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Multimodality treatment of malignant pleural mesothelioma with or without immunotherapy: does it change anything?☆

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Abstract

The purpose of this study was to investigate immunological effector cells and angiogenesis in malignant pleural mesothelioma (MPM) patients, who underwent multimodality treatments. Clinical and pathological characteristics of 57 patients, with International Mesothelioma Interest Group stage II–III MPM, who underwent two different multimodality treatments (with and without immunotherapy) between 1999 and 2008 were analyzed. CD8+, CD4+ and Foxp3+ tumor-infiltrating lymphocytes, tryptase and chymase mast cells (MCs), CD34, number of microvessels and vascular endothelial growth factor were determined by immunohistochemistry. The histology was 51 epitheliomorf and 6 biphasic. The stage was III in 41 cases and II in 16 cases. With an average follow-up of 69 months (range 9–115) 14 patients are still alive and the overall median actuarial survival is 21.4 months. Tryptase MCs, CD8+ and Foxp3+ lymphocytes had significantly increased in the interleukin 2 (IL-2) treated group. Moreover, the number of microvessels was significantly lower in IL-2 treated patients. This study indicates that immunotherapy leads to an increase in cytotoxic CD8+ lymphocytes and tryptase MCs and to a decrease of the tumoral neoangiogenesis. Changes in MPM microenvironment induced by immunotherapy may play a major role in the local control of this disease and need further investigations.

Keywords: Mesothelioma; Multimodality treatments; Pleurectomy; Chemotherapy; Immunotherapy

1. Introduction

Unfortunately mesothelioma incidence is expected to continue in the near future and still there is neither any cure nor any evidence of the optimal treatment [1]. Multimodality treatments are currently adopted in most of the centers with different extent of surgical resections [pleurectomy/decortication (PD) or extended pleuro-pneumonectomy (EPP)] [2–4], different scheduling of chemotherapy administered in a pre- or postoperative setting [5] and radiotherapy at various dosages and with different intents.

Furthermore, malignant pleural mesothelioma (MPM) is an immunogenic tumor, which response and regression has been associated to the administration of immuno-modulators [6, 7]. Immunotherapy, especially interleukin 2 (IL-2) and interferon α (IFN-α) or β (IFN-β) can induce MPM regression in animal models and in human beings [6, 7]; it can be easily combined with other therapeutic modalities. We already reported in this journal our experience on a four-modality treatment of MPM including pre- and post-operative therapy with IL-2 [8].

The purpose of this research was to investigate changes in MPM microenvironment induced by immunotherapy by means of quantitative comparison of immunological effector cells and angiogenesis between MPM patients, who underwent multimodality treatments with and without preoperative IL-2.

2. Material and methods

2.1. Patients

Clinical and pathological characteristics of 57 patients, with International Mesothelioma Interest Group (IMIG) stage II-III MPM, who underwent two different multimodality treatments (with and without immunotherapy) between 1999 and 2008 were analyzed. The first group of patients ($n=34$) underwent, between 1999 and 2004, a multimodality treatment including preoperative intrapleural IL-2, as previously described in this journal [8]. The second group of patients ($n=23$) treated between 2005 and 2008 underwent primary PD, hyperthermic intrapleural chemotherapy with epidoxorubicin (25 mg/m^2) and ciplatin (80 mg/m^2), adjuvant radiotherapy, systemic chemothera-

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py (cisplatin 80 mg/m^2 and pemetrexed 500 mg/m^2 up to six courses).

All patients were followed-up until death or up to the final date of analysis for this report (March 2009).

As regards the follow-up tests, a physical examination and CT-scan of the chest and upper abdomen were performed one month after radiotherapy and then three months thereafter. Disease progression, according to the Response Evaluation Criteria in Solid Tumours Group (RECIST), was defined as at least 20% increase in the sum of the longest diameters of target lesions. Cytological or histological proof of disease progression was rarely obtained.

Survival was calculated from the date of the diagnosis until the date of death or of the last follow-up.

