Combination therapy of tumors and infections with thymosin alpha$_1$

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A possible approach in using biological response modifiers (BRMs) in the treatment of cancer and infectious diseases is the attempt to utilize combination therapies with these agents, in order to improve their efficacy (1–3). In fact it is known that immune physiological responses involve cascades and feedback networks in which the release of one factor could modulate that of others and the expression of their receptors. Moreover, combination of BRMs could affect different immune effector cells.

Thymic hormones (THs) have been shown to stimulate the production of different cytokines by peripheral blood lymphocytes (PBL) (reviewed in 4). Thymosin α_1 (Tα_1) has been also shown to induce interferon (IFN)-α after in vivo administration (5). Moreover it has been shown that Tα_1 can upregulate the expression of high-affinity interleukin 2 (IL-2) receptors in mitogen-stimulated human PBL or large-granular-lymphocytes, and it enhances IL-2 production (6–9). Because of these interesting interactions, we hypothesized that combined treatment with THs and cytokines, a major class of compounds belonging to the category of BRMs, could be considered as a potentially interesting approach. Thus, during the recent years we have been involved in the study of combination therapy with Tα_1 and cytokines, in association with or without chemotherapy.

Our first experimental animal studies demonstrated that Tα_1 and IFN, when administered in combination, were effective in significantly restoring natural killer (NK) activity in cyclophosphamide-(CY) treated or B-16 and 3LL tumor-bearing mice (10–12). Subsequently, we clearly demonstrated the curative efficacy of combined immunotherapy in conjunction with CY, both in 3LL and Friend leukemia cell (FLC) experimental tumor models (13, 14). Moreover, we found that pre-treatment with Tα_1 in vitro or in vivo reinforces the effect of IL-2, inducing an enhancement of cytotoxic activity in spleen cells from normal as well as from tumor and/or CY-suppressed mice (15). Combined chemo-immunotherapy using Tα_1 and well tolerated doses of IL-2 after CY induced 100% regression in 3LL mice, compared with 35% using CY + IL-2 alone, and significantly reduced tumor growth in FLC-bearing mice (14, 16).

More recently we extended our study on chemoimmunotherapy with Tα_1 to experimental infections. In particular we examined the effects of the antiviral drug amantadine administered in combination with Tα_1 and IFN on mice infected with influenza A virus. The efficacy of this new chemoimmuno-

therapy protocol has been seen in the long-term survival of a high percentage of animals when compared with treatments using single agents. Moreover, chemoimmunotherapy with $T\alpha_1$ reduced viral titer in the lung and restored some immunological parameters (17). A summary of the principal results obtained in these preclinical studies is reported in table I.

The encouraging results of our preclinical studies on combination therapies with $T\alpha_1$ in treating infections and tumors in rodents, led us to explore the potential of similar protocols in humans. For this purpose, in collaboration with several clinical groups in different institutions, we have studied the effects of different regimens of combination therapy with $T\alpha_1$ in a number of Phase I/II clinical trials in various human pathologies. Many of these studies have been concluded and the results have recently been published or are in press. Among these are included a randomised not-blinded study on the effect of the combination of 3'-azido-3'deoxythymidine (AZT) + $T\alpha_1$ + IFN-α in HIV patients and two studies on the use of $T\alpha_1$ in combination with lymphoblastoid interferon in the treatment of chronic hepatitis B and chronic hepatitis C, respectively. Regarding the study on HIV patients, preliminary in vitro experiments had shown that a combination treatment with $T\alpha_1$ and IFN synergistically stimulated the cytotoxic activity against NK-sensitive target cells of PBL from HIV-infected donors and did not interfere with the antiviral activity of AZT. As a consequence we studied the effect of the AZT + $T\alpha_1$+ IFN combination regimen in patients with $CD4^+$ lymphocytes ranging from 500 to 200/mm^3. The treatment was well tolerated after 12 months of therapy and was associated with a substantial increase in the number and function of CD4 + T-cells. A similar beneficial effect was not observed in a group of HIV patients treated with AZT alone or in those in which AZT was associated with single agents (18). These data, obtained in a restricted number of patients, suggested the need for a controlled phase III double-blind clinical trial. Such a trial, involving a high number of patients in several centers, has been recently concluded and definitive results, which substantially confirms those obtained in the above mentioned pilot study, will be soon available in detail.

