The dendritic therapy with its potential applications in pancreatic cancer

S. Van Gool

Pediatric neuro-oncology, University Hospital of Leuven, Leuven, Belgium.

Introduction

Several adult tumors remain a major challenge for the patients and physicians. Amongst these tumors, pancreatic carcinoma (PC) and high grade glioma (HGG) are tumors with very poor prognosis in spite of optimal oncological treatment. During the last years, novel treatment approaches include targeted therapy, anti-angiogenesis, gene therapy and immunotherapy.

General aspects of tumor immunology

Immunotherapy is aimed to modulate the interaction between the patient's immune system and the tumor so that the immune system acquires the capacity to eradicate tumor cells (1). Tumor antigens are recognized by CD8+ cytotoxic T cells (CTL), which are activated by antigen-presenting cells that express the antigen in the context of MHC class I molecules. CD4+ T helper cells are activated by antigens presented in the context of MHC class II molecules. CD4+ T cells produce cytokines which provide help for CTL activation. Activated CD4+ T cells stimulate antigen-presenting cells (cognate interaction), again resulting in improved CTL activation. CD4+ T cells play also a role in NK cell stimulation and B cell activation (antibody production). The most potent antigen-presenting cells are dendritic cells (DC). Immature DC (DCi) reside everywhere in the body and continuously transport antigens to the lymph node T cell zone to keep peripheral tolerance via maintenance of immune suppressive regulatory T cells (Treg). Only in case of danger signals, DC become mature (DCm) and activated, and induce CD4+ and CD8+ T cell activation and effector functions to the antigens presented by the DCm.

In most tumors, tumor-specific antigens are expressed. However, for full T cell activation, besides triggering of the T cell receptor by antigens, costimulatory signals are required. The necessary costimulatory ligands to induce T cell costimulation are usually not expressed on tumor cells, thereby inducing T cell anergy instead of T cell activation. Moreover, within the tumor microenvironment, a lot of immune suppressive mechanisms are active amongst which TGF-b, PGE2 and IL-10 are well known. Besides the existence of tumor-induced Treg, T cell anergy, and immune suppressive cytokines, a whole panel of mechanisms are present within the tumor microenvironment, and all these mechanisms together make tumor immune escape possible (2,3).

Since recent years, several groups were able to produce ex vivo patient-derived DCm, and to load these cells with tumor antigens (peptides, RNA, or proteins). Most of the technical aspects of the production of the vaccines are still in debate (4). Nevertheless, a lot of early clinical experiences with immunotherapy in general and DC vaccination particularly have been obtained for different types of tumors (5). These clinical trials are mostly early phase I/II clinical trials which conclude that the therapy is feasible without major toxicity but with only very limited clinical effect (6). Injection of these vaccines are aimed to re-inforce the immune system to act against the tumor cells.

Immunotherapy for high grade glioma

Since 2000, we have set-up a complete translational clinical research program to develop immunotherapy for patients with malignant glioma. We are accepted internationally as leader in this field (7). The prognosis of malignant glioma is dismal in spite of maximal neurosurgery, radiochemotherapy and maintenance chemotherapy with Temozolomide (TMZ) (8). At relapse, the prognosis is really poor, and all patients die within 18 months in spite of TMZ treatment (9).

In vitro characteristics of the cellular product

We developed DCm loaded with lysate of high grade glioma (DCm-HGG-L). In a preclinical in vitro model, we have demonstrated that DC are able to internalize proteins of the lysate, and present tumor protein fragments in the context of MHC molecules. DCm-HGG-L are able to stimulate T cell proliferation, switch to IFN-g production and generation of MHC-restricted MHC-dependent tumor antigen-specific CTL activity (10). A delicate cytokine balance was responsible for the generation of the tumor growth-suppressive functions (11).

Correspondence to : S. Van Gool, M.D. Ph.D., Pediatric neuro-oncology, University Hospital Leuven, Herestraat 49, 3000 Leuven, Belgium. E-mail : stefaan.vangool@uzleuven.be

Presented at the meeting of the Belgian Pancreatic Club, XXIst Belgian Week of Gastroenterology, 12.02.2009.

Submission date : 08/05/2009
Acceptance date : 09/05/2009

In vivo murine GL261 orthotopic mouse model

We have developed an orthotopic mouse model with GL261 tumor cells. We first developed a method to measure the tumor load with bioluminescence imaging (12). Next the mice were treated before tumor challenge with DC loaded with GL261 RNA. This model was chosen because in the clinical setting, DC vaccination is also provided at time of minimal residual disease after resection.

In the experimental condition (treatment with DCm-GL261-RNA), half of the mice could be cured, in contrast to untreated mice or mice treated with DC loaded with RNA from splenocytes or Lewis Lung Cancer. After depletion of CD8+ effector cells, mice in the control untreated group died earlier (demonstrating some spontaneous CD8+ T cell-mediated anti-tumor effect), and none of the DCm-GL261-RNA treated animals could be cured. After depletion of Treg, all mice survived without or with DC vaccination. However, upon rechallenge, only the mice that got DC vaccination prior to first tumor challenge were protected, while none of the surviving mice after Treg depletion showed any long-term protective antitumoral immune activity. These data demonstrate that only DC vaccination leads towards immunological memory (resubmitted after minor revision).