2.2. Tumor specimens

Tumor samples were formalin-fixed and paraffin-embedded for microscopic examination. The most representative paraffin block of tumor was selected for immunohistochemical analysis. A histological and pathological diagnosis was reviewed by two pathologists (G.A. and G.F.), according to the World Health Organization (WHO) 2004 histological and immunohistochemical criteria [9]. Disagreements concerning the histological diagnosis were discussed, and after a critical discussion, a mutual agreement was reached. The surgical-pathological staging was performed, according to the tumor, node, metastasis (TNM) classification by the IMIG [10].

2.3. Immunohistochemistry

Immunohistochemical analyses were performed on 3 μm tissue sections using specific antibodies. Immunoreaction was displayed using the avidin–biotin–peroxidase complex (ABC) method. Immunostaining was done using a Benchmark immunostainer (Ventana, Tucson, AZ). In all cases, the immunohistochemical evaluation was performed independently by two pathologists (G.A. and G.F.) who were blind to the clinico-pathological characteristics and treatment of the patients.

For the tryptase and chymase immunohistochemical stainings, sections were incubated with a mouse anti-human tryptase monoclonal antibody (Chemicon International, Temecula, CA, USA) and with a mouse anti-human chymase monoclonal antibody (Chemicon International, Temecula, CA, USA). Immunostaining for tryptase and chymase was clearly visible as brown deposits within intact mast cells (MCs) and as highly localized extracellular granular material. Each pathologist counted the chymase and tryptase positive MCs at $\times$200 microscopic fields. The average of their counts in three fields was calculated.

For tumor-infiltrating lymphocyte-immunohistochemical staining, sections were incubated with the following antibodies:

- mouse anti-human Foxp3 monoclonal antibody (clone 236A/E7 diluted 1:300; Abcam, Cambridge, UK),
- mouse anti-human CD8 monoclonal antibody (clone C8/144B, ready to use for the Ventana automated slide stainer; Ventana, Tucson, AZ, USA),
- mouse anti-human CD4 monoclonal antibody (clone 1F6 diluted 1:20; Diagnostic BioSystem, Pleasanton, CA, USA).

Each pathologist counted the cells at $\times$400 microscopic fields. The average of their counts in five fields was calculated.

The microvessel count (MVC) was determined using the anti-CD34 antibody (Ventana Medical System, ready to use for the Ventana automated slide stainer) to better visualize the endothelial cells and, consequently, the microvessels. Each pathologist examined the samples and identified the area with the most intense vascularization (hot spot) under low microscopic power ($\times$10 objective lens and $\times$10 ocular lens). In this area, the number of microvessels were counted and recorded at $\times$400.

For the Vascular Endothelial Growth Factor (VEGF) expression, the sections were incubated with an anti-VEGF rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The expression of VEGF was evaluated as a percentage of positive cells in a total of at least 1000 tumor cells.

2.4. Statistical analysis

All of the statistical analyses were carried out using the Statistica software (Stat-soft). The Student's *t*-test was used to determine the differences between the two different populations: preoperative IL-2 treated patients vs. untreated patients. The ANOVA test was used to determine the association between histo-pathological and clinical characteristics. Survival curves were estimated by the Kaplan–Meier's product-limit method and were compared by using the log-rank test. The a priori level of significance was set at a $P<0.05$.

3. Results

Fifty-seven patients (49 male – 8 female) treated for MPM were analyzed. We considered two groups of patients according to the multimodality treatment, and the main difference between the two groups for the pathological correlation is that the first group received preoperative immunotherapy with IL-2 and the second group did not. Histological features of the tumors as well as other clinico-pathological characteristics of the series of patients of both groups are summarized in Table 1.

The number of tumor-infiltrating MCs was evaluated in all of the tumor samples. Regarding the tryptase MCs, they were significantly higher in the IL-2 treated group ($P=0.01$). No differences between the two groups of patients were observed for the chymase MCs (Table 2).