The trial on chronic hepatitis B was an open label study initiated to assess the safety and efficacy of lymphoblastoid IFN-α and $T\alpha_1$ in eleven patients who had failed to respond to standard IFN therapy, and in four IFN naive patients. Patients were followed-up for 12 months. Nine (60%) of the total 15 patients, including 6 (55%) of the eleven previously treated patients, responded by losing serum HBV DNA and normalising alanine aminotransferase values. Six of nine responders seroconverted to HBsAg negativity. Significant histological improvements were observed in the responders and treatment was well tolerated (19). In the case of chronic hepatitis C, which is highly resistent to monotherapy using IFN, 15 patients with serum HCV RNA positivity (13 serotype 1b) were studied in an open label trial. Among these, four patients had failed previous monotherapy with IFN and eleven patients were therapy naive. Combination therapy was administered for one year and follow-up was managed for six months. Six months after the end of treatment six patients (40%), including one with previous IFN monotherapy failure, showed a

Table I. Preclinical studies on combination therapies with thymosin α_1 and cytokines.

Cytokine combined	Experimental model	Main results	References
IFN in vivo	cyclohphosphamide treated mice	stimulation of NK activity	*Favalli* et al. 1985 (10) *Garaci* et al. 1989 (12)
IFN in vivo	B16 and 3LL tumor bearing mice	stimulation of NK activity	*Favalli* et al. 1989 (11) *Garaci* et al. 1989 (12)
IFN in vivo	cyclophosphamide treated 3LL or metastatic Friend leukemia cell tumor bearing mice	antitumor effect	*Garaci* et al. 1990 (13) *Garaci* et al. 1993 (14)
IFN in vivo	amantadine treated influenza A virus infected mice	antiviral effect	*D'Agostini* et al. 1996 (17)
IL-2 in vitro or in vivo	normal, cyclophosphamide treated, cyclophosphamide treated or not B16 or 3LL tumor bearing mice	stimulation of cytotoxic activity against NK-sensitive or NK-resistant target cells	*Garaci* et al. 1989 (12) *Mastino* et al. 1991 (15) *Mastino* et al. 1992 (16)
IL-2 in vivo	cyclophosphamide treated 3 LL or metastatic Friend leukemia cell tumor bearing mice	antitumor effect	*Mastino* et al. 1992 (16) *Garaci* et al. 1993 (13)

sustained response characterised by serum HCV RNA negativity (20). Results and treatment schedules referring to clinical trials with combined treatments using $T\alpha_1$ in infectious diseases are reported in table II.

We also promoted some clinical trials on cancer patients. In particular, the following studies have been completed: 1. a Phase II trial on sequential chemoimmunotherapy for advanced non-small cell lung cancer (NSCLC) using cisplatin, etoposide, $T\alpha_1$ and IFN-α_{2a}; 2. a Phase II controlled trial on combined treatment with $T\alpha_1$ and low-dose IFN-α after ifosfamide in NSCLC; 3. a Phase I/II trial on combination therapy with $T\alpha_1$, hrIL-2 and dacarbazine in patients with metastatic melanoma. Regarding to the trial on NSCLC patients treated using cisplatin, etoposide, $T\alpha_1$ and IFN-α_{2a}, we observed 24 responses (two complete, 22 partial) among 56 assessable patients. Median survival time was 12.6 months. The impact on the immune system of the chemoimmunotherapy regimen was indicated by a less prominent depression in NK activity in this group of patients in comparison with a group of patients treated with the same chemotherapy schedule alone. Thus, this chemoimmunotherapy regimen was demonstrated to be effective in advanced NSCLC patients with acceptable toxicity (21).

The clinical trial in NSCLC patients using ifosfamide as chemotherapy regimen gave similar results. In this case 22 patients were randomised and divided into two groups to receive ifosfamide alone or ifosfamide associated to $T\alpha_1$ + IFNα, respectively. Immunological findings showed that patients treated with ifosfamide alone experienced a significant decrease in $CD4^+$, $CD8^+$ and $CD56^+/CD16^+/CD3^-$ (NK) cells after two cycles of therapy, patients who received $T\alpha_1$ + IFN in association with chemotherapy showed no statistically significant difference in effector lymphocyte count compared to basal levels. Moreover, haematological toxicity was reduced in patients chemoimmunotherapy-treated in comparison with patients treated by chemotherapy alone. Chemoimmunotherapy induced an enhanced response rate in comparison with chemotherapy alone (33% versus 10% respectively) (22). However, the low number of cases in this study did not allow us to achieve statistically significant results on the higher efficacy of the chemoimmunotherapy regimen in comparison with chemotherapy alone.

