

Effects of the Conventional Antitumor Therapies Surgery, Chemotherapy, Radiotherapy and Immunotherapy on Regulatory T Lymphocytes in Cancer Patients

P. LISSONI¹, F. BRIVIO², L. FUMAGALLI², G. MESSINA¹, S. MEREGALLI¹,
G. PORRO¹, F. ROVELLI¹, L. VIGORÈ³, E. TISI² and G. D'AMICO⁴

*Divisions of ¹Radiation Oncology, ²Surgery, ³Immunomicrobiological Laboratory and
⁴Research Center "Fondazione Tettamanti" Clinica Pediatrica Università
Milano-Bicocca, St. Gerardo Hospital, Monza, Milan, Italy*

Abstract. *Background:* Several clinical studies have clearly demonstrated that the immune status is one a major prognostic factor for the survival time in cancer patients. However the main clinical problem is to identify the most prognostically important index within the great number of immune parameters. Recently the evaluation of regulatory T (T-reg) (CD4CD25) lymphocyte count and function with respect to the T helper (TH) (CD4) number has been shown to represent the main immune parameters capable of representing the functional status of the anticancer immunity in cancer patients. This study evaluated the influence of the four main conventional anticancer therapies (surgery, chemotherapy, radiotherapy, immunotherapy) on the CD4/CD4CD25 ratio. *Patients and Methods:* The study included 70 patients. The oncological treatments consisted of surgery in 14, chemotherapy in 36, radiotherapy in 12 and immunotherapy (subcutaneous low-dose, S.C.-low, interleukin, IL-2) in 8 patients. The normal value of the CD4/CD4CD25 ratio was greater than 4.0. *Results:* Surgery induced a significant decline in the CD4/CD4CD25 mean ratio. Radiotherapy also induced also a dramatic significant decrease in the CD4/CD4CD25 ratio, whereas the effect of both chemotherapy and immunotherapy reflected the clinical response to the treatments. The CD4/CD4CD25 mean ratio was significantly enhanced in the patients who obtained control of the neoplastic growth, whereas it diminished in progressing patients. *Conclusion:* The commonly used anticancer therapies profoundly modify the levels of amounts

of T-reg lymphocytes. Because of the fundamental role of T-reg cells in suppressing the anticancer immunity, thus diminishing survival, the monitoring of the CD4/CD4CD25 ratio could constitute an important clinical index during conventional anticancer therapies to predict the prognosis of cancer patients.

Today, it is known that the failure of an effective anticancer immune response in most metastatic cancer patients depends on the generation of several immunosuppressive events, including lymphocytopenia, namely a decline in T helper (TH) lymphocyte number and functions, decreased dendritic cell function, abnormally high blood concentrations of interleukin (IL)-6, IL-10 and transforming growth factor (TGF)-beta and progressively diminished levels of IL-2 and IL-12 (1-7). Moreover, the laboratory evidence of alterations involving immune cell function and the endogenous secretion of cytokines has appeared to show an association with a poor prognosis (8-10). Unfortunately, from a clinical point of view, the major problem is to quantify the impact of a single immune alteration on the prognosis of a neoplastic disease. Therefore, it could be very important to have an immune index, which might represent the end-result of the great number of immune anomalies occurring during the clinical course of the neoplastic disease. Recent immuno-oncological studies have demonstrated the existence within the TH lymphocyte group (CD4) of a subtype of cell expressing the alpha-chain of the IL-2 receptor (CD25), which may suppress the anticancer immune response by blocking both IL-2- and IL-12-dependent cytotoxicity, the so-called regulatory T lymphocyte (T-reg) (11-14). Therefore, the measurement of the T-reg count could constitute an immune index capable of assessing the general status of the anticancer immune response and the antitumor cytokine network in an individual cancer patient, since T-reg cells have been proven to be stimulated by TGF-beta, IL-10 and IL-6 and to determine a diminished endogenous production of both IL-

Correspondence to: Dr. Paolo Lissoni, Divisione di Radioterapia Oncologica, Ospedale S. Gerardo, 20052 Monza, Milan, Italy. Fax: +39 0392332284, e-mail: p.lissoni@hsgerardo.org

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2 and IL-12 (1-14). In the same way, T-reg cells could represent a new target for future immunoendocrinobiological therapies of human neoplasms. From an experimental point of view, markers other than CD4 and CD25 cell surface expression, such as the intracytoplasmatic content of forkhead box protein 3 (FOX p3) and cell surface expression of CD152 (15, 16) are required to better identify the T-reg cells, whereas from a clinical point of view, the evidence of CD4 and CD25 cell surface expression is generally considered sufficient to characterize a lymphocyte as a T-reg cell (17). In fact, preliminary clinical studies have demonstrated the progressive increase in the T-reg count, as detected as CD4⁺CD25⁺ lymphocytes, the metastatic disease (18-20). Therefore, it has become important to establish the effects of the common standard anticancer therapies, including surgery, chemotherapy, immunotherapy and radiotherapy on the T-reg count in cancer patients, which was the aim of the present study.