No significant associations were found between the density of tryptase and chymase positive MCs and other clinico-pathological parameters, such as age, gender, histology, performance status, and stage in both of the patients' groups.

The number of CD8 positive (CD8+) lymphocytes was significantly higher in the preoperative IL-2 group ($P=0.001$).

Evaluation of the number of Foxp3 positive (Foxp3+) lymphocytes revealed a significant difference ($P=0.006$)

Table 1
Patient characteristics according to the treatment with or without IL-2

Clinico-pathological characteristics	IL-2 Treated patients ($n=34$)	No IL-2 patients ($n=23$)
Age		
Range (years)	41–77	54–73
Mean	61	68.4
Gender		
Male	29	16
Female	5	7
Histologic subtypes		
Epithelioid	31	20
Biphasic	3	3
IMIG stage		
II	6	10
III	28	13
ECOG performance status		
0	5	5
1	25	14
2	4	4

IMIG, International Mesothelioma Interest Group; ECOG, Eastern Cooperative Oncology Group.

between the two different groups of treatment with a major number in the patients, who received preoperative IL-2 treatment.

No correlations were observed between the tumor-infiltrating lymphocytes, CD8, CD4, Foxp3, and the clinico-pathological characteristics of both groups of patients.

The mean of number of microvessels was significantly lower in the IL-2 treated patients compared with untreated patients ($P=0.000000$).

Regarding VEGF expression, there was no statistically significant difference between the two groups. Moreover, no significant correlations were observed between the MVC and VEGF expression and the patients' clinico-pathological characteristics.

With an average follow-up of 86.7 months (range 51–115) and 26 (range 9–46) for the first and second group of patients, 2 and 12 patients are still alive, respectively. The two-year actuarial survivals were 44.8% and 43.2% for the first and second group, respectively, and the difference was not statistically significant (Fig. 1). The performance status at the diagnosis significantly affected survival ($P=0.005$) (Fig. 2). There was a better survival rate in stage II MPM respect to stage III, but the difference was not statistically significant. Among the pathologic variables which were investigated, only the Foxp3+ expression (median value as cut-off) impacted favorably the outcome with better survival in patients with low Foxp3+ expression ($P=0.001$) (Fig. 3).

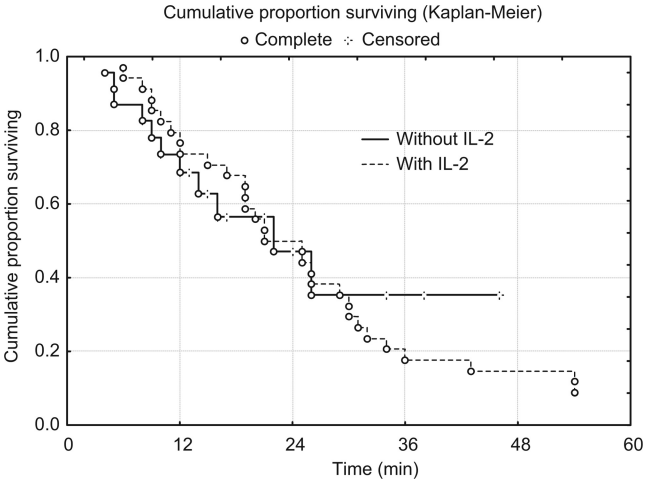

Fig. 1. Overall survival of the two groups of MPM patients, who underwent multimodality treatments with and without IL-2. MPM, malignant pleural mesothelioma.

