicin HCl
 - 100 to 200 mg carboplatin
 - 1 g 5-FU
 - Zadaxin: 1.6 mg SC days 1 to 10 after TACE treatment
- 26 historical controls
 - Matched for gender, age, Okuda staging, Child's score, serum a-fetoprotein, viral hepatitis infection
 - Treated with TACE alone
- HCC tumor variants: massive, nodular, diffuse

Results (Figure 18) — Zadaxin + TACE:

- Significantly lowered AFP levels from baseline (512 ng/mL):
 - 354 ng/mL after treatment
 - ng/mL 2 weeks post therapy
- Significantly increased CD3 count and NK-cell activity 2 weeks post treatment ($P < 0.05$)
- Significantly increased CD4/CD8 ratio
 - 1.07 ratio value before therapy
 - 1.55 ratio value 2 weeks post therapy

Figure 18. Zadaxin+TACE: HCC survival. (143)



<p>Results: 3-month survival</p> <ul style="list-style-type: none"> • 96% Zadaxin + TACE • 96% TACE 	<p>Results: 9-month survival</p> <ul style="list-style-type: none"> • 88% Zadaxin + TACE • 58% TACE • $P < 0.05$
<p>Results: 6-month survival</p> <ul style="list-style-type: none"> • 91% Zadaxin + TACE • 77% TACE • $P < 0.05$ 	<p>Results: 12-month survival</p> <ul style="list-style-type: none"> • 78% Zadaxin + TACE • 46% TACE • $P < 0.05$

Cancer

Non–Small-Cell Lung Cancer

Zadaxin has been used to treat NSCLC in several different trials. This cancer accounts for approximately 75% of lung cancer cases, and current therapies such as radiation therapy and chemotherapy give disappointing results, with 5-year survival for stage III tumors ranging from 0% to 12% after a combination of radiation and chemotherapy. (144-146)

George Washington University trial (147)

This was a double-blind, randomized trial.

Patients and protocol:

- 42 patients with localized, unresectable NSCLC
- Treatment after radiation therapy up to 1 year or until relapse
 - Placebo biweekly
 - Zadaxin 0.9 mg/m² biweekly
 - Zadaxin 0.9 mg/m² daily for 14 days followed by biweekly maintenance treatments

Results:

- Improvement of absolute T-cell levels that had been depleted by radiation in Zadaxin-treated patients
- Increase in the percentage of lymphocytes expressing the pre-T-cell and helper-/inducer-T-cell
 - Zadaxin treated patients: 90% retention of both OKT3- and OKT4-positive cells surface markers in Zadaxin-treated patients
 - Placebo-treated patients: OKT3-positive cells decreased to 78% of original value; OKT4-positive cells decreased to 64% of original value)
- Statistically significant improvement in relapse-free survival in both Zadaxin-treated groups (P = 0.04)
- Statistically significant improvement in overall survival in both Zadaxin-treated groups (P = 0.009)

Italian trials

Trial #1 (148)

- 60 patients
- Zadaxin in combination with chemotherapy (cisplatin and etoposide) and IFN α
- 55 evaluable patients
- 2 complete responses
- 22 partial responses
- Overall response rate of 44%
- Median survival was 12.6 months

Trial #2 (25)

- 22 patients
 - 10 patients randomized to chemotherapy (ifosfamide)
 - 12 patients randomized to chemotherapy followed by Zadaxin (1 mg) and low-dose IFN α (3 MIU)
- Enhanced response rate of 33% in Zadaxintreated patients
- 10% response rate with chemotherapy alone
- Increased time to progression in Zadaxintreated patients
 - Zadaxin-treated patients: 18 weeks, range 9-53 weeks
 - Ifosfamide alone: 9 weeks, range 6-18 weeks
 - P = 0.006
- CD4 and CD8 cell counts significantly depressed after 2 cycles of chemotherapy alone
- No difference in cell count in Zadaxin group
- 50% of patients treated with ifosfamide alone presenting with grade 3 or 4 myelosuppression
- 0% of Zadaxin patients presenting with grade 3 or 4 myelosuppression

Cancer

Malignant Melanoma

Malignant melanoma is resistant to most forms of therapy, with response rates to dacarbazine (DTIC), the most active single agent, of approximately 17% to 20%, without impact on patient survival. (149-151) The effects of Zadaxin in combination with chemotherapy and cytokine therapy for treatment of malignant melanoma were examined in 3 trials in Italy with comparisons to historical controls.

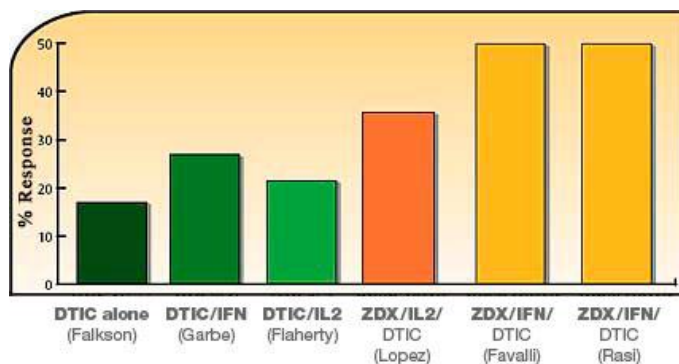
Trial #1 (152)

- 26 patients
- Zadaxin used following DTIC therapy in combination with IFN α
- World Health Organization (WHO) evaluation criteria
- Overall response rate of 50%
- Mean duration of response of 13.5 months

Trial #2 (153)

