Interleukin-2 and Lanreotide in the Treatment of Medullary Thyroid Cancer: In Vitro and In Vivo Studies

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Context: To date no efficacious treatments are available for advanced medullary thyroid carcinoma (MTC).

Objective: We investigated in vitro and in vivo a new strategy for the therapy of MTC, combining human recombinant IL-2 with lanreotide (LAN), a somatostatin analog.

Methods: The in vitro effects of LAN on the sensitivity of TT cells, a MTC cell line, to IL-2–stimulated human peripheral blood mononuclear cells were determined by a lactate dehydrogenase release assay. In addition, we evaluated the toxicity, the effects on quality of life, and the antitumor activity of sc low-dose IL-2 in combination with LAN (90 mg every 28 days) in a series of 6 patients with symptomatic and advanced MTC.

Results: The cytotoxicity of IL-2–activated peripheral blood mononuclear cells was significantly increased in TT cells treated with LAN or LAN plus IL-2 compared with that in TT cells without treatment. The therapy was well tolerated, and a statistically significant improvement of quality of life was observed in patients treated with the combination of LAN and IL-2. After 6 months of therapy, partial response and stable disease have been recorded in 2 and 3 patients, respectively, with a significant decrease in calcitonin levels in 3 patients.

Conclusions: Both in vitro and in vivo evidence suggests that the combination of LAN and IL-2 may have a role in the management of advanced and symptomatic MTC. However, these preliminary data require further validation in larger randomized trials. **(*J Clin Endocrinol Metab* 98: E1567–E1574, 2013)**

Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor developing from parafollicular C cells (1). The clinical course of patients with MTC ranges from indolent to extremely aggressive, and it is related to the stage of the disease at the time of diagnosis. Therefore, an early diagnosis is a main point in the management of MTC (2, 3).

At present, surgery is the most effective treatment for MTC (1, 4). In advanced MTC, radiotherapy and che-

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Abbreviations: AdCMVmIL2, adenoviral vectors containing murine IL-2 cDNA; CT, calcitonin; FACT-G, Functional Assessment of Cancer Therapy-General; LAN, lanreotide; LDH, lactate dehydrogenase; MTC, medullary thyroid carcinoma; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PR, partial response; QoL, quality of life; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; Treg, T regulatory cell.

motherapy play a marginal role because of the related toxicity and the limited effectiveness of these treatments in MTC (4). Somatostatin analogs have been demonstrated to control neuroendocrine symptoms and to be well tolerated, also improving the quality of life (QoL) in these patients. However, all studies regarding biological treatment in MTC have been limited to a small series of patients, and there are no consistent data showing tumor shrinkage and/or improvement of patient survival (5–8).

Recent progress in understanding the pathogenesis of MTC has led to increased interest in the development of potential therapeutic strategies, including tyrosine kinase inhibitors (sunitinib, vandetanib, cabozantinib, and others) and mammalian target of rapamycin inhibitors (everolimus) (9, 10). Several studies have proposed the use of human recombinant IL-2 in the treatment of MTC. This cytokine induced regression of MTC in both in vitro and in vivo models, promoting the proliferation of cytotoxic and T helper cells, the activation of natural killers, and enhancing their cytolytic functions as lymphokine-activated killer cells, thus stimulating both nonspecific and specific immune responses to tumor cells (11–16). Therefore, immune surveillance seems to play a relevant role in protecting against MTC. However, MTC may escape immune surveillance through secretion of bioactive molecules able to suppress immune responses via the inhibition of cytotoxic T-lymphocyte activity and the recruitment of regulatory T cells (Tregs). These effects have been described previously in neuroendocrine tumors (17). In support of this hypothesis, it was recently reported that MTC is characterized by a significant infiltration of Tregs in the tumor and metastatic lymph nodes as well as in the blood of the patients (18). Tregs, formerly known as suppressor T cells, are a subpopulation of T cells that suppress immune responses of other cells. These cells are able to control and inhibit the autologous immune reaction, which plays an important role with regard to malignant tumors. For instance, it has been shown that depletion of Tregs in mice with IL-2–transfected colon adenocarcinoma resulted in total tumor rejection (19). In patients with MTC, the number of Tregs directly correlated with the staging and degree of severity of disease (18). Therefore, an increase in Tregs may be a strategy of tumor immune evasion adopted by MTC.

Most biological functions of MTC cells are regulated by somatostatin receptors that are involved in the control of their proliferation and of the secretion of several bioactive molecules (4). In this scenario, somatostatin analogs could modulate the secretion of tumor cell–derived factors interfering with the tumor cell growth and the immune system (20).

On the basis of these considerations, in the present study, we evaluated the effects of lanreotide (LAN), a somatostatin analog, in combination with IL-2 on the growth of TT, a MTC cell line, and the effects of LAN on the sensitivity of human TT cells to IL-2–stimulated human peripheral blood mononuclear cells (PBMCs). In addition, we evaluated the toxicity, the effects on QoL, and the antitumor activity of sc low-dose human recombinant IL-2 in combination with LAN in a series of patients with advanced MTC.

