CHAPTER SEVEN

Immune Modulation with Thymosin Alpha 1 Treatment

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Contents

Abstract

Thymosin alpha 1 (Ta1) is a peptide originally isolated from thymic tissue as the compound responsible for restoring immune function to thymectomized mice. Ta1 has a pleiotropic mechanism of action, affecting multiple immune cell subsets that are involved in immune suppression. Ta1 acts through Toll-like receptors in both myeloid and plasmacytoid dendritic cells, leading to activation and stimulation of signaling pathways and initiation of production of immune-related cytokines.

Due to the immune stimulating effects of Ta1, the compound would be expected to show utility for treatment of immune suppression, whether related to aging or to diseases such as infection or cancer. Extensive studies in both the preclinical and clinical setting will be summarized in the subsequent sections. These studies have demonstrated improvements in immune system cell subsets and the potential of Ta1 for the treatment of a range of diseases.

Vitamins and Hormones, Volume 102
ISSN 0083-6729
http://dx.doi.org/10.1016/bs.vh.2016.04.003

1. INTRODUCTION

Thymosin alpha 1 (INN: thymalfasin; commonly abbreviated Ta1) is currently sold in over 30 countries worldwide under the brand name ZADAXIN®. Ta1, initially selected for its activity in restoring immune function to thymectomized mice, was the first peptide to be isolated from thymic tissue (Goldstein, Low, McAdoo, et al., 1977; Low & Goldstein, 1979; Low, Thurman, McAdoo, et al., 1979). Ta1 is an N-terminal acetylated acidic peptide of 28 amino acids with a molecular weight of 3108 Da (Low & Goldstein, 1985). Circulating Ta1 is the amino terminal proteolytic cleavage product of the chromatin-remodeling protein prothymosin (Haritos, Goodall, & Horecker, 1984), and is derived from cleavage of prothymosin by the lysosomal asparaginyl endopeptidase legumain (Sarandeses, Covelo, Diaz-Jullien, & Freire, 2003). Ta1 is a highly conserved peptide, and therefore of biological significance, as its amino acid sequence is homologous in bovine, porcine, ovine, and human species (Schulof, 1985), and similar peptides have even been found in crustaceans (Oates & Erdos, 1989).

Endogenous Ta1 serum levels measured in healthy adults by immunoassay are in the 0.1–1.0 ng/mL range (Weller, Shah, Cummings, Chretien, & Mutchnick, 1992), although the circulating concentration tends to be lower in diseased individuals and higher during pregnancy (Jevremovic et al., 1997; Sherman, Jones, Goldstein, & Naylor, 1991; Welch, Lee, Sokol, & Mutchnick, 1988; Welch, Mutchnick, Weller, & Sokol, 1987). While the highest concentrations of Ta1 are found in the thymus, the peptide has also been found in spleen, lung, kidney, brain, blood, and a number of other tissues.

A chemically synthesized version of Ta1 shows activity similar to the native peptide (Wang, Makofske, Bach, & Merrifield, 1980); this compound has been used for preclinical and clinical evaluation leading to regulatory approval.

2. MECHANISM OF IMMUNE RECONSTITUTION WITH TA1

In keeping with Ta1's original isolation as a compound responsible for reconstitution of immune function in preclinical models of immune-compromised animals, investigation of the mechanism of action at the

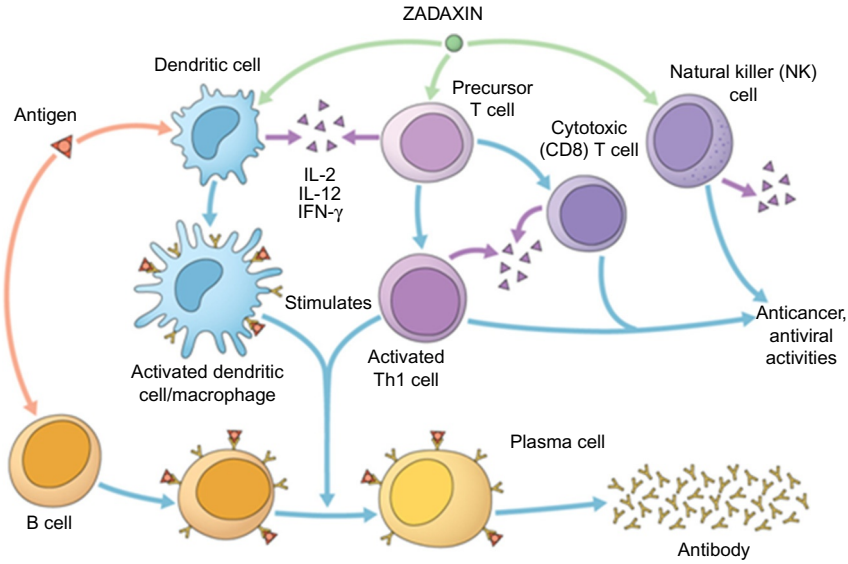


Fig. 1 Immune-stimulating mechanism of action of Ta1.

cellular level has implicated a number of intracellular cell-signaling pathways that are associated with stimulation of the immune system (Fig. 1).