DC vaccination for patients with relapsed HGG

Methods. DC were injected intradermally according to 3 different vaccination schedules (cohort A, B, C). Cohort D consisted of cohort C with addition of local application of Imiquimod at the injection site. Adverse events were registered and scored according to the National Cancer Institute Common Toxicity criteria. Univariable and multivariable analyses were performed for progression free (PFS) and overall (OS) survival with age, extent of resection, pathology and cohort as prognostic variables. *Results.* 152 patients entered these cohorts. All patients received their vaccinations in an ambulatory setting. There were no serious adverse events except in one relapsed patient with gross tumoral disease prior to vaccination, who developed repetitive vaccine-related peritumoral edema. There is a significant better PFS and OS for total resection and significant worse PFS for GBM. There is no evidence that the effect of total resection and pathology differs between the cohorts. Both in the univariable analysis (stratified on cohort) and the multivariable analysis, the effect of age differs significantly between the four cohorts : throughout the cohorts, the beneficial effect for children (HR = 0.20 ; 95%CI = 0.08-0.52 in cohort A) progressively weakened in cohort D. Considering PFS in adult patients with relapsed GBM, the vast majority of the included patients, the PFS differed significantly between the 4 cohorts (p = 0.0097) with cohort D leading to the best PFS. This difference between cohorts was not present anymore for OS. *Conclusion.* Immunotherapy for patients with relapsed HGG is feasible without major adverse events. Long-term (> 24 months up to 7 years) survival after relapse is possible in a substantial group of patients. Especially total resection seemed to be an independent, favourable prognostic variable for both PFS and OS after multivariable analysis. The vaccination schedule seems to play a role in building up the immune-mediated control mechanisms in adults with relapsed GBM. Further improvement of the PFS in these patients could be realised by stimulating in vivo the ex vivo generated DC with Imiquimod. Data from these cohorts have been published (13-15).

DC vaccination integrated in the primary treatment for GBM : surgery, radiochemotherapy, immunotherapy, maintenance chemotherapy plus boost vaccines

Methods. After (sub)total resection of glioblastoma multiforme (GBM, grade IV HGG), leukapheresis was performed prior to radiochemotherapy (RCT), and 4 weekly vaccinations with autologous GBM lysate-loaded dendritic cells were given after RCT. Boosts with GBM lysates were given during TMZm. Immunomonitoring, feasibility and toxicity and progression free (PFS) and overall (OS) survival were assessed. National Cancer Institute Common Toxicity Criteria were used to grade the adverse events. *Results.* Serious adverse events during FU included an ischemic cerebral event, epilepsia with humerus fracture after fall due to epileptic fit, grade IV thrombopenia, short bowel perforation and cerebral abces. ELISPOT for tumor antigen-reacting IFN-g-producing T cells was performed on stored blood samples at different time points and results are pending. In the pilot patients (n = 8) with an actual FU of 32 months, median PFS is 17.8 months and median OS 24.3 months, with 3-year OS of 37.5%. In the phase I/II trial, with a median FU of 11.3 months, the preliminary median PFS = 11 months and median OS = 17.3 months for the per protocol analysis (n = 53). At the time of the joint meeting, data will be available for the full study group. *Conclusion.* Tumor vaccination integrated within the standard primary postoperative treatment for patients with newly diagnosed GBM is feasible, well tolerated and possibly beneficial for patients with minimal residual tumor burden. *Perspectives.* These early experiences with postoperative radio-chemo-immunotherapy provide good hope that integrating immunotherapy as fourth treatment modality in patients with primary diagnosis of HGG might result in a better disease control.

Organisation model for patient care

The implementation of immunotherapy for HGG in Belgium via one well-defined vaccination centre has an enormous impact on the organisation of the health care for patients with HGG in general. The current experience in UZ Leuven is very obvious. Putting patients in clinical treatment optimalisation protocols as such influences survival and quality of life of patients. This is a well

known general phenomenon (16). But in the proposed structure, there is an important further impact in the health care in general. Because the vaccination centre is qualified at the highest level not only for performing the immunotherapy but also for their advanced knowledge of the field of (pediatric) neuro-oncology in general, all patients, even those who got resection in regional centres, can ultimately receive upon presentation in the vaccination centre a multidisciplinary oncological consult, eventually as second opinion, by experts in the different fields from the multimodal anticancer treatment (neurosurgery, radiotherapy, chemotherapy, immunotherapy, imaging, pathologists). The current experience in UZ Leuven definitely demonstrates that also patients who would never had come to the university hospital obtain now an organised access to the multidisciplinary consult by experts in the field, even when it has to be decided that vaccination as such is not a therapeutic option for a particular patient. These patients at least receive a personalized advise on further (novel) treatment possibilities. The organisation model guarantees thereby that only patients with the appropriate indication have access to the innovative treatment approach integrated in standard multimodal anticancer therapy, and counterbalances the potential risk of uncontrollable commercially-induced expensive "cell traffic" outside the non-profit sector.