Patients and Methods

Cell cultures and PBMCs

The TT cell line was purchased from American Type Culture Collection. Cells were grown at 37°C in Ham's F12 with Kaighn's modification medium containing 10% fetal bovine serum, 2 mM glutamine, and 10^5 U/L penicillin-streptomycin and were maintained in a humidified atmosphere of 5% CO_2. Cells were harvested with trypsin-EDTA, washed twice with PBS, and resuspended in medium. Before plating, the cells were counted microscopically using a standard hemocytometer. Human PBMCs were obtained by Ficoll-Hypaque gradient centrifugation of heparinized blood collected from healthy donors.

Reagents and drugs

Human recombinant IL-2 was kindly provided by Novartis Farma SPA, and LAN was obtained from Ipsen.

Cytotoxic activity assay

Fresh PBMCs (effector cells) and TT cells (target cells) were separately incubated with or without LAN (10 nM) and/or IL-2 (100 IU/mL) in a humidified atmosphere in 5% CO_2 at 37°C. After 24 hours, the drugs were washed off, and PBMCs were added to TT cells (5000 cells/well) at different effector to target cell ratios (1:1, 10:1, 20:1, 50:1, and 100:1) in 96-well plates. After a further 4 hours of incubation, the cytotoxic activity of PBMCs on TT cells was determined by lactate dehydrogenase (LDH) release assay (Sigma-Aldrich). The samples were centrifuged, and the supernatants were collected and incubated for 30 minutes at room temperature with the reaction mixture kit to detect LDH activity. The absorbance of the samples was measured at 490 nm on the automated plate reader (Multiwell reader; Bio-Rad). The reference wavelength was 630 nm. The percentage of cell-mediated cytotoxicity was calculated according to the equation: cytotoxicity (%) = {[(effector/target cell mix − spontaneous effector cells LDH release) − spontaneous target LDH release]/(maximum target cells LDH release − spontaneous target cells LDH release)} × 100. The experiment was performed 3 times as described previously (21).

Cell proliferation assay

Analysis of cell proliferation was performed in the presence of LAN (10 nM) and/or IL-2 (100 IU/mL) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. In brief, TT cells were seeded in 96-well plates at a density of 3.5×10^4/well in serum-containing medium and allowed to attach for 24 hours.

Table 1. Characteristics of Patients, Tumor Mass Response, Overall Survival, and Duration of Tumor Response or SD to the Treatment with LAN and IL-2

Patient	Sex	Age, y	Tumor Response	Overall Survival, mo	Duration of Response/ SD, mo	Previous Treatments	Metastatic Sites at the Time of the Enrollment (Maximum Diameter of Each Lesion)
1	F	43	PR	48	36	RT; EDX + CDDP; OCT; IFN + OCT	2 mediastinal nodes (2 and 3 cm); 1 left cervical node (3.2 cm); 2 lung metastases (2 and 3 cm)
2	M	36	PR	48+	30	RT; OCT	2 mediastinal nodes (2 and 4 cm), 1 right cervical node (4 cm), 1 lung metastasis (2.2 cm)
3	F	26	PD			OCT; IFN + OCT	1 liver metastasis (4 cm)
4	M	62	SD	39	24	OCT	2 left cervical nodes (2 and 3 cm)
5	F	43	SD	54+	24	RT; EDX + CDDP; OCT; IFN + OCT	2 liver metastases (2 and 3 cm)
6	M	32	SD	36+	18	OCT; IFN + OCT	3 mediastinal nodes (2, 3 and 3 cm)

Abbreviations: CDDP, cisplatin; EDX, epidoxorubicin; IFN, interferon-α; MR, minor response; OCT, octreotide; RT, radiotherapy.

The medium was then removed and replaced with new medium containing drugs. After 24 hours, the drugs were washed off, and fresh medium was added. After further 4 hours of incubation, cell viability was assessed with the MTT assay, as described previously (22).

Patient selection

Six patients, 3 men and 3 women, ranging in age from 26 to 62 years (mean age ± SD, 40.33 ± 12.47 years), with symptomatic and advanced MTC in tumor progression were enrolled in the present study (Table 1).

The following criteria were required for study selection: histologically confirmed, unresectable, measurable, locally advanced, or metastatic MTC; high serum calcitonin (CT) levels; disease progression within 6 months of study entry, based on radiographic images according to the Response Evaluation Criteria in Solid Tumors (RECIST) (23) and on CT doubling time shorter than 2 years; expression of somatostatin receptors in the tumor, demonstrated by a positive Octreoscan result; adequate cardiac, hematopoietic, hepatic, and renal function; and a washout time of at least 6 weeks from any previous treatment with antitumor agents (chemotherapy and biological therapy) and 3 months from radiotherapy.

All patients had previously undergone total thyroidectomy and lymphadenectomy. Persistent and refractory diarrhea was observed in 2 patients, flushing episodes in 2 patients, and weight loss and fatigue in 4 and 3 patients, respectively.

Treatment schedule

Slow-release LAN (Ipsen) was administered in a 90-mg deep sc injection every 28 days. One month after the beginning of LAN treatment, human recombinant IL-2 was administered sc in doses of 1 000 000 IU every 8 hours for 5 consecutive days, followed by 9 days of rest. Thereafter, treatment was resumed. The combined treatment was given for 11 consecutive months (total duration, 1 year). No other anticancer medications were allowed during the course of the study.

