Free-from-progression Period and Overall Short Preoperative Immunotherapy with IL-2 Increases the Survival of Pancreatic Cancer Patients Treated with Macroscopically Radical Surgery

R. CAPROTTI[1], F. BRIVIO[1], L. FUMAGALLI[1], C. NOBILI[1], L. DEGRATE[1], P. LISSONI[2], D. PAROLINI[2], G. MESSINA[2], M. COLCIAGO[3], M. SCOTTI[1] and F. UGGERI[1]

[1]Department of General Surgery, [2]Division of Radiation Oncology, San Gerardo Hospital, Monza, Milan, Italy; [3]Laboratory of Analyses, INRCA, Casatenovo, Lecco, Italy

Abstract. *Background: The treatment of pancreatic cancer is still rudimentary, even in the case of locally limited tumors, because of the high frequency of recurrence due to severe suppression of the anticancer immunity that is further amplified by surgery-induced immunosuppression, evidenced by a decline in lymphocyte numbers during the postoperative period. Previous studies in colorectal cancer demonstrated that surgery-induced lymphocytopenia may be abrogated by a brief preoperative administration of IL-2. Materials and Methods: The study included 30 consecutive patients who were randomized to be treated by radical surgery alone as a control group or by a preoperative immunotherapy with IL-2 (12 MIU/day SC for 3 consecutive days) plus surgery. Results: Mean lymphocyte numbers significantly decreased in patients treated with surgery only, whereas it significantly rose in the IL-2-treated group. After a follow-up of 36 months, both the free-from-progression period (FFPP) and the overall survival were significantly higher in patients treated with IL-2. Conclusion: These preliminary results suggest that a short-period preoperative immunotherapy with IL-2 is sufficient to modify host tumor interactions in operable pancreatic cancer, with a subsequent abrogation of postoperative lymphocytopenia and a prolongation of FFPP and overall survival time.*

Despite the great advances in the investigation of tumor biology, pancreatic adenocarcinoma remains an untreatable disease. The recent discoveries in the area of cancer biology and antitumor immunity have contributed to an understanding of the causes responsible for the biological malignancy of pancreatic cancer. In fact, the development of pancreatic cancer appears to be associated with a status of severe immunosuppression due to the generation of a high percentage of T regulator lymphocytes (T-reg) (1-4),which may suppress anticancer immunity by inhibiting the activation of both T helper-1 (TH1) and T cytotoxic lymphocytes (5). In addition, the carcinomas of the pancreas have been proven to secrete a great amount of transforming growth factor (TGF)-beta (6), which would promote tumor growth by stimulating T-reg generation (7), resulting in an immunosuppressive status. Since tumor growth may depend on both growth factor-induced tumor cell proliferation and the suppression of anticancer immunity (1-7), TGF-beta would play a key role in the clinical evolution of cancer because of its immunosuppressive effect and its involvement in the control of cell proliferation (7).

Pancreatic cancer could thus constitute a paradigmatic example of neoplasia where tumor-related variables and host immunosuppressive status have the same importance in determining an unfavourable prognosis. The severe suppression of anticancer immunity, which characterizes patients suffering from pancreatic cancer, is further aggravated by surgical treatment (8). In fact, it is known that surgery may inhibit anticancer immunity by provoking a postoperative decline in the absolute number of circulating lymphocytes (9-11), which play a fundamental role in generating an effective anticancer immune reaction; this is fundamentally an IL-2-dependent phenomenon (12). Surgery-induced immunosuppression could represent one of the main factors responsible for relapse in cancer patients treated by radical surgery, by possibly promoting the growth of micro-metastases, already existing at the time of the surgical removal of the tumor (9-12). Previous clinical studies have shown that the immunosuppressive status occurring during the postoperative period is particularly severe in patients with pancreatic cancer (11) and this

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

Key Words: Immunosurgery, immunotherapy, interleukin-2, lymphocytopenia, pancreatic cancer.

evidence could explain, at least in part, the high percentage of recurrences occurring in patients radically operated for cancer of the pancreas (13).