Patients and Methods

The study included 70 consecutive cancer patients affected by locally limited or metastatic disease, whose clinical characteristic are shown in Table I. The antitumor therapies consisted of surgery in 14, chemotherapy in 36, radiotherapy in 12 and subcutaneous (S.C.) low-dose IL-2 in the remaining 8 patients, who were suffering from advanced renal cell cancer. The surgical treatments consisted of right hemicolectomy in 6, left hemicolectomy in 5 and partial gastrectomy in 3 patients.

Chemotherapy consisted of oxaliplatin and 5-fluorouracil (5-FU) (FOLFOX regimen) in 7 patients, irinotecan and 5-FU (FOLFIRI regimen) in 6 metastatic colorectal cancer patients, cisplatin and gemcitabine in 9 patients, weekly vinorelbine in 6 metastatic non-small cell lung cancer patients and weekly taxotere in 8 metastatic breast cancer women. The clinical response to chemotherapy was evaluated according to the WHO criteria. Radiotherapy consisted of pelvic irradiation for uterine cervix carcinoma in 8 and rectal cancer in 4 patients, at a dose of 50.4 Gy by 1.8 Gy daily fractions for 5 days/week. Finally, for immunotherapy, IL-2 was injected S.C. at a daily dose of 3 mIU for 5 days/week for 4 consecutive weeks.

The patients who underwent the surgical removal of a tumor were investigated before surgery and at day 5 of the postoperative period. The patients treated by chemotherapy were evaluated before and after 3 months of treatment. Finally, the patients treated by radiotherapy or immunotherapy were analyzed before and at the end of treatment. For the immune evaluation, the venous blood samples were collected in the morning after an overnight fast. In each blood sample the absolute number of total lymphocytes, T helper lymphocytes (CD4), T-reg lymphocytes (CD4CD25) and the TH/T-reg ratio (CD4/CD4CD25) were evaluated. The CD4 and CD4CD25 cells were measured by flow cytometric analysis and monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy). The normal values obtained in our laboratory (95% confidence limits) were lower than 240/mm³ for the T-reg cells and greater than 4 for the CD4/CD4CD25 ratio. The data were reported as mean±SE and were statistically analyzed by the Student's *t*-test, the analysis of variance and the Chi-square test, as appropriate.

Table I. Clinical characteristics.

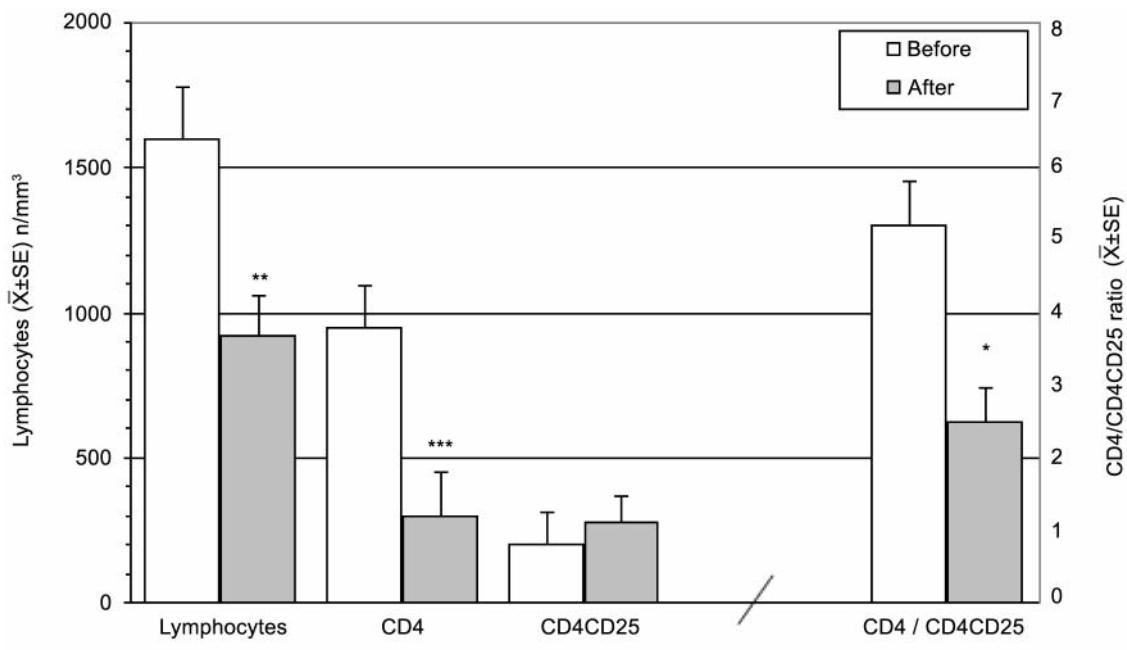
Characteristics	Number
N	70
Male / Female	39/31
Median Age (years)	61 (37-75)
Tumor Histotypes:	
Non-small cell lung cancer	15
Colorectal cancer	28
Gastric cancer	3
Breast cancer	8
Uterine cervix carcinoma	8
Renal cell cancer	8
Disease extension:	
Locally limited disease	26
Metastatic disease	44