Evaluation of treatment tolerability and symptomatic, biochemical, and tumor mass responses

Patients underwent clinical and routine biochemical examination before entry into the study and then monthly. Each evaluation included a complete physical examination, a routine biochemical profile, and the assessment of side effects. All adverse events were recorded and graded according to World Health Organization criteria (24). To assess the symptomatic response, patients were asked to complete at baseline and every 3 months the Functional Assessment of Cancer Therapy-General (FACT-G) questionnaire (version 4), as described previously (25). Serum CT levels were measured at baseline and every 3 months by using a commercial ELISA kit (USCN Life Science Inc). Disease staging was performed before the beginning of the treatment and then every 6 months throughout the treatment by neck and abdominal ultrasound, technetium-99m diphosphonate bone imaging, total body computed tomography and/or magnetic resonance imaging, and response according to RECIST (23). Changes in tumor burden were classified as follows: complete response, defined as the disappearance of all target lesions; partial response (PR), defined as at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters; progressive disease (PD), defined as at least a 20% increase in the sum of the diameter of target lesions and/or the appearance of one or more new lesions; stable disease (SD), in the case of neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

If the patients were considered responsive or with SD, treatment was protracted until PD or unacceptable toxicity. After the end of the treatment, follow-up examinations were conducted every 3 to 6 months. For each patient, the following parameters were calculated: long-term overall survival (period from the start of the treatment to the date of the death or last follow-up), duration of response (period from the time that measurement criteria are met for a complete response or PR until the first date that recurrent disease or PD is objectively documented), or duration of SD (period from the start of the treatment until the criteria for disease progression are met) (23).

The protocol was approved by the institutional ethics committee of the "S. Giovanni di Dio" Hospital of Frattamaggiore (Naples, Italy), and all patients gave informed consent.

Statistical analyses

All experiments were performed at least 3 times and gave comparable results. For statistical analysis, GraphPad Prism 3.0 (GraphPad Software) was used. The comparative statistical eval-

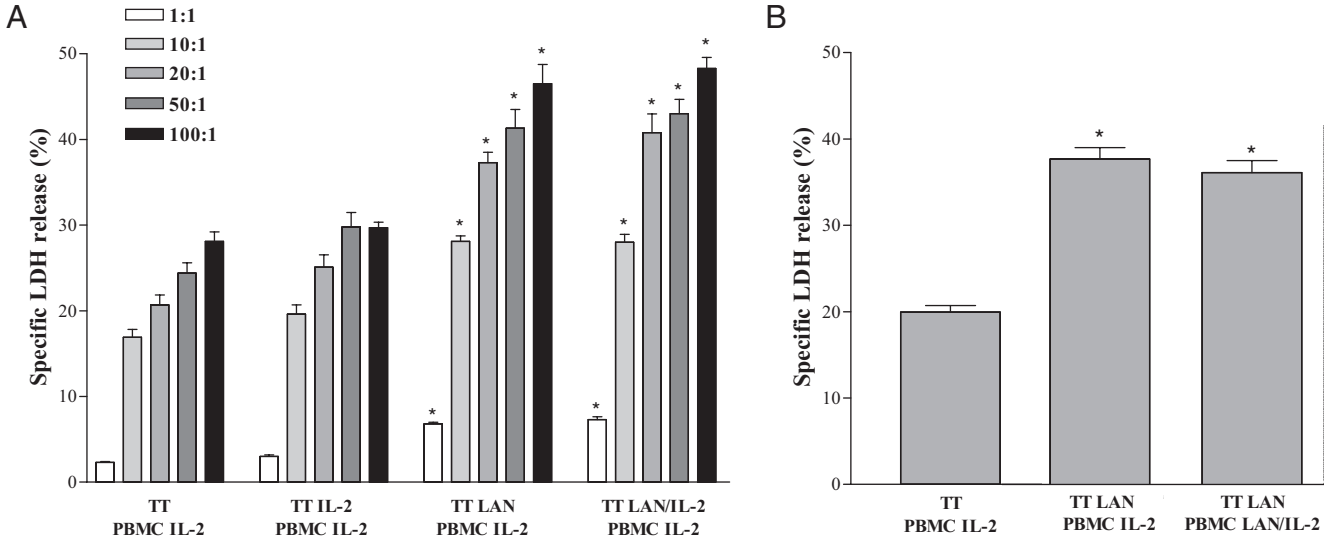

Figure 1. Cytotoxic activity evaluated by LDH release assay. A, Evaluation of the cytotoxic activity of IL-2–stimulated PBMCs (PBMC IL-2), considered as effector cells, against the human medullary thyroid cancer TT cells (target cells) treated with or without LAN, IL-2, and LAN plus IL-2 (LAN/IL-2). The experiments were performed at different effector-to-target ratios (1:1, 10:1, 20:1, 50:1, and 100:1). Each point is the mean of at least 3 different replicate experiments (±SD). *, $P < .001$ vs TT + PBMC IL-2. B, Effect of LAN on the cytotoxic activity of PBMCs. The experiments were performed at an effector-to-target ratio of 20:1. Each point is the mean of at least 3 different replicate experiments (±SD). *, $P < .001$ vs TT + PBMC IL-2.

uation among groups was first performed by ANOVA. When significant differences were found, a comparison between groups was made using the Newman-Keuls test. In all analyses, values of $P < .05$ were considered statistically significant. Data are reported as means ± SD.

