P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wcjd@public.bta.net.cn www.wjgnet.com

World J Gastroenterol 2002;8(6):1019-1022
World Journal of Gastroenterology
 ISSN 1007-9327

• GASTRIC CANCER •

# Efficacy of intraperitoneal thermochemotherapy and immunotherapy in intraperitoneal recurrence after gastrointestinal cancer resection

Qing-Guo Fu, Fan-Dong Meng, Xiao-Dong Shen, Ren-Xuan Guo

**Qing-Guo Fu, Fan-Dong Meng, Xiao-Dong Shen, Ren-Xuan Guo,** The Second General Surgery Department of The First Clinical College, China Medical University, Shenyang 110001, Liaoning Province, China
**Correspondence to:** Dr. Qing-Guo Fu, The Second General Surgery Department of The First Clinical College, China Medical University, Shenyang 110001,Liaoning Province, China. qingguofu@hotmail.com
**Telephone:** +86-24-23256666-6237
**Received** 2002-04-24 **Accepted** 2002-06-03

## Abstract

**AIM:** To investigate the prophylactic and therapeutic efficacy of intraperitoneal IL-2 immunotherapy following intraperitoneal thermochemotherapy in the metastasis and recurrence of gastric and colorectal cancer after operation.

**METHODS:** Forty-two gastric cancer patients at $T_3$II-$T_4$ $III_B$ stages and 96 patients with colorectal cancer at B to D stages admitted from January 1996 to October 1998 were randomly divided into control group (group Ⅰ, 65 cases) receiving intraperitoneal thermochemotherapy, and group Ⅱ (73 cases) receiving both intraperitoneal thermochemotherapy and intraperitoneal IL-2 immunotherapy. Distilled water at 43-45 ℃ containing 5-Fu 0.5 g/L and MMC 8 mg/L was perfused into peritoneal cavity before closure at the end of operation for 1 h, and from the third day, IL-2 10 million IU in 500 ml 0.9 % sodium chloride was intraperitoneally administrated daily for 10 times. One month after operation, all the patients underwent regular intravenous chemotherapy. Before and after the IL-2 immunotherapy, some Th1 type cytokines in the peripheral blood of the patients in the two groups were detected by ELISA, and the intraperitoneal recurrence and liver metastasis rates and the 3-year survival rate were statistically evaluated after intensive follow-up.

**RESULTS:** IL-2 intraperitoneal immunotherapy significantly elevated the level of some Th1 type cytokines ($P$<0.01 compared with that of control group), and the 3-year survival rate of group Ⅱ was 18.1 % higher and the rates of intraperitoneal recurrence and liver metastasis were 16.9 % and 6.0 % lower than those of group I significantly ($P$<0.05-0.01).

**CONCLUSION:** The combination of intraperitoneal IL-2 immunotherapy and thermochemotherapy could promote Th1 immune paradigm and enforce anti-tumor activity of bodies, which plays a positive role in preventing gastric and colorectal cancer from intraperitoneal recurrence and development.

Fu QG, Meng FD, Shen XD, Guo RX. Efficacy of intraperitoneal thermochemotherapy and immunotherapy in intraperitoneal recurrence after gastrointestinal cancer resection. *World J Gastroenterol* 2002; 8(6):1019-1022

## INTRODUCTION

Occurring frequently, that the gastrointestinal cancers spread in abdominal cavity and metastasize to the liver after resection, and in a number of cases, the lesion penetrated to serosa and implanted to peritoneum before operation. More and more clinical studies have revealed that postoperative intraperitoneal thermochemotherapy was obviously efficient in reducing the intraperitoneal recurrence and liver metastasis incidence[1-7]. Intraperitoneal thermochemotherapy can increase the sensitivity of tumor cells to chemotherapy drugs[8], and simultaneously enhance the antigenicity of tumor cells[9] which would be conducive to immunotherapy, therefore, based on this hypothesis, we conducted a clinical study on the efficacy of intraperitoneal thermochemotherapy and intraperitoneal immunotherapy involved in 42 cases of gastric cancer and 96 cases of colorectal cancer, and reported below.

## MATERIALS AND METHODS

From January 1996 to October 1998, 42 cases of gastric cancer at $T_3$II-$T_4$$III_B$ stages and 96 cases of colorectal cancer at B-D stages were randomly divided into 2 groups (control group, group I and treatment group, group II, Table 1), among whom 87 cases were males, and 51 cases females, with an age from 21 to 73 years, averaging 64.4±7.1 years.