Finally, the clinical trial in metastatic melanoma patients addressed the possibility to utilizing $T\alpha_1$ in combination with IL-2 in chemoimmunotherapy regimens. Particularly, in this study the clinical and immunological effects of chemoimmunotherapy with dacarbazine (DTIC), $T\alpha_1$ and IL-2 in 46 patients were investigated. Objective responses were obtained in 15 (36%) of 42 patients evaluated. Two patients experienced complete responses, and stable disease was observed in five. The median time progression was 5.5 and median survival was eleven months. $T\alpha_1$ treatment did not add side effects to those predominantly caused by IL-2 and the regimen was tolerated reasonably well, indicating that the combination DTIC + $T\alpha_1$ + IL-2 is active in the treatment of advanced melanoma with acceptable toxicity (23). The characteristics of clinical trials using chemoimmunotherapy treatments with $T\alpha_1$ in cancer patients are summarised in table III.

Table II. Clinical trials with combination therapies using thymosin α_1 and cytokines in human infections.

Infectious pathology	Treatment schedule	Main results	References
HIV infection	AZT (500 mg/day) daily, $T\alpha_1$ (1 mg) followed by IFN-α (2 MU) twice weekly for 52 weeks	increase in number and functions of $CD4^+$ lymphocytes	*Garaci* et al. 1994 (18)
Chronic hepatitis B	loading dose (first week) $T\alpha_1$ (1 mg) daily x 4 dd followed by a single dose of IFN-α (3 MU); maintenance dose (second and subsequent 25 weeks): $T\alpha_1$ (1 mg) followed by IFN-α (3 MU) twice weekly	60% response at 1 year after the end of treatment	*Rasi* et al. 1996 (19)
Chronic hepatitis C	loading dose (first week) and maintenance dose (second and subsequent 51 weeks): the same as for chronic hepatitis B	40% response at 6 months after the end of treatment	*Rasi* et al. 1996 (20)

Table III. Clinical trials with combination therapies using thymosin α_1 and cytokines, associated to chemotherapy, in cancer.

Tumor	Treatment schedule	Main results	References
Advanced non-small cell lung cancer	chemotherapy: cisplatin 100 mg/m^2) day 1, etoposide (120 mg/m^2) days 1–3; immunotherapy: Tα_1 (1 mg) days 8–11 and 15–18 followed by IFN-α (3 MU) days 11 and 18, courses repeated every 3 weeks for a maximum of 6 courses	43% overall response, median survival time in responding patients: 15.7 months; improvement of immunological status in comparison with chemotherapy alone	*Garaci* et al. 1995 (21)
Advanced non-small cell lung cancer	chemotherapy: ifosfamide (3 g/m^2) plus Mesna (400 mg) day 1 and 2; immunotherapy: Tα_1 (1 mg) days 8–11 and 15–18 followed by IFN-α (3 MU) days 11 and 18, courses repeated every 3 weeks for a maximum of 6 courses	66% overall response, median survival time in responding patients: 24 weeks; improvement of immunological status in comparison with chemotherapy alone	*Salvati* et al. 1996 (22)
Metastatic melanoma	chemotherapy: dacarbazine (850 mg/m^2) day 1; immunotherapy: Tα_1 (2 mg) days 4–7, IL-2 (18 X 10^6 IU/m^2/d) days 8–12, courses repeated every 3 weeks for a maximum of 6 courses	36% overall response, median survival time in responding patients: 13.3 months; no overlapping toxicity between chemotherapy and immunotherapy	*Lopez* et al. 1994 (23)

In conclusion our preclinical studies and the subsequent clinical trials performed in collaboration with several clinical centers suggest that combination therapies with $T\alpha_1$ are effective with acceptable toxicity in some infections and in some malignancies. As a consequence the following step in these studies should be the confirmation of the results in Phase III randomised trials. However, additional information on the mechanisms involved in the synergisms between $T\alpha_1$ and cytokines and more efficacious regimens are also needed in order to further improve the response rates obtainable with such a therapeutic approach.

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