Results

Effect of LAN on IL-2–stimulated PBMC-mediated cytotoxicity against TT cells

We first investigated the effect of LAN on the cytotoxic activity of PBMCs, stimulated by human recombinant IL-2, against the human MTC TT cell line, using different effector to target ratios (Figure 1). Fresh PBMCs (effector cells) and TT cells (target cells) were separately incubated with or without LAN (10 nM) and/or IL-2 (100 IU/mL). After 24 hours of incubation with the different drugs, these were washed off and PBMCs were added to TT cells, and after an additional 4 hours of incubation, the cytotoxic activity of PBMCs on TT cells was determined.

The cytotoxicity of IL-2–activated PBMCs, evaluated as LDH release, was significantly higher in TT cells treated with LAN (10 nM) or LAN plus IL-2 for 24 hours than in TT cells without treatment ($P < .001$) (Figure 1A). On the other hand, TT incubation with only IL-2 did not change the cytotoxic activity of IL-2–activated PBMCs (Figure 1A). In addition, incubation of PBMCs with LAN did not reduce the cytotoxic activity induced by IL-2–activated PBMCs (Figure 1B). Similar results were obtained using ^{51}Cr release as an assay to evaluate the sensitivity of tumor cells to immunological effectors (Supplemental Figure 1 published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Under the same experimental conditions, LAN, IL-2, or LAN plus IL-2 did not induce significant growth inhibition of TT cells, as evaluated by the MTT assay ($P > .05$; Supplemental Figure 2), thus excluding direct antiproliferative effects of LAN and/or IL-2 on TT cells.

Patient characteristics and treatment tolerability

From a group of 23 patients with MTC, 6 subjects were enrolled in our study. The characteristics of enrolled patients with MTC are summarized in Table 1. Five of 6 patients completed 1 year of treatment with LAN plus human recombinant IL-2. One patient (patient 3) discontinued the therapy after 6 months because of the progression of the disease. The treatment was generally well tolerated, and only minor adverse events were recorded (Table 2). Most of the side effects were transient and did not necessitate treatment discontinuation. Fever was easily controlled by oral paracetamol, and nausea and vom-

Table 2. Toxicity of the Treatment With LAN and IL-2

	No. (%) of Patients		
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**
Fever	3 (50.0)	2 (33.3)	1 (16.7)
Asthenia	3 (50.0)	2 (33.3)	1 (16.7)
Nausea	3 (50.0)	1 (16.7)	
Vomiting	2 (33.3)	1 (16.7)	
Headache	2 (33.3)	1 (16.7)	
Abdominal pain	1 (16.7)		

iting were responsive to the conventional antiemetic medications. Side effects of more than grade 3 according to the World Health Organization were not observed.

Symptomatic, biochemical, and tumor mass response

Figure 2 reports the effect of treatment with LAN and IL-2 on the QoL evaluated through the FACT-G questionnaire. We observed a statistically significant improvement in the QoL as measured by the FACT-G questionnaire already at 3 months from the beginning of therapy ($P < .01$). This finding suggests a clinically relevant impact of this new schedule in patients with MTC. The maximal percent increase in mean FACT-G values from baseline was +26.5% after 12 months of therapy ($P < .001$). Interestingly, in patients who did not achieve an objective response, a significant and considerable amelioration of their FACT-G score was recorded.

Indeed, at the time of enrollment, all patients showed neuroendocrine symptoms (diarrhea and flushing) and/or general symptoms (fatigue and weight loss) unresponsive to conventional therapy. In 4 of 6 patients a significant amelioration of the symptoms was recorded at 3 months after the beginning of the treatment and was still evident after 1 year from the enrollment.

In patients 1, 2, and 4, a marked reduction in CT levels was recorded after 3 months of treatment and was maintained after 6 months from the beginning of the treatment. The maximal percent decreases in CT levels from the basal values were 76%, 61%, and 54%, in patients 1, 2, and 4, respectively (Figure 3). The differences from the basal levels were statistically significant ($P < .05$).

After 6 months of treatment, PR and SD occurred in 2 and 3 patients, respectively, which were still detectable after 6 additional months and for a long term. In patient 4, the response classified as SD according to RECIST criteria was almost a PR, considering that a 28% reduction in the sum of the diameters of cervical node metastases was observed compared with the baseline sum diameters. PD was observed in 1 patient after 6 months of treatment (Table 1). An example of a significant tumor mass response to LAN plus IL-2 therapy is given in Figure 4, demonstrating the shrinkage of some lung and node lesions in 2 patients.

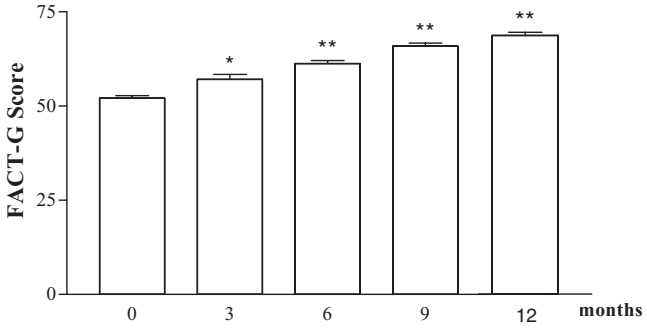

Figure 2. Effects of treatment with LAN and IL-2 on the QoL in patients with MTC. The QoL was evaluated through the FACT-G questionnaire. *, $P < .01$; **, $P < .001$ vs baseline.