### *Method*

**Therapeutic method** Radical operation was performed on 35 gastric cancer patients and the B-$C_2$ stage colorectal cancer patients, and palliative operation on 4 gastric cancer and colorectal patients of D-stage. The localized mesenteric and peritoneal infiltration lesions were removed as clear as possible or electrically burned if the lesions were at feasible locus. Before closure under general anesthesia, 4000 ml distilled water at 43-45 ℃ containing 5-Fu 0.5 g/l and MMC 8 mg/l was perfused in 4 equal volumes into peritoneal cavity, 1000 ml per quarter for an hour. Ice bags were put at groins, axilla and lateral chest and with ice cap on the head. Patients with heart, kidney, lung diseases or diabetes were not accepted in the study. Blood pressure, pulse, ECG and saturation of oxygen in blood were closely monitored during the treatment. On the 3rd day after operation, Group II was treated with IL-2, 1 million u dissolved in 0.9 % sodium chloride 500 ml, through trocars fastened in the abdominal wall. Patients were directed to change body positions to help defuse the drug. The puncture spots were adjusted to the tumor sites, and the therapy was carried out once a day and 10 times in all.

Both groups were administered intravenous chemical therapy from the 1st month after operation, which lasted one year. Routine of blood and urine, function of liver and kidney, CT, and B-ultrasound were performed regularly.

**Evaluation of patients' immune function** Both before and after intraperitoneal thermochemotherapy and immunotherapy,

**Table 1** The clinicopathological stages and surgical procedures in each group

| Groups | *n* | Male | Female | Age(yrs) | Stages(n) | Radical | Non-radical |
|---|---|---|---|---|---|---|---|
| Group I | 65 | 39 | 26 | 62.5±6.6 | - | 57 | 8 |
| Gastric cancer | 19 | 12 | 7 | 61.7±5.5 | $T_3$ II (9)$T_4$III$_A$(7)$T_4$III$_B$(3) | 16 | 3 |
| Colorectal cancer | 46 | 27 | 19 | 64.4±3.9 | B(20)C1(12)C2(9)D(5) | 41 | 5 |
| Group II | 73 | 48 | 25 | 65.4±8.7 | - | 62 | 11 |
| Gastric cancer | 23 | 16 | 7 | 67.1±7.6 | $T_3$ II (11)$T_4$III$_A$(8)$T_4$III$_B$(4) | 19 | 4 |
| Colorectal cancer | 50 | 32 | 18 | 63.3±5.2 | B(22)C1(13)C2(8)D(7) | 43 | 7 |

Control group: (1) Gastric cancer: papilloadenocarcinoma, 7 cases; tuboadenocarcinoma, 6 cases; lowly-differentiated adenocarcinoma, 2 cases; mucoadenocarcinoma, 1 case; signet ring cell carcinoma, 2 cases; and undifferentiated carcinoma 1 case; (2)Colorectal cancer: highly and intermediately differentiated adenocarcinoma, 29 cases; mucoadenocarcinoma, 12 cases; and undifferentiated carcinoma 5 cases. Therapy group: (1) Gastric cancer: papilloadenocarcinoma, 9 cases; tuboadenocarcinoma, 6 cases; lowly-differentiated adenocarcinoma, 2 cases; mucoadenocarcinoma, 2 cases; signet ring cell carcinoma, 2 cases; and undifferentiated carcinoma 2 cases; Colorectal cancer: highly and intermediately differentiated adenocarcinoma, 33 cases; mucoadenocarcinoma, 12 cases; and undifferentiated carcinoma 5 cases.

**Table 2** The levels of some Th1 cytokines in peripheral blood of patients before and after immunotherapy (pg / ml)

| Groups *n* | The level of the cytokines | | | | | | *P* |
|---|---|---|---|---|---|---|---|
| | IL-2 | | TNF-β | | IFN-γ | | |
| | Pre-therapy | Post-therapy | Pre-therapy | Post-therapy | Pre-therapy | Post- therapy | |
| group I (65) | 10.2±3.7 | 9.5±3.8 | 25.3±7.4 | 24.9±4.5 | 29.5±6.9 | 27.7±7.3 | >0.25 |
| group II (73) | 13.5±6.7 | 38.4±6.2 | 18.0±4.6 | 55.4±10.1 | 27.4±7.1 | 77.1±18.2 | <0.01 |

serum levels of several Th1 type cytokines (IL-2, TNF-β, IFN-γ) were detected with ELISA techniques in both groups to contrast results and evaluate the anti-tumor immune activity of the patients in two groups. The ELISA Kit was bought from Bangding Biotechnic Company in Beijing, and the results were recognized with a mean value of A450nm.

