

Combination of Local, Non-viral *IL12* Gene Therapy and Systemic Paclitaxel Chemotherapy in a Syngeneic ID8 Mouse Model for Human Ovarian Cancer

MARGIT MARIA JANÁT-AMSBURY¹, JAMES WILLIAM YOCKMAN²,
MATTHEW LINLEY ANDERSON¹, DIRK GÜNTER KIEBACK¹ and SUNG WAN KIM²

¹*Department of Obstetrics and Gynecology, Division of Gynecologic Oncology,
Baylor College of Medicine, Dryden Rd., Suite 1100, Houston, 77030 Texas;*

²*University of Utah, CCCD, 205 Bio-Polymer, 20 South 2030 East, Salt Lake City 84112, Utah, U.S.A.*

Abstract. *The in vivo feasibility of the previously established ID8 and ID8-VEGF ovarian cancer models for non-viral IL-12 gene delivery by itself or in combination with paclitaxel chemotherapy, was investigated in C57BL/6 black mice. The syngeneic mouse ovarian epithelium (MOSE) cancer cell line and its more aggressive variant, a VEGF-modified strain, were used to perform these experiments. Tumor growth and survival were observed in C57/BL6 mice, inoculated with both ID8 substrains. The superiority of IL-12 gene therapy in comparison to conventional paclitaxel chemotherapy in terms of tumor size and survival was demonstrated.*

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death among women in the United States and Northern and Western Europe (1). As the fourth leading cause of death among all cancers of women in Western countries, around 25,500 women were diagnosed with this disease in the United States in 2004 (2). Despite high response rates to cytoreductive surgery followed by platinum-based chemotherapy, the relapse rate is high, and consequently, for patients with advanced disease, the 5-year survival rate is approximately 25% to 35% (3, 4). Therefore, this tumor has the highest mortality rate among female reproductive tract malignancies. Ovarian cancer cells from the primary tumor subsequently disperse into the whole abdomen through the physiological flow of peritoneal fluid.

Correspondence to: Margit Maria Janát-Amsbury, MD, Ph.D., Baylor College of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Faculty Center, Dryden Road, Suite 1143, Houston, Texas 77030, U.S.A. Tel: 001 713 798 5072, Fax: 001 713 798 7957, e-mail: amsbuy@bcm.edu

Key Words: IL-12, gene therapy, ovarian cancer, *in vivo* ID8 mouse model.

These cells start forming solid secondary lesions and result in ascites formation. Because the site of disease recurrence is usually within the peritoneal cavity, intraperitoneal (*i.p.*) consolidation strategies may offer advantages by focusing treatment or the site of residual disease, while minimizing systemic toxicities (5, 6).

Paclitaxel (PCT) has demonstrated significant antitumor efficacy in clinical trials against a broad variety of tumors and has become one of the chemotherapeutic agents of choice for treating metastatic breast cancer, ovarian cancer and other cancer entities (7, 8). PCT has clinically significant, sometimes limiting, toxic side-effects at the predominantly used dose levels (9). With its current vehicle Cremophor EL (CEL), PCT presents a number of serious concerns with clinical implications, such as severe anaphylactoid hypersensitivity reactions and peripheral neuropathy (10, 11). It is now well recognized that alternative formulations need to be pursued to allow better control of the toxicity and avoid pharmacological interactions, such as those related to the use of CEL (12). One such alteration is the use of taxanes, such as taxotere or a different delivery system.

Interleukin-12 (IL-12) is one of the most potent experimental antitumor and antimetastatic cytokines (13, 14). It may overcome PCT-mediated T cell suppression (15). Systemically administered, rIL-12 protein has been accompanied by dose-dependent toxicities, which limit the treatment intensity and cause clinical complications (16). Local non-viral gene delivery of relatively low levels of IL-12 to the tumor site by direct injection induced an antitumoral effect without causing systemic toxicity (17), whereas untreated animals did not show these effects and responded like control animals. Cationic lipids and polymers are increasingly being considered for *in vivo* gene delivery, due to their relative stability, greater carrying capacity and ease of large-scale production (18, 19).

The use of a non-viral, polymeric gene delivery system controls the molecular composition and simplifies manufacturing and analysis. Medically used polymers are known to possess a lower antigenic potential. The transgene can be up to 7 kDa in size and still be transported efficiently into cellular targets.