Immunotherapy for pancreatic cancer

Although we have no direct experience in the treatment of patients with pancreatic cancer (PC), we can offer our technology to the Belgian Pancreatic Club, and set-up a collaborative effort for this group of patients. Because we face also in PC with a variety of known and unknown antigens, similar to HGG, our SOP of producing clinically effective DCm loaded with lysate might be of high value for these patients. The rationale for exploring immunotherapy for patients with PC has been reviewed (17-19) and is based on the response to four critical questions. This mini-review is derived from a PubMed search with Mesh terms "Pancreatic neoplasms" (Majr) AND ("Immunotherapy, Active" OR "Dendritic cells") without limitations.

Is there a spontaneous interaction between pancreatic cancer and the immune system

High frequencies of functional tumor-reactive T cells have been found in PC patients (20). Some of these T cells are responsive to mesothelin (21,22) or to HER-2/Neu peptides (23). A spontaneous specific immune recognition of PC by patient-derived CD4 and CD8 T cells could be improved by IFN-γ (24). In case of extensive infiltration of T cells and DC, the prognosis of patients with PC seems improved (25). However, in most of the tissues, DC infiltration is rather minimal (26). If present, DC are sequestered in human PC by IL-8 (27). PC cells also produce IL-6 and G-CSF which suppress DC differentiation and activation (28). DC cultured in cytokines secreted by PC cells obtained a tolerogenic phenotype (29). MUC1 mucins interact with differentiating monocytes and induce also tolerogenic DC (30). Other studies similarly report on impaired function of circulating DC in patients with PC (31). After surgery, the function and count of circulating DC in patients with PC is improved (32). Phagocytosis of apoptotic PC cells by functional DC has been described (33). Finally, there are case reports which described prolonged survival with massive lymphocyte and DC infiltration after immune stimulation with rIL-2 demonstrating a potential redirecting of the immune response to the tumor cells (34).

Preclinical evidence of DC as functional antigen-presenting cells in the context of immunotherapy against pancreatic cancer

DC pulsed with PC total tumor RNA generated specific anti-PC T cell function (35). DC pulsed with tumor-derived RNA and CA 19-9 induced NK-like T cell activation that could overcome resistance of tumor cells (36). Other studies showed DC loading with apoptotic pancreatic tumor cells to be superior to lysate loading for cross-priming to activate CD8+ T cells and NK cells (37). Nevertheless, tumor cell lysate-pulsed human DC were able to induce T cell responses against PC cells (38). Functional DC could be generated ex vivo from patients who had surgical procedure for PC (39). In some conditions, the use of patient-derived CA 19-9 containing serum increased the immunostimulatory effect of the DC (40). This was further enhanced by using CA 19-9 protein (41). Other studies are exploring the use of allogeneic DC to generate peptide-specific CTL (42).

Preclinical evidence of immunotherapy for pancreatic cancer

Preclinical in vivo models of active specific immunotherapy have been generated in mice, rats and hamsters. Activation of DC by CD40L transfected in tumor cells could induce tumor suppression in an orthotopic mouse model of PC, suggesting that in vivo immune responses might induce tumor control (43). DC pulsed ex vivo with heat-treated tumor lysate could induce antitumor immunity in a murine PC model (44). DC pulsed with alpha-galactosylceramide induced antitumor immunity against PC (45). RNA-pulsed DC could also induce antitumor immunity in the pancreatic murine tumor model (46). The combination of lysate pulsed DC vaccination and anti-angiogenic treatment was efficient to induce tumor control in murine PC (47). DC pulsed with tumor lysate and transfected with adenoviral vector encoding IL-18 gene resulted in a specific and effective immune response against PC (48). DC-tumor cell fusion, combined with the activation signal SEB induced MUC1-specific T cells to enhance survival in a spontaneous PC tumor model (49). Finally, DC loaded with

apoptotic tumor cells and used as vaccine in combination with gemcitabine increased the survival of murine PC (50). As an alternative to the generation of an in vivo T cell immune response, telomerase-specific T cells were stimulated ex vivo with telomerase peptide pulsed DC and were able to induce tumor control in a syngeneic PC mouse model (51). In a subcutaneous model of rat duct-like PC, intratumoral injection of Flt3 ligand led to increased activation of DC and NK cells resulting in moderate reduction of tumor growth (52). In hamsters, DC loaded with lysate served as effective immunotherapy against established pancreatic cancer (53). DC pulsed with tumor lysate were also able to induce antitumor effect against peritoneal dissemination of the hamster PC (54).