Results

The immune variations induced by surgery are illustrated in Figure 1. The mean numbers of both total lymphocytes and TH lymphocytes significantly decreased during the postoperative period ($p<0.025$ and $p<0.01$, respectively), whereas the T-reg mean count increased with respect to the presurgical values without, however without statistically significant difference. In contrast, the TH/T-reg mean ratio observed in the postoperative period was significantly lower than that found before surgery ($p<0.05$).

Figures 2 and 3 illustrate the immune effects induced by chemotherapy in the overall patients and in relation to their clinical response, respectively. The clinical responses consisted of complete response (CR) in 3 (8%), partial response (PR) in 8 (22%) and stable disease (SD) in 15 (42%), whereas the remaining 10 patients (28%) had progressive disease (PD). As shown in Figure 2, no significant variation was seen after chemotherapy in the mean number of total lymphocytes, TH lymphocyte and T-reg cells, whereas an increase in the TH/T-reg value occurred after chemotherapy, even though the difference was not statistically significant. In contrast, by considering the immune changes in relation to the clinical response (Figure 3), in patients with disease control (DC), including CR, PR and (SD), irrespectively of the type of chemotherapeutic regimen, a significant decline in the mean number of T-reg occurred after chemotherapy ($p<0.001$), whereas it increased in those with PD with respect to the values found prior to chemotherapy, however without statistically significant difference. In the same way, the mean value of the TH/T-reg ratio significantly increased after chemotherapy in the patients who achieved DC ($p<0.001$), whereas it decreased (non-significantly) in the patients with PD.

Figure 4 illustrates the immune changes induced by pelvic irradiation. A dramatic statistically significant



* $p<0.05$, ** $p<0.025$, *** $p<0.01$ vs. before surgery

Figure 1. Effects of oncological surgery on the mean value of total lymphocytes, T helper lymphocytes (CD4), regulatory T lymphocytes (CD4CD25) and the CD4/CD4CD25 ratio.

