

# Thymosin Apha 1 – A Peptide Immune Modulator with a Broad Range of Clinical Applications

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## Abstract

Thymosin alpha 1 (thymalfasin, Ta1), the active ingredient in ZADAXIN®, is a peptidic biological response modifier which activates various cells of the immune system. Ta1 is therefore expected to have clinical benefits in disorders where immune responses are impaired or ineffective, such as acute and chronic infections, cancers, and vaccine non-responsiveness, all of which are hallmarks of an impaired or inadequate immune response. Importantly, Ta1 acts without overstimulation of cytokine production and is generally well tolerated; it has an excellent safety profile and does not appear to induce the side effects and toxicities commonly associated with agents in this class such as interferon-alpha and interleukin-2.

Ta1 has been shown to act through Toll-like receptors, linking to intracellular cell-signaling pathways and leading to stimulation of the immune system, including T helper cells, cytotoxic T cells, and natural killer cells.

Clinical studies with Ta1 have been conducted and shown evidence of benefit in subjects with acute or chronic viral or bacterial infections, including subjects with severe sepsis, infections after bone marrow transplant, chronic hepatitis B or C, or acquired immune deficiency syndrome. Ta1 has also shown promise in clinical evaluation as an adjunctive treatment in subjects suffering from various types of cancer, including hepatocellular carcinoma, non-small-cell lung cancer, melanoma, renal, breast and gastric cancers; and in subjects receiving vaccines (geriatric subjects receiving influenza vaccine and subjects with renal failure receiving influenza or hepatitis vaccine).

**Keywords:** Thymosin alpha 1; ZADAXIN; Immune modulation; Sepsis; Hepatitis; Cancer; Vaccination

## Introduction

The peptide thymosin alpha 1 (thymalfasin, Ta1; commercial name ZADAXIN®) is a peptide of 28 amino acids (Figure 1) which was originally isolated from thymic tissue as one of the compounds responsible for reconstitution of immune function in thymectomized animal models [1,2].

Endogenous Ta1 can be detected in serum, where levels measured in healthy adults by immunoassays are in the 0.1 to 1.0 ng/mL range [3,4]. The circulating concentration of Ta1 tends to be lower in diseased individuals and higher during pregnancy [5-8]. Ta1, found in highest concentrations in the thymus, has also been found in spleen, lung, kidney, brain, blood, and a number of other tissues.

Ta1 is a biological response modifier which activates various cells of the immune system, and is therefore expected to have clinical benefits in disorders where immune responses are impaired or ineffective. These disorders include acute and chronic infections (including severe sepsis, infections after bone marrow transplant, lung infections including Chronic Obstructive Pulmonary Disorder (COPD), hepatitis B and C, and HIV), cancers (HCC, lung cancer, and melanoma), and vaccine non-responsiveness, all of which are hallmarks of an impaired or inadequate immune response. Although originally isolated from bovine tissue, the identical synthetic peptide has been evaluated in clinical studies and gained marketing approval in several countries, most notably in China.

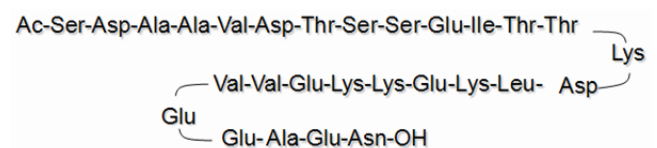
## Mechanism of Action

Investigation of the mechanism of action of Ta1 at the cellular level has shown both immune modulating and direct-acting effects (Figure 2). Ta1 interacts with intracellular Toll-Like Receptors (TLR; a family of proteins that mediate innate immunity) and may gain access to the cell by crossing the membrane unassisted, as it can fold into a structured helix in organic solvents [9,10].

## Immune stimulating effects

Ta1's interaction with TLR9 and TLR2 on Dendritic Cells (DCs) and precursor T cells leads to a stimulation of intracellular cell-signaling pathways, including NFkappaB and p38 MAPK [10-12], and results in:

- Increased Natural Killer (NK) activity [13-25]
- A shift of T helper (Th or CD4) cells to the Th1 cell subset [11,26-28]
- Increased expression of Th1 type cytokines such as Interleukin (IL) 2 [24,29,30,31], and Interferon (IFN)-alpha [24,29-34]
- Increased levels of Cytotoxic T (Tc1 or CD8) cells [15,16,22,23,25,35,36]



**Figure 1:** Primary structure of Ta1. Thymosin alpha 1 (Ta1) is a synthetic 28 amino acid peptide, acetylated at the N terminus.

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- Increased activation of DCs [36-40]

These immunological effects can explain Ta1's effectiveness in indications where a stimulated or enhanced immune response is desirable for health outcome, including viral, bacterial, and fungal infections and cancers, as well as vaccination of immunocompromised subjects (e.g., the elderly or those with renal failure). NK cells, and CD4 helper Th1 and CD8 cytotoxic Tc1 cells acting in concert, lead to killing of virally-infected cells and tumors [41-43]. Activated DCs also provide anti-viral and anti-tumor responses [44-49], as well as killing of bacterial and fungal infections. Activated DCs also lead to more efficient antigen presentation [50] for increased production of antibodies, an effect of Ta1 [51-53] which is especially important for the elderly who do not respond well to vaccination due to being immunosuppressed.

Ta1 also increases lymphocytic infiltration to sites of disease [15], and a correlation between tumor-infiltrating lymphocytes and prognosis for patients with stage IV cancer has been documented [54]. Moreover, Ta1 can reduce apoptosis of immune cells, as shown in mouse [55,56] and human [57,58] thymocytes, and increase stem cell expansion in immunosuppressed mice [59-61]. Additionally, Ta1 leads to increased expression of the thymopoietic cytokines IFN-alpha, IL-7, and IL-15 [37,57].

### Immune dampening effects

Importantly, it has also been shown that Ta1 stimulates activity of Indoleamine-2,3-Dioxygenase (IDO) in plasmacytoid DCs [37,40,62]. Stimulation of IDO leads to an increase in FoxP3 IL-10 producing regulatory T cells, and this increase leads to feedback inhibition of

cytokine production, hence dampening immune response to prevent a pro-inflammatory cytokine storm and possibly autoimmune phenomena (Figure 3).

### Direct-acting effects

Ta1 has also been shown to increase expression of proteins on the surface of virally-infected or tumor cells, including those that mediate antigen presentation such as Major Histocompatibility (MHC) Class I, MHC Class II, and beta-2 microglobulin [36,63], as well as tumor-specific antigens [64,65]. Immune escape by virally-infected and tumor cells has been correlated with down-regulation of antigen-presenting molecules [66,67].

Ta1 treatment also increases intracellular Glutathione (GSH) [68], important for anti-viral effects, and importantly has been shown to directly inhibit the *in vitro* growth of virally infected and cancer cells [69,70].

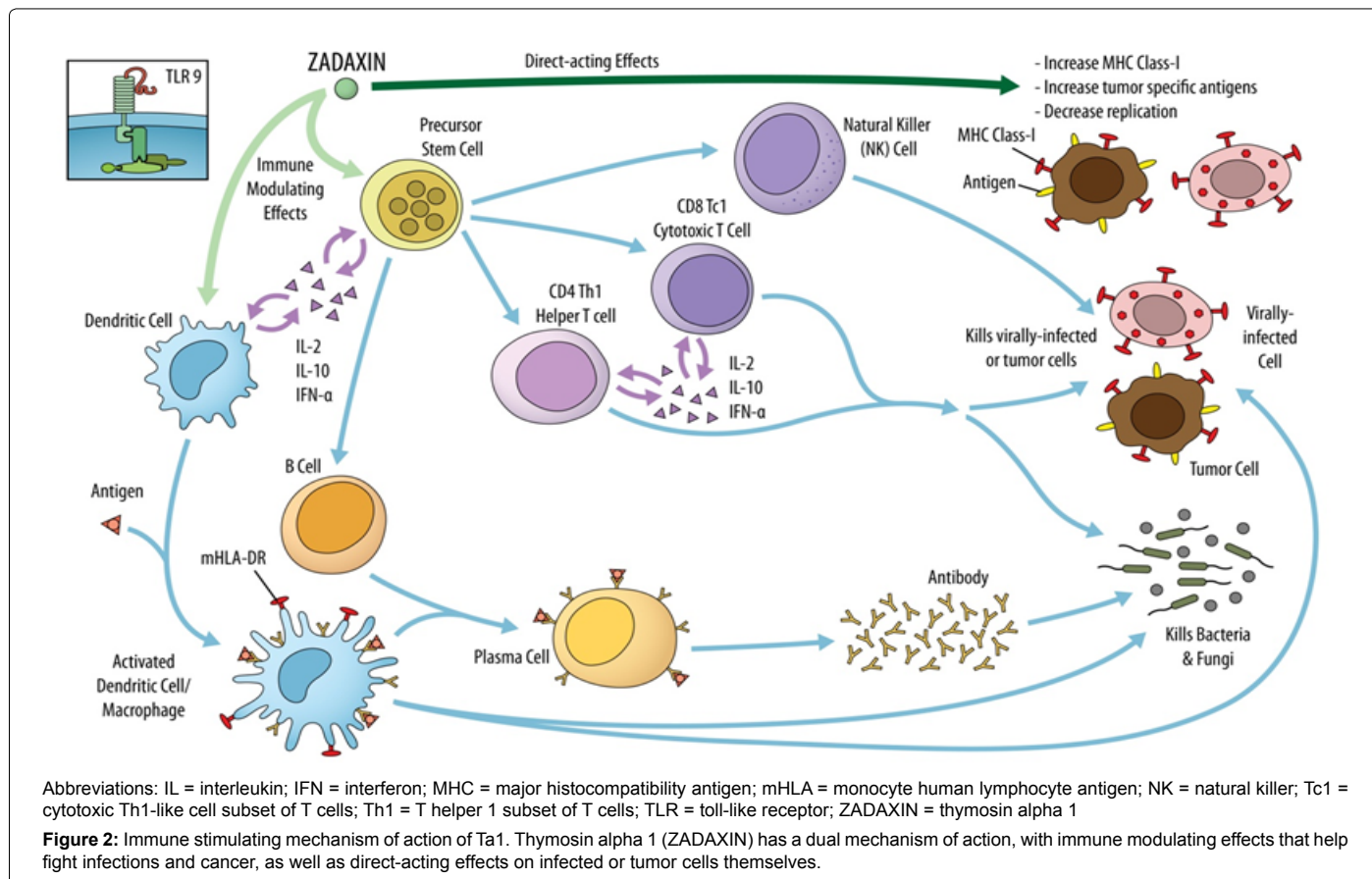
A number of previous review articles [71-80] address the history, biological effects, and potential therapeutic indications for Ta1.