At present, the only molecule which has been proven to correct the lymphocytopenia is IL-2, representing the main growth factor for lymphocytes, including T lymphocytes and natural killer (NK) cells (14) and the stimulation of lymphocyte proliferation would constitute the main mechanism responsible for the antitumor activity of IL-2 in the immunotherapy of cancer (15). Moreover, the preoperative administration of IL-2 for only few days prior to surgery was effective in preventing surgery-induced lymphocytopenia (16). In addition, the abrogation of surgery-induced lymphocyte decline has been shown to improve the prognosis of patients with colorectal cancer in whether treated by radical or palliative surgery (17). The therapeutic impact of IL-2 presurgical administration remains to be better defined in gastric cancer (18), despite its efficacy in preventing the postoperative lymphocytopenia. Finally, the prevention of postoperative lymphocyte decline by IL-2 presurgical immunotherapy was associated with clear lymphocyte and eosinophil intratumoral infiltration in colorectal cancer patients (17), which, in contrast, was less evident in patients with gastric carcinoma (18). Preliminary clinical studies have suggested that preoperative injection of IL-2 may also prevent surgery-induced lymphocytopenia in patients with pancreatic cancer (19). On this basis, a preliminary controlled study was planned to investigate the immunobiological effects of IL-2 presurgical immunotherapy and its impact on the clinical course of the neoplastic disease in pancreatic cancer patients treated by macroscopically radical surgery. The first endpoint of the study was the evaluation of the overall survival time (OS) and the second endpoint was the assessment of the free-from-progression period (FFPP), by assessing these parameters with the perioperative variations in lymphocyte count.

Materials and Methods

The study included 30 consecutive patients undergoing surgery for cancer of the head of pancreas who were randomized to be treated with surgery alone as a control group or with a preoperative immunotherapy of IL-2 plus surgery. The experimental protocol, which was approved by the Ethical Committee, was explained to each patient and written consent was obtained. Eligibility criteria were as follows: histologically proven locally limited pancreatic adenocarcinoma, head of the pancreas as the site of the primary tumor, no distant organ metastasis, no double tumor, macroscopically radical surgery and no chronic concomitant treatment with drugs influencing the immune system. The clinical characteristics of the two groups of patients are reported in Table I.

In accordance with our previous investigations (16-19), IL-2 was subcutaneously (*s.c.*) injected at a dose of 6 MIU twice/day for 3 consecutive days prior to surgery, operating within 48 hours from the last IL-2 injection. This corresponds to the period of rebound lymphocytosis after an initial decline in circulating lymphocyte number, due to extravascular migration (20). Moreover, patients receiving IL-2 were concomitantly treated with the pineal hormone melatonin (MLT) at a dose of 20 mg/day orally in the evening on the basis of previous clinical experimental studies which had demonstrated the capacity of MLT to enhance the anticancer immunobiological activity of IL-2 and reduce its toxicity (21-23). To evaluate the postoperative variations, the lymphocyte count was measured before surgery and at day 7 of the postoperative period.

Table I. *Clinical characteristics of 30 pancreatic cancer patients treated by surgery alone (control group) or surgery plus IL-2 pre-operative immunotherapy (IL-2 group).*

Characteristic	Control Group	IL-2 Group
n 14	16	
M / F	9/5	10/6
Median age (years)	67 (49-76)	65 (47-77)
Median performance status (Karnofsky's score)	90 (80-100)	90 (80-100)
Clinical stage		
IA	2	1
IB	3	2
IIA	1	0
IIB	8	13
Microscopically residual disease (R1)	6 (43 %)	8 (50%)

After the surgical treatment, patients were monitored for a minimum follow-up period of 36 months. Radiological examinations, including CT scan and/or NMR and/or PET, were repeated every 3 months. Moreover, both patients with minimal persistence of disease at histological examination and those with recurrence after histologically documented radical surgery underwent chemotherapy of gemcitabine at a dose of 1000 mg/m^2 at days 1, 8 and 21, for at least 9 consecutive injections. Finally, patients with histological persistence of disease or relapsed patients with good clinical status were concomitantly treated with cisplatin at a dose of 25 mg/m^2. Data were statistically analyzed by the chi-square test, Student's *t*-test and the analysis of variance, as appropriate. Moreover, the survival curves were plotted according to the Kaplan-Meier method and statistically evaluated by the log-rank test.