Ta1 has been shown to be a Toll-like receptor (TLR)-9 and TLR-2 agonist (Romani, Bistoni, Montagnoli, et al., 2007; Romani, Bistoni, Perruccio, et al., 2006; Romani, Moretti, Fallarino, Bozza, et al., 2012). The TLRs are a family of proteins that mediate innate immunity; stimulation of one or more TLRs by a TLR agonist can enhance the adaptive immune response which is critical for fighting viral, bacterial, and fungal infections and cancers, as well as stimulation of humoral immunity for vaccine effectiveness. Ta1 affects TLRs in both myeloid and plasmacytoid dendritic cells (DCs), the professional antigen-presenting cells, leading to activation and stimulation of signaling pathways and initiation of production of immune-related cytokines that fight infections (Romani, Bistoni, Gaziano, et al., 2004; Serafino, Pierimarchi, Pica, et al., 2012).

The effects of Ta1 on precursor T cells leads to an increase in the number of activated T helper (Th) cells (CD4 T cells) (Bozza, Gaziano, Bonifzi, et al., 2007; Peng, Chen, Yu, et al., 2008) and a shift toward the Th1 subclass (Cursaro, Margotti, Favarelli, et al., 1998; Gramenzi et al., 2005). This shift leads to increased expression of Th1-type cytokines such as interleukin-2 (IL-2) (Serrate, Schulof, Leondaridis, Goldstein, & Sztein, 1987; Sztein & Serrate, 1989; Sztein, Serrate, & Goldstein, 1986; Yao, Doan, Zhang,

et al., 2007), and interferon (IFN)-alpha (Hsia, Sarin, Oliver, & Goldstein, 1989; Leichtling, Serrate, & Sztein, 1990; Serrate et al., 1987; Svedersky, Hui, May, McKay, & Stebbing, 1982; Sztein & Serrate, 1989; Sztein et al., 1986; Yao et al., 2007). The activated DCs and Th1 cells then act in concert to kill bacterial, fungal, or viral infections or tumor cells and lead to the stimulation of differentiation of specific B cells to antibody-producing plasma cells and an improvement in response to vaccines by stimulation of antibody production (Effros, Casillas, & Walford, 1988; Ershler, Hebert, Blow, Granter, & Lynch, 1985; Ershler, Moore, & Socinski, 1984). Further, Ta1 acting through TLR9 leads to stimulation of the NFkappaB and p38 MAPK pathways (Peng et al., 2008; Romani et al., 2004; Zhang, Chan, Dragoi, et al., 2005), both of which play critical roles in the maturation of DCs (Arrighi, Rebsamen, Rousset, Kindler, & Hauser, 2001; Iijima, Yanagawa, & Onoe, 2003) and in antigen presentation by DCs (Yoshimura, Bondeson, Brennan, Foxwell, & Feldmann, 2001). Ta1 leads to increased expression of the thymopoetic cytokines IFN-alpha, IL-7, and IL-15 (Knutsen, Freeman, Mueller, Roodman, & Bouhasin, 1999; Weller et al., 1992).

The wide-ranging effects of Ta1 include a reduction in apoptosis of immune cells, as shown in mouse (Baumann, Badamchian, & Goldstein, 1997; Osheroff, 1981) and human (Ho, Stehle, Dietz, Hunstein, & Hoffbrand, 1985; Knutsen et al., 1999) thymocytes, and stem cell expansion or differentiation in immunosuppressed mice (Hu, Low, & Goldstein, 1981; Ohta, Sueki, Yoneyama, Tezuka, & Yagi, 1983; Ohta, Tezuka, Tamura, & Yagi, 1985). Ta1 treatment also leads to an increase in intracellular glutathione (GSH) (Palamara, Bue, Savini, et al., 1998), important for antiviral effects, and to directly inhibit the in vitro growth of certain cancer cells (Moody, Badamchian, & Goldstein, 1998; Moody, Fagarasan, Zia, Cesnjaj, & Goldstein, 1993).

In addition to its effects on DCs and T helper cells, Ta1 also stimulates innate immunity, including natural killer (NK) cells and macrophages, additionally supporting its antiviral and anticancer activities. NK-cell activity has been shown to be increased by Ta1 in a variety of model systems, including infections (mice with herpes simplex virus (HSV) (Shiau, Wu, & Huang, 1988) or influenza (D'Agostini, Palamara, Favalli, et al., 1996), as well as various cancers in mice and rats and polymorphonuclear blood cells from human subjects (Ni et al., 2015; Serrate et al., 1987; Umeda, Sakamoto, Nakamura, Ishitsuka, & Yagi, 1983). In human monocyte-derived macrophages, Ta1 helps implement pathogen internalization and phagocytosis (Serafino et al., 2014).

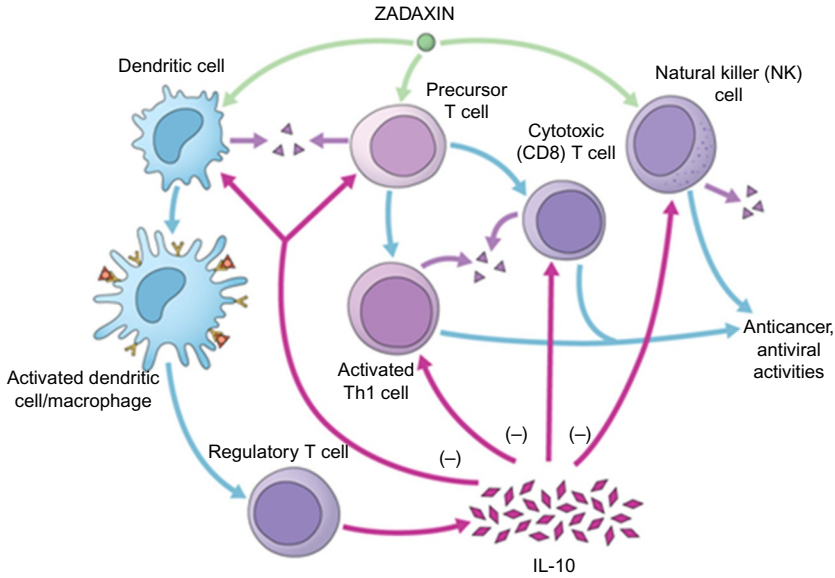


Fig. 2 Immune-modulating mechanism of action of Ta1.