- 20 patients
- Stage III or IV unresectable metastatic melanoma
- Zadaxin used following DTIC therapy in combination with IFN α
- Up to 9 cycles of therapy
- 50% overall response to therapy
 - 25% complete response
 - 25% partial response
- Median survival time 11.5 months (range 6 to 83+)
- Median time to progression 5.5 months (range 3 to 83+)
- 35% survival >12 months (7/20)
- 15% disease free after more than 3 years (3/20)

Figure 19. Effect of Zadaxin on metastatic melanoma. The trials involving Zadaxin were Lopez, (148,154) Favalli, (152) and Rasi. (153) Percent response is as defined in each study. (148-150,152-155)



Trial #3 (154)

- 42 evaluable patients
- Zadaxin administered following DTIC therapy in combination with IL-2
- 36% objective response rate
 - 2 complete responses
 - 13 partial responses
- Median time to progression 5.5 months
- Median survival 11 months

Historical controls have shown response rates of about 27% with DTIC and IFN α (149) and about 22% with DTIC and IL-2. (155) Thus, the Zadaxin-treated patients showed greater overall response over these other regimens, namely DTIC with IFN or IL-2 ([Figure 19](#)).

Cancer

Key Takeaways

- In HCC, Zadaxin in combination with TACE doubles survival rate at 7 months.
- Zadaxin plus TACE significantly increases survival post treatment compared with TACE alone.
- In NSCLC, Zadaxin treatment following radiation therapy increases T-cell levels and significantly improves overall survival.
- Zadaxin helps to maintain CD4 and CD8 cell counts during and after chemotherapy.
- In malignant melanoma, Zadaxin plus IFN more than doubles the response rate to DTIC alone.
- Zadaxin has not been shown to add to side effects from front-line cancer treatments.

Toxicity and Safety

Toxicity in Animals and in Vitro

In vivo preclinical studies have included single dose toxicity, 2-week, 13-week, and 26-week multiple-dose toxicity, special immune toxicity, and reproductive toxicity. Zadaxin has also been tested for genotoxicity in in vivo and in vitro systems. In these studies, Zadaxin has not demonstrated any drug-related adverse toxicity at the doses tested. A maximum tolerated dose has not yet been achieved in single or repeat-dose studies.

Single-dose toxicity studies in mice, rats, and marmosets tested subcutaneous doses up to 20 mg/kg – over 800 times the currently used daily human dose (23 to 25 $\mu\text{g}/\text{kg}$ for a 1.6-mg dose). Repeat dose studies in mice, rats, and marmosets tested daily SC injections up to 6 mg/kg/day (over 200 times the daily human dose of 1.6 mg) for 13 weeks or up to 1 mg/kg/day for 26 weeks.

Safety in Humans

Since 1979, Zadaxin has been evaluated in more than 3,000 patients in over 70 clinical studies. Administration has been in daily doses ranging from 0.6 to 9.6 mg/m² and 1 mg to 16 mg, primarily administered subcutaneously on a biweekly schedule, for treatment periods ranging from 1 day to 18 months. No serious adverse experiences have been observed. Zadaxin has been shown to be well tolerated even in patients with poor performance status, including those with decompensated liver disease, renal disease requiring hemodialysis, primary immunodeficient individuals, and elderly patients as old as 101 years.

The lack of significant side effects with Zadaxin is in sharp contrast to other major immune response modulators such as IFN and IL-2. The side effects and toxicities of the latter drugs make them difficult for most patients to tolerate. For example, IFN results in flu-like side effects (fever, chills, malaise, and headaches) and IL-2 causes significant edema in the lungs and elsewhere.

References

1. Wara D, Goldstein A, Doyle N, Ammann A. Thymosin activity in patients with cellular immunodeficiency. *N Engl J Med.* 1975;292:70-74.
2. Goldstein AL, Low T, McAdoo M, et al. Thymosin alpha 1: isolation and sequence analysis of an immunologically active thymic polypeptide. *Proc Natl Acad Sci U S A.* 1977;74:725-729.
3. Wang SS, Makofske R, Bach A, Merrifield RB. Automated solid phase synthesis of thymosin alpha 1. *Int J Pept Protein Res.* 1980;15:1-4.
4. Low TLK, Goldstein AL. The chemistry and biology of thymosin II. Amino acid sequence analysis of thymosin alpha 1 and polypeptide beta 1. *J Biol Chem.* 1979;254:987-995.
5. Low TLK, Thurman GB, McAdoo M, et al. The chemistry and biology of thymosin. I. Isolation, characterization, and biological activities of thymosin alpha 1 and polypeptide beta 1 from calf thymus. *J Biol Chem.* 1979;254:981-986.
6. Oates K, Erdos M. Biochemical identification of thymosin alpha-1: its phylogenetic distribution and evolutionary implications. *Comp Biochem Phys.* 1989;94-B(4):759-763.
7. Weller FE, Shah U, Cummings GD, Chretien PB, Mutchnick MG. Serum levels of immunoreactive thymosin alpha 1 and thymosin beta 4 in large cohorts of healthy adults. *Thymus.* 1992;19:45-52.
8. Jevremovic M, Kartaljevic G, Jelusic V, Vodnik T, Pesic M, Filipovic S. Determination of thymosin alpha 1 with enzyme-immunoassay in colorectal cancer patients. *Arch Oncol.* 1997;5:193-194.
9. Sherman K, Jones C, Goldstein A, Naylor P. Low thymosin alpha-1 concentrations in patients chronically infected with the hepatitis B virus. *Viral Immunol.* 1991;4:195-199.
10. Welch R, Mutchnick M, Weller F, Sokol R. Maternal and fetal circulating levels of thymosin alpha 1 during parturition. *Am J Reprod Immunol Microbiol.* 1987;13:125-127.
11. Welch R, Lee H, Sokol R, Mutchnick M. Amniotic fluid thymosin alpha 1 levels increase during gestation. *Am J Reprod Immunol Microbiol.* 1988;17:96-97.
12. Gomez-Marquez J, Segade F. Prothymosin alpha is a nuclear protein. *Fed Eur Biochem Soc.* 1988;226:217-219.
13. Watts JD, Cary PD, Crane-Robinson C. Prothymosin alpha is a nuclear protein. *FEBS Lett.* 1989;245(1-2):17-20.
14. Gallego R, Rosón E, García-Caballero T, et al. Prothymosin alpha expression in lymph nodes and tonsils: an optical and ultrastructural study. *Acta Anat (Basel).* 1992;143:219-222.
15. Mori M, Barnard GF, Staniunas RJ, Jessup JM, Steele GD, Jr., Chen LB. Prothymosinalpha mRNA expression correlates with that of c-myc in human colon cancer. *Oncogene.* 1993;8:2821-2826.
16. Pavlov N, Evstafieva A, Rubtsov Y, Vartapetian A. Human prothymosin alpha inhibits division of yeast *Saccharomyces cerevisiae* cells, while its mutant lacking nuclear localization signal does not. *FEBS Lett.* 1995;366:43-45.