Active specific immunotherapy in clinical practice

There are only a few clinical trials published yet, which show mainly the feasibility and toxicity of active specific immunotherapy. The first series of trials used peptides with adjuvants to generate immune responses. Four studies report on the clinical and immunological responses induced by ras peptide vaccination (55-58). Immune responses could also be measured in patients treated with personalized peptide vaccinations even combined with gemcitabine treatment (59). As an alternative to peptides, lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene have been used for the treatment of pancreatic adenocarcinoma (60). However, the optimal adjuvant to present tumor antigens are the DC. Injection of immature DC in advanced PC refractory to gemcitabine was feasible (61). Adoptive immunotherapy for PC using MUC1 peptide-pulsed DC and activated T cells was feasible and effective for PC (62). Immunotherapy with DC loaded with CEA mRNA was also reported as feasible and safe (63). Autologous DC pulsed with allogeneic tumor lysate was not toxic and resulted in an immunological anti-tumor response in a single patient (64). To our knowledge, there is no trial yet reported on immunotherapy with autologous mature DC loaded with autologous tumor lysate. Of note, two manuscripts report on DC vaccination for neuroendocrine PC (65,66).

Conclusion

We have established a world leading translational research program on the development and improvement of active specific immunotherapy for HGG based on autologous DC loaded with autologous tumor lysate. We are running larger-scale phase I/II clinical trials for children and adults with (relapsed) HGG according to a newly developed model of patient care that include advanced personalised cellular therapy. A review of the literature provide a strong rationale on the potential applicability of autologous DC loaded with autologous tumor lysate for patients with pancreatic cancer. These three facts strongly invite us to set-up a collaborative effort to implement DCm-PC-L into an early phase I/II clinical trial. Maximal surgical reduction of the tumor load thereby obtaining enough tumor material for the production of the lysate and thereby modulating the immune suppressive intratumoral milieu might be a prerequisite for optimal immune therapeutic efficacy.

References

1. VAN GOOL S.W., VAN DEN HOVE L., CEUPPENS J.L. Activation of the immune system in cancer patients. *Med. Pediatr. Oncol.*, 2000.
2. MELVOLD R.W., STICCA R.P. Basic and tumor immunology : a review. *Surg. Oncol. Clin. N. Am.*, 2007, **16** : 711-35, vii.
3. BHARDWAJ N. Harnessing the immune system to treat cancer. *J. Clin. Invest.*, 2007, **117** : 1130-1136.
4. TUYAERTS S., AERTS J.L., CORTHALS J., NEYNS B., HEIRMAN C., BRECKPOT K., THIELEMANS K., BONEHILL A. Current approaches in dendritic cell generation and future implications for cancer immunotherapy. *Cancer Immunol. Immunother.*, 2007, **56** : 1513-1537.
5. FIGDOR C.G., DE VRIES I.J.M., LESTERHUIS W.J., MELIEF C.J. Dendritic cell immunotherapy : mapping the way. *Nat. Med.*, 2004, **10** : 475-480.
6. ROSENBERG S.A., YANG J.C., RESTIFO N.P. Cancer immunotherapy : moving beyond current vaccines. *Nat. Med.*, 2004, **10** : 909-915.
7. DE VLEESCHOUWER S., RAPP M., SORG R.V., STEIGER H.J., STUMMER W., VAN GOOL S., SABEL M. Dendritic cell vaccination in patients with malignant gliomas : current status and future directions. *Neurosurgery*, 2006, **59** : 988-999.
8. STUPP R., MASON W.P., VAN DEN BENT M.J., WELLER M., FISHER B., TAPHOORN M.J., BELANGER K., BRANDES A.A., MAROSI C., BOGDAHN U., CURSCHMANN J., JANZER R.C., LUDWIN S.K., GORLIA T., ALLGEIER A., LACOMBE D., CAIRNCROSS J.G., EISENHAUER E., MIRIMANOFF R.O. ; EUROPEAN ORGANISATION FOR RESEARCH AND TREATMENT OF CANCER BRAIN TUMOR AND RADIOTHERAPY GROUPS ; NATIONAL CANCER INSTITUTE OF CANADA CLINICAL TRIALS GROUP. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Eng. J. Med.*, 2005, **352** : 987-996.
9. BRADA M., HOANG-XUAN K., RAMPLING R., DIETRICH P.Y., DIRIX L.Y., MACDONALD D., HEIMANS J.J., ZONNENBERG B.A., BRAVO-MARQUES J.M., HENRIKSSON R., STUPP R., YUE N., BRUNER J., DUGAN M., RAO S., ZAKNOEN S. Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. *Ann. Oncol.*, 2001, **12** : 259-66.
10. DE VLEESCHOUWER S., ARREDOUANI M., ADE M., CADOT P., VERMASSEN E., CEUPPENS J.L., VAN GOOL S.W. Uptake and presentation of malignant glioma tumor cell lysates by monocyte-derived dendritic cells. *Cancer Immunol. Immunother.*, 2005, **54** : 372-382.
11. DE VLEESCHOUWER S., SPENCER LOPES I., CEUPPENS J.L., VAN GOOL S.W. Persistent IL-10 production is required for glioma growth suppressive activity by Th1-directed effector cells after stimulation with tumor lysate-loaded dendritic cells. *J. Neurooncol.*, 2007, **84** : 131-140.
12. MAES W., DEROOSE C., REUMERS V., KRYLYSHKINA O., GIJSBERS R., BAEKELANDT V., CEUPPENS J., DEBYSER Z., VAN GOOL S.W. In vivo bioluminescence imaging in an experimental mouse model for dendritic cell based immunotherapy against malignant glioma. *J. Neuro-Oncol.*, 2009, **91** : 127-39.
13. DE VLEESCHOUWER S., VAN CALENBERGH F., DEMAEREL P., FLAMEN P., RUTKOWSKI S., KAEMPGEN E., WOLFF J.E., PLETS C., SCIOT R., VAN GOOL S.W. Transient local response and persistent tumor control of recurrent malignant glioma treated with combination therapy including dendritic cell therapy. *J. Neurosurg.*, 2004, **100** (5 Suppl. Pediatrics) : 492-497.
14. RUTKOWSKI S., DE VLEESCHOUWER S., KAEMPGEN E., WOLFF J.E., KÜHL J., DEMAEREL P., WARMUTH-METZ M., FLAMEN P., VAN CALENBERGH F., PLETS C., SÖRENSEN N., OPITZ A., VAN GOOL S.W. Surgery and adjuvant dendritic cell-based tumour vaccination for patients with relapsed malignant glioma, a feasibility study. *Br. J. Cancer*, 2004, **91** : 1656-1662.
15. DE VLEESCHOUWER S., FIEUWS S., RUTKOWSKI S., VAN CALENBERGH F., VAN LOON J., GOFFIN J., SCIOT R., WILMS G.,