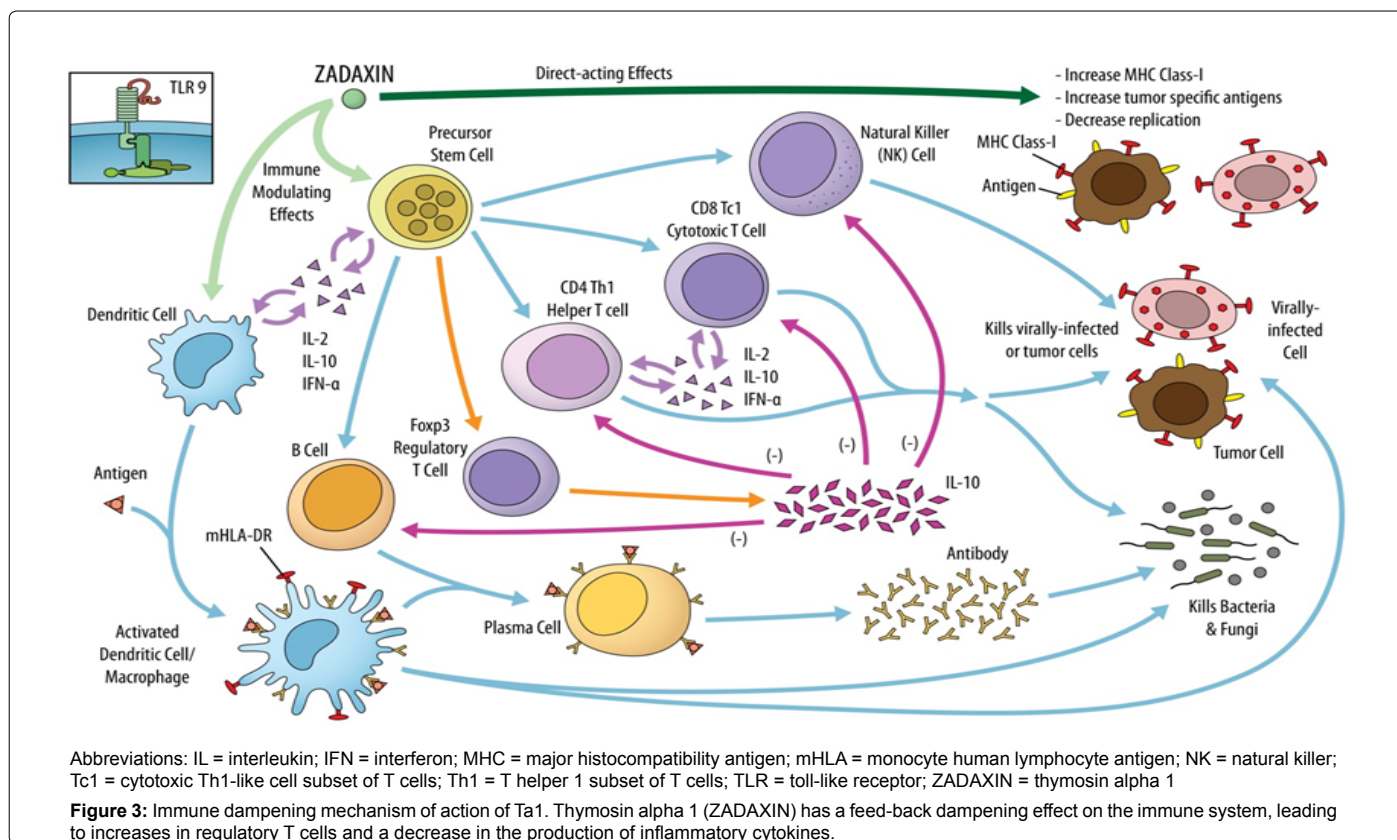
### Ta1 Studies in Infectious Disease

Ta1 has been evaluated for prevention and treatment of many different acute and chronic infectious diseases, including bacterial, fungal, and viral infections, as outlined in the studies described below.

### Sepsis

In the US, severe sepsis is still one of the leading causes of death and accounts for an estimated 750,000 new cases every year and





about 10% of admissions to intensive care units [81]. The mortality rates for patients with sepsis, severe sepsis and septic shock has been approximately reported to be 16, 20 and 46%, respectively. A large number of experimental agents including numerous anti-inflammatory and immuno-modulatory drugs have been tried, but few, if any, have yet been found to significantly reduce the risk of death as single agents. Although bacteria play a significant role in the onset of sepsis, in many cases the primary cause of death is due to the paralysis and dysregulation of the immune system and the severe pro-inflammatory response due to a cascade of inflammatory cytokines and chemokines that are multi-organ in nature.

In severe sepsis, sepsis-induced immunosuppression is increasingly recognized as the overriding immune dysfunction in these vulnerable patients [82] and there is now agreement that many patients with severe sepsis survive the first critical hours of the syndrome but eventually die later in a state of immunosuppression that is illustrated by the patients' difficulty to fight the primary bacterial infection, decreased resistance to secondary nosocomial infections, and reactivation of viral infections [83]. Consequently, an immunostimulatory therapy such as Ta1 could be used to restore immune functions in the most immunodepressed patients.

The use of Ta1 in treatment of 361 subjects diagnosed with severe sepsis was recently evaluated in a large, prospective, multi-center, single-blind, randomized, and controlled trial in China [84] which was conducted in the Intensive Care Units (ICUs) of six tertiary, teaching hospitals. The trial was registered with ClinicalTrials.gov, number NCT00711620.

Within 28 days after enrollment, 47 of 181 patients in the Ta1 treatment group (26.0%) and 63 of 180 patients in the control group

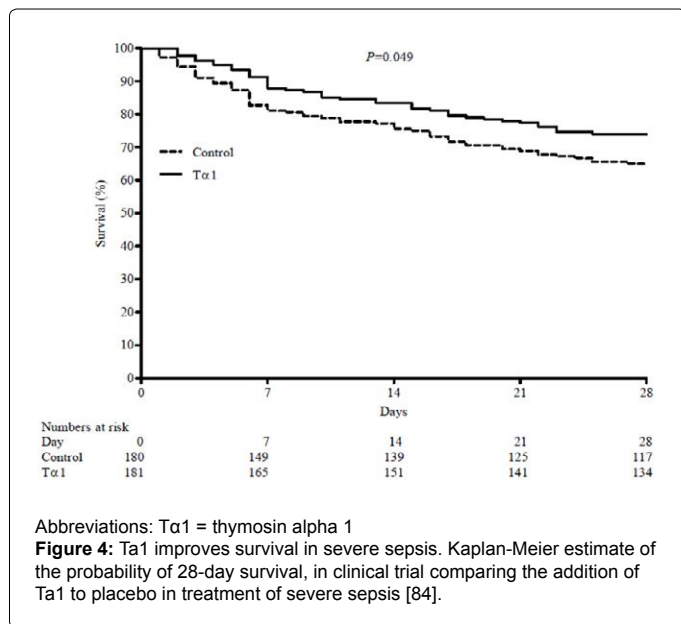
(35.0%) expired. The relative risk of death in the Ta1 group as compared to the control group was 0.74 (95% Confidence Interval [CI] 0.54 to 1.02) with a  $p$  value of 0.062 in the nonstratified analysis. There was a 9.0% (95% CI -0.5 to 18.5%) absolute reduction in mortality in the Ta1 group. Patients in the Ta1 group survived longer after enrollment than the control group (log rank,  $p=0.049$ ; Figure 4). Greater improvements in Monocyte Human Lymphocyte Antigen (mHLA)-DR were observed in patients in the Ta1 group on day 3 (mean difference in mHLA-DR changes between the two groups was 3.9%, 95% CI 0.2 to 7.6%,  $p=0.037$ ) and day 7 ( $p=0.017$ ). No serious drug-related adverse events were recorded.