References

17. Gomez-Marquez J, Segade F, Dosil M, Pichel JG, Bustelo XR, Freire M. The expression of prothymosin alpha gene in T lymphocytes and leukemic lymphoid cells is tied to lymphocyte proliferation. *J Biol Chem.* 1989;264:8451-8454.
18. Smith MR, al-Katib A, Mohammad R, et al. Prothymosin alpha gene expression correlates with proliferation, not differentiation, of HL-60 cells. *Blood.* 1993;82:1127-1132.
19. Contreas CN, Mutchnick MG, Palmer KC, et al. Cellular levels of thymosin immunoreactive peptides are linked to proliferative events: evidence for a nuclear site of action. *Proc Natl Acad Sci U S A.* 1990;87:3269-3273.
20. Calvo J, Guerrero J, Goberna R. Interaction of thymic peptide thymosin alpha 1 with VIP receptors in rat intestinal epithelial cells: comparison with PHI and secretin. *Gen Pharmacol.* 1989;20:503-505.
21. Calvo JR, Goberna R, Guerrero JM. Interaction of thymic peptide thymosin alpha 1 with vasoactive intestinal peptide (VIP) receptors. *Biosci Rep.* 1986;6:727-733.
22. Grottesi A, Sette M, Palamara T, Rotilio G, Garaci E, Paci M. The conformation of peptide thymosin alpha 1 in solution and in a membrane-like environment by circular dichroism and NMR spectroscopy: a possible model for its interaction with the lymphocyte membrane. *Peptides.* 1998;19:1731-1738.
23. Knutsen AP, Freeman JJ, Mueller KR, Roodman ST, Bouhasin JD. Thymosin-alpha1 stimulates maturation of CD34+ stem cells into CD3+4+ cells in an in vitro thymic epithelia organ coculture model. *Int J Immunopharmacol.* 1999;21:15-26.
24. Mutchnick MG, Appelman HD, Chung HT, et al. Thymosin treatment of chronic hepatitis B: a placebo-controlled pilot trial. *Hepatology.* 1991;14:409-415.
25. Salvati F, Rasi G, Portalone L, Antilli A, Garaci E. Combined treatment with thymosin alpha 1 and low-dose interferon-alpha after ifosfamide in non-small cell lung cancer: a phase II controlled trial. *Anticancer Res.* 1996;16:1001-1004.
26. D'Agostini C, Palamara AT, Favalli C, et al. Efficacy of combination therapy with amantadine, thymosin alpha 1 and alpha/beta interferon in mice infected with influenza A virus. *Int J Immunopharmacol.* 1996;18:95-102.
27. Hadden JW, Saha A, Sosa M, Hadden EM. Immunotherapy with natural interleukins and/or thymosin alpha 1 potently augments T-lymphocyte responses of hydrocortisonetreated aged mice. *Int J Immunopharmacol.* 1995;17:821-828.
28. Di Francesco P, Pica F, Gaziano R, Favalli C, Garaci E. In vivo recovery of natural killer cell activity by the association of thymosin a 1 and cytokines during cocaine administration. *Med Sci Res.* 1994;22:41-42.
29. Favalli C, Mastino A, Jezzi T, Grelli S, Goldstein AL, Garaci E. Synergistic effect of thymosin alpha 1 and alpha beta-interferon on NK activity in tumor-bearing mice. *Int J Immunopharmacol.* 1989;11:443-450.