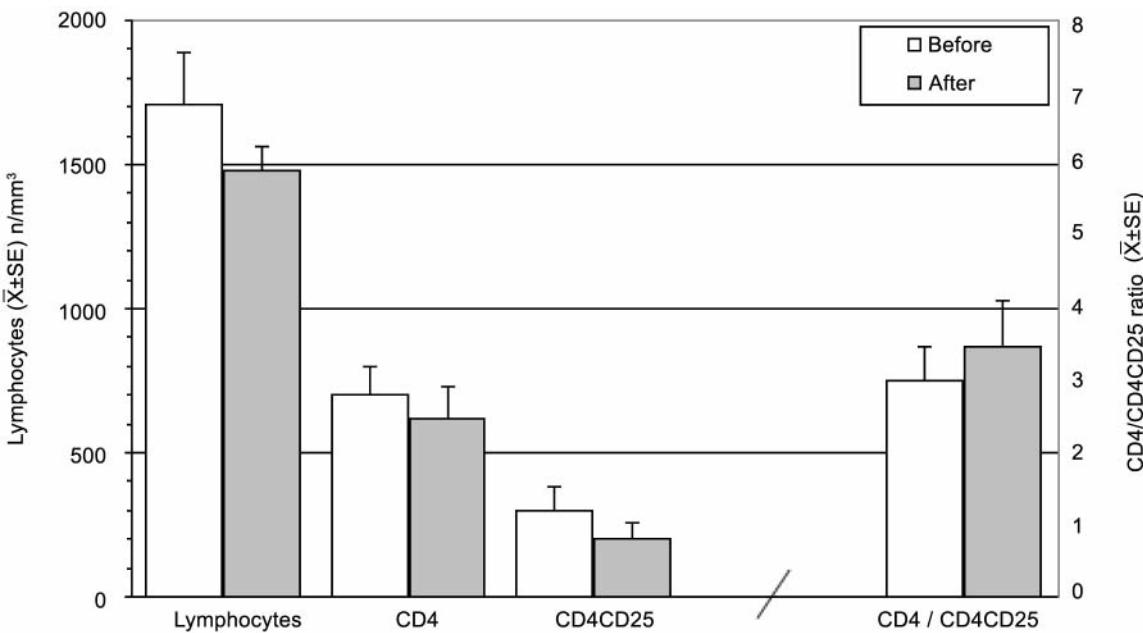
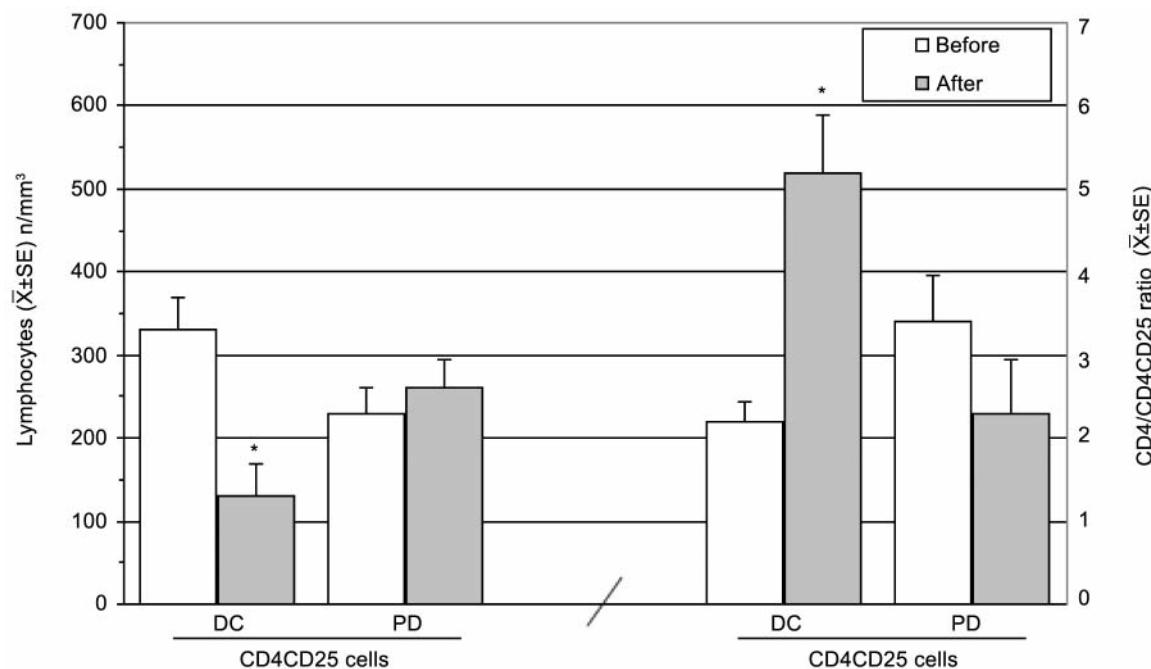


Figure 2. Effects of chemotherapy on the mean value of total lymphocytes, T helper lymphocytes (CD4), regulatory T lymphocytes (CD4CD25) and the CD4/CD4CD25 ratio.