Discussion

The immune system plays a major role in limiting the development of cancer cells. However, tumors often avoid surveillance by the immune system through several mechanisms (26). During the past few years, significant progress in understanding the molecular basis of the immune response to cancer aided in the development of effective immunotherapy. Immunological manipulation with IL-2 seems to induce a severe immune response against several tumors, including MTC (14–16).

Lausson et al (12) showed that coinjection of transfected MTC cells, engineered to secrete IL-2, and parental cells in Wag/Rij rats induced the rejection of implanted tumor cells. Both retroviral and adenoviral vectors represent an alternative approach to the use of xenogeneic cells for local interleukin delivery (14). In small MTCs (≤ 30 mm^3), intratumor injection of replication-defective adenoviral vectors containing murine IL-2 cDNA (AdCMVmIL2) induced tumor regression in 69% of animals. The treatment of large tumors (>30 mm^3) with AdCMVmIL2 led to the stabilization of the tumor size in 71% of cases. In addition, a massive infiltration of T cells and natural killer cells was detected in tumors treated with AdCMVmIL2 (14), and a long-lasting state of antitumor immunity was observed in responsive mice (16).

In a clinical trial, 20 patients with solid cancers (4 of them with MTC) were treated with dendritic cell–based vaccination. Autologous dendritic cells were pulsed with autologous tumor lysate and matured with TNF-α. These cells were injected into the patient's growing lymph node. IL-2 was injected sc at a dose of 20,000 IU/kg for 12 days after each vaccination. A biochemical response was observed in all patients with MTC, whereas a decrease in tumor mass was detected in 2 of 4 patients with MTC (13).

Several studies evaluated the possibility of enhancing the antitumor effects of IL-2 in MTC by the combination with other drugs or therapeutic agents. Cressent et al (11) described a potent immunological antitumor response when a mixture of MTC cells and IL-2– and/or IL-4–secreting cells was implanted into syngenic rats, inducing a major rejection of tumor cells with a synergistic effect between both cytokines. Zhang et al (15) demonstrated efficacious antitumor activity by infecting rat MTC cells with an adenoviral vector expressing IL-2 and herpes sim-

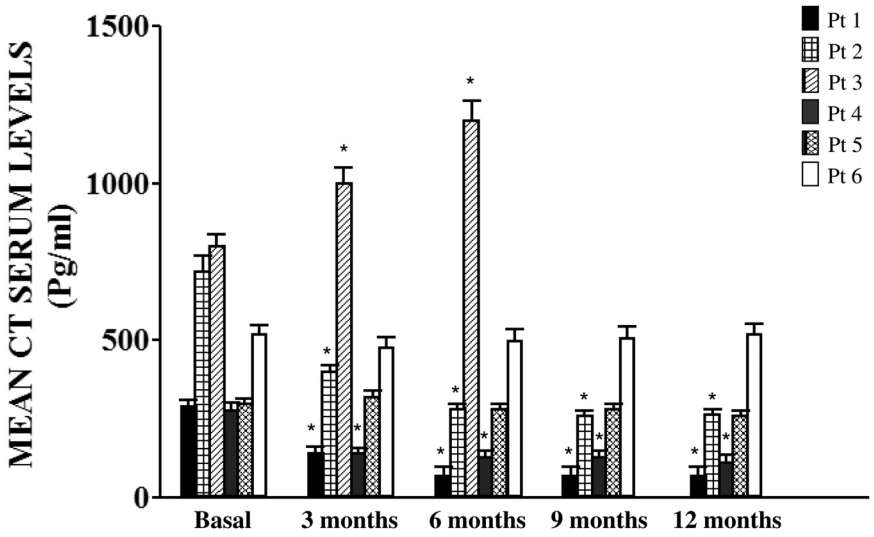

Figure 3. Serum CT levels before and after the beginning of the treatment. The CT levels were determined 3 different times for each serum sample, and the bars show the SDs of the different determinations. Statistical significance was evaluated comparing the different values at the different time points vs the basal levels before the beginning of the therapy. *, $P < .05$.

plex viral thymidine kinase, a suicide gene that transforms nucleoside analogs such as ganciclovir into toxic molecules. Infected cells lost their tumorigenicity when transplanted into syngenic rats.