Importantly, it has also been shown that Ta1 stimulates activity of indoleamine-2,3-dioxygenase (IDO) in plasmacytoid DCs (Romani, Moretti, et al., 2012; Romani et al., 2007). 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 proinflammatory cytokine storm (Fig. 2).

In summary, due to its pleiotropic mechanisms on affected immune cells, Ta1 could be useful in treatment of subjects who are immunosuppressed, whether the suppression is a result of infection, cancer, renal disease, or age. The utility of treatment with Ta1 for these indications has been investigated in animal model systems and clinical studies, as outlined later.

3. PRECLINICAL STUDIES OF TA1 IN IMMUNE SUPPRESSED ANIMALS

3.1 Animal Models of Infectious Disease

Microbial infections lead to multiple pathways of immune suppression, so that infections can lead to chronic, debilitating, and often fatal conditions. Ta1 treatment has been investigated in animal models of both acute and chronic viral, bacterial, and fungal infections.

Improvements in in vitro immune responses to mitogens by mouse lymphocytes were seen after treatment of aged mice immune suppressed by hydrocortisone treatment (Hadden, Saha, Sosa, & Hadden, 1995). As expected from these effects, Ta1 treatment provided statistically significant protection against a variety of acute lethal bacterial and fungal infections in animals immunosuppressed with 5-flurouracil (5-FU), including *Serratia marcescens*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Candida albicans* (Ishitsuka, Umeda, Nakamura, & Yagi, 1983). Ta1 also prolonged survival of mice, and prevented the increased susceptibility to infection, in animals infected with *C. albicans* and immune suppressed with either cyclophosphamide or morphine treatment (Bistoni, Marconi, Frati, Bonmassar, & Garaci, 1982; Di Francesco et al., 1994). Recent studies have investigated the efficacy of Ta1 against *Aspergillus fumigatus* in more detail, including the evaluation of knockout mice to determine the cellular pathways involved in the increased survival, decreased infectious agent burden, and increased T cells and NK-cell activity that are seen (Romani et al., 2004). Ta1 has shown benefit in treatment of generalized sepsis in several mouse models, both as a monotherapy and in combination with dexamethasone (Xiang, Li, Zhao, Li, & Li, 2014).

With respect to acute viral infections, Ta1 treatment has also led to increased survival, in mice infected with HSV (Shiau et al., 1988), influenza (D'Agostini et al., 1996; Effros et al., 1988), and cytomegalovirus (Bozza et al., 2007). Studies with mice infected with influenza, for example, showed that Ta1 in combination with IFN increased survival, reduced viral titer, and stimulated NK cells; Ta1 in combination with both IFN and the antiviral agent amantadine led to statistically significant increases in survival, NK-cell activity, CD4 and CD8 counts, and cytotoxic activity in the lung, while viral titers in the lung were reduced (D'Agostini et al., 1996).

Chronic infections have also been shown to respond positively to treatment with Ta1, with improved immune parameters and decreased viral loads, in models for hepatitis B virus (HBV) (Gerin, Korba, Cote, & Tennant, 1992; Tennant et al., 1993) hepatitis C virus (HCV) (Cursaro et al., 1998), and human immunodeficiency virus (HIV) (Garaci, Rocchi, Perroni, et al., 1994).

3.2 Animal Models of Cancer

Ta1 has been shown to have beneficial effects in several experimental models of cancer. When given in combination with chemotherapy and either IL-2

or IFN, Ta1 has shown not only to increase cytotoxic responses of T cells and NK-cell activity, but also to reduce tumor size and, importantly, to increase survival in models of colorectal cancer (Rasi, Silecchia, Sinibaldi-Vallebona, et al., 1994; Silecchia, Guarino, Sinibaldi-Vallebona, et al., 1999), melanoma (King & Tuthill, 2015; Pica, Fraschetti, Matteucci, Tuthill, & Rasi, 1998), non-small cell lung cancer (NSCLC) (Moody et al., 1993), lung carcinoma (Garaci, Mastino, Pica, & Favalli, 1990; Mastino, Favalli, Grelli, et al., 1992), erythroleukemia (Garaci et al., 1993), leukemia (Umeda et al., 1983), and fibrosarcoma (Tomazic, Sacasa, Loftus, Suter, & Elias, 1988).

In colorectal cancer, for example, Ta1 treatment in combination with 5-FU and IL-2 led to significantly improved median survival time compared to the 5-FU control (70.0 ± 8.2 days vs 54.6 ± 5.3 days, $p < 0.0001$) (Rasi et al., 1994). In addition to these positive effects on survival, there were also greatly reduced growth of liver metastases, reduced liver invasion (20% vs 60% in control), and reduced extra-hepatic spread. In addition, the improvement of survival time in these rats from the addition of Ta1 treatment allowed for a second cycle of treatment to be provided (Silecchia et al., 1999), which led to a further significant increase in survival time (81% survival at 100 days vs 39% with 5-FU alone or 44% for 5-FU plus IL-2).