DEMAEREL P., WARMUTH-METZ M., SOERENSEN N., WOLFF J.E., WAGNER S., KAEMPGEN E., VAN GOOL S.W. Clinical experience of postoperative adjuvant dendritic cell-based immunotherapy in a large group of patients with relapsed glioblastoma multiforme. *Clin. Cancer Res.*, 2008, **14** : 3098-3104.
16. BLEYER W.A. Cancer in older adolescents and young adults : epidemiology, diagnosis, treatment, survival, and importance of clinical trials. *Med. Pediatr. Oncol.*, 2002, **38** : 1-10.
17. KAWAKAMI Y., OKADA T., AKADA M. Development of immunotherapy for pancreatic cancer. *Pancreas*, 2004, **28** : 320-325.
18. BRUSERUD O., GLENJEN N. A critical review of T cell targeting immunotherapy in pancreatic cancer : only a hypothesis or a real hope for the patients ? *Expert. Opin. Biol. Ther.*, 2002, **2** : 693-701.
19. KAUFMAN H.L., DI V.J. JR., HORIG H. Immunotherapy for pancreatic cancer : current concepts. *Hematol. Oncol. Clin. North Am.*, 2002, **16** : 159-97, viii.
20. SCHMITZ-WINNENTHAL F.H., VOLK C., Z'GRAGGEN K., GALINDO L., NUMMER D., ZIOUTA Y., BUCUR M., WEITZ J., SCHIRRMACHER V., BÜCHLER M.W., BECKHOVE P. High frequencies of functional tumor-reactive T cells in bone marrow and blood of pancreatic cancer patients. *Cancer Res.*, 2005, **65** : 10079-10087.
21. THOMAS A.M., SANTARSIERO L.M., LUTZ E.R. *et al.* Mesothelin-specific CD8(+) T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. *J. Exp. Med.*, 2004, **200** : 297-306.
22. LI M., BHARADWAJ U., ZHANG R., ZHANG S., MU H., FISHER W.E., BRUNICARDI F.C., CHEN C., YAO Q. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. *Mol. Cancer Ther.*, 2008, **7** : 286-296.
23. PEREZ S.A., SOTIROPOULOU P.A., SOTIRIADOU N.N., MAMALAKI A., GRITZAPIS A.D., ECHNER H., VOELTER W., PAWELEC G., PAPAMICHAIL M., BAXEVANIS C.N. HER-2/neu-derived peptide 884-899 is expressed by human breast, colorectal and pancreatic adenocarcinomas and is recognized by in-vitro-induced specific CD4(+) T cell clones. *Cancer Immunol. Immunother.*, 2002, **50** : 615-624.
24. SCHMITZ-WINNENTHAL F.H., ESCOBEDO L.V., BECKHOVE P., SCHIRRMACHER V., BUCUR M., ZIOUTA Y., VOLK C., SCHMIED B., KOCH M., ANTOLOVIC D., WEITZ J., BÜCHLER M.W., Z'GRAGGEN K. Specific immune recognition of pancreatic carcinoma by patient-derived CD4 and CD8 T cells and its improvement by interferon-gamma. *Int. J. Oncol.*, 2006, **28** : 1419-1428.
25. FULEIHAN R., AHERN D., GEHA R.S. CD40 ligand expression is developmentally regulated in human thymocytes. *Clin. Immunol. Immunopathol.*, 1995, **76** : 52-58.
26. DALLAL R.M., CHRISTAKOS P., LEE K., EGAWA S., SON Y.I., LOTZE M.T. Paucity of dendritic cells in pancreatic cancer. *Surgery*, 2002, **131** : 135-138.
27. FEIJOO E., ALFARO C., MAZZOLINI G., SERRA P., PEÑUELAS I., ARINA A., HUARTE E., TIRAPU I., PALENCIA B., MURILLO O., RUIZ J., SANGRO B., RICHTER JA., PRIETO J., MELERO I. Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8. *Int. J. Cancer*, 2005, **116** : 275-281.