Materials and Methods

Polymer synthesis. The facilitating agent, PPC (MPEG methoxypolyethylenimine-PEI branched methoxypolyethyleneglycol-Cholesterol), was synthesized as a white to yellowish granular powder, which was dissolved in water at 4.5 mg/mL prior to complex formation with the biological substance pHIL-12-005. PPC has a molecular weight of about 4000, PEI (Polyethylenimine) has a low molecular weight of only 1.8 kDa, while the molecular weights of PEG and cholesterol are 0.55 kDa and ~0.5 kDa, respectively (US patent number 6,696,038). The components PEI-cholesterol-chloroformate and methoxypolyethyleneglycol (MPEG) were reacted in chloroform under constant stirring for 4 h at room temperature (20-25°C). The mix was acidified and the PPC was acetone-precipitated and vacuum-dried. Its properties are comparable to WSLP (water-soluble lipopolymer).

Polymer synthesis. The polymer PCC was kindly provided by Expression Genetics, Inc. (US patent number 6,696,038).

Preparation of p2CMVmIL-12. The p2CMVmIL-12 plasmid was constructed by using two subunits (mIL-12p35 and mIL-12p40) of IL-12 ligated into the pCI vector (Promega Corp, Burlingame, CA, USA) each under the transcriptional control of a separate CMV promoter, as previously described (20).

Preparation of mIL-12/PPC complexes. The final drug product consists of 25 µg of p2CMVmIL-12 plasmid DNA forming a complex with the lipopolymer (PPC) gene carrier at a 20:1 N/P ratio prepared in 5% glucose.

ID8 mouse ovarian surface epithelial cell line. ID8, a cell line derived from the spontaneous *in vitro* malignant transformation of C57BL/6 mouse ovarian surface epithelial cells, was maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 4% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml transferrin, and 5 ng/ml sodium selenite (Roche, Indianapolis, IN, USA) in a 5% CO₂ atmosphere at 37°C. ID8 cells were generously provided by Dr. G. Coukos, the University of Pennsylvania, USA.

Tumor generation and treatment.

***In vivo* tumor inoculation.** Subconfluent wild-type ID8 cells (Figure 1b) and their VEGF-transfected subtype (Figure 1c) were trypsinized, washed twice and harvested by centrifugation at 1,000 xg for 5 min. A single-cell suspension was prepared in phosphate-buffered saline (PBS) and mixed with an equal volume of cold Matrigel (BD Biosciences, Bedford, MA, USA) at 10 mg/ml. For flank injections, a total volume of 0.5 ml containing 5x10⁶ ID8 and ID8/VEGF-transfected cells were injected subcutaneously into the flank of 6- to 8-week-old C57BL/6 mice. All procedures were

performed according to established guidelines of NIH and University of Utah Animal Care Committee.

The tumors were measured every 3-4 days using a digital Vernier caliper along the longest width (*W*) and the corresponding perpendicular length (*L*) and the tumor volume ($L \times (0.5W)^2$) was calculated (21). The volume, the area under the efficiency curve for each given time point and the overall area for each group were calculated using the formula: (current tumor volume + previous tumor volume)/2 x (current measurement time point - previous measurement time point). Fifty µl of Genexol-PM (paclitaxel formulation using polymeric micelles, Samyang Corp., Korea) solution were injected intravenously (*i.v.*) into the tail vein at 16 mg/kg following a biweekly schedule. This regimen is well established and closely resembles clinical dosing.

The p2CMVmIL-12/PPC complexes were prepared in 5% glucose, concentrated to a final concentration of 0.5 µg DNA/µl using a Centricon 100,000 MW concentrator (Millipore Corp., MA, USA), and then 50 µl of the complexes were injected locally at the tumor site of C57BL/6 mice at a dose of 25 µg pDNA/mouse on a weekly schedule. The mice were treated with either Genexol-PM alone, or p2CMVmIL-12/PPC alone, or in combination. The tumor volume and survival were compared to uninjected controls. Naked p2CMVmIL-12 and polymer/empty-plasmid controls had also been compared in earlier experiments (data not shown).

Statistical analysis. Data were analyzed by analysis of variance and Student's *t*-test. Data were considered statistically significant if *p* values were <0.05.