Studies with a mouse model of melanoma showed that combination of increasing doses of Ta1 with chemotherapy and IFN chemotherapy led to significantly increased time to relapse, decreased the tumor growth rate, and improved survival in a Ta1 dose-dependent fashion (Pica et al., 1998). In fact, the addition of a single cycle of Ta1 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 Ta1 treatment in animal models of cancer, immune parameters were also improved. Splenocytes from treated mice showed markedly increased cytotoxic activities, and the tumor-induced reduction in percentages of CD3 and CD4 cells was reversed to nontumor levels (Pica et al., 1998).

The beneficial effects of Ta1 have also been seen when the compound is used as a sole therapy: Ta1 monotherapy has shown to prevent lung carcinogenesis in mice injected with a chemical carcinogen (Moody et al., 1993, 1998, 2000) and breast cancer in rats (Moody, Tuthill, Badamchian, & Goldstein, 2002). In addition, recent studies have shown that Ta1 monotherapy and/or in combination with anti-PD-1 antibody was able to significantly reduce lung metastases, and also to significantly reduce tumor growth of the highly metastatic melanoma clone B16F10;

widely used in studying the mechanisms of metastasis and in evaluating cancer therapeutics (King & Tuthill, 2015).

3.3 Animal Models of Improvement in Vaccine Response

As expected from the effects of Ta1 on antigen-presenting DCs, administration of Ta1 increases antibody response to vaccination. For example, while older mice (23 months old) have a significantly lower response to vaccination than young mice (2–3 months), when mice were treated with Ta1 not only was their antibody response to tetanus toxoid significantly increased ($p < 0.05$), but the response of older mice was restored to levels seen in young animals (Ershler et al., 1985). In a similar study comparing response to influenza virus infection in old mice (>24 months), Ta1 treatment was shown to increase IgM titer along with virus-specific cytotoxic T-cell responses (Effros et al., 1988).

The ability of Ta1 to enhance response to a pandemic influenza vaccine was tested with the 2008 seasonal trivalent vaccine. Studies in both older mice and ferrets showed an improvement in antibody titers when Ta1 was given along with the vaccine (Tuthill et al., 2010). Administration of Ta1 twice, 7 days prior to vaccination and again on the day of vaccination, was found to be the optimal dosing regimen, leading to improvement in response to all three strains of influenza tested. The titer of antibody was greater when determined 21 days after vaccination, and persisted when evaluated 42 days after vaccination. The improvement in titer was seen whether animals were given a vaccine booster or not.

4. CLINICAL STUDIES OF TA1 IN IMMUNE SUPPRESSION

As anticipated from the immune-modulating mechanism of action of Ta1 and the positive effects seen in animal models as described above, Ta1 has proven to be a useful therapeutic in a wide range of clinical indications. Over 4400 subjects have been enrolled in US, European and Chinese clinical trials investigating the use of Ta1, including primary treatment for subjects with acute infections, such as seen in severe sepsis, and for chronic infections including chronic hepatitis B (CHB), chronic hepatitis C (CHC), and HIV; as an adjunct treatment for cancers, including melanoma, hepatocellular carcinoma (HCC), and NSCLC; and as an enhancement to both hepatitis B and influenza vaccines in immune-depressed individuals. Results from some of these trials will be discussed later; based on these clinical studies Ta1 (trade name ZADAXIN) was first licensed in

Italy in 1993 for use as a vaccine enhancer, and has since obtained market approvals in over 30 countries in Asia, Latin and South America and Eastern Europe as a mono or combination therapy for CHB and CHC, as an adjuvant therapy for chemotherapy-induced immune suppression, and as a vaccine-enhancement agent.

To date, over 17 million doses of ZADAXIN (estimated as >350,000 individuals exposed) have been administered postmarket. ZADAXIN is generally well tolerated and has an excellent safety profile. Over the past 20 years, adverse experiences have been infrequent and mild.

4.1 Clinical Studies in Primary Immune Deficiency

The first clinical evaluation of Ta1 was a physician-sponsored study of 10 individuals with congenital immune deficiencies including hypogammaglobulinemia, ataxia telangectasia, Down's syndrome, and chronic mucocutaneous candidiasis (unpublished data). The trial was open-label and uncontrolled; the age of 7 subjects ranged from infancy to 16 years. Data were analyzed for eight subjects who had completed 6 months of treatment, and improvement in some parameters of T-lymphocyte function were observed in five of these subjects. No adverse experiences associated with Ta1 were reported.

A single child with DiGeorge anomaly was also treated with Ta1 (Gupta, Aggarwal, & Nguyen, 1997). Blood cells were taken from this 13-month-old infant before and after 3 months of treatment with Ta1 and examined for evidence of lymphocyte apoptosis compared to an age-matched healthy control. Prior to treatment with Ta1, the subject showed increased apoptosis (increased Fas and FasL, decreased Bcl-2 in both CD4 and CD8 cells, increased DNA fragmentation); after treatment with Ta1 the proportion of lymphocytes undergoing apoptosis decreased. The T-cell responses (response to mitogen) and B-cell function (specific antibody formation) also improved after treatment, and the subject showed a marked clinical improvement evidenced by a significant decrease in infection. No adverse experiences associated with Ta1 were reported.