**Method of follow-up** The follow-up was made by a group of experienced doctors. Patients were checked regularly at a 3-6 month interval after operation in the outpatient department. Checking items included general physical examination such as supraclavicular lymph nodes and anus digital palpation, blood and urine routine, liver and kidney function, serum CEA, B-ultrasound of liver and spleen, also CT when necessary. We managed to keep in corresponding and phonic touch with these patients. Patient' s situation and tumor status were determined according to the clinical manifestation and associated examinations. The death time and cause were defined and recorded carefully. Those who lost to follow-up were also recognized as dead.

### *Statistical methods*

The result of cytokine detection was analyzed with Student *t* test, the recurrent rate in abdominal cavity and metastasis rate in liver with $\chi^2$ test,and 3-year-survival rate with survival curve.

## RESULTS

### *The changes of some Th1 cytokine levels in the peripheral blood of the patients after intraperitoneal immunotherapy with IL-2*

The levels of IL-2, TNF-β, INF-γ in the peripheral blood of the patients who received IL-2 intraperitoneal therapy were obviously increased as compared with the control group. The difference was significant ($P$<0.01). And there were no significant changes in the levels of the same cytokines in control group ($P$>0.25, Table 2).

### *The effect of IL-2 intra-peritoneal immunotherapy*

The 3-year follow-up ratio of the cases was 91.3 %, the result is shown in Table 3.

**Table 3** The therapeutic efficiency of intraperitoneal thermochemotherapy combined with IL-2 immunotherapy

| Groups | *n* | Intraperitoneal recurrent rate (%) | Hepatic metastasis rate(%) | 3-year survival rate(%) |
|---|---|---|---|---|
| Group I | 65 | 29.2(19 / 65) | 16.9(11 / 65) | 47.7(31 / 65) |
| Group II | 73 | 12.3(9 / 73)[b] | 10.9(8 / 73)[a] | 65.8(48 / 73)[a] |

[a]$P$<0.05 *vs* control group, [b]$P$<0.01 *vs* control group

Based on the comparison of intra-peritoneal recurrence rate, hepatic metastasis rate and 3-year survival rate, we could draw a conclusion that intraperitoneal thermochemotherapy combined with immunotherapy was effective in decreasing intraperitoneal recurrence and hepatic metastasis rate and raising 3-year survival rate ($P$<0.01-0.05 contrasted with control group). In our study, 4 cases were lost in group I and 8 in group II, and they were calculated as dead cases. Intraperitoneal spread, metastasis in liver and lung, uncontrollable hydrothorax and hydroperitoneum and dyscrasia at the end of advanced-stage cancer were accounted

for the death. In group I, only one patient with gastric carcinoma who received palliative operation survived for 2 years, while there were 2 cases in group II. And 4 (4/5) colorectal cancer cases of D-stage and 3 (3/7) in group II died from intraperitoneal spread. Although the number of cases was not big enough for statistical study, the therapeutic effect was indicated in some degree.

## DISCUSSION

Nowadays in most of formal hospitals, there are no technological difficulties with the radical operation of gastrointestinal cancer. Thus, how to raise the survival rate and the life quality of these patients depends much on the compound therapy following operation. Although routine chemotherapy (intravenously or orally) could help inhibit the liver metastasis, intraperitoneal spread and recurrence, its effect is still not satisfactory. In recent ten years, a large number of clinical studies have proved that postoperative intra-abdominal thermochemotherapy has exerted obvious therapeutic effect in inhibiting the recurrence of gastrointestinal cancer in abdominal cavity and liver metastasis[10-15], which is routinely applied in many hospitals. Intra-abdominal chemotherapy can be given at any time, but it can cause peritonitis, abdominal pain, and sometimes overlapped at short interval with intravenous or oral chemotherapy. Meanwhile, being a single therapy, it would bring about severe adverse effect following a long-term administration. On the other hand, intraperitoneal thermochemotherapy should be administered under general anesthesia and could not be applied repeatedly. Although its therapeutic effect is among the best, the low frequency of administration is its unavoidable defect. Based on this idea, more research should be made to seek a compound strategy with complementary therapeutic effect[16-24].