28. BHARADWAJ U., LI M., ZHANG R., CHEN C., YAO Q. Elevated interleukin-6 and G-CSF in human pancreatic cancer cell conditioned medium suppress dendritic cell differentiation and activation. *Cancer Res.*, 2007, **67** : 5479-5488.
29. BELLONE G., CARBONE A., SMIRNE C., SCIRELLI T., BUFFOLINO A., NOVARINO A., STACCHINI A., BERTETTO O., PALESTRO G., SORIO C., SCARPA A., EMANUELLI G., RODECK U. Cooperative induction of a tolerogenic dendritic cell phenotype by cytokines secreted by pancreatic carcinoma cells. *J. Immunol.*, 2006, **177** : 3448-3460.
30. MONTI P., LEONE B.E., ZERBI A., BALZANO G., CAINARCA S., SORDI V., PONTILLO M., MERCALLI A., DI CARLO V., ALLAVENA P., PIEMONTI L. Tumor-derived MUC1 mucins interact with differentiating monocytes and induce IL-10highIL-12low regulatory dendritic cell. *J. Immunol.*, 2004, **172** : 7341-7349.
31. YANAGIMOTO H., TAKAI S., SATOI S., TOYOKAWA H., TAKAHASHI K., TERAKAWA N., KWON A.H., KAMIYAMA Y. Impaired function of circulating dendritic cells in patients with pancreatic cancer. *Clin. Immunol.*, 2005, **114** : 52-60.
32. TAKAHASHI K., TOYOKAWA H., TAKAI S., SATOI S., YANAGIMOTO H., TERAKAWA N., ARAKI H., KWON A.H., KAMIYAMA Y. Surgical influence of pancreatectomy on the function and count of circulating dendritic cells in patients with pancreatic cancer. *Cancer Immunol. Immunother.*, 2006, **55** : 775-784.
33. SHIMAMURA H., SUNAMURA M., TSUCHIHARA K., EGAWA S., TAKEDA K., MATSUNO S. Irradiated pancreatic cancer cells undergo both apoptosis and necrosis, and could be phagocytized by dendritic cells. *Eur. Surg. Res.*, 2005, **37** : 228-234.
34. NOBILI C., DEGRATE L., CAPROTTI R., FRANCIOSI C., LEONE B.E., ROMANO F., DINELLI M., UGGERI F. Prolonged survival of a patient affected by pancreatic adenocarcinoma with massive lymphocyte and dendritic cell infiltration after interleukin-2 immunotherapy. Report of a case. *Tumori*, 2008, **94** : 426-430.
35. KALADY M.F., ONAITIS M.W., EMANI S., ABDUL-WAHAB Z., PRUITT S.K., TYLER D.S. Dendritic cells pulsed with pancreatic cancer total tumor RNA generate specific antipancreatic cancer T cells. *J. Gastrointest. Surg.*, 2004, **8** : 175-181.
36. ZISKE C., MÄRTEN A., SCHÖTTKER B., BUTTGEREIT P., SCHAKOWSKI F., GORSCHLÜTER M., VON RÜCKER A., SCHEFFOLD C., CHAO N., SAUERBRUCH T., SCHMIDT-WOLF I.G. Resistance of pancreatic carcinoma cells is reversed by coculturing NK-like T cells with dendritic cells pulsed with tumor-derived RNA and CA 19-9. *Mol. Ther.*, 2001, **3** : 54-60.
37. SCHNURR M., SCHOLZ C., ROTHENFUSSER S., GALAMBOS P., DAUER M., RÖBE J., ENDRES S., EIGLER A. Apoptotic pancreatic tumor cells are superior to cell lysates in promoting cross-priming of cytotoxic T cells and activate NK and gammadelta T cells. *Cancer Res.*, 2002, **62** : 2347-2352.
38. SCHNURR M., GALAMBOS P., SCHOLZ C., THEN F., DAUER M., ENDRES S., EIGLER A. Tumor cell lysate-pulsed human dendritic cells induce a T-cell response against pancreatic carcinoma cells : an in vitro model for the assessment of tumor vaccines. *Cancer Res.*, 2001, **61** : 6445-6450.