4.2 Clinical Studies in Infectious Disease

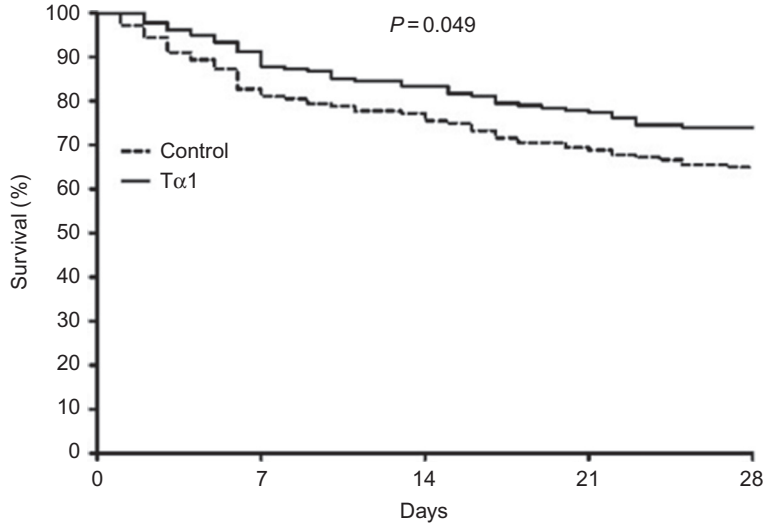
4.2.1 Acute Infections

Subsequent to these early physician-sponsored studies, Ta1 showed promise in larger clinical studies of acute infections, including severe sepsis (Chen, 2007; Li, Zhou, Qiang, et al., 2009; Wu, Zhou, Liu, et al., 2013; Zhao,

Cao, Fei, et al., 2007), severe acute pancreatitis (Wang, Li, et al., 2011), acute respiratory distress syndrome (ARDS) (Ji, Li, Sun, et al., 2007; Sun, Liu, Chen, et al., 2006), infectious episodes in chronic obstructive pulmonary disease (Li, Wang, et al., 2007; Zheng et al., 2008), and lung infections (Huang, Yang, Pen, et al., 2006; Li, Xu, Zhang, et al., 2007).

Severe sepsis, a syndrome defined as the presence of two or more features of systemic inflammation (such as fever or hypothermia, leucocytosis or leukopenia, tachycardia, tachyponea or supranormal minute ventilation), has historically been thought to be a manifestation of over-stimulation of the immune system in response to infection and thus was originally treated with immune suppressive drugs. Severe sepsis begins with a bacterial or fungal infection, which triggers immune signaling and subsequent release of noxious mediators, including proinflammatory cytokines and reactive oxygen free radicals that cause direct tissue injury. It is a remarkably diverse and heterogeneous condition, ranging in severity from mild systemic inflammation without significant clinical consequences to multisystem failure in septic shock with a high mortality rate. Also occurring very early on, however, is a compensatory antiinflammatory response leading to lymphocyte apoptosis, monocyte hyporesponsiveness and a state of immune suppression including a change to the T helper cell type 2 antiinflammatory cytokine phenotype (LaRosa, 2002). The pathophysiologic response in a given patient is often determined by the infectious insult, as well as the patient's age, comorbid illnesses and genome (Hotchkiss & Karl, 2003). In fact, only a small minority of previously well young patients die from an overwhelming proinflammatory cytokine storm in severe sepsis, and thus the use of immune-stimulatory therapies, in contrast to previous use of immune suppressive treatments, has recently been recommended, particularly in the elderly or with patients with serious coexisting illnesses in whom immunosuppression is identified or predicted (Angus & van der Poll, 2013).

Supporting this idea, several small clinical studies demonstrated the benefit of Ta1 in the treatment of severe sepsis, showing higher 28-day cumulative survival rates and improvements in other secondary endpoints (Gupta et al., 1997; Li et al., 2009; Zhao et al., 2007). A large (361 subjects), prospective, multicenter, single-blind, randomized, placebo-controlled trial was therefore conducted to further evaluate the concept (Wu et al., 2013). Twenty-eight-day mortality from any cause was 26% in the Ta1-treated group, vs 35% in control group ($p = 0.062$ nonstratified analysis, $p = 0.049$ log-rank; Fig. 3). The relative risk of death in the Ta1 group compared to control was 0.74 (95% confidence interval [CI] 0.54–1.02). There



Numbers at risk					
Day	0	7	14	21	28
Control	180	149	139	125	117
Tα1	181	165	151	141	134

Fig. 3 Ta1 improves survival in severe sepsis.

was a 9.0% (95% CI −0.5% to 18.5%) absolute reduction in mortality in the Ta1-treated subjects. As a measure of immune response, the levels of HLA-DR were evaluated and greater improvements were seen in subjects treated with Ta1 ($p = 0.037$).

Another study in acute infection is also of particular interest. Renal transplant patients with ARDS due to CMV infection were given Ta1 treatment at the time that they presented with low CD4 counts, and were treated until the CD4 levels returned to normal (Ji et al., 2007). Mortality was decreased from 50% (7/14) in control subjects, to 22% (7/32) in subjects treated with Ta1. All subjects were being treated with immunosuppressive drugs on admission; those treated with Ta1 were able to have the doses of immune suppressants decreased. Furthermore, in the survivors, acute cellular rejection of the renal graft was seen in only 12% of the Ta1 group vs 28.5% in the control group; renal transplant success rate was significantly higher (78% vs 50%) in the Ta1 treatment group. Finally, Ta1 treatment also significantly increased the number of CD4 and CD8 lymphocytes ($p < 0.05$), suggesting that repairing cellular immunity with Ta1 reinforces resistance to CMV.