In addition to the trial described above, Ta1 has been used in many studies in combination with standard of care, and also in combination with trypsin Protease Inhibitors (PI) in addition to standard of care, for treatment of severe sepsis. Studies and their results are summarized in Table 1.

### Infections after bone marrow transplant

While bone marrow transplant offers benefit for treatment of hematological malignancies, next to relapse the main cause of death in these subjects is a 35–40% Transplant-Related Mortality (TRM) which is largely due to infections (primarily cytomegalovirus and *Aspergillus*) [94].

As animal models have shown efficacy for Ta1 in prevention and treatment of *Aspergillus* infections after bone marrow transplant [11,38], a phase 2 clinical study was conducted in Italy to evaluate the use of Ta1 after HLA matched or haploidentical stem cell transplant in 75 subjects (Perruccio 2010 and Romani 2012) [95,96]. This study found very promising results:



- The cumulative incidence of TRM (mostly infection-related) was 32% in control subjects but only 7% in subjects treated with Ta1 ( $p=0.02$ )
- Event-free survival was increased in Ta1 subjects (40% versus 20% in controls;  $p=0.02$ )
- Ta1 treatment was a significant independent factor predicting a lower incidence of TRM ( $p=0.04$ ), which tended to provide better survival ( $p=0.09$ )
- Ta1 administration was associated with increased T cell counts and earlier appearance of functional pathogen-specific T cell responses (against *Aspergillus*, *Candida*, cytomegalovirus, varicella-zoster virus, herpes simplex virus, and *Toxoplasma*).

### Other studies in acute infections

Chronic Obstructive Pulmonary Disease (COPD; including chronic bronchitis and emphysema) is a lung ailment characterized by persistent blockage of airflow from the lungs. COPD is caused by noxious particles of gas, commonly tobacco smoking, which triggers an abnormal inflammatory response in the lungs. Symptoms consist of shortness of breath and low airflow and are poorly reversible. The World Health Organization (WHO) predicts that COPD will become the third leading cause of death worldwide by the year 2030 (<http://www.who.int/respiratory/copd/en>). There is no cure, only management guidelines for medicines which can help dilate major air passages of the lungs.

Several studies have evaluated the use of Ta1 in COPD. In China, Li reported a study with 108 elderly COPD in-patients who were having acute episodes. The subjects were treated with standard of care and half were randomized to also receive Ta1 [97]. They found that clinical efficacy was increased, hospital stay was shortened, and incidence of double infection was decreased ( $p<0.05$ ). Immune parameters were also significantly improved, more so than in the control group ( $p<0.01$ ), and there were no adverse effects seen. Another study in China was conducted in 80 subjects with COPD, half of whom received 10 treatments of Ta1 before being followed for 6 months [98]. They found the number of patients with an acute exacerbation of symptoms when

evaluated at 3 months or 6 months post-treatment was significantly lower in the group of subjects treated with Ta1 (from 11 down to 4 at 3 months, and from 15 down to 7 at 6 months;  $p<0.05$ ). The duration of the acute exacerbations were shorter in the subjects treated with Ta1, evaluated both at 3 and 6 months after treatment (from 10.5 days down to 4.5 at 3 months, and 14.5 down to 7.6 days at 6 months;  $p<0.05$ ), while immune cell parameters were significantly increased in the Ta1 treatment group ( $p<0.05$ ).

Acute Respiratory Distress Syndrome (ARDS), a sudden failure of the respiratory system characterized by rapid breathing, difficulty getting enough air into the lungs, and low blood oxygen levels, can

Study ID	# Subjects Enrolled	Summary of Findings
<b>Subjects treated with Ta1</b>		
Wu 2013 [84]	367	<ul style="list-style-type: none"> <li>• 28-day mortality decreased (26% vs. 35%; <math>p=0.062</math> or <math>0.049</math>)</li> <li>• Relative risk of death decreased (0.74)</li> <li>• mHLA-DR increased more</li> </ul>
Chen 2007 [85]	42	<ul style="list-style-type: none"> <li>• 28-day cumulative survival increased (18/21 vs. 12/21 placebo; <math>p&lt;0.01</math>)</li> <li>• Shortened length of mechanical ventilation, use of antibiotics, stay in ICU</li> </ul>
Zhao 2007 [86]	42	<ul style="list-style-type: none"> <li>• Improvement in lymphocytes, mHLA-DR, IL-10 (<math>p&lt;0.05</math>)</li> <li>• Decrease in inflammation marker IL-6</li> </ul>
Li 2009a [87]	91	<ul style="list-style-type: none"> <li>• Improvement in Marshall and APACHE II scores and immune parameters</li> <li>• Shortened length of mechanical ventilation and stay in ICU</li> </ul>
Wang 2010 [88]	24	<ul style="list-style-type: none"> <li>• Subjects had severe acute pancreatitis</li> <li>• Decreased 28-day infection rate (<math>p=0.012</math>)</li> <li>• Decreased sepsis (<math>p=0.036</math>)</li> <li>• Shorter duration of ICU stay (<math>p=0.014</math>)</li> <li>• Decreased rate of surgery (<math>p=0.036</math>)</li> </ul>
<b>Subjects treated with Ta1 plus PI</b>		
Lin 2007 [89]	342	<ul style="list-style-type: none"> <li>• 28-day mortality decreased (25.1 vs. 38.3; <math>p=0.0088</math>)</li> <li>• 90-day mortality decreased (37.1 vs. 52.1; <math>p=0.0054</math>)</li> <li>• APACHE II decreased (<math>p=0.0382</math>)</li> <li>• mHLA-DR increased</li> </ul>
Zhang 2008[90]	120	<ul style="list-style-type: none"> <li>• 28-day survival increased (51.7 vs. 33.9; <math>p=0.086</math>)</li> <li>• APACHE II improved (<math>p&lt;0.001</math>)</li> <li>• Shortened length of mechanical ventilation, use of dopamine, stay in ICU (<math>p&lt;0.001</math>)</li> <li>• Incidence of shock decreased (<math>p=0.026</math>)</li> </ul>
Li 2009b [91]	56	<ul style="list-style-type: none"> <li>• 28-day survival increased (78 vs. 60%; <math>p&lt;0.05</math>)</li> <li>• APACHE II improved</li> </ul>
Chen 2009 [92]	114	<ul style="list-style-type: none"> <li>• 28-day survival increased (54.1 vs. 35.4; <math>p=0.078</math>)</li> <li>• 60-day survival increased (54.1 vs. 28.2; <math>p=0.045</math>)</li> <li>• 90-day survival increased (47.4 vs. 20.0; <math>p=0.033</math>)</li> <li>• APACHE II improved significantly</li> <li>• Shorter stay in ICU, duration of supportive ventilation, dopamine, and antimicrobial therapy</li> </ul>
Huang 2009[93]	70	<ul style="list-style-type: none"> <li>• 28-day survival increased (63.9 vs. 41.2; <math>p&lt;0.05</math>)</li> <li>• Average survival time increased from 245 to 342 days</li> <li>• mHLA-DR increased more</li> </ul>

Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit; mHLA = monocyte human leukocyte antigen; PI = protease inhibitor; Ta1 = thymosin alpha 1; vs. = versus

**Table 1:** Summary of sepsis studies

occur in anyone who is critically ill. ARDS has a high mortality rate, up to 40%, although this is significantly improved from the rate of 50 to 70 percent just 20 years ago [99]. In 21 renal transplant subjects in China who had ARDS requiring mechanical ventilation due to severe pneumonia (resulting either from CMV (43%), bacterial (33%), or fungal (5%) etiology), the benefit of the addition of Ta1 was evaluated [100]. The results showed that 13 of 21 patients (61.9%) who received Ta1 survived a significant increase over historical controls. In a follow-up trial in China, 46 renal transplant patients in which CMV pulmonary infection led to ARDS either received Ta1 (32 subjects) or did not (14 controls). In this study [101] the death rate was significantly reduced in the Ta1 treatment group (21.9% versus 50%), the renal transplant success rate was significantly higher (78% versus 50%), and the number of CD4 and CD8 lymphocytes were significantly increased ( $p < 0.05$ ), suggesting that repairing cellular immunity can reinforce resistance to CMV.

*Pseudomonas aeruginosa* infections are a major clinical problem in the lungs of immunocompromised and aging populations and account for about 10% of nosocomial infections (<http://textbookofbacteriology.net/themicrobialworld/Pseudomonas.html>). A Chinese study evaluated 42 critically ill hospitalized patients with tracheotomies who were treated with Ta1 (1.6 mg daily for 7 days, compared to untreated control; [102]). A significant decrease in lung infection rate was seen in the subjects who received Ta1 than the control subjects. The Ta1 treated subjects also had significant improvements in white blood cell count, C-reactive protein, TNF $\alpha$ , and IL-6 levels. In a follow up study, a group of 35 older Chinese patients with drug-resistant *P. aeruginosa* pneumonia were treated with Ta1 (1.6 mg daily for 5 days, in combination with Sulperazone) and it was found [103] that Ta1 treatment improved clinical symptoms (cure of pneumonia in 71.4% of subjects compared to 52.4%,  $p < 0.05$ ), and the bacterial load was decreased significantly greater than control subjects ( $p < 0.05$ ). Ta1 also led to improved immunological function and reduced inflammation ( $p < 0.01$  compared to control).