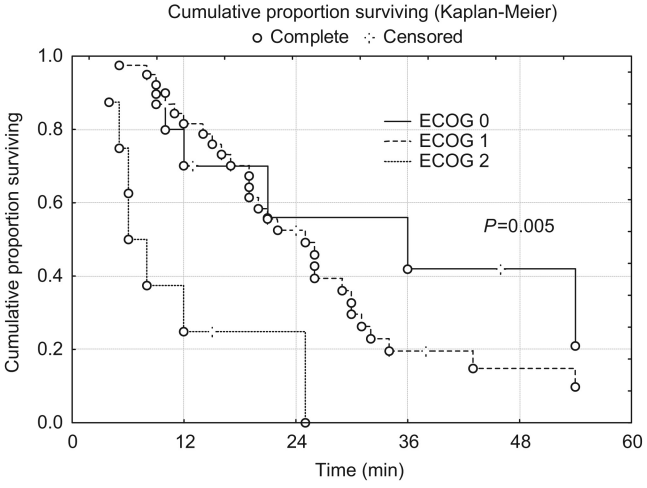

Fig. 2. Survival according to the patients' performance status at the diagnosis.

4. Discussion

Different multimodality treatments have been proposed for the treatment of MPM with both palliation and care intent [2–5]. As a matter of fact, there is no scientific evidence about the best treatment to offer to MPM patients and, unfortunately, survival, as well as quality of life, is still poor.

Table 2
Correlation of pathological parameters between the 2 MPM patient groups

Pathological parameters (mean±S.D.)	IL-2 Treatment	No IL-2 treatment	*P*-value
Tryptase	29.61±46.03	4.26±6.70	0.01
Chymase	25.95±19.14	20.07±8.55	0.17
Foxp3	10.43±10.23	3.58±5.87	0.006
CD4	63.33±44.38	48.62±34.16	0.21
CD8	88.04±27.16	58.05±38.07	0.001
CD34 – Microvessel count	56.47±23.26	130.43±49.57	0.000000
VEGF	38.43±31.17	44.34±37.27	0.52

MPM, malignant pleural mesothelioma; VEGF, vascular endothelial growth factor.

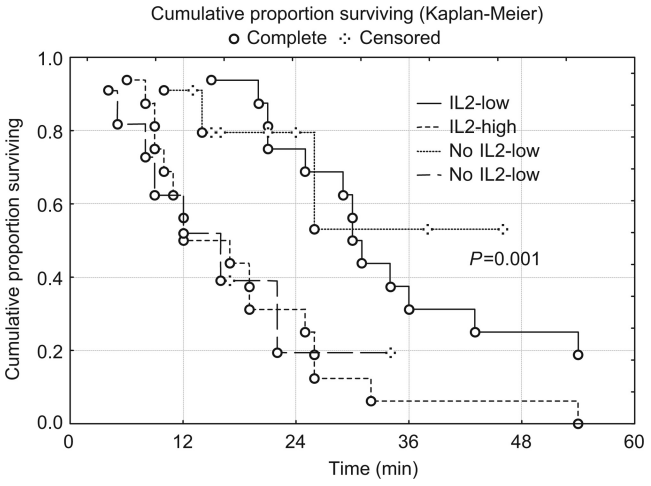

Fig. 3. Survival according to the Foxp3+ expression (median value as cut-off).

In this study, we had the possibility of investigating surgical specimens of two series of patients, who underwent different multimodality treatments in different periods. Considering the pathological characteristics the main difference between the two groups was that the first group underwent intrapleural preoperative IL-2, while the second did not.

We observed a statistically significant increase of tryptase positive MCs in the IL-2 treated group of patients.

We have previously demonstrated in a series of IL-2 treated patients with MPM that tumor-infiltrating tryptase MCs predict a better clinical outcome [11]. In the present study, we observed that all of the MPM patients showed infiltrating tryptase MCs in the tumor microenvironment, even at different levels, supporting the hypothesis for an important role of tryptase MCs, independent of the IL-2 preoperative treatment.

Regarding the tumor-infiltrating lymphocytes, we observed a statistically significant increase in both CD8 and Foxp3+ lymphocytes in the IL-2 treatment group. It has already been demonstrated that CD8+ T-cells play a pivotal role in mediating local tumor immunity throughout antigen-specific tumor cell killing [12] and that in human MPM high levels of CD8+ tumor-infiltrating lymphocytes are associated with a better prognosis, and with a lower incidence of mediastinal lymph node metastasis [13]. If IL-2 increases the number of CD8+ lymphocytes and enhances CD8+ cytotoxic lymphocyte activity this is a valid rationale for using IL-2 in order to improve the survival of MPM patients.