Somatostatin analogs (octreotide and lanreotide) have been used in the therapy of advanced and symptomatic MTC (5–8). Somatostatin analogs inhibit neuroendocrine tumor cell growth through the inhibition of the release and activity of growth-promoting hormones or factors, inhibition of angiogenesis, and direct antimitotic effects by way of somatostatin receptors (27, 28). Somatostatin and relative receptors are expressed in human lymphoid organs and seem to modulate the immune system (20, 29, 30). However, the role of somatostatin in the control of the immune system in patients with tumors is not completely clear, and there are contradictory results in literature. Some studies showed that natural somatostatin and/or somatostatin analogs enhance the proliferative response of T cells (31), induce marked secretion of IL-2, IL-4, IL-10, and interferon-γ in T helper cells (32), increase IL-2 secretion in human colonic lamina propria mononuclear cells (33), and enhance the adhesion of T cells to fibronectin, collagen type IV, laminin, and β_1 integrins, promoting cytotoxic T lymphocyte activation (34, 35). In addition, Beckner et al (36) showed that somatostatin stimulates the DNA synthesis of human T lymphocytes and differentiation into lymphokine-activated killer cells and enhances the cytolytic lymphokine-activated killer activity induced by IL-2, through inhibition of adenylate cyclase and activation of protein kinase C. On the other hand, several authors reported that somatostatin inhibits the proliferation of human T lymphocytes stimulated by phytohemagglutinin, concanavalin A, or alloantigens (37, 38) and the natural killer activity induced by IL-2 (39).

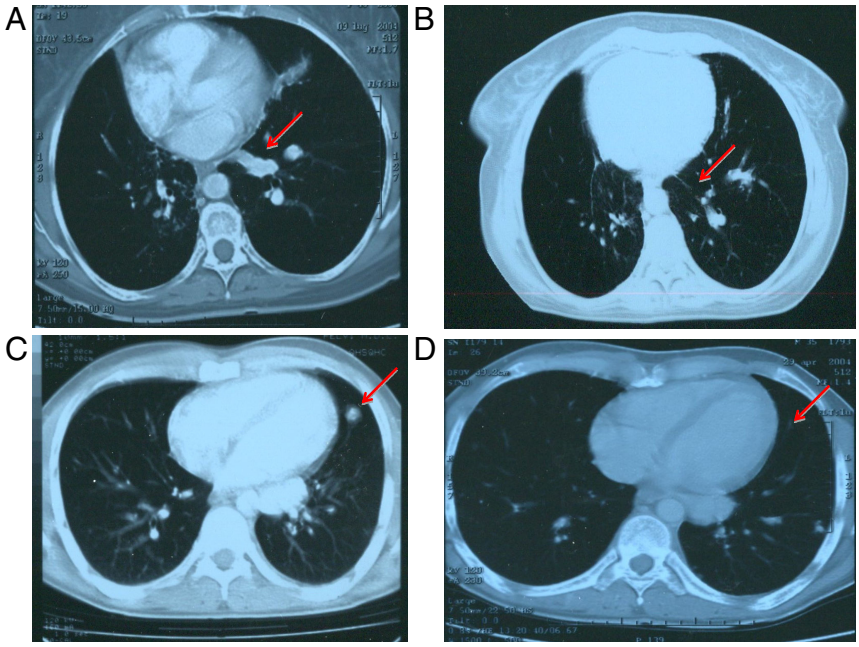

Figure 4. Chest computed tomography scans in patients 1 (A and B) and 2 (C and D), before (A and C) and 6 months from the beginning of the therapy (B and D) with LAN and sc low-dose human recombinant IL-2. A dramatic shrinkage of metastatic lesions (red arrows) was observed in both patients after biological therapy.

In the present article, we have investigated the potential role of the combination of LAN plus human recombinant IL-2 in the treatment of MTC through both in vitro and in vivo studies. We observed that the cytotoxicity of IL-2–activated PBMCs, measured as both LDH and ^{51}Cr release, was significantly higher in TT cells incubated with LAN than in TT cells without any treatment, whereas neither treatment of PBMCs with LAN nor treatment of TT cells with IL-2 had any effect on the cytolytic activity of IL-2–stimulated PBMCs. These data suggested that LAN may increase the sensitivity of MTC TT cells to IL-2–activated PBMCs. In fact, under the same experimental

conditions, we observed no clear growth inhibitory effects of the different agents, either alone or in combination, when added to MTC.

In addition, we treated 6 patients having progressing symptomatic and advanced MTC with the combination of LAN and sc low-dose human recombinant IL-2. The therapy was well tolerated, and only minor adverse events were recorded.

A significant improvement in QoL, as measured by the FACT-G questionnaire, was observed after 3 months of treatment and was still evident after long-term treatment. Interestingly, in patients who did not achieve an objective response, a significant amelioration of the relative FACT-G score was recorded. This seemed to be related to the improvement in tumor-associated symptoms.

Surprisingly, this treatment showed inhibitory effects even on tumor mass. After 6 months of therapy, PR and SD have been recorded in 2 and 3 patients, respectively, whereas a decrease in CT levels was observed in 3 patents. All these effects were still detectable after 6 additional months. These interesting results are also supported by the fact that, at the time of the enrollment, all patients showed both radiological and biochemical tumor progression and exhibited neuroendocrine symptoms (diarrhea and flushing) and/or general symptoms (fatigue and weight loss) unresponsive to conventional therapy. In 4 of 6 patients, a significant amelioration of the symptoms was recorded during combined treatment.