Severe Acute Respiratory Syndrome (SARS) is caused by a novel corona virus that was first identified in Asia in 2003 and quickly spread globally to many countries in Europe, North America and South America before it was contained. After a near pandemic of the virus between November 2003 and July 2003, about 8000 cases were reported with 77 deaths worldwide ([http://www.who.int/csr/sars/country/table2004\\_04\\_21/en/endex.html](http://www.who.int/csr/sars/country/table2004_04_21/en/endex.html)). Treatment of SARS included antipyretics with supportive ventilation and oxygen, since antibiotics were shown to be ineffective. The last reported case of SARS was in 2004. Many persons used Ta1 prophylactically during that outbreak, and an animal model demonstrated efficacy against the virus. In addition, there were several interesting published reports in China suggesting that Ta1 treatment may have contributed to controlling development of the disease [104-106] and there were no adverse events reported in over 40 subjects included in these reports.

Ta1 has also shown been used in treatment of a patient infected with the highly pathogenic avian influenza H5N1 strain [107]. After a complex treatment regimen with Ta1, the subject had improvement in CD4 T cells and appeared to be cured of the influenza infection.

### Chronic Hepatitis B (CHB)

Hepatitis B is an infectious inflammatory disease of the liver caused by the Hepatitis B Virus (HBV). This disease has caused epidemics in many parts of Asia and Africa and is considered an endemic in China. 2 billion people worldwide have been infected by HBV and about

600,000 persons die every year due to consequences of HBV (<http://www.who.int/mediacentre/factsheets/fs204/en/index.html>). Typically, most adults will clear an HBV infection without need for medication, but for chronically infected individuals, treatment with anti-virals may be necessary to reduce the risk of cirrhosis or liver cancer. These treatments last from six months to a year, depending on medication and genotype. Chronic HBV 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 is thought to explain development of chronic HBV infection.

Interest in using Ta1 for treatment of CHB was based on the fact that its immunomodulating effects can trigger maturation in lymphocytes, augmentation of T-cell function, and promotion of reconstitution of immune defects. In addition, Ta1 has been shown to be effective in the woodchuck hepatitis model for CHB [108,109]. Many clinical studies have investigated the safety and efficacy of Ta1 for the treatment of CHB, both as monotherapy or in combination treatment with IFN-alpha or nucleoside analogs. These studies have shown that Ta1 can promote disease remission in 26% to 41% of the subjects treated and has led to commercial approval for this indication in several countries including China.

Two independent meta-analyses of the hepatitis B Ta1 monotherapy studies have been conducted. The first one [110] included 435 subjects who had been entered into randomized, controlled studies of Ta1 monotherapy for CHB (Table 2) [34,111-113]. The analysis demonstrated a statistically significant benefit in favor of Ta1 therapy inducing a sustained virological response over placebo (Odds Ratio [OR] = 2.87; 95% CI 1.58-5.22;  $p = 0.0005$ ). The same meta-analysis also demonstrated a trend in favor of Ta1 compared with IFN-alpha for sustained virological response (OR = 2.62; 95% CI 0.80-8.56).

A second meta-analysis was conducted [114] which included 199 subjects [112,115-117]. The ORs [95% CI] of the virological response, biochemical response and complete response of Ta1 over IFN-alpha at the end of 6 months treatment were not significant, but were quite impressive at the end of 6 month's follow-up (3.71 [2.05, 6.71], 3.12 [1.74, 5.62] and 2.69 [1.47, 4.91], respectively). These data showed that compared with IFN-alpha, the benefit of Ta1 was not immediately significant at the end of therapy, but virological, biochemical and complete responses had a tendency to increase or accumulate gradually after the therapy.

Figure 5 demonstrates typical results seen in these CHB studies. This was a multicenter, randomized, controlled study evaluating Ta1 versus IFN-alpha2b in 33 difficult-to-treat subjects infected with the hepatitis B precore mutant virus, who are characterized by being HBeAg negative and HBV DNA positive [112]. At the end of the follow up period, Ta1 treatment led to a complete response rate of 41% ( $p < 0.05$ ), compared to 25% in the IFN-alpha2b-treated group and only 7% in untreated subjects.

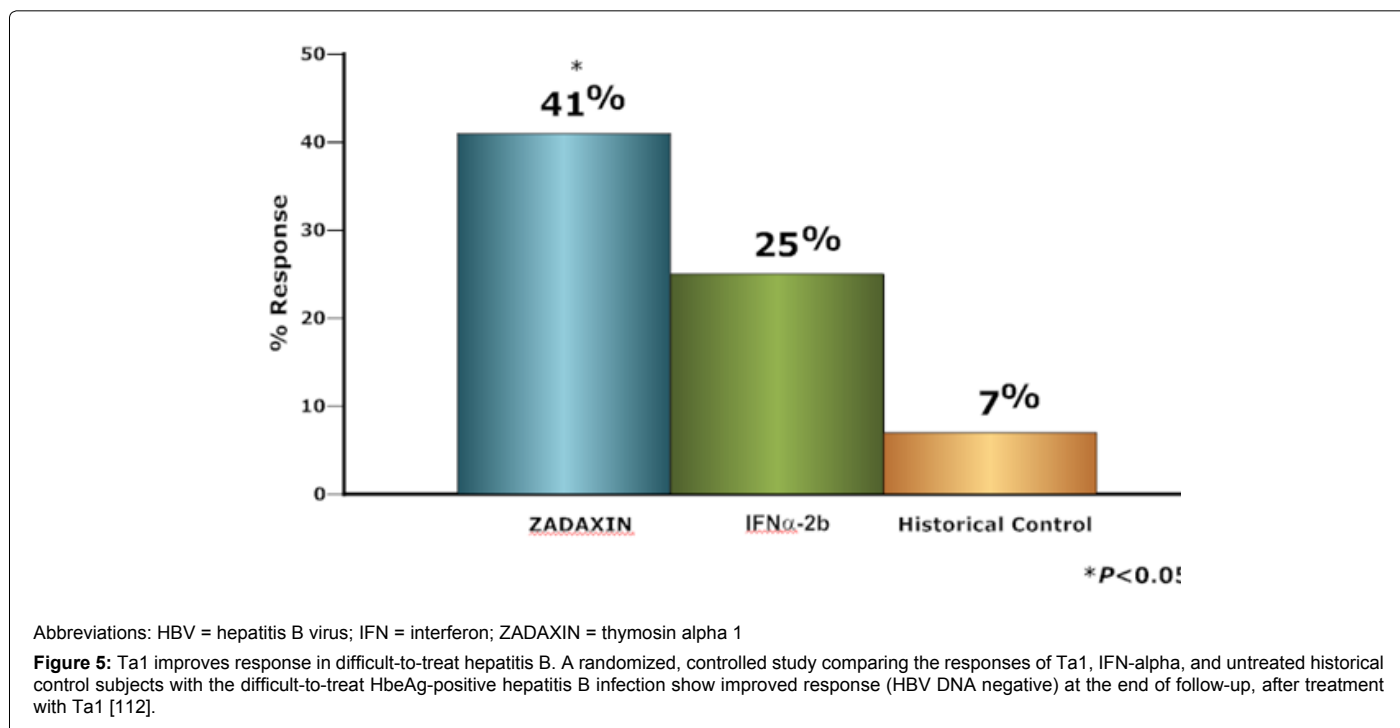
In addition to the studies included in the meta-analyses, several other clinical trials were conducted [118,119] including an open-labeled, randomized, multicenter, controlled study sponsored investigating the safety and efficacy of Ta1 in 316 Japanese subjects with CHB [120]. Ta1 monotherapy exhibited equal efficacy when administered at a dose of either 0.8 mg or 1.6 mg; the rate of normalization of ALT was 32% and 37%, respectively ( $p > 0.05$ ); clearance of HBV DNA was 26% and 30% ( $p > 0.05$ ); clearance of HBeAg was 23% and 23% ( $p > 0.05$ ); and the appearance of anti-HBeAg at 72 weeks was 18% and 22% ( $p > 0.05$ ). At

Study Design	Study County	Subjects Enrolled	Clinical Outcome (at end of follow up)		
Randomized, placebo-controlled double-blind [34]	United States Phase 2	20	Ta1Placebo	83% 25%	$p \leq 0.04$
Multicenter, randomized, placebo-controlled double-blind [111]	United States Phase 3	99	Ta1Placebo	24% 12%	$p < 0.11$
Multicenter, randomized, controlled [112]	Italy Phase 3	33	Ta1IFN-alpha2b	41% 25% 7%	$p = 0.025$
Multicenter, randomized, controlled open-label [113]	Taiwan Phase 3	158	Ta1 (6 mo Rx)	36%	
			Ta1 (12 mo Rx) Control	27% 25%	
		98 <sup>1</sup>	Ta1 (6 mo Rx) Ta1 (12 mo Rx) Control	41% 38% 9%	$p < 0.004$ (6 mo vs. Control)
Meta-analysis [110]	United States, Taiwan, Italy	223	Ta1 (6 mo Rx) Control	36% 19%	$p = 0.04$

Abbreviations: CHB = chronic hepatitis B; IFN = interferon; Ta1 = thymosin alpha 1; vs. = versus

<sup>1</sup>Data from the Chang Gung Memorial Hospital, the major site of the study

Table 2: Meta-analysis of Ta1 CHB monotherapy studies.