References

30. Favalli C, Jezzi T, Mastino A, Rinaldi- Garaci C, Riccardi C, Garaci E. Modulation of natural killer activity by thymosin alpha 1 and interferon. *Cancer Immunol Immunother.* 1985;20:189-192.
31. Mastino A, Favalli C, Grelli S, Innocenti F, Garaci E. Thymosin alpha 1 potentiates interleukin 2-induced cytotoxic activity in mice. *Cell Immunol.* 1991;133:196-205.
32. Serrate S, Schulof R, Leondaridis L, Goldstein AL, Sztein MB. Modulation of human natural killer cell cytotoxic activity, lymphokine production, and interleukin 2 receptor expression by thymic hormones. *J Immunol.* 1987;139:2338-2343.
33. Garaci E, Rocchi G, Perroni L, et al. Combination treatment with zidovudine, thymosin alpha 1, and interferon alpha in human immunodeficiency virus infection. *Int J Clin Lab Res.* 1994; 24:23-28.
34. Kawarabayashi N, Seki S, Hatsuse K, et al. Decrease of CD56(+)T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. *Hepatology.* 2000;32:962-969.
35. Sztein M, Serrate S, Goldstein A. Modulation of interleukin-2 receptor expression on normal human lymphocytes by thymic hormones. *Proc Natl Acad Sci U S A.* 1986;83:6107-6111.
36. Sztein M, Serrate S. Characterization of the immunoregulatory properties of thymosin alpha 1 on interleukin-2 production and interleukin-2 receptor expression in normal human lymphocytes. *Int J Immunopharmacol.* 1989;11:789-800.
37. Svedersky L, Hui A, May L, McKay P, Stebbing N. Induction and augmentation of mitogen-induced immune interferon production in human peripheral blood lymphocytes by N-desacetylthymosin alpha 1. *Eur J Immunol.* 1982;12:244-247.
38. Hsia J, Sarin N, Oliver JH, Goldstein AL. Aspirin and thymosin increase interleukin-2 and interferon-gamma production by human peripheral blood lymphocytes. *Immunopharmacology.* 1989;17:167-173.
39. Leichtling KD, Serrate SA, Sztein MB. Thymosin alpha 1 modulates the expression of high affinity interleukin-2 receptors on normal human lymphocytes. *Int J Immunopharmacol.* 1990;12:19-29.
40. Koziel MJ. Cytokines in viral hepatitis. *Semin Liver Dis.* 1999;19:157-169.
41. Foschi F, Gramenzi A, Castelli E, et al. Soluble CD30 serum levels in HCV-positive chronic active hepatitis: a surrogate marker of disease activity? *Cytokine.* In press.
42. Andreone P, Cursaro C, Gramenzi A, et al. In vitro effect of thymosin-alpha1 and interferon-alpha on Th1 and Th2 cytokine synthesis in patients with chronic hepatitis C. *J Viral Hepat.* 2001;8:194-201.
43. Baumann CA, Badamchian M, Goldstein AL. Thymosin alpha 1 antagonizes dexamethasone and CD3-induced apoptosis of CD4+CD8+ thymocytes through the activation of cAMP and protein kinase C dependent second messenger pathways. *Mech Age Develop.* 1997;94:85-101.

References

44. Baumann CA, Badamchian M, Goldstein AL. Thymosin alpha 1 is a time and dose dependent antagonist of dexamethasone-induced apoptosis of murine thymocytes in vitro. *Int J Immunopharmacol.* 2000;22:1057-1066.
45. Roy R, Singh SM, Shanker A. Mechanism of thymocyte apoptosis induced by serum of tumor-bearing host: the molecular events involved and their inhibition by thymosin alpha-1. *Int J Immunopharmacol.* 2000;22:309-321.
46. Gupta S, Aggarwal S, Nguyen T. Accelerated spontaneous programmed cell death in lymphocytes in DiGeorge Syndrome. *J Allergy Clin Immunol.* 1997;99(1):S3(#11).
47. Giuliani C, Napolitano G, Mastino A, et al. Thymosin a-1 regulates MHC class I expression in FRTL-5 cells at transcriptional level. *Eur J Immunol.* 2000;30:778-786.
48. Hicklin DJ, Marincola FM, Ferrone S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. *Mol Med Today.* 1999;5:178-186.
49. Rinaldo CR. Modulation of major histocompatibility complex antigen expression by viral infection. *Am J Pathol.* 1994;144:637-650.
50. Colledge D, Wang Y, Tuthill C, Locarnini S. Antiviral effects of thymosin alpha 1 versus leukocytic interferon in primary cultures of duck hepatocytes persistently infected with duck HBV in vitro. Paper presented at: Second International Conference on Therapies for Viral Hepatitis, 1997; Kona, Hawaii.
51. Palamara A, Bue M, Savini P, et al. Thymosin alpha 1 inhibits Sendai virus replication: involvement of intracellular redox state. Paper presented at: 6th International Expert Forum of Immunotherapy and Gene Therapy; May 1998; Florence, Italy.
52. Moody TW, Leyton J, Zia F, Tuthill C, Badamchian M, Goldstein AL. Thymosin alpha 1 is chemopreventive for lung adenoma formation in A/J mice. *Cancer Lett.* 2000;155:121-127.
53. Moody TW, Fagarasan M, Zia F, Cesnjaj M, Goldstein AL. Thymosin alpha 1 downregulates the growth of human non-small cell lung cancer cells in vitro and in vivo. *Cancer Res.* 1993;53:5214-5218.
54. Moody T, Badamchian M, Goldstein A. Thymosin alpha 1 prevents lung carcinogenesis. *FASEB J.* 1998;12: A1457.
55. Moody T, Tuthill C, Badamchian M, Goldstein A. Thymosin alpha 1 inhibits mammary carcinogenesis in Fisher rats. *Cancer Res.* Submitted August 2001.
56. Herzenberg LA, De Rosa SC, Dubs JG, et al. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci U S A.* 1997;94:1967-1972.
57. Ciriolo MR, Palamara AT, Incerpi S, et al. Loss of GSH, oxidative stress, and decrease of intracellular pH as sequential steps in viral infection. *J Biol Chem.* 1997;272:2700-2708.
58. Palamara AT, Perno CF, Ciriolo MR, et al. Evidence for antiviral activity of glutathione: in vitro inhibition of herpes simplex virus type 1 replication. *Antiviral Res.* 1995;27:237-253.