The therapeutic responses recorded in our study are probably due to the sensitization of MTC cells to IL-2–activated effectors induced by LAN. However, the possibility of in vivo effect on the cellular components of the tumor microenvironment and/or inhibition of secretion of bioactive molecules promoting cell growth cannot be excluded. Moreover, the potentiation of the cytolytic activity of IL-2–activated PBMCs on TT cells induced by LAN could be, at least in part, due to the decreased expression/secretion of tumor-derived bioactive molecules, as recently suggested by Ameri and Ferone (20). The presence of high number of Tregs in MTC strongly support the hypothesis that MTC cells can orchestrate an escape strategy from the immune system and that antisecretogog agents, such as somatostatin analogs, could be useful in interrupting this circuit (18).

It is also worth mentioning that IL-2 has been used in the adjuvant setting of several tumors (40). In view of the above, we cannot exclude a potential role of IL-2 plus somatostatin analogs not only in advanced MTC but also as adjuvant therapy for this tumor.

In conclusion, in vitro and in vivo evidence suggests that the combination of LAN and IL-2 may have a role in the management of advanced and symptomatic MTC. The combination of low-dose IL-2 and LAN is able to induce objective responses in patients with advanced and symptomatic MTC refractory to previous treatments. The therapy does not induce significant toxicity and improves the QoL of the patients. However, these preliminary data require further validation in larger randomized trials and a better understanding of the molecular mechanisms involved in the modulation of IL-2–activated PBMC cytotoxicity through the somatostatin system. The recent development of new subtypes of selective agonists and pan-somatostatin receptor agonists (SOM230) might help in gaining a better understanding of the molecular interactions between the immune and somatostatin systems with potential therapeutic applications.

Acknowledgments

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References

1. **Kloos RT, Eng C, Evans DB, et al.** Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid*. 2009;19:565–612.
2. **Colombo C, Verga U, Mian C, et al.** Comparison of calcium and pentagastrin tests for the diagnosis and follow-up of medullary thyroid cancer. *J Clin Endocrinol Metab*. 2012;97:905–913.
3. **Vitale G, Ciccarelli A, Caraglia M, et al.** Comparison of two provocative tests for calcitonin in medullary thyroid carcinoma: omeprazole vs pentagastrin. *Clin Chem*. 2002;48:1505–1510.
4. **Vitale G, Caraglia M, Ciccarelli A, Lupoli G, Abbruzzese A, Tagliaferri P.** Current approaches and perspectives in the therapy of medullary thyroid carcinoma. *Cancer*. 2001;91:1797–1808.
5. **Frank-Raue K, Ziegler R, Raue F.** The use of octreotide in the treatment of medullary thyroid carcinoma. *Horm Metab Res Suppl*. 1993;27:44–47.
6. **Lupoli G, Cascone E, Arlotta F, et al.** Treatment of advanced medullary thyroid carcinoma with a combination of recombinant interferon α-2b and octreotide. *Cancer*. 1996;78:1114–1118.
7. **Modigliani E, Cohen R, Joannidis S, et al.** Results of long-term continuous subcutaneous octreotide administration in 14 patients with medullary thyroid carcinoma. *Clin Endocrinol (Oxf)*. 1992;36:183–186.