72 weeks from baseline, both treatment groups still showed significant improvements in these parameters.

Because of its excellent safety profile, Ta1 may be combined with other therapies to enhance their efficacy without increasing their toxicity. Therefore, clinical trials to improve the effectiveness of other CHB monotherapies by the addition of Ta1 have also been explored. An open label study in Italy tested the combination of low-dose lymphoblastoid IFN-alpha and Ta1 in 15 subjects [121]. Most of the subjects had already failed IFN-alpha2b therapy, and yet Ta1 treatment showed an overall sustained response of 60% (9/15), including a sustained response in 55% (6/11) of previous IFN-alpha2b treatment failures (compared to the historical retreatment rate of 10%).

A study in Turkey compared IFN-alpha2b monotherapy with Ta1/IFN-alpha2b combination therapy in 52 subjects) [122,123]. At the

end of 52 weeks, response rates (HBV DNA negative and normal ALT) were 88% and 70%, respectively, for the Ta1 combination therapy and IFN-alpha2b alone. At the end of 78 weeks, response rates (HBV DNA negative and normal ALT) were 74% and 40% respectively ( $p=0.002$ ). The Ta1 treatment group retained this response 18 months after completion of therapy (71.4%) while the IFN-alpha2b alone sustained response had dropped to 10% ( $p=0.0003$ ). These results suggest that the addition of Ta1 to IFN-alpha2b improves response rates and reduces relapses in difficult-to-treat precore mutant HBV infection. 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-alpha would be expected to be no better than 10%.

Several other studies also investigated the combination of Ta1 with IFN-alpha [124,125].

The combination of Ta1 with nucleoside analogs has also been tested in several clinical trials [126-128]. For example, a study was conducted in 11 immune-tolerant Chinese subjects with vertically transmitted CHB whose high HBV DNA and ALT levels made them highly unlikely to undergo spontaneous seroconversion to negative HBV DNA and HBeAg [129]. Treatment included Ta1, famciclovir, and lamivudine; the combination was well tolerated and 64% of subjects had a complete virological response. These data support the hypothesis that a combination of Ta1 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.

### Chronic Hepatitis C (CHC)

Hepatitis C is recognized as a global health problem, with an estimated worldwide prevalence of more than 185 million infected by the Hepatitis C Virus (HCV) and no available vaccine [130]. 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 HCC. They both induce hepatocellular damage, whether through direct cytotoxicity or through induction of immune mechanisms that lead to hepatocellular necrosis. A covalent conjugate of recombinant IFN-alpha with a Polyethylene Glycol (PEG) moiety has been approved in combination with ribavirin and other direct-acting antiviral agents, for the treatment of CHC.

Several studies investigated the therapeutic effect of Ta1 in combination with IFN-alpha for treatment of CHC. Three of these studies, two phase 2 studies in Italy [131,132] and a phase 3 study in the United States [133] were analyzed independently using pooled and meta-analysis techniques. Results showed that Ta1 in combination with IFN-alpha was safe and significantly superior to IFN-alpha alone at the end of treatment ( $p=0.01$ ) [134].

Ta1 in combination with PEG-IFN-alpha has also been investigated in clinical trials. Two multicenter, double-blind, placebo-controlled phase 3 studies were conducted in the US, evaluating Ta1 in combination with PEG-IFN-alpha2a in 534 subjects with non-cirrhotic CHC and in 527 subjects with compensated cirrhosis, none of whom had responded to previous IFN-alpha or IFN-alpha plus ribavirin therapy. Ta1 was well tolerated overall, but the studies showed only a small effect on sustained viral responses in this subject population. Other studies have shown benefit from Ta1 when it was added to a combination of PEG-IFN-alpha and ribavirin treatment, however: a phase 2 trial in Mexico in 40 CHC subjects who were non-responders to previous therapy with IFN-alpha plus ribavirin [135,136] had an end-of-treatment response rate of 53% and a sustained response rate of 21%, an improvement over historical comparison studies.

A large European randomized, double-blind study with the same combination treatment of Ta1, PEG-IFN-alpha, and ribavirin was recently conducted in 553 subjects who had failed to respond to previous PEG-IFN-alpha/ribavirin treatment [137]. Results showed that 13% of Ta1-treated subjects achieved sustained responses at week 72, versus 11% in the placebo group ( $p=0.407$ ). In the prospectively defined secondary population of subjects who completed the full course of 48 weeks of triple combination treatment, the primary endpoint achieved statistical significance for the difference in sustained viral response between the Ta1 treated group and the placebo group ( $p=0.048$ ).

### Human Immunodeficiency Virus (HIV)

Acquired Immunodeficiency Syndrome (AIDS) is a disease of

the human immune system caused by HIV infection. As the disease progresses, the immune system will slowly weaken, leading to a higher chance of infections that would not regularly occur in healthy patients. There is no cure or vaccine for HIV; however, antiretroviral treatments have shown to slow the course of the disease. Approximately 34 million people are living with HIV infection globally, while AIDS is considered a pandemic worldwide, having claimed more than 25 million lives (<http://www.who.int/mediacentre/factsheets/fs360/en/index.html>). Ta1 in combination with IFN-alpha and AZT was investigated for treatment of HIV in several studies [138,139] and several measures of response, including greater increases in CD4 cells, were seen in subjects given Ta1.

### Ta1 use in Treatment of Cancers

The therapeutic usefulness of Ta1 has been examined in several types of cancers. 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. Immune modulation (e.g., with IL-2) has shown promising results for treatment of human cancers [140-142], and the results summarized below show that Ta1 also demonstrates usefulness in treating certain forms of cancer, although with none of the side effects of IL-2. Ta1 treatment has shown benefit to cancer patients in multiple different ways:

- Increase in effectiveness of chemotherapy, likely due to increases in tumor infiltrating lymphocytes, upregulation of anti-tumor T cells, and enhanced expression of cell-surface markers, as described above under Ta1's mechanism of action
- Decrease in side effects of chemotherapy, possibly due to increases in regulatory T cells and subsequent decreases in proinflammatory cytokines
- Improvement in quality of life, as expected from responses to and decreases in side effects from chemotherapy

### Hepatocellular carcinoma (HCC)

HCC is a primary malignancy of the liver responsible for over 600,000 deaths annually [143]. It is the third leading cause of cancer deaths worldwide, with the highest incidence in Asia and Africa due to the high prevalence of HBV and HCV. Symptoms include weight loss, signs of decompensated liver disease and right upper quadrant pain. Although surgical resection or liver transplant may benefit some patients, due to the advanced stage of cancer and liver disease at initial diagnosis, surgery may not be a viable option. Standard treatments for patients who cannot receive a liver transplant involve radiation and chemotherapies. Systemic chemotherapy results are at best dismal, although a number of reports have provided encouraging perspectives for regional chemotherapy. 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. For patients who have either failed TACE or who present with more advanced HCC, sorafenib (a multikinase inhibitor with activity against Raf-1, B-Raf, vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor, and c-Kit receptors, among other kinases) is now considered first-line treatment and has shown a clinically relevant improvement in time to progression and in survival.

Based on the immune-modulating mechanism of action of Ta1, it is expected to be of benefit for use in combination with either sorafenib or TACE, with no addition of toxicity. A pilot study was conducted in Italy in 12 subjects with HCC to determine whether Ta1 could increase

response to treatment with TACE in comparison to historical controls [144]. The addition of Ta1 to TACE led to a statistically significant increase in survival (82% versus 41%;  $p < 0.05$ ), and significant increases in immune parameters (cytotoxic T cells and NK cells).

A Chinese study of 32 patients being treated with TACE for HCC also demonstrated that the addition of Ta1 improved response [145,146], with a significant increase in survival compared to historical controls at 6, 9, and 12 months ( $p < 0.05$ ; Figure 6). Ta1 treatment also significantly lowered alfa fetoprotein levels and increased CD3 cells, CD4/CD8 ratio, and NK cells ( $p < 0.05$ ).

Cheng et al. also evaluated the addition of Ta1 to TACE after tumor resection or partial hepatectomy, in a study in China of 57 subjects with HCC randomized to different treatment groups [147,148]. The addition of Ta1 significantly improved survival (10 versus 7 months;  $p = 0.002$ ) and delayed time to tumor recurrence ( $p = 0.039$ ).

Cheng also evaluated the addition of Ta1 plus lamivudine combination therapy after tumor resection or partial hepatectomy in a study of 81 subjects with hepatitis B infection and HCC [149-151]. In this study, the addition of Ta1/lamivudine combination treatment resulted in a statistically significant increase in time to tumor recurrence (10.0 versus 6.5 months;  $p = 0.0032$ ), median survival time (12.5 versus 6.0 months;  $p = 0.0023$ ), and HBV-DNA clearance (100% versus 4%;  $p = 0.0000$ ) and seroconversion to HBeAg (73% versus 7.5%;  $p < 0.05$ ).