References

59. Palamara AT, Perno CF, Aquaro S, Bue MC, Dini L, Garaci E. Glutathione inhibits HIV replication by acting at late stages of the virus life cycle. *AIDS Res Hum Retroviruses*. 1996;12:1537-1541.
60. De Rosa SC, Zaretsky MD, Dubs JG, et al. N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest*. 2000;30:915-929.
61. Neri S, Ierna D, Antoci S, Campanile E, D'Amico RA, Noto R. Association of alpha-interferon and acetyl cysteine in patients with chronic C hepatitis. *Panminerva Med*. 2000;42:187-192.
62. Gerin JL, Korba BE, Cote PJ, Tennant BC. A preliminary report of a controlled study of thymosin alpha 1 in the woodchuck model of hepatitis B virus infection. In: Block T, ed. *Innovations in Antiviral Development and the Detection of Virus Infection*. Philadelphia, Pa: Jefferson Medical; 1992:121-123.
63. Tennant BC, Korba BE, Baldwin BH, Goddard LA, Hornbuckle WE, Cote PJ. Treatment of chronic woodchuck hepatitis virus infection with thymosin alpha-1 (TA1). *Antiviral Res*. 1993;20(suppl 1):163.
64. Gangemi J, Korba B, Tennant B, Ueda H, Jay G. Antiviral and anti-proliferative activities of alpha interferons in experimental hepatitis B virus infections. *Antiviral Therapy*. 1996;1:64-70.
65. Tennant B. Manuscript in preparation. November 2001.
66. Ishitsuka H, Umeda Y, Nakamura J, Yagi Y. Protective activity of thymosin against opportunistic infections in animal models. *Cancer Immunol Immunother*. 1983;14:145-150.
67. Shiau AL, Wu CL, Huang KY. The effect of thymosin on experimental herpes simplex virus infections. *J Formosan Med Assoc*. 1988;87:34-42.
68. Effros RB, Casillas A, Walford RL. The effect of thymosin alpha 1 on immunity to influenza in aged mice. *Aging: Immunology and Infectious Disease*. Vol 1. New York, NY: Mary Ann Liebert, Inc; 1988:31-40.
69. Bistoni F, Marconi P, Frati L, Bonmassar E, Garaci E. Increase of mouse resistance to candida albicans infection by thymosin alpha 1. *Infect Immun*. 1982;36:609-614.
70. Di Francesco P, Gaziano R, Casalnuovo IA, Belogi L, Palamara AT, Favalli C. Combined effect of fluconazole and thymosin alpha 1 on systemic candidiasis in mice immunosuppressed by morphine treatments. *Clin Exp Immunol*. 1994;97:347-352.
71. Hadden EM, Malec P, Sosa M, Hadden JW. Mixed interleukins and thymosin fraction V synergistically induce T lymphocyte development in hydrocortisone-treated aged mice. *Cell Immunol*. 1992;144:228-236.
72. Rasi G, Silecchia G, Sinibaldi-Vallebona P, et al. Anti-tumor effect of combined treatment with thymosin alpha 1 and interleukin-2 after 5-fluorouracil in liver metastases from colorectal cancer in rats. *Int J Cancer*. 1994;57:701-705.

References

73. Mastino A, Favalli C, Grelli S, et al. Combination therapy with thymosin alpha 1 potentiates the antitumor activity of interleukin-2 with cyclophosphamide in the treatment of the Lewis Lung carcinoma in mice. *Cancer Res.* 1992; 50:493-499.
74. Garaci E, Pica F, Mastino A, Palamara AT, Belardelli F, Favalli C. Antitumor effect of thymosin alpha-1 interleukin-2 or thymosin a-1/interferon ab following cyclophosphamide in mice injected with highly metastatic Friend erythroleukemia cells. *J Immunother.* 1993;13:7-17.
75. Umeda Y, Sakamoto A, Nakamura J, Ishitsuka H, Yagi Y. Thymosin alpha 1 restores NK-cell activity and prevents tumor progression in mice immunosuppressed by cytostatics or X-rays. *Cancer Immunol Immunother.* 1983;15:78-83.
76. Tomazic V, Sacasa C, Loftus A, Suter C, Elias G. Thymic factor-induced reduction of pulmonary metastases in mice with FSA-1 fibrosarcoma. *Clin Expl Metastasis.* 1988;6:17-25.
77. Silecchia G, Guarino E, Sinibaldi-Vallebona P, et al. Efficacy of repeated cycles of chemo-immunotherapy with thymosin alpha 1 and interleukin-2 after intraperitoneal 5-fluorouracil delivery. *Cancer Immunol Immunother.* 1999; 48:172-178.
78. Pica F, Frascchetti M, Matteucci C, Tuthill C, Rasi G. High doses of thymosin alpha 1 enhance the anti-tumor efficacy of combination chemo-immunotherapy for murine B16 melanoma. *Anticancer Res.* 1998;18:3571-3578.
79. Product Information - Intron A. In: Arky R, ed. *Physicians' Desk Reference.* Vol 51. Montvale, NJ: Medical Economics Company; 1999:2513.
80. Weiss K. Safety profile of interferon-alpha therapy. *Semin Oncol.* 1998;25(1 suppl 1):9-13.
81. Hunt C, Dominitz J, Philips Bute B, Waters B, Blasi U, Williams D. Effect of interferon alpha treatment of chronic hepatitis C on health-related quality of life. *Dig Dis Sci.* 1997;42:2482-2486.
82. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology.* 2001;33:1527-1532.
83. Malik AH, Lee WM. Hepatitis B therapy: the plot thickens. *Hepatology.* 1999;30:579-581.
84. Garaci E, Milanese G, Vella S, et al. A randomized controlled study for the evaluation of the activity of a triple combination of zidovudine, thymosin alpha 1 and interferon alpha in HIV-infected individuals with CD4 counts between 200 and 500 cells/mm³. *Antiviral Therapy.* 1998;3:103-111.
85. Shen SY, Corteza QB, Josselson J, et al. Age-dependent enhancement of influenza vaccine responses by thymosin in chronic hemodialysis patients. In: Goldstein AL, ed. *Biomedical Advances in Aging.* New York, NY: Plenum Press; 1990:523-530.
86. Chan HL, Tang JL, Sung JY. Thymosin a1 for the treatment of chronic hepatitis B virus (HBV) infection: a meta-analysis. Paper presented at: Digestive Disease Week 2001; May 20-23, 2001; Atlanta, GA.