Results

Paclitaxel dose determination. *In vitro* cytotoxicity assays showing the sensitivity of ID8 cells towards Paclitaxel was as had been reported in our previous studies. Further comparisons between Taxol and Genexol-PM had demonstrated no difference in *in vitro* cytotoxicity (22).

IL-12 was shown to be as efficacious against aggressive VEGF-modified ID8 cells as PCT. The dosing strategy for systemic Genexol-PM was determined by *i.v.* injections of 16 mg/kg every 2 weeks for a total of three injections given on days 1, 14 and 28 for ID8 cell injected animals and for a total of two injections on days 1 and 14 for ID8-VEGF-injected animals. The treatment groups received either Genexol-PM only, p2CMVmIL-12/PPC only or both. Similar results had been obtained regarding tumor growth and survival with either weekly or biweekly injections in various *in vivo* tumor models (23). p2CMVmIL-12/PPC performed similarly to 16 mg/kg PCT alone in terms of influence on tumor size, but showed superiority in animals inoculated with the less aggressive ID8 variant (Figure 1 a, b, c). IL-12 resulted in an increased survival in mice bearing the more aggressive VEGF-modified variant of ID8 as compared to the untreated controls (Figure 1 d). There was no difference between the treatment groups on comparing IL-12, PCT and their combination.

The combined approach of local IL-12 gene delivery with systemic PCT treatment, led to a significant (*p*<0.05)