39. PIEMONTI L., MONTI P., ZERBI A., BALZANO G., ALLAVENA P., DI C., V. Generation and functional characterisation of dendritic cells from patients with pancreatic carcinoma with special regard to clinical applicability. *Cancer Immunol. Immunother.*, 2000, **49** : 544-550.
40. MÄRTEN A., ZISKE C., SCHÖTTKER B., RENOTH S., WEINECK S., BUTTGEREIT P., SCHAKOWSKI F., KLINGMÜLLER D., SCHEFFOLD C., VON RÜCKER A., SAUERBRUCH T., SCHMIDT-WOLF I.G. Increase in the immunostimulatory effect of dendritic cells by pulsing with serum derived from pancreatic and colorectal cancer patients. *Int. J. Colorectal. Dis.*, 2000, **15** : 197-205.
41. MÄRTEN A., SCHÖTTKER B., ZISKE C., WEINECK S., BUTTGEREIT P., HUHN D., SAUERBRUCH T., SCHMIDT-WOLF I.G. *et al.* Increase of the immunostimulatory effect of dendritic cells by pulsing with CA 19-9 protein. *J. Immunother.*, 2000, **23** : 464-472.
42. PEIPER M., GOEDEGEBUURE P.S., EBERLEIN T.J. Generation of peptide-specific cytotoxic T lymphocytes using allogeneic dendritic cells capable of lysing human pancreatic cancer cells. *Surgery*, 1997, **122** : 235-241.
43. SERBA S, SCHMIDT J, WENTZENSEN N, RYSCHICH E, MÄRTEN A. Transfection with CD40L induces tumour suppression by dendritic cell activation in an orthotopic mouse model of pancreatic adenocarcinoma. *Gut*, 2008, **57** : 344-351.
44. KIM H.S., CHOO Y.S., KOO T., BANG S., OH T.Y., WEN J., SONG S.Y. Enhancement of antitumor immunity of dendritic cells pulsed with heat-treated tumor lysate in murine pancreatic cancer. *Immunol. Lett.*, 2006, **103** : 142-148.
45. NAGARAJ S., ZISKE C., STREHL J., MESSMER D., SAUERBRUCH T., SCHMIDT-WOLF I.G. Dendritic cells pulsed with alpha-galactosylceramide induce anti-tumor immunity against pancreatic cancer in vivo. *Int. Immunol.*, 2006, **18** : 1279-1283.
46. SCHMIDT T., ZISKE C., MÄRTEN A., ENDRES S., TIEMANN K., SCHMITZ V., GORSCHLÜTER M., SCHNEIDER C., SAUERBRUCH T., SCHMIDT-WOLF I.G. Intratumoral immunization with tumor RNA-pulsed dendritic cells confers antitumor immunity in a C57BL/6 pancreatic murine tumor model. *Cancer Res.*, 2003, **63** : 8962-8967.
47. MIYAZAKI J., TSUZUKI Y., MATSUZAKI K., HOKARI R., OKADA Y., KAWAGUCHI A., NAGAO S., ITOH K., MIURA S. Combination therapy with tumor-lysate pulsed dendritic cells and antiangiogenic drug TNP-470 for mouse pancreatic cancer. *Int. J. Cancer*, 2005, **117** : 499-505.
48. TANG Z.H., QIU W.H., WU G.S., YANG X.P., ZOU S.Q., QIU F.Z. The immunotherapeutic effect of dendritic cells vaccine modified with interleukin-18 gene and tumor cell lysate on mice with pancreatic carcinoma. *World J. Gastroenterol.*, 2002, **8** : 908-912.
49. MC CONNELL E.J., PATHANGEY L.B., MADSEN C.S., GENDLER S.J., MUKHERJEE P. Dendritic cell-tumor cell fusion and staphylococcal enterotoxin B treatment in a pancreatic tumor model. *J. Surg. Res.*, 2002, **107** : 196-202.
50. BAUER C., BAUERNFEIND F., STERZIK A., ORBAN M., SCHNURR M., LEHR HA., ENDRES S., EIGLER A., DAUER M. Dendritic cell-based vaccination combined with gemcitabine increases survival in a murine pancreatic carcinoma model. *Gut*, 2007, **56** : 1275-1282.