References

87. Chien RN, Liaw YF, Chen TC, Yeh CT, Sheen IS. Efficacy of thymosin alpha1 in patients with chronic hepatitis B: a randomized, controlled trial. *Hepatology*. 1998;27:1383-1387.
88. Andreone P, Cursaro C, Gramenzi A, et al. A randomized controlled trial of thymosin alpha 1 versus interferon alpha treatment in patients with hepatitis B e antigen antibody — and hepatitis B virus DNA — positive chronic hepatitis B. *Hepatology*. 1996;24:774-777.
89. Mutchnick MG, Lindsay KL, Schiff ER, Cummings GD, Appelman HO. Thymosin alpha 1 treatment of chronic hepatitis B: a multicenter, randomized, placebo-controlled double blind study. *Gastroenterology*. 1995;108:A1127.
90. Locarnini SA. Challenges for development of antiviral agents for hepatitis B. Paper presented at: Second International Conference on Therapies for Viral Hepatitis; 1997; Kona, Hawaii.
91. Malaguarnera M, Restuccia S, Motta M, Ruello P, Trovato B, Pistone G. Interferon, cortisone, and antivirals in the treatment of chronic viral hepatitis: a review of 30 years of therapy. *Pharmacotherapy*. 1997;17:998-1005.
92. Woo MH, Burnakis TG. Interferon alfa in the treatment of chronic viral hepatitis B and C. *Ann Pharmacother*. 1997;31:330-337.
93. Saracco G, Rizzetto M. Recent results in the treatment of chronic B virus hepatitis. *Biomed Pharmacother*. 1995;49:55-57.
94. Lok A, Wu P-C, Lai C-L, Leung E. Long-term follow-up in a randomised controlled trial of recombinant alpha2-interferon in Chinese patients with chronic hepatitis B infection. *Lancet*. 1988;2:298-302.
95. Lok AS, Wu PC, Lai CL, et al. A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. *Gastroenterology*. 1992;102:2091-2097.
96. Liaw YF, Lin SM, Chen TJ, Chen RN, Sheen IS, Chu CM. Beneficial effect of prednisolone withdrawal followed by human lymphoblastoid interferon on the treatment of chronic type B hepatitis in Asians: a randomized controlled trial. *J Hepatol*. 1994;20:175-180.
97. Lopez-Alcorocho J, Vartolome J, Cotonat T, Carreno V. Efficacy of prolonged interferon alpha treatment in chronic hepatitis B patients with HBeAb: comparison between 6 and 12 months of therapy. *J Vir Hep*. 1997;4(suppl 1):27-32.
98. Garaci E, Mastino A, Pica F, Favalli C. Combination treatment using thymosin alpha 1 and interferon after cyclophosphamide is able to cure Lewis lung carcinoma in mice. *Cancer Immunol Immunother*. 1990;32:154-160.
99. Rasi G, Mutchnick MG, Di Virgilio D, et al. Combination low-dose lymphoblastoid interferon and thymosin alpha 1 therapy in the treatment of chronic hepatitis B. *J Viral Hep*. 1996;3:191-196.
100. Saruc M, Yuceyar H, Kucukmetin N, Bayar C. Comparison of interferon a2b monotherapy with the combination of thymosin a1 and interferon a2b in the treatment of anti-HBe-positive chronic hepatitis B in Turkey. Paper presented at: Digestive Disease Week 2001; May 20-23, 2001; Atlanta, GA.