The intraperitoneal thermochemotherapy can increase the sensitivity of tumor cells to chemotherapy drugs and kill even more tumor cells than routine administration,and can efficiently lower the incidence of intraperitoneal recurrence and liver metastasis[24-27]. More importantly, thermal effect can increase the antigenicity of tumor cells and facilitate the expression of tumor antigens (such as heat shock proteins), which is conducive to immune effector cells to recognize and kill the tumor cells[28-30].

IL-2 is an effective anti-tumor cytokine, and it can induce and promote the activation and proliferation of T lymphocytes, increase the tumor-killing effect of effector cells, such as TIL, CTL, LAK and NK, and improve the general anti-tumor immune function of the body[31-37]. There are many lymph nodes and abundant lymphatic network in the abdominal cavity, and lots of lymph organs in the intestinal wall. When a high concentration of IL-2 is administered into the abdominal cavity and act on those lymphatic tissues and organs, the proliferation and killing capacity of lymphocytes is efficaciously promoted, under the background that the antigenicity of residual cancer cells has already increased due to the thermal effect, the anti-tumor effect of IL-2 would be maximized. The lymphocytes activated by IL-2 spreading with blood circulation will kill the metastatic foci in liver or other sites. In this study, the level of major Th1 cytokines in peripheral blood was significantly increased after immunotherapy, demonstrating that IL-2 could activate anti-tumor immune effect cells, induce the production of Th1 cytokines, enhance the anti-tumor immune function of the body, and kill tumor cells. In the immunotherapy group, the intra-abdominal recurrence and the incidence of liver metastasis were decreased by 16.9 % and 6.0 %, respectively as compared with the control group, which support the point that IL-2 immunotherapy combined with intraperitoneal thermochemotherapy is effective and applicable, and that it is appropriate to perform immunotherapy after thermochemotherapy, which may be a more scientific and reasonable strategy than other combinations and may contribute to the immunotherapeutic function and the complementation of the two therapies. Because IL-2 could promote the proliferation of lymphocytes and the latter might be inhibited by chemotherapy drugs, we did not combine IL-2 with chemotherapy drugs. This is worth of further research. During immunotherapy, most patients could tolerate and no obvious side-effect was observed. The common side-effect was the increase of body temperature (4 cases reached 38.8 ℃ and others in the range of 38.2-38.6 ℃) and physical cooling could take effect. Fever is another common side-effect of IL-2, which may disappear after the withdrawal of IL-2.

In this study, we found that, in the patients with peritoneal infiltration, the removal of the tumor as complete as possible during operation combining with thermochemotherapy and immunotherapy could produce satisfactory therapeutic effect. Four patients of this type survived for more than 3 years. So the intra-abdominal therapy for the gastrointestinal cancers should be paid enough attention, even to the intraperitoneal metastasis and infiltration in certain degree, resection or partial resection should be performed as completely as possible other than giving up. Immediate postoperative thermochemotherapy and immunotherapy could also improve the prognosis of some patients.

In conclusion,intra-abdominal metastasis of gastrointestinal cancer is an important factor in affecting the prognosis of the patients. In our study, the intraperitoneal thermochemotherapy and intraperitoneal immunotherapy have displayed a promising therapeutic and prophylactic effect, and research is need on this compound therapy upon our observation.

## REFERENCES


1 **Beaujard AC**, Glehen O, Caillot JL, Francois Y, Bienvenu J, Panteix G, Garbit F, Grandclement E, Vignal J, Gilly FN. Intraperitoneal chemohyperthermia with mitomycin C for digestive tract cancer patients with peritoneal carcinomatosis. *Cancer* 2000; **88**:2512-2519

2 **Kim JY**, Bae HS. A controlled clinical study of serosa-invasive gastric carcinoma patients who underwent surgery plus intraperitoneal hyperthermo-chemo-perfusion (IHCP). *Gastric Cancer* 2001; **4**: 27-33

3 **Witkamp AJ**, de Bree E, Kaag MM, Boot H, Beijnen JH, van Slooten GW, van Coevorden F, Zoetmulder FA. Extensive cytoreductive surgery followed by intra-operative hyperthermic intraperitoneal chemotherapy with mitomycin-C in patients with peritoneal carcinomatosis of colorectal origin. *Eur J Cancer* 2001; **37**: 979-984

4 **Sugarbaker PH**, Yonemura Y. Palliation with a glimmer of hope: management of resectable gastric cancer with peritoneal carcinomatosis. *Hepatogastroenterology* 2001; **48**: 1238-1247

5 **Elias DM**, Ouellet JF. Intraperitoneal chemohyperthermia: rationale, technique, indications, and results. *Surg Oncol Clin N Am* 2001; **10**: 915-933