A further report by Cheng described the effect of Ta1 on subjects with HCC and portal vein tumor thrombus, being treated with surgery and TACE [152]. 84 subjects were randomized in various treatment groups, and it was seen that the addition of Ta1 improved median survival time (10 versus 7 months) and 1-year survival (44% versus 15%), although the results were not significant.

In a study in the US, Gish also showed an improvement in response to TACE with the addition of Ta1 [153]; in 25 subjects randomized to TACE with or without Ta1, the addition of Ta1 led to improvements in response (57% versus 46%) and median overall survival (110 weeks versus 57 weeks).

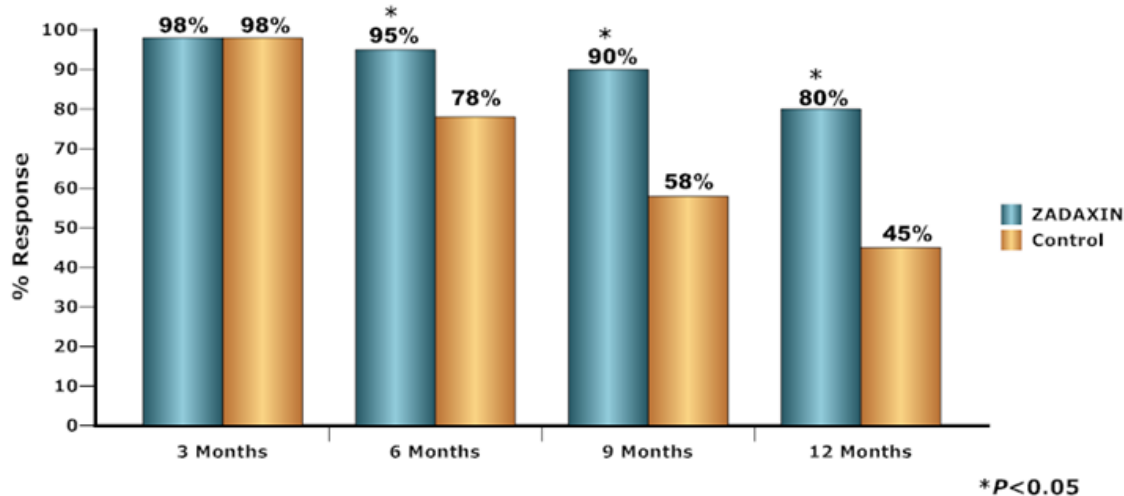
## Non-small-cell lung cancer

Lung cancer is estimated to be the most common form of cancer worldwide and to have the highest mortality rate (<http://www.cancerresearchuk.org/cancer-info/cancerstats/world/lung-cancer-world/>). Non-Small-Cell Lung Carcinoma (NSCLC) is any type of epithelial lung cancer excluding small cell lung carcinoma. NSCLC accounts for approximately 80% of all lung cancers with nearly 40% of the cases having already metastasized and at time of diagnosis (<http://lungcancer.about.com/od/typesoflungcancer/a/Where-Does-Lung-Cancer-Spread.htm>). NSCLC is typically treated by surgical resection due to the low efficacy of chemotherapy. Since NSCLC cannot be cured via current treatment modalities, palliative care is an important part of the standard treatment regimen.

Ta1 has been used to treat NSCLC in several different studies. The first randomized, double-blind trial with Ta1 was conducted in the US in 42 subjects with NSCLC being treated with radiation [154]. The addition of Ta1 after radiation therapy led to statistically significant improvement in relapse-free survival ( $p = 0.04$ ), overall survival ( $p = 0.009$ ), and immune cell function and numbers, which had been depleted by radiation treatment ( $p = 0.04$ ).

Several studies in Italy reported that the addition of Ta1 to cisplatin/etoposide or ifosfamide chemotherapy in subjects with NSCLC led to an improvement in response and immune parameters compared (overall response rate of 44% and median survival of 12.6 months, Garaci 1995; time to progression of 18 weeks versus 9 weeks,  $p = 0.006$ , Salvati 1996) [155,156]. The toxicity of the chemotherapy was decreased as well, as the percentage of subjects presenting with grade 3 or 4 myelosuppression was greatly decreased (0% versus 50%).

Multiple clinical studies in China were also conducted; the first evaluated the addition of Ta1 to cisplatin/vinorelbine (NP) chemotherapy in NSCLC [157]. In a study of 69 subjects with stage III or IV cancer randomized to NP or NP with Ta1, after 4 monthly cycles of treatment the addition of Ta1 led to improvement in efficacy (from 21.2% to 38.9%;  $p < 0.05$ ), median survival time (from 10.4 to 19.3 months), survival rates at 1, 2, 3, and 4 years post-treatment ( $p < 0.05$ ), and lymphocyte subsets ( $p < 0.05$ ).



Abbreviations: HCC = hepatocellular carcinoma; TACE = transarterial chemoembolization; ZADAXIN = thymosin alpha 1

**Figure 6:** Ta1 improves survival in HCC treated with TACE. In a study with 32 Chinese patients being treated with TACE for HCC, the addition of Ta1 to the treatment (1.6 mg daily for 10 days) provided a significant increase in survival compared to historical controls at 6, 9, and 12 months after TACE [145, 146].



Hou reported a study with 68 subjects, also comparing NP alone with NP plus Ta1 [158]. The addition of Ta1 led to an improvement in clinical symptoms (from 47.1 to 73.5%;  $p < 0.05$ ), Quality Of Life (QOL) as determined by Karnovsky Performance Score (KPS; increased from 29.4 to 55.9%;  $p < 0.05$ ), and decreases in side effects from chemotherapy, as the percent of subjects suffering adverse effects (nausea and vomiting; depression in white blood cells and platelets) were decreased with Ta1 treatment ( $p < 0.05$ ). The time to progression also increased from 5.8 to 7.1 months but this was not significant.

In another comparison study, 57 NSCLC subjects were given Ta1 in addition to cisplatin/naelbine chemotherapy [159]. The investigators reported improvements in immune parameters (increases in CD3 T cells, CD4 Th1 helper T cells, CD4/CD8 ratio, and NK cells;  $p < 0.05$ ) and an increase in efficacy along with no toxic side effects of treatment.

Sun also reported improvement in immune parameters in 68 subjects with NSCLC being treated with gemcitabine and cisplatin [160], and Liang saw significant improvements in QOL in a study with 58 elderly subjects comparing gemcitabine with gemcitabine plus Ta1 [161].

The 5 Chinese studies described above were recently included in a meta-analysis of randomized controlled trials in lung cancer using Cochrane criteria [162]. The meta-analysis concluded that the addition of Ta1 to cisplatin/vinorelbine led to an increase in overall tumor response rate (OR 1.86; 95% CI 1.08-3.20), tumor control rate (OR 3.06; CI 1.36-6.88), 1-year survival rate (OR 3.05; CI 1.34-6.96), and QOL (OR 3.39; CI 1.54-7.47).

## Melanoma

Melanoma is a cancer of the skin caused by a malignant tumor of melanocytes found predominantly in adults. Melanoma results in more than 75% of deaths caused by all skin cancers (<http://www.cancer.org/cancer/cancercauses/sunandvexposure/skin-cancer-facts>). If diagnosed early, patients with localized melanoma may be cured with surgery; however, there is no current cure for malignant melanoma after the cancer has metastasized. The average survival of patients with melanoma that has spread outside the local area is only about 8 months, with a 10 year survival rate of less than 10% [163]. Early diagnosis of melanoma is essential for improving survivability. The World Health Organization has reported 48,000 deaths relating to melanoma worldwide [164].

Two small pilot studies showed beneficial effects compared to historical control when Ta1 treatment was added to Dacarbazine (DTIC) chemotherapy combined with IL-2 [165] or IFN-alpha [166].

These positive effects led to the conduct of a large, randomized study in Europe in 488 subjects with metastatic melanoma (Table 3 and Figure 7; Maio 2010) [167]. Addition of Ta1 to DTIC and IFN-alpha led to no further toxicity above the chemotherapy alone, but provided an increase in median Overall Survival (OS), Progression-Free Survival (PFS; significant for a dose of 3.2 mg Ta1 plus DTIC compared to

control,  $p = 0.04$ ), tumor response ( $p < 0.05$ ), and duration of response. Clinical benefit rates were significantly higher for subjects given 3.2 mg Ta1 plus DTIC compared to control ( $p = 0.009$ ).

## Other cancers

Ta1 has been given to subjects with lung, gastric, or breast cancer in 2 separate studies in China. Ta1 was given to 60 subjects in each study during one of two cycles of chemotherapy and QOL was evaluated. Treatment with Ta1 provided a significant improvement in all measures of QOL (appetite,  $p = 0.0001$ ; sleep,  $p = 0.0002$ ; fatigue,  $p = 0.001$ ; daily activity,  $p = 0.0001$ ; overall feeling,  $p = 0.001$ ; depression,  $p = 0.003$ ) [168] as well as a lowered occurrence of infections during chemotherapy and increased immune parameters [169].