A recent phase 2 clinical study was conducted in yet another acute infection setting: evaluating the use of Ta1 after HLA matched or haploidentical stem cell transplant in 75 subjects (Perruccio, Bonifazi, Topini, et al., 2010; Romani, Aversa, Garaci, & Velardi, 2012). This study found quite promising results. The cumulative incidence of transplant-related mortality (TRM; the majority of which was CMV or *Aspergillus* 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 (40% vs 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 ($p = 0.09$). And, as expected from its mechanism of action, Ta1 administration was associated with increased T-cell counts and earlier appearance of functional pathogen-specific T-cells responses (against *Aspergillus*, *Candida*, CMV, varicella-zoster virus, HSV, and *Toxoplasma*).

4.2.2 Chronic Infections

Chronic infections resulting from infection with HBV, HCV, and HIV are considered hallmarks of immune suppression, resulting from the myriad pathways of immune system evasion that the viruses have evolved. During the course of infection with HBV, for example, the virus disrupts innate immunity pathways (including TLR signaling, DCs, NK, and NKT cells) early during infection, which then also compromises the quality of the adaptive immune response, including a decrease in the number and function of HBV-specific T lymphocytes (Busca & Kumar, 2014). Although most adults are able to clear the acute infection, those individuals who have impaired cellular immune mechanisms, including the young, do not effectively clear HBV-infected hepatocytes and chronic infection results, correlated with a greatly increased risk for developing cirrhosis, liver failure, and HCC. About one-third of the world's population will be infected by HBV at one point in their lives; CHB has caused epidemics in many parts of Asia and Africa and is considered an endemic in China, affecting up to 240 million persons worldwide (WHO, 2015).

Interest in using Ta1 for treatment of CHB was based on its immunomodulating effects, primarily the improved maturation of lymphocytes and augmentation of T-cell function. Clinical studies with Ta1 have resulted in disease remission in 26–41% of the subjects treated. An independent meta-analysis of 435 subjects entered into randomized, controlled studies of Ta1 monotherapy for CHB 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; $p = 0.0005$) (Chan, Tang, & Sung, 2001). The same study also demonstrated a trend in favor of Ta1 treatment compared with IFN for sustained virological response (OR = 2.62; 95% CI 0.80–8.56). Other trials have shown that the addition of Ta1 to other agents, such as IFN and nucleoside analogs, can lead to improved responses (Lau et al., 1999; Lim, Wai, Lee, et al., 2006; Saruc, Yuceyar, Kucukmetin, Demir, & Kandiloglu, 2002; Saruc, Ozden, et al., 2002).

4.3 Clinical Studies in Cancer

Most cancer patients have clearly depressed cellular immunity, and progression of cancer appears to be related to impaired suppression of the tumors by the immune system. Immune-based therapies are emerging as efficacious treatments for cancer, including antibodies or compounds that manipulate cellular signals to induce better recognition of cancer cells by the immune system, such as anti-CTLA-4, which can enable T-cell activation via stimulation of CD80 and CD86 (Acharya & Jeter, 2013); anti-PD-L1, which can reverse immunosuppression (John, Westwood, Darcy, & Kershaw, 2013); or anti-CD47, which enhances phagocytosis of cancer cells (Weissman & Majeti, 2012). General immune modulation (eg, with IL-2) has also shown promising results for treatment of human cancers (Balch et al., 1997; Richards, Gale, Mahta, & Lestingi, 1999; Rosenberg, Mul, Spiess, Reichert, & Schwarz, 1985). A robust host immune response appears to be useful or necessary for the complete clearance of tumor cells, and the establishment of effective antitumor immune responses may be essential to the success of treatment (Burkholder et al., 1845).

Ta1 treatment has been shown to provide several benefits to cancer patients, including an increase in effectiveness of chemotherapy, a decrease in treatment side effects, and an overall improvement in quality of life (QOL), without the types of adverse events seen with IL-2 or IFN.

4.3.1 Melanoma

Melanoma is a cancer of the skin caused by a malignant tumor of melanocytes found predominantly in adults, responsible for more than 75% of deaths caused by skin cancers, one of the most common types of cancer. 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 approximately 7.5 months, with a 90% mortality rate after 5 months (American Cancer Society, 2009).

Pilot studies showed that Ta1 treatment resulted in an improvement in response, time to progression, and overall survival compared to historical controls in subjects with metastatic melanoma being treated with dacarbazine (DTIC) chemotherapy plus IL-2 (Lopez et al., 1994) or IFN (Rasi, Terzoli, Izzo, et al., 2000). A significant protection against chemotherapy-induced reductions in NK activity and CD4 T cells ($p < 0.001$) was also seen.