6 **Chen J**, Wang S, Xu H. Curative effect of radical gastrectomy combined with peritoneal lavage with thermal hypoosmotic solution in treatment of gastric cancer. *Zhonghua Yixue Zazhi* 2001; **81**: 730-732

7 **Takahashi I**, Emi Y, Hasuda S, Kakeji Y, Maehara Y, Sugimachi K.Clinical application of hyperthermia combined with anticancer drugs for the treatment of solid tumors. *Surgery* 2002; **131**: S78-84

8 **Chen ZX**, Chen JP, Chen Z, Peng DS, Zhen JX, Tan JS. Treatment of cancerous ascites and radical gastrectomy with intraperitoneal hyperthermic double distilled water and cis-diaminodichloro-platinum perfusion. *China Natl J New Gastroenterol* 1997; **3**: 246-248

9 **Rau B**, Gaestel M, Wust P, Stahl J, Mansmann U, Schlag PM, Benndorf R. Preoperative treatment of rectal cancer with radiation, chemotherapy and hyperthermia: analysis of treatment efficacy and heat-shock response. *Radiat Res* 1999; **151**: 479-488

10 **Feng GG**, Zhou XG, Yu BM. Prevention of metastasis to liver by using 5-FU intraperitoneal chemotherapy in nude mice inoculated with human colonic cancer cells. *China Natl J New Gastroenterol* 1996; **2**: 134-135

11 **Cavaliere F**, Perri P, Di Filippo F, Giannarelli D, Botti C, Cosimelli M, Tedesco M, Principi F, Laurenzi L, Cavaliere R. Treatment of peritoneal carcinomatosis with intent to cure. *J Surg Oncol* 2000; **74**: 41-44

12 **Ceelen WP**, Hesse U, de Hemptinne B, Pattyn P. Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intra-abdominal cancer. *Br J Surg* 2000; **87**: 1006-1015

13 **Fujimura T**, Yonemura Y, Nakagawara H, Kitagawa H, Fushida S, Nishimura G, Miyazaki I, Shibata K. Subtotal peritonectomy with chemohyperthermic peritoneal perfusion for peritonitis carcinomatosa in gastrointestinal cancer. *Oncol Rep* 2000; **7**: 809-814

14 **Sugarbaker PH**, Yonemura Y. Clinical pathway for the management of resectable gastric cancer with peritoneal seeding: best palliation with a ray of hope for cure. *Oncology* 2000; **58**: 96-107

15 **Piso P**, Bektas H, Werner U, Schlitt HJ, Kubicka S, Bornscheuer A, Manns M, Klempnauer J. Improved prognosis following peritonectomy procedures and hyperthermic intraperitoneal chemotherapy for peritoneal carcinomatosis from appendiceal carcinoma. *Eur J Surg Oncol* 2001; **27**: 286-290

16 **Sobat H**, Juretic A, Samija M. Combined modality therapy of rectal cancers. *Ann Oncol* 1999; **10**: 99-103

17 **Cavaliere F**, Di Filippo F, Cosimelli M, Aloe L, Arcuri E, Anza M, Callopoli A, Di Lauro L, Morace E, Botti C, Natoli S, Tedesco M, Giunta S, Cavaliere R. The integrated treatment of peritoneal carcinomatosis. A preliminary experience. *J Exp Clin Cancer Res* 1999; **18**: 151-158

18 **Yonemura Y**, Fujimura T, Fushida S, Fujita H, Bando E, Nishimura G, Miwa K, Endou Y, Tanaka M, Sasaki T. A new surgical approach (peritonectomy) for the treatment of peritoneal dissemination. *Hepatogastroenterology* 1999; **46**: 601-609

19 **Samel S**, Singal A, Becker H, Post S. Problems with intraoperative hyperthermic peritoneal chemotherapy for advanced gastric cancer. *Eur J Surg Oncol* 2000; **26**: 222-226

20 **Rau B**, Wust P, Tilly W, Gellermann J, Harder C, Riess H, Budach V, Felix R, Schlag PM. Preoperative radiochemotherapy in locally advanced or recurrent rectal cancer: regional radiofrequency hyperthermia correlates with clinical parameters. *Int J Radiat Oncol Biol Phys* 2000; **48**: 381-391

21 **Shido A**, Ohmura S, Yamamoto K, Kobayashi T, Fujimura T, Yonemura Y. Does hyperthermia induce peritoneal damage in continuous hyperthermic peritoneal perfusion? *World J Surg* 2000; **24**: 507-511