Regulatory T-cells, a subset of CD4+CD25+Foxp3+ T-lymphocytes (Treg lymphocytes), are mediators with the functional ability to regulate/suppress tumor immunity [12]. In our study, we observed a significantly higher number of positive Treg cells in the group of IL-2 treated patients, similarly to what happened to patients with melanoma and renal carcinoma, who underwent IL-2 therapy [14].

Several studies have correlated IL-2 therapy with angiogenesis showing that CD8+ lymphocytes play a role in reducing tumor-associated vascularity [15]. In the present report, the number of microvessels was significantly lower in IL-2 treated patients and VEGF expression was lower in IL-2 treated patients, but the difference with untreated patients was not significant. Our results suggest an anti-angiogenetic mechanism mediated by IL-2 in MPM, which may represent a supplementary anticancer action.

The two groups of patients were sequential, consequently the follow-up and the survival outcome was real for the first series and actuarial and short for the second. Despite this major bias, the survival between the two groups was not statistically significant.

In both group of patients, we observed a significative better survival when Foxp3+ lymphocytes were low. Regulatory T-cells, a subset of CD4+CD25+Foxp3+ T-lymphocytes, are mediators with the functional ability to regulate/suppress tumor immunity [12]. In this study, we observed a significantly higher number of positive Treg cells in the group of IL-2 treated patients compared with untreated patients and, observing a better survival in the case of lower number of Foxp3+ T-lymphocytes, we may deduce that depletion of the Treg subset lymphocytes may enhance the ability of IL-2 to boost host immunity against cancer.

In conclusion, by comparing the clinical-pathological characteristics of MPM patients, who underwent preoperative immunotherapy and patients who did not, we verified the changes in MPM microenvironment induced by immunotherapy and we demonstrated that treatment of MPM with intra-pleural IL-2 leads, on one hand to an increase in tryptase positive MCs, and in CD8+ and Foxp3+ lymphocytes, and on the other hand to an inhibition of the tumoral angiogenesis.

These findings give new insights into the complex anti-tumoral mechanisms mediated by IL-2 in malignant mesotheliomas, which are useful if IL-2 is to be successfully used in the multimodality treatment of MPM patients. Further studies are required to confirm the functional properties of the effector, or regulatory phenotypes, since the anti-cancer effect of IL-2 as well as other immunostimulators is much more complex than we actually know.

References

[1] Treasure T, Tan C, Lang-Lazdunski L, Waller D. The MARS trial: mesothelioma and radical surgery. Interac CardioVas Thorac Surg 2006;5:58–59.

[2] Martino D, Pass HI. Integration of multimodality approaches in the management of malignant pleural mesothelioma. Clin Lung Cancer 2004;5:290–298.

[3] Sugarbaker DJ, Flores RM, Jaklitsch MT, Richards WG, Strauss GM, Corson JM, DeCamp MM Jr, Swanson SJ, Bueno R, Lukanich JM, Baldini EH, Mentzer SJ. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117:54–63.

[4] Flores RM, Pass HI, Seshan VE, Dycoco J, Zakowski M, Carbone M, Bains MS, Rusch VW. Extrapleural pneumonectomy versus pleurectomy/decortication in the surgical management of malignant pleural mesothelioma: results in 663 patients. J Thorac Cardiovasc Surg 2008;135:620–626.

[5] Weder W, Kestenholz P, Taverna C, Bodis S, Lardinois D, Jerman M, Stahel RA. Neoadjuvant chemotherapy followed by extrapleural pneumonectomy in malignant pleural mesothelioma. J Clin Oncol 2004;22:3451–3457.

[6] Boutin C, Viallat JR, Van Zandwijk N, Douillard JT, Paillard JC, Guerin JC, Mignot P, Migueres J, Varlet F, Jehan A. Activity of intrapleural recombinant gamma interferon in malignant masothelioma. Cancer 1991;67:2033–2037.