A large randomized study was subsequently conducted in 488 subjects with metastatic melanoma (Fig. 4) (Maio et al., 2010). In this study, addition of various doses of Ta1 to DTIC, with or without IFN, led to an increase in median overall survival (9.4 months for Ta1-treated subjects vs 6.6 months for control; $p = 0.08$), progression-free survival (16 months for Ta1 vs 9 months, $p = 0.06$; significant for the subset of subjects given 3.2 mg Ta1 plus DTIC, $p = 0.04$), a significant improvement in tumor response (10.3 or 12.1% in subjects treated with 3.2 mg Ta1 with DTIC with or without IFN, vs 4% in the control group; $p < 0.05$), and an increase in duration of

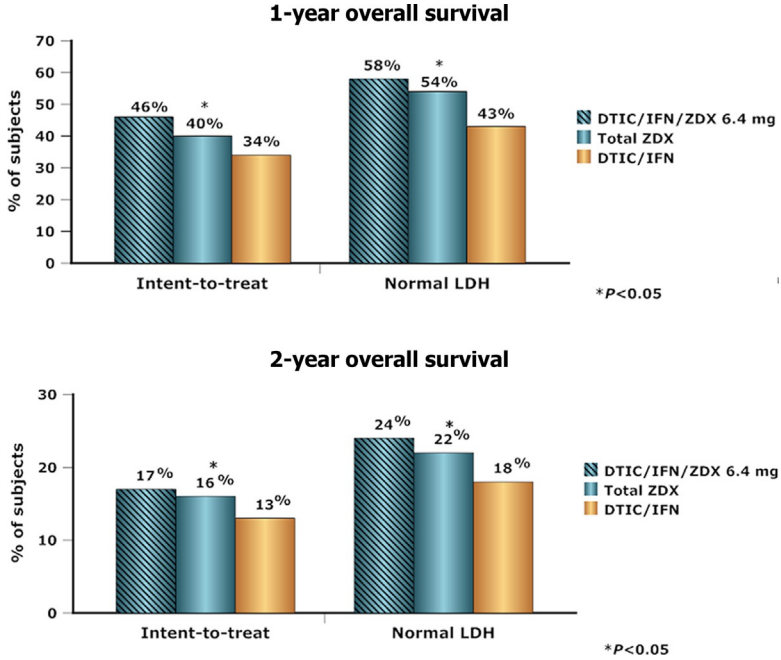


Fig. 4 Ta1 improves survival in Stage IV melanoma.

response (from a range of only 4.4–8.4 months in the control, to a range of 1.9–23.2 for Ta1-treated subjects). The clinical benefit rates were significantly higher for subjects given 3.2 mg Ta1 plus DTIC compared to control ($p = 0.009$), and no additional toxicity was seen with the addition of Ta1.

4.3.2 Hepatocellular Carcinoma

HCC is a primary malignancy of the liver affecting over 500,000 people worldwide. 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 (El-Serag & Rudolph, 2007). 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 when used in combination with either sorafenib or TACE in treatment of HCC, with no addition of toxicity. Pilot studies were conducted to evaluate the benefit of Ta1 after TACE, which demonstrated significant improvement in survival compared to historical controls at 6, 9, and 12 months after TACE with or without Ta1 (91% compared to 77%, 88% compared to 58%, and 78% compared to 46%, respectively; $p < 0.05$) (Li, 2001; Stefanini, Foschi, Castelli, et al., 1998; Zhang, 2000). Significantly increased cytotoxic T cells, CD3 cells, CD4/CD8 ratio, and NK cells were also seen.

Further controlled studies confirmed these results, some showing only a trend toward survival (Cheng et al., 2005b; Gish, Gordon, Nelson, Rustgi, & Rios, 2009). But one study showed significantly improved survival (10 vs 7 months; $p = 0.002$) and delayed time to tumor recurrence

($p = 0.039$) compared to TACE treatment controls (Cheng et al., 2004a, 2004b).

Subjects with HCC and concurrent HBV infection were evaluated for the addition of Ta1 treatment to lamivudine after tumor resection or partial hepatectomy (Cheng et al., 2005a, 2006; Cheng, Ding, et al., 2005). In this study, the addition of Ta1/lamivudine combination treatment resulted in statistically significant increases in time to tumor recurrence (10.0 vs 6.5 months; $p = 0.0032$), median survival time (12.5 vs 6.0 months; $p = 0.0023$), and HBV-DNA clearance (100% vs 4%; $p = 0.0000$) and seroconversion to HBeAg (73% vs 7.5%; $p < 0.05$).

4.3.3 Non-Small Cell Lung Cancer

Lung cancer is the most common form of cancer worldwide and has the highest mortality rate (World Cancer Research Fund International, 2012). NSCLC is any type of epithelial lung cancer excluding small cell lung carcinoma. NSCLC accounts for approximately 85% of all lung cancers with more than half of the cases having already metastasized at time of diagnosis. 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.

Clinical studies have shown notable improvements after treatment with Ta1 in combination with chemotherapy or radiation, including statistically significantly improvement in survival time and survival rates compared to chemotherapy or radiation alone, even in subjects with stages III or IV cancers (Garaci et al., 1995; Ma, Lin, Mei, & Ping, 2006; Salvati, Rasi, Portalone, Antilli, & Garaci, 1996; Schulof, Lloyd, Cleary, et al., 1985). As expected, these studies also demonstrated increases in immune cell numbers and function, and decreased toxicity from chemotherapy.

4.3.4 Quality of Life in Cancer

A large number of studies conducted with Ta1 in various cancers have shown improvements in QOL, as expected from an agent that counteracts the immune suppression that results from the cancers themselves, the chemotherapy utilized, and other confounding factors such as age.

In NSCLC, the Karnovsky performance score (KPS) measurement of QOL was increased from about 30–60% ($p < 0.05$) (Hou, 2007; Liang, Zhou, & Yang, 2010), and immune parameters were significantly improved (Shi, Ding, & Yang, 2007; Sun, Gao, Liu, & Li, 2009). The significant

improvements in side effects evaluated included an abrogation of decreases in white blood cell counts and platelets, and decreases in the number of subjects experiencing nausea and vomiting.