Results

As shown in Table I, the two groups of patients were comparable for the overall main prognostic variables, including clinical stage, node involvement, age, performance status (PS) and percentage of histological persistence of disease. The perioperative changes in mean lymphocyte numbers are illustrated in Figure 1. Mean lymphocyte numbers significantly decreased during the postoperative period in control patients ($p<0.05$ *vs.* presurgery). On the contrary, in patients concomitantly treated by IL-2, the mean lymphocyte number significantly increased in the postsurgical period ($p<0.01$ *vs.* presurgery), and that for the IL-2 group was significantly higher with respect to the values found in the control patients ($p<0.005$).

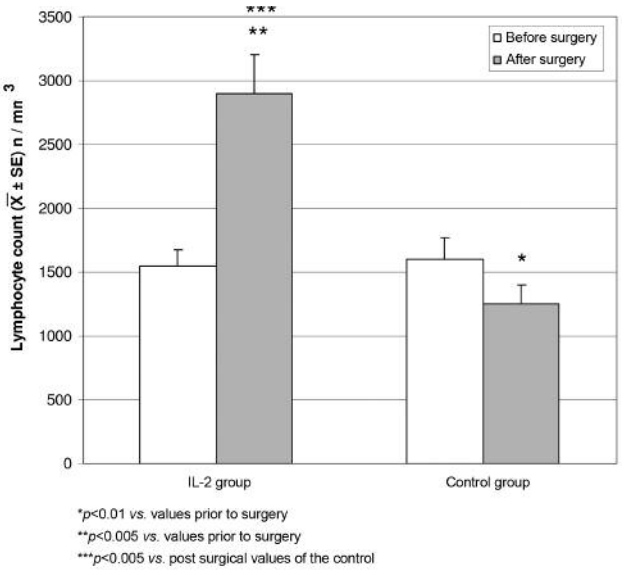

Figure 1. *Mean number of lymphocytes observed before surgery and at day 7 of the postoperative period in pancreatic cancer patients treated with surgery alone (Control group) or IL-2 immunotherapy plus surgery (IL-2 group).*

FFPP and OS found in the two groups of patients are illustrated in Figures 2 and 3, respectively. The duration of FFPP and OS for patients pre-treated with IL-2 were significantly longer with respect to those achieved in patients treated with surgery only ($p<0.01$ and $p<0.05$, respectively). No intratumoral infiltration of lymphocytes or eosinophils occurred in patients treated with surgery alone, nor in those preoperatively treated with IL-2.

Finally, no IL-2-related toxicity occurred, but patients pre-treated with IL-2 showed a better postoperative recovery in terms of clinical conditions and reduction in the duration of hospitalization than those who underwent surgery alone.

Discussion

According to our previous investigations (16-19), IL-2 presurgical immunotherapy may also completely abrogate surgery-induced lymphocytopenia also patients with pancreatic carcinoma, as well as previously described for both colorectal and gastric carcinomas. Moreover, in agreement with the clinical results previously reported for colorectal cancer patients and in contrast to those more controversially reported in gastric cancer, this study would suggest that a preoperative immunotherapy with IL-2 may improve the clinical course of the pancreatic cancer in terms of both FFPP and OS. Therefore, particularly because of its unfavourable prognosis, presurgical immunotherapy with IL-2 could represent a simple but effective clinical strategy to improve the prognosis of pancreatic cancer patients undergoing macroscopical radical surgery.