22 **Abe T**, Sakaguchi Y, Ohno S, Ikeda Y, Kitamura K, Maehara Y, Sugimachi K.Apoptosis and p53 overexpression in human rectal cancer; relationship with response to hyperthermo-chemoradiotherapy. *Anticancer Res* 2001; **21**: 2115-2120

23 **Kunisaki C**, Shimada H, Nomura M, Akiyama H, Takahashi M, Matsuda G. Lack of efficacy of prophylactic continuous hyperthermic peritoneal perfusion on subsequent peritoneal recurrence and survival in patients with advanced gastric cancer. *Surgery* 2002; **131**: 521-528

24 **Zhang GQ**, Qing SH, Zhou ZD, Qi DL, Hou BH. Animal experiment study of toxin of regional and system of MMC intraperitoneal hyperthemotherapy perfusion. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 592-593

25 **Sayag-Beaujard AC**, Francois Y, Glehen O, Sadeghi-Looyeh B, Bienvenu J, Panteix G, Garbit F, Grandclement E, Vignal J, Gilly FN. Intraperitoneal chemo-hyperthermia with mitomycin C for gastric cancer patients with peritoneal carcinomatosis. *Anticancer Res* 1999; **19**: 1375-1382

26 **Hou BH**, Qing SH,Dong FY,Qi DL,Zhang GQ,Zhao F,Yao XQ, Peng M. Effects of continuous hyperemia peritoneal perfusion chemotherapy on peritoneal implantation of human colonic cancer cells in nude mice. *Shijie Huaren Xiaohua Zazhi* 2000;**8**:650-653

27 **Luo F**, Sun JL, Ren DM, Cai D, Shen M. Effect of hyperthermia on telomerase activity and genes expression in human gastric cancer cell line. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 1261-1264

28 **Okamoto M**, Tazawa K, Kawagoshi T, Maeda M, Honda T, Sakamoto T, Tsukada K. The combined effect against colon-26 cells of heat treatment and immunization with heat treated colon-26 tumour cell extract. *Int J Hyperthermia* 2000; **16**: 263-273

29 **Sinha P**, Poland J, Schnolzer M, Celis JE, Lage H. Characterization of the differential protein expression associated with thermoresistance in human gastric carcinoma cell lines. *Electrophoresis* 2001; **22**: 2990-3000

30 **Wang XY**, Kazim L, Repasky EA, Subjeck JR. Characterization of heat shock protein 110 and glucose-regulated protein 170 as cancer vaccines and the effect of fever-range hyperthermia on vaccine activity. *J Immunol* 2001; **166**: 490-497

31 **Han DM**, Zhu XN, Huang ZG, Wang JJ, Cheng J, Fan EZ, Pian YS, Li Y, Zhang W. The observation on treatment effects of local adoptive immunotherapy in 33 cases with head and neck cancer. *Zhonghua Zhongliu Zazhi* 1997; **19**: 454-456

32 **Gravis G**, Viens P, Vey N, Blaise D, Stoppa AM, Olive D, Maraninchi D. Pilot study of immunotherapy with interleukin-2 after autologous stem cell transplantation in advanced breast cancers. *Anticancer Res* 2000; **20**: 3987-3991

33 **Rosenberg SA**. Progress in human tumour immunology and immunotherapy. *Nature* 2001; **411**: 380-384

34 **Buzio C**, Andrulli S, Santi R, Pavone L, Passalacqua R, Potenzoni D, Ferrozzi F, Giacosa R, Vaglio A.Long-term immunotherapy with low-dose interleukin-2 and interferon-alpha in the treatment of patients with advanced renal cell carcinoma. *Cancer* 2001; **92**: 2286-2296

35 **Mantovani G**, Maccio A, Madeddu C, Massa E, Mudu MC, Mulas C, Gramignano G, Massidda S, Murgia V, Lusso MR, Mura L. Immunotherapy (recombinant interleukin 2), hormone therapy (medroxyprogesterone acetate) and antioxidant agents as combined maintenance treatment of responders to previous chemotherapy. *Int J Oncol* 2001; **18**: 383-391

36 **Rosenberg SA**. Progress in the development of immunotherapy for the treatment of patients with cancer. *J Intern Med* 2001; **250**: 462-475

37 **Atkins MB**. Interleukin-2: clinical applications. *Semin Oncol* 2002; **29**: 12-17


**Edited by** Ma JY