References

101. Lau G, Yuen S, Kwok A, Lai S, Lim W, Lam S. Six-months follow-up on a 26 week trial of thymosin a 1 plus famciclovir in the treatment of Chinese immune tolerant adult patients with chronic hepatitis B. *Gastroenterology*. 1999;116:A339.
102. Leung Y, So T. Treatment of chronic hepatitis B using thymosin alpha 1 and a combination of two nucleoside analogues, lamivudine and famciclovir. *Hepatology*. 1998;28:216A.
103. Suoglu OD, Elkabes B, Sokucu S, Saner G. Thymosin alpha and lamivudine combination therapy in pediatric patients with chronic hepatitis B infection unresponsive to previous treatments: a pilot study. *Gut*. 2001;49(suppl III). Abstract 1639.
104. Davis GL, Esteban-Mur R, Rustgi V, et al, for the International Hepatitis Interventional Therapy Group. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. *N Engl J Med*. 1998;339:1493-1499.
105. Sherman KE, Sjogren M, Creager RL, et al. Combination therapy with thymosin alpha1 and interferon for the treatment of chronic hepatitis C infection: a randomized, placebo-controlled double-blind trial. *Hepatology*. 1998;27:1128-1135.
106. Rasi G, DiVirgilio D, Mutchinick MG, et al. Combination thymosin a1 and lymphoblastoid interferon treatment in chronic hepatitis C. *Gut*. 1996;39:670-683.
107. Moscarella S, Buzzelli G, Monti M, et al. Treatment with interferon-alpha and thymosin alpha 1 of naive patients affected by chronic hepatitis C. Paper presented at: 4th International Meeting on Hepatitis C Virus and Related Viruses; 1997; Kyoto, Japan.
108. Sherman K, Sherman S. Interferon plus thymosin a-1 treatment of chronic hepatitis C infection: a meta-analysis. In: Schinazi R, Sommadossi J, Thomas H, eds. *Therapies for Viral Hepatitis*. London, England: International Medical Press; 1998:379-383.
109. Kullavanuaya P, Treeprasertsuk S, Thong-Ngam D, Chaermthai K, Gonlachanvit S, Suwanagool P. The combined treatment of interferon alpha-2a and thymosin alpha 1 for chronic hepatitis C: the 48 weeks end of treatment results. *J Med Assoc Thai*. 2001;84(suppl 1):S462-468.
110. Bonkovsky H. Therapy of hepatitis C: other options. *Hepatology*. 1997;26:1435-1515.
111. Garaci E, Rocchi G, Perroni L, et al. Combined therapy with zidovudine-thymosin alpha 1 - alpha interferon in the treatment of HIV-infected patients. Paper presented at: Second International Symposium on Combination Therapies; 1992; Sicily, Italy.
112. de Weck A, Kristensen F, Joncourt F, Bettens F, Walker C, Wang Y. Lymphocyte proliferation, lymphokine production, and lymphocyte receptors in ageing and various clinical conditions. *Springer Semin Immunopathol*. 1984;7:273-289.
113. Makinodan T, Peterson W. Relative antibody-forming capacity of spleen cells as a function of age. *Proc Natl Acad Sci U S A*. 1962;48:234-238.
114. Wade AW, Szewczuk MR. Aging, idiotype repertoire shifts, and compartmentalization of the mucosal-associated lymphoid system. *Adv Immunol*. 1984;36:143-188.
115. Ershler W, Moore A, Socinski M. Influenza and aging: age-related changes and the effects of thymosin on the antibody response to influenza vaccine. *J Clin Immunol*. 1984;4:445-454.

References

116. Ershler W, Moore A, Hacker M, Ninomiya J, Naylor P, Goldstein A. Specific antibody synthesis in vitro. II. Age-associated thymosin enhancement of antitetanus antibody synthesis. *Immunopharmacology*. 1984;8:69-77.
117. Bramwell SP, Tsakiris DJ, Briggs JD, et al. Dinitrochlorobenzene skin testing predicts response to hepatitis B vaccine in dialysis patients. *Lancet*. 1985;1:1412-1415.
118. Dammin G, Couch N, Murray J. Prolonged survival of skin homografts in uremic patients. Paper presented at: Second Tissue Homotransplantation Conference; 1957; New York, NY.
119. Lawrence HS. Uremia: nature's immunosuppressive device [editorial]. *Ann Intern Med*. 1965;62:166-170.
120. Revie D, Shen S, Ordonez J, et al. T-cell subsets and status of hepatitis B surface antigen and antibody in end-stage renal disease patients. *Kidney Int*. 1985;27:150.
121. Sanders CV Jr, Luby JP, Sanford JP, Hull AR. Suppression of interferon response in lymphocytes from patients with uremia. *J Lab Clin Med*. 1971;77:768-776.
122. Stevens CE, Alter HJ, Taylor PE, Zang EA, Harley EJ, Szmunes W. Hepatitis B vaccine in patients receiving hemodialysis: immunogenicity and efficacy. *N Engl J Med*. 1984;311:496-501.
123. Grob P, Binswanger U, Zaruba K, et al. Immunogenicity of a hepatitis B subunit vaccine in hemodialysis and in renal transplant recipients. *Antiviral Res*. 1983;3:43-52.
124. Crosnier J, Jungers P, Courouc AM, et al. Randomised placebo-controlled trial of hepatitis B surface antigen vaccine in French haemodialysis units: I, medical staff. *Lancet*. 1981;1:455-459.
125. Ershler W, Hebert J, Blow A, Granter S, Lynch J. Effect of thymosin alpha one on specific antibody response and susceptibility to infection in young and aged mice. *Int J Immunopharmacol*. 1985;7:465-471.
126. Ravagnan G, Falchetti R, Di Francesco P, et al. Effect of thymosin alpha 1 on cocaine-induced inhibition of T-cell dependent murine immune response [abstract]. Paper presented at: Second International Symposium on Combination Therapies; 1992; Sicily, Italy.
127. Gravenstein S, Ershler WB, Drumaskin S, Schwab R, Weksler ME. Anti-influenza antibody response: augmentation in elderly "non-responders" by thymosin alpha 1. *Gerontologist*. 1986;26:150A.
128. Gravenstein S, Duthie EH, Miller BA, et al. Augmentation of influenza antibody response in elderly men by thymosin alpha one. A double-blind placebo-controlled clinical study. *J Am Geriatr Soc*. 1989;37:1-8.
129. McConnell L, Gravenstein S, Roecker E, Spencer S, Simon G, Ershler W. Augmentation of influenza antibody levels and reduction in attack rates in elderly subjects by thymosin alpha 1. *The Gerontologist*. 1989;29:188A.
130. Shen S, Josselson J, McRoy C, Sadler J, Chretien P. Effect of thymosin alpha 1 on heptavax-B vaccination among hemodialysis patients. *Kidney Int*. 1987:217.