Ta1 was given to 26 subjects in China with pancreatic cancer [170] after intra-arterial chemotherapy with gemcitabine, 5-FU, mitomycin-c and octreotide. Subjects randomized to treatment with Ta1 had statistically significant increases in immune function (CD3, CD4, CD4/CD8 ratio;  $p < 0.05$ ) while control subjects had decreases in these parameters. Ten subjects from each group underwent subsequent surgery, and those treated with Ta1 had no severe post-surgical infections compared to 3 in the control group, a faster recovery time, and fewer side effects (neutropenia 8% versus 39%; nausea and vomiting 31% vs 39%; diarrhea 8% vs 23%; fever 8% vs 23%).

The effect of treatment with Ta1 was evaluated in 30 elderly subjects in China with advanced malignant cancer (lung, liver, stomach, colon, rectal; Yang 2003) [171], and showed a significant improvement in QOL (KPS) score ( $p < 0.01$ ) and immune parameters ( $p < 0.05$ ). Another study in a similar population of 60 subjects evaluated the addition of Ta1 to standard chemotherapy [172] and showed a significant increase in survival, from 6 to 24 months for 43% survival ( $p < 0.01$ ), a significant increase in QOL (KPS) score ( $p < 0.01$ ), and improvements in immune function (CD4/CD8 ratio and NK cells;  $p < 0.05$ ). The effect of Ta1 addition was so strong that at 24 months, when 43% survival was seen in the Ta1-treated group, the control group of subjects in the study had no survivors at all.

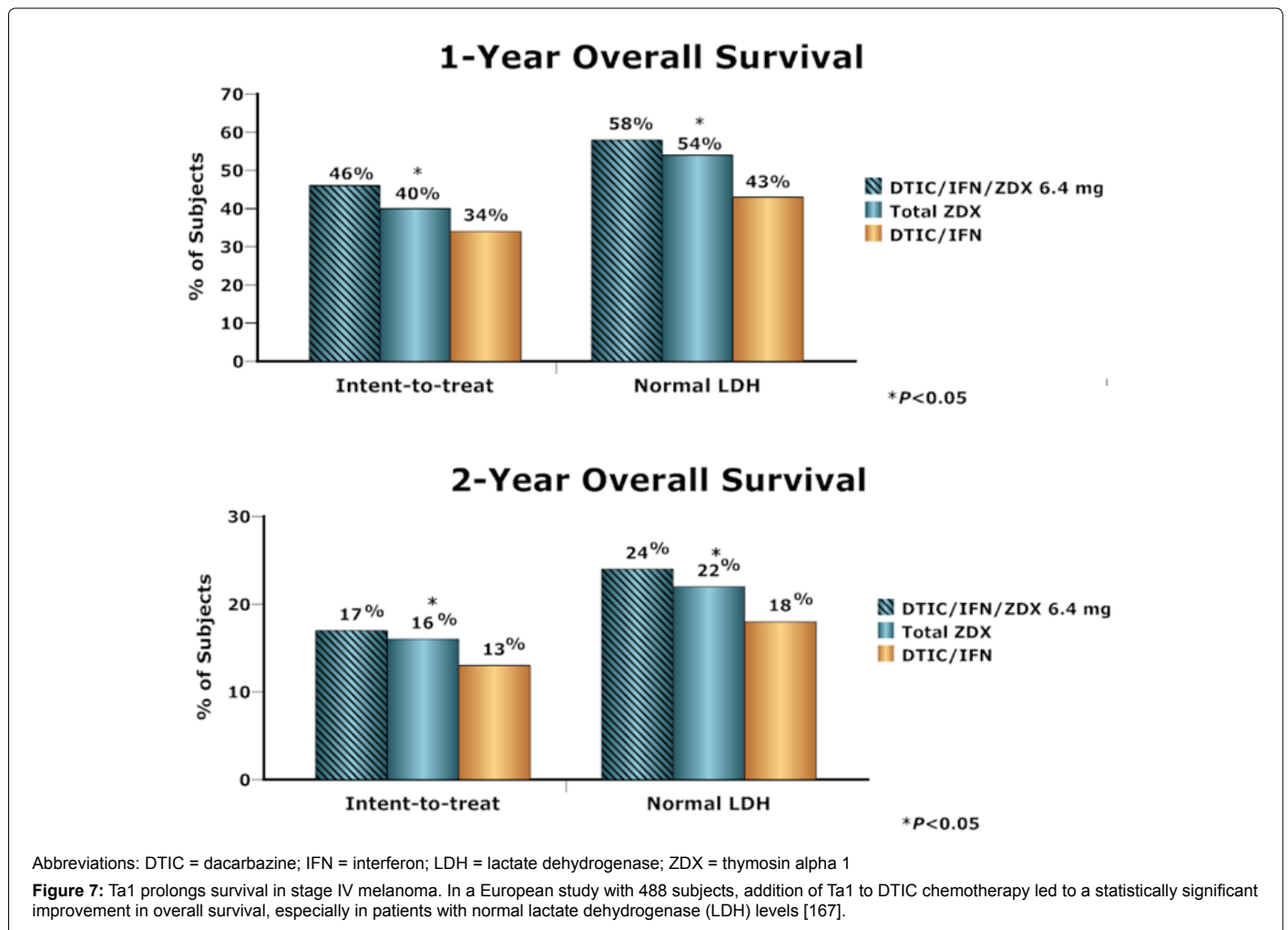
Twenty-two subjects in China with lung or breast cancer who had neurotoxicity after treatment with chemotherapy (grades 2-4) were treated with Ta1 during one of their chemotherapy cycles [173,174]. After this combination treatment 10 of the subjects (45.4%) had a decrease in neurotoxicity scores (from 2 and 3 down to 0 and 1).

A pilot Chinese study evaluated the effect of Ta1 on QOL in 16 post-menopausal women undergoing treatment for breast cancer with aromatase inhibitors who had joint symptoms and pain related to the treatment [175]. After treatment with Ta1, all subjects reported statistically significant improvement in worst pain scores (decreasing from a score of 5.7 to 3.4;  $p < 0.001$ ), pain severity (from 3.9 to 2.9;  $p < 0.01$ ), and pain-related functional interference (4.2 to 1.8;  $p < 0.001$ ) using the Brief Pain Inventory (BPI) scale. Significant improvement was also seen in the Western Ontario and McMaster Universities

	DTIC IFN 1.6 mg Ta1 (N=97)	DTIC IFN 3.2 mg Ta1 (N=97)	DTIC IFN 6.4 mg Ta1 (N=98)	DTIC Ta1 (3.2 mg) (N=99)	Combined Total Ta1 (N=391)	DTIC IFN (Control) (N=95)
Median overall survival (months)	9.3	8.6	10.3	9.3	9.4	6.6
Progression-free survival 6 months (%)	12	21	13	16	16	9
Overall tumor response number (%)	7 (7.2%)	10 (10.3%)	6 (6.1%)	12 (12.1%)	35 (9.0%)	4 (4.1%)

Abbreviations: DTIC = dacarbazine; IFN = interferon; N = number; Ta1 = thymosin alpha 1

**Table 3:** Ta1 improves OS, PFS, and tumor response in stage IV melanoma.



Osteoarthritis Index (WOMAC;  $p < 0.001$ ) and Functional Assessment of Cancer Therapy-General physical well-being (FACT-G;  $p < 0.001$ ).

### Ta1 use in Vaccine Enhancement

Decreased antibody response to T-cell dependent antigens, particularly in the elderly, may be one factor that accounts for insufficient efficacy of certain vaccination programs (e.g., influenza). 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 [176,177]. In the elderly, for example, analysis of a specific antibody response after vaccination has been shown to be impaired when compared with response in young subjects [52,178,179]. 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 [179-183]. Several studies have reported poor antibody response after hepatitis B vaccination in hemodialysis patients [184-186].

Since Ta1 can enhance T-cell-dependent specific antibody production, the addition of Ta1 to vaccination protocols for immunocompromised individuals should be effective. In vaccinated elderly individuals, *in vitro* influenza antibody synthesis was augmented with the addition of Ta1 [52]. Enhancement of specific

antibody responses to tetanus toxoid and sheep red blood cells also was observed by the *in vivo* administration of Ta1 to older mice or mice immunosuppressed with cocaine [51,53]. These experimental data support that Ta1, when appropriately administered with a vaccine, could enhance immune function and antibody response in individuals with a compromised immune system.

Six clinical studies have been completed that evaluated the efficacy of Ta1 as an adjuvant for influenza or hepatitis B vaccines in subjects immunocompromised due to age or hemodialysis. When compared with vaccine plus placebo, administration of Ta1 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 Ta1 is safe for administration to immunocompromised subjects, including the elderly, and no serious adverse effects were observed in any of the studies.

### Elderly subjects

The immune enhancing effect of Ta1 for influenza vaccination was first examined in a pilot study with 9 elderly subjects (ages 65–99 years) at the University of Wisconsin and Cornell Medical Center [187]. In this study, 67% (6/9) of Ta1 subjects responded with high levels of anti-influenza antibodies, compared to a historical typical revaccination response rate of only 10%.