[7] Kruklitis RJ, Singhal S, Delong P, Kapoor V, Sterman DH, Kaiser LR, Albelda SM. Immuno-gene therapy with interferon-beta before surgical debulking delays recurrence and improves survival in a murine model of malignant mesothelioma. J Thorac Cardiovasc Surg 2004;127:123–130.
[8] Lucchi M, Chella A, Dini P, Melfi FMA, Ambrogi MC, Fino L, Fontanini G, Mussi A. A phase II study of intrapleural immuno-chemotherapy, pleurectomy/decortication, radiotherapy, systemic chemotherapy and long-term sub-cutaneous IL-2 in stage II-III malignant pleural mesothelioma. Eur J Cardiothorac Surg 2007;31:529–534.
[9] Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC. World Health Organization classification of tumours. Pathology and genetics of tumours of the lung, pleura, thymus and heart. Lyon, France: IARC Press; 2004.
[10] Rush VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995;108:1122–1128.
[11] Alì G, Boldrini L, Lucchi M, Mussi A, Corsi V, Fontanini G. Tryptase mast cells in malignant pleural mesothelioma as an independent favorable prognostic factor. J Thorac Oncol 2009;4:348–354.
[12] Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer 2005;5:263–274.
[13] Anraku M, Cunningham KS, Yun Z, Tsao MS, Zhang L, Keshavjee S, Johnston MR, de Perrot M. Impact of tumor-infiltrating T cells on survival in patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2008;135:823–829.
[14] Ahmadzadeh M, Rosenberg SA. IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients. Blood 2006; 107:2409–2414.
[15] Jackaman C, Bundell CS, Kinnear BF, Smith AM, Filion P, van Hagen D, Robinson BW, Nelson DJ. IL-2 intratumoral immunotherapy enhances CD8+ T-cells that mediate destruction of tumor cells and tumor-associated vasculature: a novel mechanism for IL-2. J Immunol 2003; 171:5051–5063.

Conference discussion

Dr. Rea (*Moderator*): For the invited discussant, unfortunately we don't have Dr. Opitz, but she sent us the comment which Walter Weder will read.

Dr Weder (*Zurich, Switzerland*): The authors have been doing research in the field of immunotherapy for malignant pleural mesothelioma, especially intrapleural and subcutaneous treatment with interleukin-2, for many years. Malignant pleural mesothelioma is known to be associated with immunologic dysfunction and to be sensitive to various forms of immunotherapy, including IL-2. Rational strategies for immune intervention require an understanding of the relationship between the immune system and the microenvironment of the malignancy, and therefore, this paper represents an interesting contribution to this topic.

In the current study, the authors compare two groups of patients with malignant pleural mesothelioma in stages II–III over a 10-year period, consecutively-treated with multimodality treatment, including or not including immunotherapy. The authors concentrate especially on changes of the tumor microenvironment by immunohistochemical analysis of immunologic effector cells, such as CD8, CD4, Foxp3 tumor-infiltrating lymphocytes, and tryptase and chymase mast cells, and assess the angiogenesis, such as microvessel density and VEGF, and perform quantitative analysis between the group treated with IL-2 or not treated. Furthermore, the prognostic influence on overall survival of these factors is assessed.

The major findings are that patients treated with IL-2 have a significant higher expression of tumor-infiltrating tryptase mast cells, CD8, Foxp3 lymphocytes, and a significant lower number of microvessels is observed. Besides low Foxp3 expression, none of the pathological factors analyzed had a prognostic impact on overall survival.

This result is in contrast to the previous findings of your group, where tumor-infiltrating tryptase mast cells after interleukin-2 preoperative induction therapy have been shown to predict improved, better clinical outcome. Your co-worker, Alì, presented this in the Journal of Thoracic Oncology in 2007. So please clarify this discrepancy between the two papers.