References

131. Shen S, Josselson J, McRoy C, Sadler J, Chretien P. Effects of thymosin alpha 1 on peripheral T-cell and Heptavax-B vaccination in previously non-responsive hemodialysis patients. *Hepatology*. 1987;7:1120.
132. Morton D, Economou J. Cancer immunology and immunotherapy. In: Haskell C, ed. *Cancer Treatment*. 3rd ed. Philadelphia, Pa: WB Saunders Company; 1990:102-119.
133. Rosenberg SA, Mul JJ, Spiess PJ, Reichert CM, Schwarz SL. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. *J Exp Med*. 1985;161:1169-1188.
134. Balch CM, Reintgen DS, Kirkwood JM, Houghton A, Peters L, Aug KK. Cutaneous Melanoma. In: DeVita V, Hellman S, Rosenberg S, eds. *Cancer: Principles and Practice of Oncology*. 5th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 1997:1947-1994.
135. Richards J, Gale D, Mahta N, Lestingi T. Combination of chemotherapy with interleukin-2 and interferon alfa for treatment of metastatic melanoma. *J Clin Oncol*. 1999;17:651-657.
136. Lee R. Neoplasms and other masses. *Diagnostic Liver Pathology*. Vol 1. St. Louis, MO: Mosby; 1994:487-499.
137. Hodgson H. Primary hepatocellular carcinoma. In: Blumgart L, ed. *Surgery of the Liver and Biliary Tract*. Vol 2. 2 ed. Edinburgh, United Kingdom: Churchill Livingstone; 1994:1341-1363.
138. Carr B, Flickinger J, Lotze M. Hepatobiliary Cancers. In: deVita V Jr, Hellman S, Rosenberg S, eds. *Cancer: Principles and Practice of Oncology*. Vol 1. 5 ed. Philadelphia, Pa: Lippincott-Raven Publishers; 1997:1088-1109.
139. Beasley R. Hepatitis B as the etiologic agent in hepatocellular carcinoma: Epidemiologic considerations. *Hepatology*. 1982;2(suppl):21.
140. Wogan G. Aflatoxins and their relationship to hepatocellular carcinoma. In: Okuda K, Peters R, eds. *Hepatocellular Carcinoma*. New York, NY: John Wiley & Sons; 1976:25.
141. Kew M. Hepatitis C virus and hepatocellular carcinoma. *FEMS Microbiol Rev*. 1994;14:211-219.
142. Stefanini GF, Foschi FG, Castelli E, et al. Alpha-1-thymosin and transcatheter arterial chemoembolization in hepatocellular carcinoma patients: a preliminary experience. *Hepatogastroenterology*. 1998;45:209-215.
143. Li ZS. HCC patients treated with TACE combined with thymalfasin: one year follow up. Paper presented at: Shanghai International Oncology Conference; 2001.
144. Feld R, Ginsberg RJ, Payne DG, Shepherd FA. Lung. In: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, eds. *Clinical Oncology*. 2nd ed. New York, NY: Churchill Livingstone; 2000.
145. Boring CC, Squires TS, Tong T. Cancer statistics, 1992 [published erratum appears in *CA Cancer J Clin* 1992;42:127-128]. *CA Cancer J Clin*. 1992;42:19-38.

References

146. Boring CC, Squires TS, Tong T. Cancer statistics, 1993. *CA Cancer J Clin.* 1993;43:7-26.
147. Schulof RS, Lloyd MJ, Cleary PA, et al. A randomized trial to evaluate the immunorestorative properties of synthetic thymosin alpha 1 in patients with lung cancer. *J Biol Response Modifiers.* 1985;4:147-158.
148. Lopez M, Di Lauro L, Vici P, et al. Biological agents and chemotherapy in the treatment of solid tumors. Paper presented at: Third International Symposium on Combination Therapies; 1993; Houston, Texas.
149. Garbe C, Kreuser E. Combined treatment of metastatic melanoma with interferons and cytotoxic drugs. *Semin Oncol.* 1992;19(suppl 4):63-69.
150. Falkson C, Falkson G. Improved results with the addition of recombinant interferon alpha-2b to dacarbazine in treatment of patients with metastatic malignant melanoma. *Proc Am Assn Cancer Res.* 1990;31:A1185.
151. Houghton A, Legha S, Bajorin D. Chemotherapy for metastatic melanoma. In: Balch C, Houghton A, Sober A, eds. *Cutaneous Melanoma.* Philadelphia, Pa: Lippincott; 1992:498-508.
152. Favalli C. Combination therapy in malignant melanoma. Paper presented at: Third International Symposium on Combination Therapies; 1993; Houston, Texas.
153. Rasi G, Terzoli E, Izzo F, et al. Combined treatment with thymosin alpha 1 and low-dose interferon alpha after dacarbazine in advanced melanoma. *Melanoma Res.* 2000;10:189-192.
154. Lopez M, Carpano S, Cavaliere R, DiLauro L, Ameglio F, Vitelli G. Biochemotherapy with thymosin alpha 1, interleukin-2 and dacarbazine in patients with metastatic melanoma: clinical and immunological effects. *Ann Oncol.* 1994;5:741-746.
155. Flaherty L, Redman B, Chabot G, et al. A phase I-II study of dacarbazine in combination with outpatient interleukin-2 in metastatic malignant melanoma. *Cancer.* 1990;65:2471-2477.
156. Rost K, Wierich W, Masayuki F, Tuthill C, Horwitz D, Herrmann W. Pharmacokinetics of thymosin alpha 1 after subcutaneous injection of three different formulations in healthy volunteers. *Int J Clin Pharmacol Ther.* 1999; 37:51-57.
157. Schulof RS, Lloyd M, Cox J. An evaluation of two different schedules of synthetic thymosin alpha 1 administration in patients with lung cancer: preliminary results. In: Goldstein A, ed. *Thymic Hormones and Lymphokines.* New York, NY: Plenum Press; 1984:601-613.
158. Food and Drug Administration Medical Officers Review. EPIVIR-HBV New Drug Application. 1998;26.
159. Data on file. SciClone Pharmaceuticals, San Mateo, CA.