The mechanisms responsible for the improvement in the duration of FFPP and OS induced by IL-2 preoperative immunotherapy need to be better investigated and understood. However, they would depend at least in part on the abrogation of surgery-induced postoperative lymphocytopenia, with a consequent more effective anticancer immune response against possible micrometastases, which are involved in determining the recurrence of the neoplastic disease after macroscopically radical removal of the primary tumor, as previously described for colorectal carcinoma (16-19). In contrast to the results observed for colorectal cancer, in patients with pancreatic cancer the abrogation of surgery-induced lymphocytopenia obtained by IL-2 preoperative immunotherapy was not associated with a concomitant evident lymphocyte infiltration within the tumor mass, which would further contribute to counteract tumor cell proliferation and dissemination. Therefore, further mechanisms other than tumor lymphocyte infiltration have to be identified in order to explain the favourable impact of IL-2 presurgical immunotherapy on the prognosis of operable pancreatic cancer.

In conclusion, the study shows that IL-2 preoperative immunotherapy, by modifying host-tumor relation, may improve the clinical course of surgically treated pancreatic cancer patients, in terms of both free-from-progression period and overall survival time.

References

1 Hansel DE, Kern SE and Hruban RH: Molecular pathogenesis of pancreatic cancer. Annu Rev Genomics Hum Genet *4*: 237-256, 2003.

2 Siegel PM and Massague J: Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer *3*: 807-821, 2003.

3 Elliott RL and Blobe GC: Role of transforming growth factor-beta in human cancer. J Clin Oncol *23*: 2078-2093, 2005.

4 Furukawa T, Sunamura M and Horii A: Molecular pathogenesis of pancreatic cancer. Cancer Sci *97*: 1-7, 2006.

5 Birebent B, Lorho R, Lechartier H, de Guilbert S, Alizadeh M, Vu N, Beauplet A, Robillard N and Semana G: Suppressive properties of human CD4+CD25+ regulatory T cells are dependent on CTLA-4 expression. Eur J Immunol *34*: 3485-3496, 2004.

6 Truty MJ and Urrutia R: Transforming growth factor-beta: what every pancreatic surgeon should known. Surgery *141*: 1-6, 2007.

7 Chen W, Jin W, Hardegen N, Lei K, Li L, Marinos N, McGrady G and Wahl SM: Conversion of peripheral CD4+CD25- naïve T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med *198*: 1875-1886, 2003.

8 Akiyoshi T, Koba F, Arinaga S, Miyazaki S, Wada T and Tsuji H: Impaired production of interleukin-2 after surgery. Clin Exp Immunol *59*: 45-49, 1985.

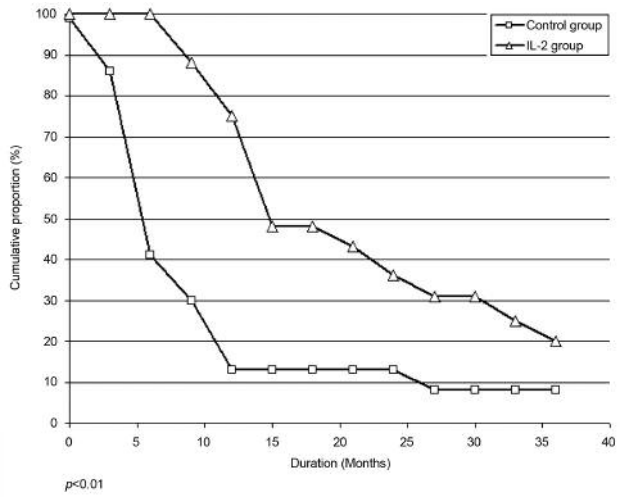

Figure 2. *Free-from-progression period in pancreatic cancer patients treated with macroscopically radical surgery alone (Control group) or IL-2 immunotherapy plus surgery (IL-2 group).*

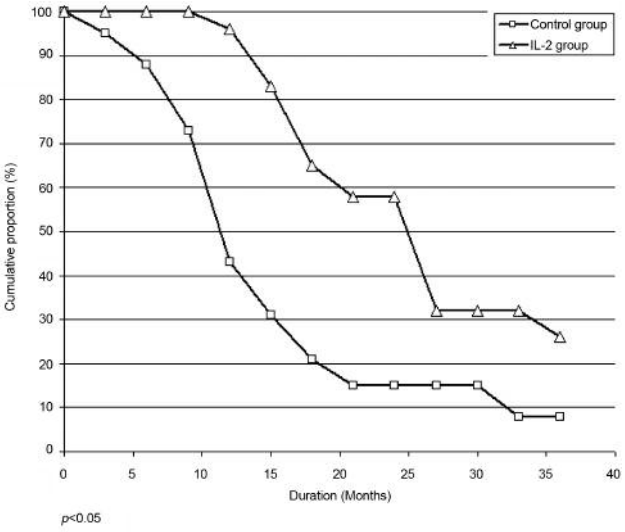

Figure 3. *Overall survival curves for patients with pancreatic cancer treated with surgery alone (Control group) or IL-2 immunotherapy plus surgery (IL-2 group).*