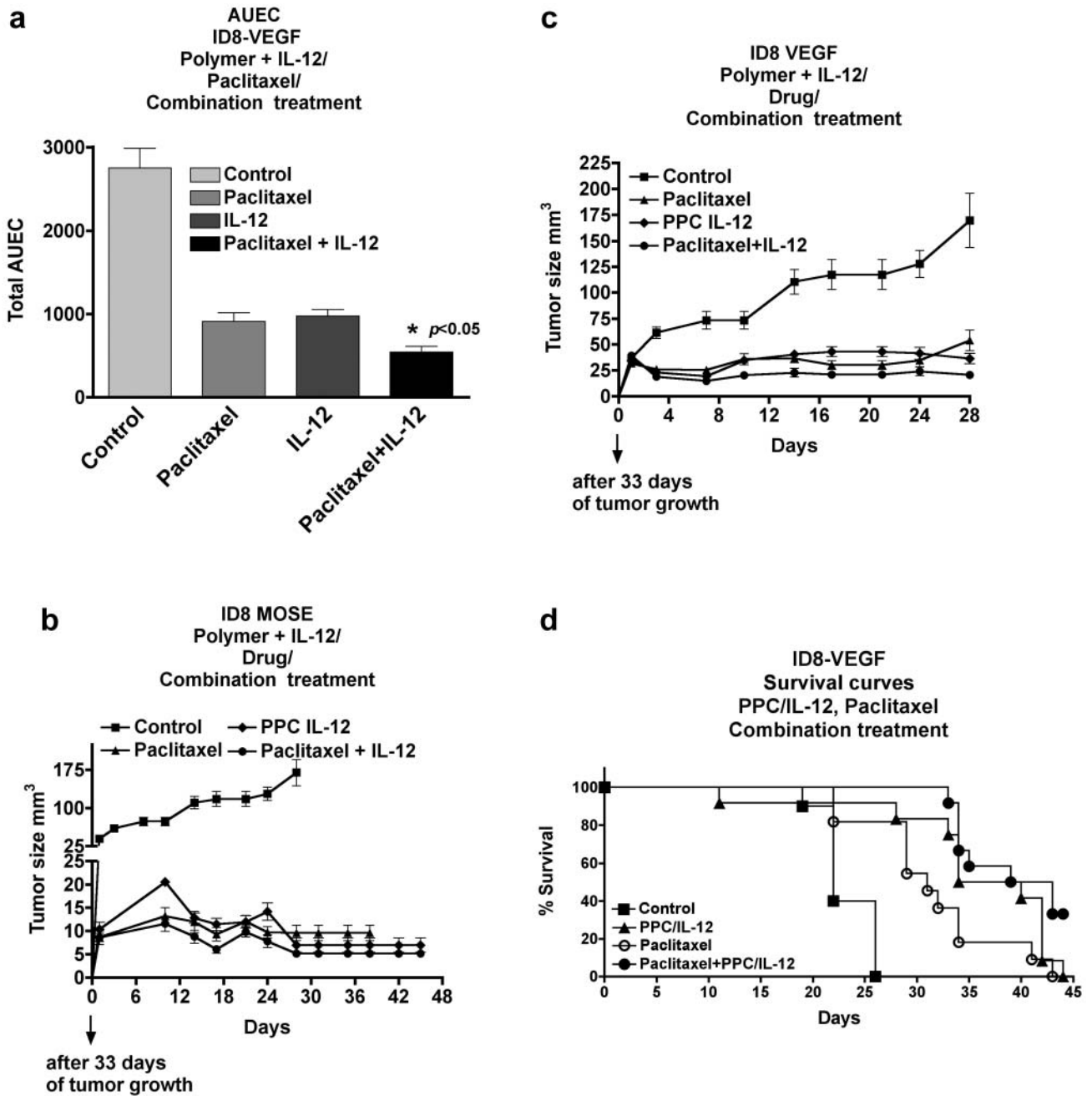


Figure 1. a) Effect of Paclitaxel, IL-12 and combination treatment of ID8-VEGF-modified ovarian cancer cells in vivo in a C57BL/6 black immunocompetent mouse strain, expressed as AUEC (area under efficiency curve). Whereas each treatment (Paclitaxel or IL-12) separately showed a significant difference compared to the untreated control group, the combination treatment including both Paclitaxel and IL-12. Survival curve showing the differences in survival for Paclitaxel, IL-12 and Paclitaxel+IL-12 treated C57BL/6 black mice, inoculated with ID8-VEGF-modified mouse ovarian cancer cells. Whereas 100% of the control animals were dead around day 26, 80% of the Paclitaxel only treated and 90% of the IL-12 only treated animals were still alive on day 26. Forty % of the animals, which were treated with the combination of IL-12 and Paclitaxel were still alive at day 45. b) In vivo tumor growth of unmodified ID8 tumor cells was inhibited equally through Paclitaxel, IL-12 or a combination of both. Tumor sizes did not exceed a size of 5 mm³ after day 30 for the animals treated with IL-12+Paclitaxel. Eight mm³ were not exceeded after day 30 for IL-12 only treated animals and 10 mm³ were not exceeded after day 30 for Paclitaxel only treated animals. c) In vivo tumor growth of VEGF-modified-ID8 tumor cells was inhibited equally through Paclitaxel, IL-12 or a combination of both. Tumor sizes did not exceed 23 mm³ for a period of time of 28 days for the animals treated with IL-12+Paclitaxel. Forty-five mm³ were not exceeded over the same time period for IL-12 only treated animals and a tumor size <50 mm³ was reached after day 28 for Paclitaxel only treated animals.

additive effect, when PCT was given every 14 days at a dose of 16 mg/kg. As compared to the control group, previous experiments had shown that PCT combined with the delivery of an empty plasmid (pCI₂; Promega Corp., Madison, WI, USA) showed no improvement over PCT or p2CMVmIL-12/PPC complexes alone, though all performed better than the uninjected controls ($p < 0.05-0.001$). An almost 50% increase in survival over 46 days was demonstrated with the combined therapy, as well as with p2CMVmIL-12/PPC by itself, as compared to the untreated controls. Genexol-PM was able to shrink the tumor size, although it showed non-significant 1-week survival deficit when compared to the other treatment groups.

Discussion

The anti-tumor activity of local, nonviral IL-12 gene delivery was equivalent to systemic PCT administration in an immunocompetent ID8 ovarian carcinoma model, based upon experiments involving tumor size, as well as survival data (Figure 1 a-d).