8. **Vitale G, Tagliaferri P, Caraglia M, et al.** Slow release lanreotide in combination with interferon-α2b in the treatment of symptomatic advanced medullary thyroid carcinoma. *J Clin Endocrinol Metab*. 2000;85:983–988.
9. **Faggiano A, Ramundo V, Dicitore A, et al.** Everolimus is an active agent in medullary thyroid cancer: a clinical and in vitro study. *J Cell Mol Med*. 2012;16:1563–1572.
10. **Kapiteijn E, Schneider TC, Morreau H, Gelderblom H, Nortier JW, Smit JW.** New treatment modalities in advanced thyroid cancer. *Ann Oncol*. 2012;23:10–18.
11. **Cressent M, Pidoux E, Cohen R, Modigliani E, Roth C.** Interleukin-2 and interleukin-4 display potent antitumour activity on rat medullary thyroid carcinoma cells. *Eur J Cancer*. 1995;31A:2379–2384.
12. **Lausson S, Fournes B, Borrel C, Milhaud G, Treilhou-Lahille F.** Immune response against medullary thyroid carcinoma (MTC) induced by parental and/or interleukin-2-secreting MTC cells in a rat model of human familial medullary thyroid carcinoma. *Cancer Immunol Immunother*. 1996;43:116–123.
13. **Stift A, Friedl J, Dubsky P, et al.** Dendritic cell-based vaccination in solid cancer. *J Clin Oncol*. 2003;21:135–142.
14. **Zhang R, Baunoch D, DeGroot LJ.** Genetic immunotherapy for medullary thyroid carcinoma: destruction of tumors in mice by in vivo delivery of adenoviral vector transducing the murine interleukin-2 gene. *Thyroid*. 1998;8:1137–1146.
15. **Zhang R, DeGroot LJ.** An adenoviral vector expressing functional heterogeneous proteins herpes simplex viral thymidine kinase and human interleukin-2 has enhanced in vivo antitumor activity against medullary thyroid carcinoma. *Endocr Relat Cancer*. 2001;8:315–325.
16. **Zhang R, Minemura K, De Groot LJ.** Immunotherapy for medullary thyroid carcinoma by a replication-defective adenovirus transducing murine interleukin-2. *Endocrinology*. 1998;139:601–608.
17. **Katsenelson NS, Shurin GV, Bykovskaia SN, Shogan J, Shurin MR.** Human small cell lung carcinoma and carcinoid tumor regulate dendritic cell maturation and function. *Mod Pathol*. 2001;14:40–45.
18. **Müller S, Poehnert D, Müller JA, et al.** Regulatory T cells in peripheral blood, lymph node, and thyroid tissue in patients with medullary thyroid carcinoma. *World J Surg*. 2010;34:1481–1487.
19. **Imai H, Saio M, Nonaka K, et al.** Depletion of $CD4^+CD25^+$ regulatory T cells enhances interleukin-2-induced antitumor immunity in a mouse model of colon adenocarcinoma. *Cancer Sci*. 2007;98:416–423.
20. **Ameri P, Ferone D.** Diffuse endocrine system, neuroendocrine tumors and immunity: what's new? *Neuroendocrinology*. 2012;95:267–276.
21. **Correale P, Botta C, Cusi MG, et al.** Cetuximab ± chemotherapy enhances dendritic cell-mediated phagocytosis of colon cancer cells and ignites a highly efficient colon cancer antigen-specific cytotoxic T-cell response in vitro. *Int J Cancer*. 2012;130:1577–1589.
22. **Vitale G, Zappavigna S, Marra M, et al.** The PPAR-γ agonist troglitazone antagonizes survival pathways induced by STAT-3 in recombinant interferon-β treated pancreatic cancer cells. *Biotechnol Adv*. 2012;30:169–184.
23. **Eisenhauer EA, Therasse P, Bogaerts J, et al.** New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228–247.
24. **Miller AB, Hoogstraten B, Staquet M, Winkler A.** Reporting results of cancer treatment. *Cancer*. 1981;47:207–214.
25. **Vitale G, Fonderico F, Martignetti A, et al.** Pamidronate improves the quality of life and induces clinical remission of bone metastases in patients with thyroid cancer. *Br J Cancer*. 2001;84:1586–1590.
26. **Zitvogel L, Tesniere A, Kroemer G.** Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat Rev Immunol*. 2006;6:715–727.
27. **Gatto F, Hofland LJ.** The role of somatostatin and dopamine D2 receptors in endocrine tumors. *Endocr Relat Cancer*. 2011;18:R233–R251.
28. **Zatelli MC, Piccin D, Tagliati F, et al.** Selective activation of somatostatin receptor subtypes differentially modulates secretion and viability in human medullary thyroid carcinoma primary cultures: potential clinical perspectives. *J Clin Endocrinol Metab*. 2006;91:2218–2224.
29. **Ferone D, van Hagen PM, Semino C, et al.** Somatostatin receptor distribution and function in immune system. *Dig Liver Dis*. 2004;36(suppl 1):S68–S77.
30. **Ferone D, van Hagen PM, Pivonello R, Colao A, Lamberts SW, Hofland LJ.** Physiological and pathophysiological role of somatostatin receptors in the human thymus. *Eur J Endocrinol*. 2000;143(suppl 1):S27–S34.
31. **Nordlind K, Mutt V.** Influence of β-endorphin, somatostatin, substance P and vasoactive intestinal peptide on the proliferative response of human peripheral blood T lymphocytes to mercuric chloride. *Int Arch Allergy Appl Immunol*. 1986;80:326–328.
32. **Levite M.** Neuropeptides, by direct interaction with T cells, induce cytokine secretion and break the commitment to a distinct T helper phenotype. *Proc Natl Acad Sci USA*. 1998;95:12544–12549.
33. **Nio DA, Moylan RN, Roche JK.** Modulation of T lymphocyte function by neuropeptides. Evidence for their role as local immunoregulatory elements. *J Immunol*. 1993;150:5281–5288.
34. **Talme T, Ivanoff J, Hägglund M, Van Neerven RJ, Ivanoff A, Sundqvist KG.** Somatostatin receptor (SSTR) expression and function in normal and leukaemic T-cells. Evidence for selective effects on adhesion to extracellular matrix components via SSTR2 and/or 3. *Clin Exp Immunol*. 2001;125:71–79.
35. **Levite M, Cahalon L, Hershkoviz R, Steinman L, Lider O.** Neuropeptides, via specific receptors, regulate T cell adhesion to fibronectin. *J Immunol*. 1998;160:993–1000.
36. **Beckner SK, Farrar WL.** Potentiation of lymphokine-activated killer cell differentiation and lymphocyte proliferation by stimulation of protein kinase C or inhibition of adenylate cyclase. *J Immunol*. 1988;140:208–214.
37. **Casnici C, Lattuada D, Perego C, Franco P, Marelli O.** Inhibitory effect of somatostatin on human T lymphocytes proliferation. *Int J Immunopharmacol*. 1997;19:721–727.
38. **Lattuada D, Casnici C, Crotta K, et al.** Inhibitory effect of pasireotide and octreotide on lymphocyte activation. *J Neuroimmunol*. 2007;182:153–159.
39. **Sirianni MC, Annibale B, Fais S, Delle Fave G.** Inhibitory effect of somatostatin-14 and some analogues on human natural killer cell activity. *Peptides*. 1994;15:1033–1036.
40. **Eklund JW, Kuzel TM.** A review of recent findings involving interleukin-2-based cancer therapy. *Curr Opin Oncol*. 2004;16:542–546.