9 Akiyoshi T, Koba F, Arinaga S, Wada T and Tsuji H: *In vitro* effect of interleukin-2 on depression of cell-mediated immune response after surgery. Jpn J Surg *15*: 375-378, 1985.

10 Brivio F, Lissoni P, Tisi E, Barni S, Tancini G, Rescaldani R and Nociti V: Effect of a preoperative therapy with interleukin-2 on surgery-induced lymphocytopenia in cancer patients: Oncology *49*: 215-218, 1992.

11 Brivio F, Lissoni P, Brivio O, Marsili MT, Alderi G and Lavorato F: Timing and schedule of perioperative immunotherapy with interleukin-2 in oncological surgery. J Emerg Surg Intensive Care *19*: 59-62, 1996.

12 Grimm EA, Mazumder A, Zhang HZ and Rosenberg SA: Lymphokine-activated killer cell phenomenon. J Exp Med *155*: 1823-1841, 1982.

13 Friess H, Kleeff J, Kork M and Buchler MW: Molecular aspects of pancreatic cancer and future perspectives. Dig Surg *16*: 281-290, 1999.

14 Whittington R and Faulds D: Interleukin-2. Drugs *46*: 446-483, 1993.

15 Atzpodien J and Kirchner H: Cancer, cytokines, and cytotoxic cells: interleukin-2 in the immunotherapy of human neoplasms. Klin Wochenschr *68*: 1-11, 1990.

16 Brivio F, Lissoni P, Barni S, Tancini G, Ardizzoia A and Erba L: Effect of preoperative course of interleumin-2 on surgical and immunological variables in patients with colorectal cancer. Eur J Surg *159*: 43-47, 1993.

17 Brivio F, Fumagalli L, Lissoni P, Nardone A, Nespoli L, Fattori L, Denova M, Chiarelli M and Nespoli A: Preoperative immunoprophilaxis with interleukin-2 may improve the prognosis in radical surgery for colorectal cancer stage B, C. Anticancer Res *26*: 599-604, 2006.

18 Cerea K, Romano F, Ferrari-Bravo A, Motta V, Uggeri F, Brivio F and Fumagalli L: Phase I-b study on prevention of surgery-induced immunodeficiency with preoperative administration of low-dose subcutaneous interleukin-2 in gastric cancer patients. J Surg Oncol *78*: 32-37, 2001.

19 Brivio F, Lissoni P, Fumagalli L, Brivio O, Lavorato F, Rescaldani R, Conti A, Roselli MG, Maestroni G and Barni S: Preoperative neuroimmunotherapy with subcutaneous low-dose interleukin-2 and melatonin in patients with gastrointestinal tumors: its efficacy in preventing surgery-induce lymphocytopenia. Oncol Rep *2*: 597-599, 1995.

20 Fumagalli L, Lissoni P, Di Felice G, Meregalli S, Valsuani G, Mengo S and Rovelli F: Pretreatment serum markers and lymphocyte response to interleukin-2 therapy. Br J Cancer *80*: 407-411, 1999.

21 Regelson W and Pierpaoli W: Melatonin:a rediscovered antitumor hormone? Cancer Invest *5*: 379-385, 1987.

22 Maestroni GJM: The immunoneuroendocrine role of melatonin. J Pineal Res *14*: 1-10, 1993.

23 Brzezinski A: Melatonin in humans. N Engl J Med *336*: 186-195, 1997.

Received November 15, 2007
Revised January 4, 2008
Accepted February 19, 2008