Novel approaches using polymers for local drug delivery have also shown promising results in metastasized breast cancer or malignant gliomas. Direct intratumoral injections of biodegradable, thermal gels/PCT resulted in efficacies equivalent to the maximum tolerated systemic dosage at doses that were 10-fold lower, with an excellent control of the release (24, 25). Local delivery aims at reducing the exposure of healthy tissue to antitumor agents, while increasing the drug concentration at the tumor site. In previous experiments only a negligible amount of IL-12 was found in the blood stream, thus, avoiding the risk of systemic toxicity of the IL-12 cytokines (19). IL-12 exerted potent antitumor effects in experimentally-induced and spontaneous tumors (26), related to the induction of T and NK cells (27, 28). As a result, IFN- γ (interferon-gamma) production was greatly enhanced. T cell development along the Th1 pathway was promoted (29) and had anti-angiogenic effects (30). IFN- γ affected mammary carcinoma metastasis (31) by acting on the host cells that secondarily produced factors to diminish tumor growth. Phagocytic cells are the main cell population involved in IFN- γ -mediated innate immunity. Neutrophils and macrophages are active phagocytic cells with tumoricidal properties, releasing incompletely reduced oxygen intermediates, such as nitric oxide (NO) (32, 33). Macrophage-depleted mice demonstrated significantly increased numbers of metastatic cells in the lungs after inoculation with 4T1 tumor cells (31). IFN- γ activates the macrophage production of NO, directly killing tumor cells. Consequently, macrophage-depleted mice have depressed NO production and show enhanced proliferation of metastatic tumors (7). NK and NKT cells are also critical components of natural immunity against

metastatic tumors (34). IFN- γ activates NKT cells, which produce IL-12, through a feedback mechanism to induce more IFN- γ . This primes macrophage populations for enhanced NO production. The systemic administration of IL-12 causes severe toxicity secondary to the necessity to administer supraphysiological concentrations to achieve adequate levels at the tumor site (16). Here, the local administration of IL-12 was focused on, using a non-viral vector system, circumventing these complications. The use of this delivery system was supported by earlier experiments on IL-12 levels, which were determined to be 3-fold higher for complexed IL-12 compared to naked IL-12. Those increased levels persisted for at least 5 days (35). Our previous work demonstrated the effects of repeated *IL12* gene delivery on survival (20). In contrast to the general belief that chemotherapy negatively affects the immune system, PCT also exerts positive effects on the NO pathway (7, 36). PCT increased the IFN- γ concentration indirectly by mimicking the bacterial lipopolysaccharide, activating dependent and independent pathways (37), and may also help increase transfection efficiencies through its antimetabolic function (38). A complicating factor in PCT chemotherapy has been the use of the solubilizer CEL. The pharmacokinetic behavior of CEL is dose-independent, although its clearance is greatly influenced by the duration of the infusion (39). This is important since CEL affects the activity of various drugs by changing the concentration of free drug through micellar encapsulation (6). CEL, therefore, modifies the toxicity profile of certain anticancer agents (40). Most solid tumors show extensive angiogenesis, hypervascularization, defective vascular architecture, and impaired lymphatic drainage/recovery systems compared to normal tissues. Tumors exhibit particularly enhanced vascular permeability, sustaining an adequate supply of nutrients and oxygen for rapid tumor growth. The enhanced vascular permeability and accumulation of plasma components in the interstitium occur also in macromolecules (MW >45 kDa). Many biocompatible water-soluble polymers show this effect. They are not cleared rapidly from the sites of the lesion and can remain localized for up to a week. This concept of enhanced permeability and retention (EPR) in solid tumors has become a gold standard in antitumor drug delivery (41-43). Genexol-PM's drug encapsulation is essential for optimal diffusion within the tumor environment and it increases drug retention compared to CEL. The EPR effect is responsible for the selective targeting of locally delivered lipid- or polymer-conjugated anticancer drugs. In the past success of systemic chemotherapy has been very modest and always accompanied by significant toxicity. Combining a systemic chemotherapeutic regimen with local IL-12 gene therapy may reduce systemic risks. A reduction in the primary tumor volume can be used to predict a reduction in

micrometastatic tumor volume resulting in clinical benefit. Using IL-12 as a component of a combined regimen may create an immune memory, potentially preventing the recurrence of disease and increasing the overall survival of the patient. The toxicity profile of non-viral vectors appears to be favorable. Most toxicity problems are related to unencapsulated DNA or protein fragments. This can be circumvented by improved encapsulation to achieve a balance between delivery and release. Using a polymeric solubilizer for PCT instead of the commercially used CEL (44), may achieve a higher component of free drug and an enhanced EPR effect. The long-term controlled release effect of this reduction in systemic side effects is observed as well in macromolecular drugs. We have previously been able to substantiate these findings (45).

The immunocompetent ID8 mouse ovarian cancer tumor cell line used in this study and the different antitumoral effects of IL-12 may provide a model for immunotherapy and gene therapy studies related to human ovarian cancer. Local gene therapy resulted in improved survival and can be combined with traditional chemotherapy. Polymeric delivery systems offer advantages for both treatment modalities with improved drug and gene delivery systems. It is probable that no single vector system is optimal for all gene therapy applications.