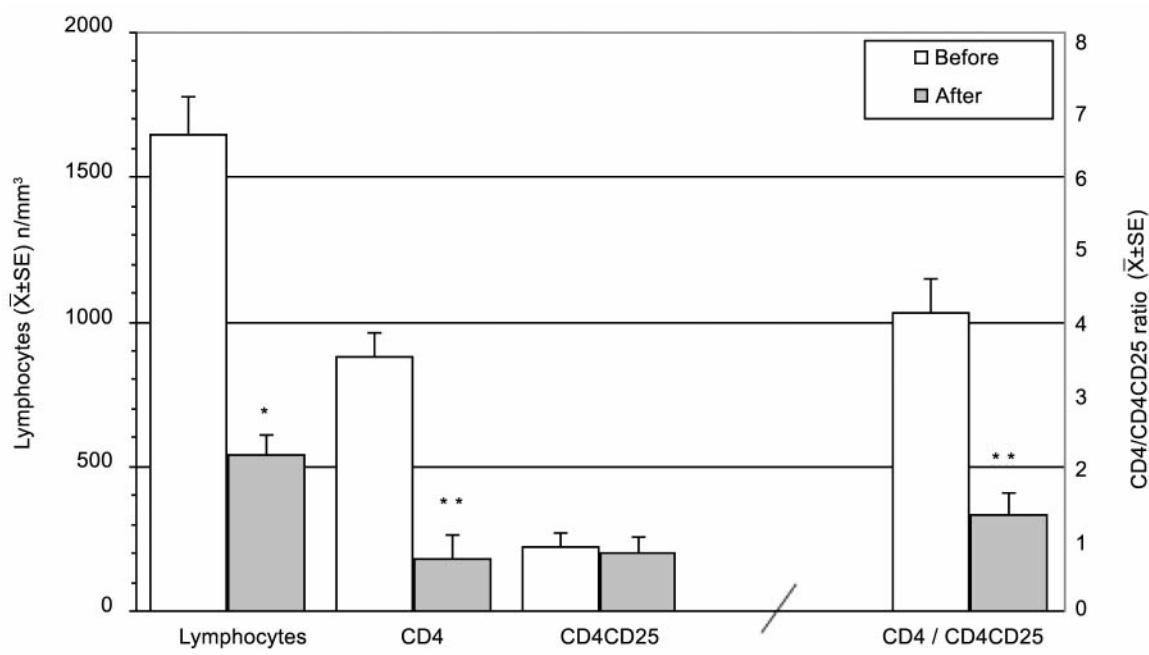
decrease occurred after radiotherapy in the mean number of both total lymphocytes ($p<0.005$) and CD4⁺ cells ($p<0.001$), whereas no substantial change was observed in

the mean number of T-reg lymphocytes. The mean value of the CD4/CD4CD25 ratio significantly decreased after radiotherapy ($p<0.001$).



* $p < 0.001$ vs. before chemotherapy

Figure 3. Changes in CD4CD25 cell mean number and CD4/CD4CD25 mean ratio after chemotherapy in relation to the clinical response (DC: disease control; PD: progressive disease).



* $p < 0.005$, ** $p < 0.001$ vs. before radiotherapy

Figure 4. Effects of radiotherapy on the mean value of total lymphocytes, T helper lymphocytes (CD4), regulatory T lymphocytes (CD4CD25) and the CD4/CD4CD25 ratio.