51. SCHMIDT J., RYSCHICH E., SIEVERS E., SCHMIDT-WOLF I.G., BUCHLER M.W., MÄRTEN A. Telomerase-specific T-cells kill pancreatic tumor cells in vitro and in vivo. *Cancer*, 2006, **106** : 759-764.
52. RYSCHICH E., HUSZTY G., WENTZENSEN N., SCHMIDT E., KNAEBEL HP., ENCKE J., MÄRTEN A., BÜCHLER MW., SCHMIDT J. Effect of Flt3 ligand gene transfer in experimental pancreatic cancer. *Int. J. Colorectal. Dis.*, 2007, **22** : 215-223.
53. AKIYAMA Y., MARUYAMA K., NARA N., HOJO T., CHENG J.Y., MORI T., WILTROUT R.H., YAMAGUCHI K. *et al.* Antitumor effects induced by dendritic cell-based immunotherapy against established pancreatic cancer in hamsters. *Cancer Lett.*, 2002, **184** : 37-47.
54. TAKIGAWA Y., AKIYAMA Y., MARUYAMA K., SUGIYAMA K., UCHIDA E., KOSUGE T., UEDA M., KITAJIMA M., YAMAGUCHI K. Antitumor effect induced by dendritic cell (DC)-based immunotherapy against peritoneal dissemination of the hamster pancreatic cancer. *Cancer Lett.*, 2004, **215** : 179-186.
55. GJERTSEN M.K., BUANES T., ROSSELAND A.R., BAKKA A., GLADHAUG I., SØREIDE O., ERIKSEN J.A., MØLLER M., BAKSAAS I., LOTHE R.A., SAETERDAL I., GAUDERNACK G. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant : Clinical and immunological responses in patients with pancreatic adenocarcinoma. *Int. J. Cancer*, 2001, **92** : 441-450.
56. GJERTSEN M.K., BJORHEIM J., SAETERDAL I., MYKLEBUST J., GAUDERNACK G. Cytotoxic CD4+ and CD8+ T lymphocytes, generated by mutant p21-ras (12Val) peptide vaccination of a patient, recognize 12Val-dependent nested epitopes present within the vaccine peptide and kill autologous tumour cells carrying this mutation. *Int. J. Cancer*, 1997, **72** : 784-790.
57. GJERTSEN M.K., SAETERDAL I., THORSBY E., GAUDERNACK G. Characterisation of immune responses in pancreatic carcinoma patients after mutant p21 ras peptide vaccination. Br J Cancer 1996, **74** : 1828-1833.
58. GJERTSEN M.K., BAKKA A., BREIVIK J., SAETERDAL I., SOLHEIM B.G., SØREIDE O., THORSBY E., GAUDERNACK G. Ex vivo ras peptide vaccination in patients with advanced pancreatic cancer : results of a phase I/II study. *Int. J. Cancer*, 1996, **65** : 450-453.
59. YANAGIMOTO H., MINE T., YAMAMOTO K., SATOI S., TERAKAWA N., TAKAHASHI K., NAKAHARA K., HONMA S., TANAKA M., MIZOGUCHI J., YAMADA A., OKA M., KAMIYAMA Y., ITOH K., TAKAI S. Immunological evaluation of personalized peptide vaccination with gemcitabine for pancreatic cancer. *Cancer Sci.*, 2007, **98** : 605-611.
60. JAFFEE E.M., ABRAMS R., CAMERON J., DONEHOWER R., DUERR M., GOSSETT J., GRETEN T.F., GROCHOW L., HRUBAN R., KERN S., LILLEMOE KD., O'REILLY S., PARDOLL D., PITT H.A., SAUTER P., WEBER C., YEO C. A phase I clinical trial of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene for the treatment of pancreatic adenocarcinoma. *Hum. Gene Ther.*, 1998, **9** : 1951-1971.
61. IRISAWA A., TAKAGI T., KANAZAWA M., OGATA T., SATO Y., TAKENOSHITA S., OHTO H., OHIRA H. Endoscopic ultrasound-guided fine-needle injection of immature dendritic cells into advanced pancreatic cancer refractory to gemcitabine : a pilot study. *Pancreas*, 2007, **35** : 189-190.
62. KONDO H., HAZAMA S., KAWAOKA T., YOSHINO S., YOSHIDA S., TOKUNO K., TAKASHIMA M., UENO T., HINODA Y., OKA M. Adoptive immunotherapy for pancreatic cancer using MUC1 peptide-pulsed dendritic cells and activated T lymphocytes. *Anticancer Res.*, 2008, **28** (1B) : 379-387.
63. MORSE M.A., NAIR S.K., BOCZKOWSKI D., CUMMING R.I., VASOVIC L., GILBOA E., LYERLY H.K. The feasibility and safety of immunotherapy with dendritic cells loaded with CEA mRNA following neoadjuvant chemoradiotherapy and resection of pancreatic cancer. Int J Gastrointest Cancer, 2002, **32** : 1-6.
64. TAN G., WANG Z.Y., WANG X.G., CHENG L., YIN S. [Immunotherapeutic effects of beta-elemene combined with interleukin-23 gene-modified dendritic cells on murine pancreatic carcinoma]. *Ai Zheng*, 2006, **25** : 1082-1086.
65. SCHOTT M., FELDKAMP J., LETTMANN M., SIMON D., SCHERBAUM W.A., SEISSLER J. Dendritic cell immunotherapy in a neuroendocrine pancreas carcinoma. *Clin. Endocrinol. (Oxf.)*, 2001, **55** : 271-277.
66. SCHOTT M., SEISSLER J., FELDKAMP J., VON SCHILLING C., SCHERBAUM W.A. Dendritic cell immunotherapy induces antitumour response in parathyroid carcinoma and neuroendocrine pancreas carcinoma. *Horm. Metab. Res.*, 1999, **31** : 662-664.