Acknowledgements

We kindly thank Expression Genetics Inc., USA, and Samyang Corp., Korea, for supporting this research.

References

- Runnebaum IB and Stickeler E: Epidemiological and molecular aspects of ovarian cancer risk. *J Cancer Res Clin Oncol* 127: 73-79, 2001.
- Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E *et al*: Cancer statistics, 2004. *CA Cancer J Clin* 54(1): 8-29, 2004
- McGuire WP, Brady MF and Ozols RF: The Gynecologic Oncology Group experience in ovarian cancer. *Ann Oncol* 10: 29-34, 1999.
- Piccart M, Bertelsen K, Stuart G *et al*: Long-term follow-up confirms a survival advantage of the paclitaxel-cisplatin (TP) regimen over the cyclophosphamide-cisplatin (CP) combination in advanced ovarian cancer (AOC). *Ann Oncol* 13: 109 (abstr 395), 2002.
- Markman M: Role of intraperitoneal chemotherapy in the front-line setting. *J Clin Oncol* 21: 145-148, 2003
- Barakat RR, Almadrone L, Venkatramon ES *et al*: A phase II trial of intraperitoneal cisplatin and etoposide as consolidation therapy in patients with stage II-IV epithelial ovarian cancer following negative surgical assessment. *Gynecol Oncol* 69: 17-22, 1998
- Mullins DW, Burger CJ and Elgert KD: Paclitaxel enhances macrophage IL-12 production in tumor-bearing hosts through nitric oxide. *J Immunol* 162: 6811-6818, 1999.
- Arbuck SG, Dorr A and Friedman MA: Paclitaxel (Taxol) in breast cancer. *Hematol Oncol Clin North Am* 8: 121-140, 1994.
- Loos WJ *et al*: Preclinical evaluation of alternative pharmaceutical delivery vehicles for paclitaxel. *Anticancer Drugs* 13: 767-775, 2002.
- Dorr RT: Pharmacology and toxicology of Cremophor EL diluent. *Ann Pharmacother* 28: S11-S14, 1994.
- Weiss RB *et al*: Hypersensitivity reactions from taxol. *J Clin Oncol* 8: 1263-1268, 1990.
- Gelderblom H, Verweij J, Nooter K and Sparreboom A: Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer* 37: 1590-1598, 2001.
- Brunda MJ *et al*: Antitumor and antimetastatic activity of interleukin 12 against murine tumors. *J Exp Med* 178: 1223-1230, 1993.
- Brunda MJ and Gately MK: Interleukin-12: potential role in cancer therapy. *In: Important Advances in Oncology*. Devita V, Hellman S and Rosenberg S (eds.), Lippincott Williams & Wilkens. Publishers, Philadelphia, PA, pp. 3-18, 1995.
- Mullins DW, Koci MD, Burger CJ and Elgert KD: Interleukin-12 overcomes paclitaxel-mediated suppression of T-cell proliferation. *Immunopharmacol Immunotoxicol* 20: 473-492, 1998.
- Cohen J: IL-12 deaths: explanation and a puzzle. *Science* 270: 908, 1995.
- Rakhmilevich AL, Janssen K, Hao Z, Sondel PM and Yang NS: Interleukin-12 gene therapy of a weakly immunogenic mouse mammary carcinoma results in reduction of spontaneous lung metastases *via* a T-cell-independent mechanism. *Cancer Gene Ther* 7: 826-838, 2000.
- Han S, Mahato RI, Sung YK and Kim SW: Development of biomaterials for gene therapy. *Mol Ther* 2: 302-317, 2000.
- Han S, Mahato RI and Kim SW: Water-soluble lipopolymer for gene delivery. *Bioconjugate Chem* 12: 337-345, 2001.
- Yockman JW, Maheshwari A, Han S and Kim SW: Tumor regression by repeated intratumoral delivery of water soluble lipopolymer/p2CMVIL-12 complexes. *J Controlled Release* 87: 177-186, 2003.
- Tomayko MM and Reynolds CP: Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol* 24(3): 148-154, 1989.
- Janát-Amsbury MM, Yockman JW, Lee M, Kern S, Furgeson DY, Bikram M and Kim SW: Combination of local, nonviral IL-12 gene therapy and systemic paclitaxel treatment in a metastatic breast cancer model. *Mol Ther* 9(6): 829-836, 2004.
- Zentner GM *et al*: Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J Controlled Release* 72: 203-215, 2001.
- Tannock IF, Lee CM, Tunggal JK, Cowan DS and Egorin MJ: Limited penetration of anticancer drugs through tumor tissue: a potential cause of resistance of solid tumors to chemotherapy. *Clin. Cancer Res* 8: 878-884, 2002.
- Sampath P *et al*: Camptothecin analogs in malignant gliomas: comparative analysis and characterization. *J Neurosurg* 98: 570-577, 2003.
- Cavallo F *et al*: Immune events associated with the cure of established tumors and spontaneous metastases by local and systemic interleukin 12. *Cancer Res* 59: 414-421, 1999.
- Seder RA, Gazzinelli R, Sher A and Paul WE: Interleukin 12 acts directly on CD4⁺ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc Natl Acad Sci USA* 90: 10188-10192, 1993.