This study was followed by a double-blind, randomized, placebo-controlled study in 90 male veterans >64 years of age (mean age 77.3 years; age range 65 to 99 years) at the Wisconsin Veterans' Administration Medical Center [188]. The study showed that 69% of Ta1 subjects were effectively immunized to the trivalent influenza vaccine versus 52% of placebo subjects ( $p=0.023$ ). The difference between treatment groups was greater in subjects older than 77 years. There was, as expected, a relationship between antibody levels and age ( $p<0.039$ ); antibody levels declined with age in placebo subjects but remained stable with age in Ta1-treated subjects, and in fact antibody levels in Ta1-treated older subjects were increased to levels seen in younger subjects.

Based on these encouraging results, a larger study was conducted at George Washington University with 330 subjects vaccinated with the trivalent influenza vaccine (B/Ann Arbor, A/H3N2 Leningrad and A/H1N1 Taiwan). As reported [189], there were greater A/H1N1 Taiwan antibody levels in subjects receiving 8 doses of Ta1 than subjects receiving only 4 injections or none ( $p=0.015$ ). Importantly, there was a lower incidence of influenza disease in subjects receiving the 8 doses of Ta1 compared to those who did not receive Ta1 ( $p=0.002$ ; Figure 8). The greatest effect was observed in subjects 80 years or older.

### Hemodialysis subjects

Three separate randomized, double-blinded, placebo controlled studies evaluated the adjuvant effect of Ta1 on vaccines in subjects immunocompromised by chronic renal failure and undergoing hemodialysis.

A study in the US was conducted in 23 subjects on hemodialysis who were nonresponders to a course of Heptavax™ hepatitis B vaccination. The subjects were revaccinated with the three-course vaccine, and half the subjects were given Ta1 after each vaccine [190,191]. After 3 months, there was a significant difference in the number of patients producing clinically significant antibody titers between the Ta1-treated and placebo subjects (64% versus 17%;  $p<0.002$ ). After 12 months, 45% of Ta1-treated subjects had retained sustained, clinically significant antibody titers, while 0% of placebo-treated subjects did ( $p<0.002$ ).

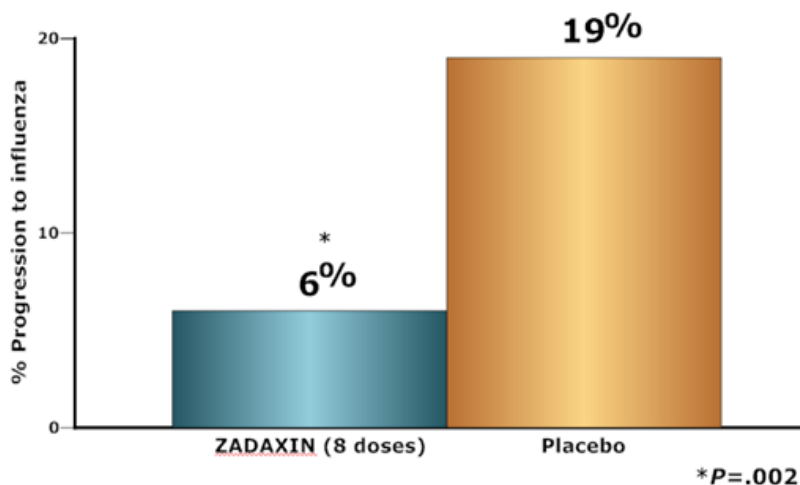
Another US study with 97 hemodialysis subjects was conducted

with monovalent A/Taiwan/1/86 (H1N1) influenza vaccine [192]. At 4 weeks post vaccination, 71% of Ta1-treated subjects had responded compared to only 43% of placebo-treated subjects ( $p<0.002$ ). At 8 weeks post vaccination, 65% of Ta1-treated subjects were still protected with circulating levels of antibody; however the placebo response had decreased to only 24% ( $p<0.001$ ).

In a recent study conducted in Italy, the effect of Ta1 on vaccination response to 2009 H1N1 monovalent, adjuvant influenza vaccine (Focetria®, Novartis) in 82 subjects with end-stage renal disease on chronic hemodialysis was evaluated [193]. The Ta1 treatment regiment was simplified compared to the previous studies, however, as there were only 2 injections of Ta1, given 7 days prior to vaccination and on the day of vaccination (rather than the previous 4 or 8 injections). The dose was higher (3.2 or 6.4 mg, compared to the previous 1.6 mg) in hope that that the fewer injections could still provide benefit. The study showed that response to vaccination was significantly increased in both Ta1-treated groups compared to placebo (Figure 9). These results are particularly striking in light of the fact that Ta1 provided a strong benefit in combination with a vaccine that already contained an adjuvant.

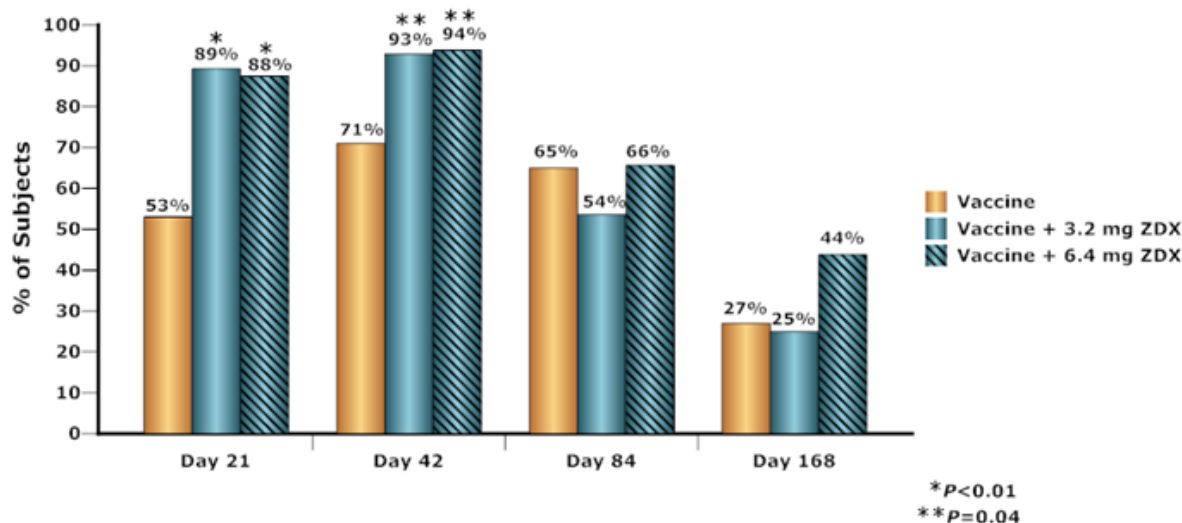
### Conclusions

Since its discovery in 1979, Ta1 has been evaluated in over 80 clinical studies and administered to more than 230,000 patients in post-marketing experience. Administration has been in daily doses ranging from 0.6 to 9.6 mg/m<sup>2</sup> and 1 mg to 16 mg, primarily administered subcutaneously on a biweekly schedule, for treatment periods ranging from 1 day to 18 months. Ta1 has been well tolerated and has not been associated with any significant side effects. Ta1 has been administered without adverse incidents to elderly subjects (up to 101 years old), children (as young as 13 months), and immunocompromised patients. Ta1 has been shown to be well tolerated even in patients with poor performance status, including those with liver disease, renal disease requiring hemodialysis, and primary immunodeficient individuals. The lack of significant side effects with Ta1 is in sharp contrast to other major immune response modulators such as IFN-alpha and IL-2. The side effects and toxicities of the latter drugs make them difficult



Abbreviations: ZADAXIN = thymosin alpha 1

**Figure 8:** Lower incidence of influenza in subjects who received Ta1 after influenza vaccine. In a study with 330 subjects vaccinated with the trivalent influenza vaccine (B/Ann Arbor, A/H3N2 Leningrad, A/H1N1 Taiwan), there was a lower incidence of subsequent influenza disease in subjects receiving 8 doses of Ta1 compared to those who did not receive Ta1 [189].



Abbreviations: ESRD = end-stage renal dialysis; ZDX = thymosin alpha 1

**Figure 9:** Ta1 improves response to H1N1 2009 influenza vaccine. A study with 120 adults with end-stage renal disease (ESRD) on chronic dialysis were given the egg-derived 2009 H1N1 monovalent vaccine (Focetria®, Novartis) with or without Ta1 (7 days prior to vaccination as well as on the day of vaccination) [193]. Those treated with Ta1 achieved a marked increase in seroconversion which lasted up to 42 days after vaccination.

for most patients to tolerate. For example, IFN-alpha results in flu-like side effects (fever, chills, malaise, and headaches), neutropenia, thrombocytopenia and depression. IL-2 causes significant edema (capillary-leak syndrome) in the lungs and elsewhere. Thus, while Ta1 is one of only a few immunomodulators that have been approved for human use, it does not appear to induce any of the side effects and toxicities commonly associated with agents in this class, resulting from the interesting TLR9-based mechanism of action as described above. Taken together, the clinical data reviewed here show that Ta1 has great promise as an immune modulator for benefit of a wide array of clinical indications.

Conflicts of interest: Both Cynthia Tuthill and Robert King work at SciClone Pharmaceuticals, Inc., the company that holds commercial rights for ZADAXIN thymosin alpha 1.

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