Subjects with lung, gastric, or breast cancer were given Ta1 treatment during one of two cycles of chemotherapy, and QOL was evaluated. Ta1 provided a significant improvement in all measures of QOL (appetite, sleep, fatigue, daily activity, overall feeling, and depression), lowered occurrences of infections during chemotherapy, increased immune parameters (Chen et al., 2000; Luna et al., 2000), and decreased neurotoxicity (An, Liu, Fang, & Wu, 2004; Liu & An, 2003). Elderly subjects with advanced malignant cancer (lung, liver, stomach, colon, and rectal) also showed significant improvement in KPS and immune parameters (Yang, Lu, & Huang, 2003), and a second study in the same patient population also showed a significant increase in survival (median survival improved from 6 to 24 months; $p < 0.01$); the effect was so pronounced that at 24 months, when there was a 43.3% survival in the Ta1-treated group, the control group of subjects in the study had no survivors at all (Wang, Zhen, et al., 2010).

Improvements in QOL have also been observed in treatment of subjects with pancreatic cancer (Ni et al., 2001) and postmenopausal women undergoing treatment for breast cancer with aromatase inhibitors who had joint symptoms and pain related to the treatment (Zhang, Tang, & Zhao, 2010). In the latter study, subjects treated with Ta1 showed significant improvement in the Western Ontario and McMaster Universities Osteoarthritis Index ($p < 0.001$) and Functional Assessment of Cancer Therapy-General physical well-being ($p < 0.001$), and 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 scale.

4.4 Clinical Studies in Vaccine Enhancement

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 (Makinodan & Peterson, 1962; Rosenberg et al., 1985; Wade & Szewczuk, 1984). 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 (Bramwell, Tsakiris, Briggs, et al., 1985).

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 (eg, influenza). Diminished antibody responses have also been reported in patients with end-stage renal disease (ESRD). The evidence for impairment of cell-mediated immunity in hemodialysis patients has been attributed to incompetence in T-cell-mediated immune responses (Dammin, Couch, & Murray, 1957; Lawrence, 1965; Revie, Shen, Ordonez, et al., 1985; Sanders, Luby, Sanford, & Hull, 1971). Several studies have reported poor antibody response after hepatitis B vaccination in hemodialysis patients (Crosnier, Jungers, Courouc, et al., 1981; Grob, Binswanger, Zaruba, et al., 1983; Stevens, Alter, Taylor, Zang, et al., 1984).

Ta1 had been shown to improve T-cell-dependent specific antibody production in animal models (Effros et al., 1988; Ershler et al., 1985); in vitro studies also demonstrated that influenza antibody synthesis was augmented in cells from vaccinated elderly individuals (Ershler et al., 1984).

Clinical studies have evaluated the benefit of Ta1 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 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 (Carraro et al., 2012; Gravenstein, Duthie, Miller, et al., 1989; Gravenstein, Ershler, Durmaskin, Schwab, & Weksler, 1986; McConnell et al., 1989; Shen, Corteza, Josselson, et al., 1990; Shen, Josselson, McRoy, Sadler, & Chretien, 1987a, 1987b). These studies also confirm that Ta1 is safe for administration to immunocompromised subjects, as no serious adverse effects were observed in any of the studies.

The most recent study evaluated the addition of Ta1 as an enhancer of the immunogenicity of the 2009 H1N1 monovalent vaccine (Focetria®, Novartis) in adults with ESRD on chronic dialysis (Carraro et al., 2012). Ta1 was given twice, 1 week before and on the day of vaccination, and was administered at two different doses (40 subjects received 3.2 mg and 42 subjects received 6.4 mg). Results show that subjects who were treated with either dose of Ta1 achieved a marked and significant increase in their antibody titers compared to placebo; the percent of subjects who seroconverted by 21 days after vaccination was 88% and 89% for the Ta1-treated subjects, respectively, but only 53% for the placebo group ($p < 0.01$). The percent of subjects who were seroprotected by 21 days after

vaccination were 94% and 93%, vs 77%, respectively, for these treatment groups. The positive effects from Ta1 were maintained at 42 days after vaccination, although by 84 days the differences were smaller and only the higher dose of Ta1 showed a continued improvement over vaccine alone.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Immune suppression occurs during aging, and is a hallmark of infectious disease and cancer. The cell-signaling pathways involved are particular targets of Ta1's mechanism of action, and can explain the utility of Ta1 shown for treatment of immune-sensitive diseases, including both acute and chronic viral, bacterial, and fungal infections, multiple types of cancers, and vaccine enhancement. For example, Ta1 has shown promise in treatment of severe sepsis, and in clinical studies monotherapy with Ta1 was as effective as or better than IFN in treatment of HBV, especially in populations with low response rates to IFN (eg, vertically transmitted HBV; previous nonresponders to other therapy; immune tolerant patients with high HBV-DNA levels). For treatment of cancer, Ta1 has shown utility in clinical trials in melanoma, HCC, and NSCLC, in which the addition of Ta1 to treatment regimen has led to significantly increased survival. Ta1 can decrease toxicity from chemotherapy and significantly improve QOL in cancer patients. Ta1 improves response to vaccines, as shown in elderly persons and subjects with end-stage renal disease. Patients receiving Ta1 report few serious drug-related toxicities during treatment, even in combination with other agents, making Ta1 particularly attractive for evaluation in immune-depressed patients from all causes.

ACKNOWLEDGMENTS

Robert King is an employee, and Cynthia Tuthill is a consultant, for SciClone Pharmaceuticals.

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