- 28 Chan SH et al: Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J Exp Med* 173: 869-879, 1991.
- 29 Wolf SF, Sieburth D and Sypek J: Interleukin 12: a key modulator of immune function. *Stem Cells* 12: 154-168, 1994.
- 30 Strasly M et al: IL-12 inhibition of endothelial cell functions and angiogenesis depends on lymphocyte-endothelial cell cross-talk. *J Immunol* 166: 3890-3899, 2001.
- 31 Pulaski BA, Smyth MJ and Ostrand-Rosenberg S: Interferon-gamma-dependent phagocytic cells are a critical component of innate immunity against metastatic mammary carcinoma. *Cancer Res* 62: 4406-4412, 2002.
- 32 Debrick JE, Campbell PA and Staerz UD: Macrophages as accessory cells for class I MHC-restricted immune responses. *J Immunol* 147: 2846-2851, 1991.
- 33 Shtrichman R and Samuel CE: The role of gamma interferon in antimicrobial immunity. *Curr Opin Microbiol* 4: 251-259, 2001.
- 34 Smyth MJ et al: Differential tumor surveillance by natural killer (NK) and NKT cells. *J Exp Med* 191: 661-668, 2001.
- 35 Mahato RI, Lee M, Han S, Maheshwari A and Kim SW: Intratumoral delivery of p2CMVmIL-12 using water-soluble lipopolymers. *Mol Ther* 4: 130-138, 2001.
- 36 Wiseman LR and Spencer CM: Paclitaxel: an update of its use in the treatment of metastatic breast cancer and ovarian and other gynaecological cancers. *Drugs Aging* 12: 305-334, 1998.
- 37 Perera PY et al: CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression. *J Immunol* 166: 574-581, 2001.
- 38 Nair RR, Rodgers JR and Schwarz LA: Enhancement of transgene expression by combining glucocorticoids and anti-mitotic agents during transient transfection using DNA-cationic liposomes. *Mol Ther* 5: 455-462, 2002.
- 39 Sparreboom A et al: Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. *Cancer Res* 59: 1454-1457, 1999.
- 40 Szebeni J, Muggia FM and Alving CR: Complement activation by Cremophor EL as a possible contributor to hypersensitivity to paclitaxel: an *in vitro* study. *J Natl Cancer Inst* 90: 300-306, 1998.
- 41 Maeda H and Matsumura Y: Tumorotropic and lymphotropic principles of macromolecular drugs. *Crit Rev Ther Drug Carrier Syst* 6: 193-210, 1989.
- 42 Matsumura Y and Maeda H: A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 46: 6387-6392, 1986.
- 43 Noguchi Y et al: Early phase tumor accumulation of macromolecules: a great difference in clearance rate between tumor and normal tissues. *Jpn J Cancer Res* 89: 307-314, 1998.
- 44 Kang WK et al: Interleukin 12 gene therapy of cancer by peritumoral injection of transduced autologous fibroblasts: outcome of a phase I study. *Hum Gene Ther* 12: 671-684, 2001.
- 45 Maeda H, Fang J, Inutsuka T and Kitamoto Y: Vascular permeability enhancement in solid tumor: various factors, mechanisms involved and its implications. *Int Immunopharmacol* 3: 319-328, 2003.

Received March 20, 2006

Revised May 17, 2006

Accepted May 22, 2006