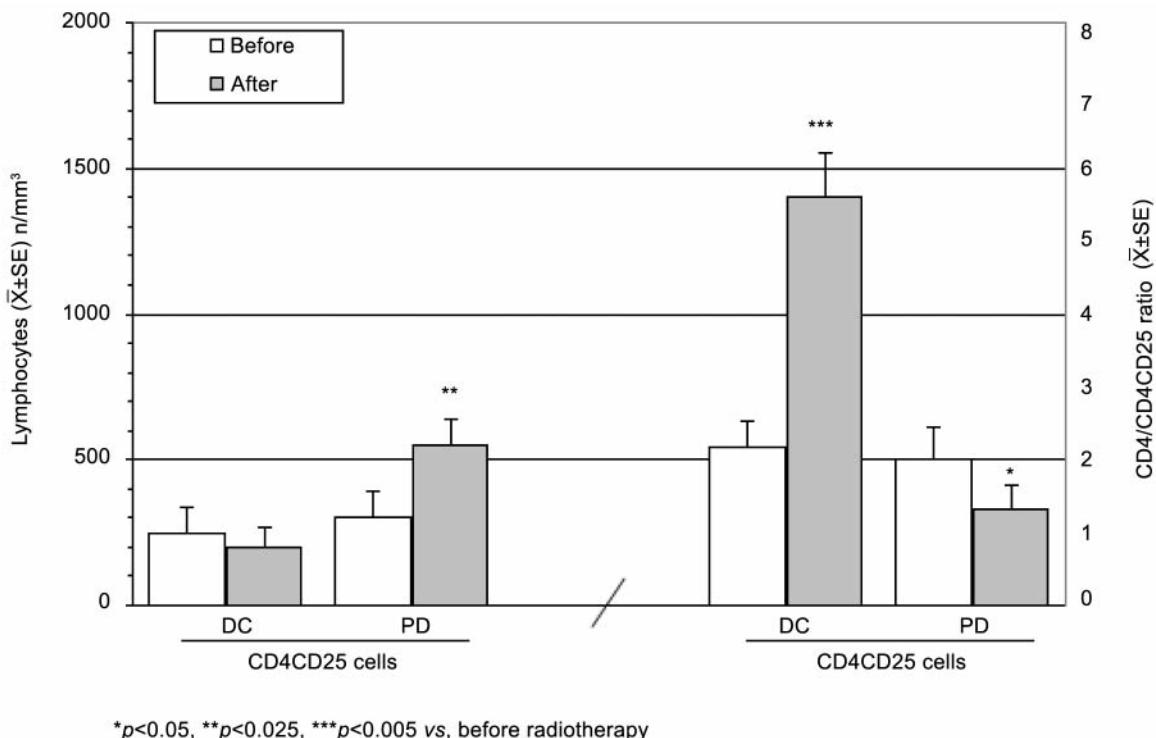


Figure 5. Changes in CD4CD25 cell mean number and CD4/CD4CD25 mean ratio during IL-2 immunotherapy in relation to the clinical response (DC: disease control; PD: progressive disease).

Finally, Figure 5 illustrates the immune changes observed during IL-2 immunotherapy in relation to the clinical response. A PR was achieved in 2/8 (25%) of the patients, 3 other patients had SD, whereas the remaining 3 patients had PD, thus DC (PR+SD) was obtained in 5/8 (63%). In the patients with DC, IL-2 induced a non significant decline in the mean number of T-reg cells, whereas a statistically significant increase occurred in the mean value of the CD4/CD4CD25 ratio ($p<0.005$). In contrast, in the progressing patients, immunotherapy induced a significant increase in the mean number of T-reg cells ($p<0.025$) and a significant decrease in the CD4/CD4CD25 ratio mean values ($p<0.05$).

Discussion

This study confirmed that previous preliminary clinical data (18-20) showing that metastatic cancer is often characterized by an abnormal increase in T-reg cell count and by a consequent decline in the TH/T-reg ratio, generally due to both T-reg increase and TH lymphocyte decline. Moreover, even though limited to a relatively low number of patients, this study clearly demonstrated that the various classical anticancer treatments may induce evident variations of T-reg generation, in terms of both absolute number and percentage with respect to the TH lymphocyte count. As far as the

surgical treatment was concerned, T-reg lymphocytes appeared to be the only immune cells whose number increased after surgery, whereas the mean number of both total lymphocytes and TH cells showed an evident decline in the postoperative period. The behaviour of the T-reg cells was also different from that of the other immune cells during radiotherapy, since their number was not substantially decreased by radiotherapy, suggesting that T-reg cells may represent the most radioresistant lymphocytes. The effect of chemotherapy on T-reg generation requires particular attention since it appeared to be different in relation to the clinical response. In fact, the T-reg cell number decreased in the patients with objective tumor regression or SD, whereas it was enhanced in the patients who had PD. Moreover, a chemotherapy-induced increase in the TH/T-reg ratio has been proven to predict the efficacy of chemotherapy, in terms of control of tumor growth. In the same way, IL-2-induced T-reg variations also depended on the efficacy of treatment, since the T-reg count decreased in the patients with DC, whereas it was enhanced in those with PD, thus suggesting that IL-2 may either stimulate or inhibit T-reg generation. In fact, experimental studies have demonstrated that the effect of IL-2 on T-reg production is dependent on the presence of TGF-beta, with stimulatory activity only in the presence of high levels of TGF-beta (19-20). Therefore, the definition of

IL-2 as the growth factor of T-reg lymphocytes proposed by some authors (12) does not seem to be appropriate. Obviously, the relatively low number of patients evaluated in this study in relation to the various anticancer therapies did not allow definite conclusions. However, according to these preliminary results, the T-reg cell count and its percentage with respect to TH cell number could represent very important immune parameters for monitoring the clinical course of a neoplastic disease. Therefore, these results justify further longitudinal studies to better establish the influence of standard anticancer therapies on T-reg production and its possible prognostic significance. In particular, further studies, by concomitantly evaluating FOXP3 and CD152- antigens, will be required to better identify T-reg cell population.

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