The fact that Foxp3 expression favorably influences overall survival when it is low, but was observed to be high in the patient treated with IL-2 was interpreted by the authors with the assumption that depletion of T-regulatory lymphocytes may enhance the ability of IL-2 to boost host immunity against cancer. The role of T-regulator cells in MPM is controversially discussed. Human malignant pleural mesothelioma tumors are reported to contain high levels of Foxp3, CD4, CD25, and regulatory cells, such as published in the European Respiratory Journal by Hegmans in 2006; however, the presence of Foxp3 tumor-infiltrating lymphocytes did not affect survival in other groups. Recent work has been published by the group of Robinson investigating in a murine model why T-cell response fails, in order to better design immunotherapy. They come to the conclusion that the inability of the activated immune system to attack malignant pleural mesothelioma appears not to be substantially mediated by CD25 and CD4 and T-regulatory cells. They could demonstrate that removal of CD25 and T-regulatory cells from the tumor site and lymphoid organs did not alter tumor growth with or without IL-2 immunotherapy.

Besides these more basic research issues, there are some questions regarding your study. Although pathological quantitative analysis is well designed in your study, with two independent pathologists blinded to the treatment and average counts of different positive fields of the antibodies used is calculated, it is not mentioned where the positive controls have been performed. Please clarify this.

Another question is, at which time point and where are these biopsies assessed? Furthermore, it is described that patients underwent thoracoscopic pleural biopsy for inclusion into the study. Have the biopsies been compared before and after treatment? This would be interesting to assess the effect of IL-2 therapy. Another interesting input would be the assessment of these cells in the regional lymph nodes, if they have been resected during pleurectomy/decortication.

The most critical point of the study is the comparison of the two groups in terms of prognostic relevant factors, since the difference between the two groups is not only the preoperative intrapleural IL-2 therapy but also the postoperative regimen.

Furthermore, the treatment of the historical patient group with IL-2 is described as previously reported in this journal, the European Journal, but there is a difference between the patient group described in the previous article, 49 patients, and in this study, 34 patients. So what happened to these 15 patients which are missing?

Dr. Lucchi: I will try to answer the questions point by point. First, recently we published in the JTO about the prognostic value of tryptase mast cells, but that study concerned 60 patients who were treated with preoperative immunotherapy. So considering that group which was larger and homogeneous, from a pathological point of view, tryptase mast cells were prognostic factors. In this smaller group and considering the two modalities of treatment, it was not significant. But, as you will note from reading the paper, this series is homogeneous from the clinical point of view, and we excluded stage I as well as sarcomatoid mesothelioma and EPP procedures.

Concerning why we treated many more patients and we analyzed only 34 is because we did a lot of investigations on the biology of the tumor and the specimens are not available anymore. So this is our mistake. As surgeons, we should preserve pathological material, especially frozen, of the disease as much as possible, because I think it's an important heritage to leave to the next researchers. So unfortunately, that's what we could do, we just had that material.

Concerning the value of the Foxp3 lymphocytes, as a surgeon, I had to study a little bit about immunological factors. Foxp3, a subset of lymphocytes, is implied also in immune disease. Where it is low, there may be immune disease; where it is high, you have no possibility of immune disease, and sometimes you can also have tumors, and the percentage of Foxp3 lymphocytes is higher in the oncological patient. What happens with interleukin-2 is that high doses of interleukin-2 improves the host immunity but, on the other hand, it also increases the level of Foxp3. So there is a conflicting action between the immune system, stimulated by the interleukin-2, and Foxp3 lymphocytes which protect the tumor from the host immunity. Actually, we have no possibility to decrease the level of Foxp3 lymphocytes, but it would be interesting to have the means to do that.

Interleukin-2 as an immunotherapy is not the best, absolutely. It's what we had some years ago, and actually, research in immunotherapy in cancer and, above all, in mesothelioma, slackened because there is no interest in developing and scheduling new immunotherapies. Furthermore, these drugs are quite expensive and hospitals don't agree with spending so much money for a disease with ominous prognosis.