Cancer Immunol Immunother (2000) 49:116–122

SYMPOSIUM SERIES

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Local cytokine therapy of cancer: interleukin-2, interferons and related cytokines

Received: 28 October 1999 / Accepted: 2 December 1999

Abstract Local therapy with interleukin-2 (IL-2) and other cytokines may be a very effective way to treat cancer. This was the theme of the First Symposium on Local Cytokine Therapy of Cancer: Interleukin-2, Interferons and Related Cytokines, in Hamburg, 29 April–1 May 1999. The abstracts are published in *Anticancer Research* 19: 1995–2016 (1999) [1]. Here we present a report.

Introduction

Local therapy of malignancies using interleukin-2 (IL-2), interferons (IFN), and related tumour-inhibitory cytokines, or genetically modified cell vaccines carrying inserted genes coding for these cytokines, represents a prospective modality of cancer treatment. Since the first preclinical studies [2, 4, 14] and clinical trials [8, 17, 29] with local administration of IL-2 and with tumour vaccines containing an inserted IL-2 gene and producing IL-2 [5], which were performed more than a decade ago, this modality of cancer therapy has been extensively developed.

Exposing tumour tissues or lymph nodes to continuous high doses of cytokines and avoiding high intravascular IL-2 levels reduce toxicity significantly. Regional application allows achievement of local tissue doses that cannot be reached by systemic therapy because of the dose-dependent toxicity induced by intravascular cytokines, especially IL-2. Clinical presentations of this concept at the symposium included repeated tumour instillation, injection, inhalation, intrasplenic and intrahepatic application, and transdermal ultradeformable carriers. The process of defining optimal schedules, doses, and treatment intervals is still in its infancy, as shown by the diversity of schedules used. However, some approaches already have clinical significance, and it is becoming obvious that effective antitumour therapy need not be toxic.

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Genetically modified vaccines

Thomas Blankenstein (Berlin) discussed the sequence of events leading to tumour immunity and focused his presentation on direct and indirect T cell priming by antigen-presenting cells and dendritic cell vaccines [17]. As key events in this process, the $CD4^+$ T-cell-mediated help in the activation of $CD8^+$ cytotoxic T lymphocytes as well as co-ordinated and time-dependent expression of IFNγ were considered.

Jan Bubeník (Prague) described IL-2 gene therapy of surgical minimal residual tumour disease, which had substantially contributed to the reduced recurrence rate of the tumours after operation. The effect of IL-2 therapy was studied in two preclinical models of tumours transplanted in syngeneic mice, 3-MC-induced sarcoma MC12, and HPV 16 E6/E7 oncogene-induced carcinoma MK16. Mice were inoculated s.c. with tumour cells and, when the tumours reached 8–10 mm in diameter, they were excised. The recurrence rate of the MC12 sarcoma was approximately 30% and that of the MK16 carcinoma was 80%. When, 2–5 days after surgery, the mice were injected s.c. to the site of the tumour resection with irradiated IL-2-producing tumour vaccine, 95% of mice with recurrences of the MC12 sarcoma and 56% of mice with recurrences of the MK16 carcinoma were completely cured. These results suggest that surgical minimal residual tumour disease is suitable for local gene therapy with IL-2-producing tumour vaccines. In addition to the efficacy of MC12-tumour-specific vaccines in the MC12 sarcoma system, in the MK16 carcinoma experiments also, non-specific, IL-2-producing tumour vaccines, which did not share any tumour-rejection antigen with

the MK16 cells and served only as a local source of IL-2 production, were efficient [6, 31].

Ralf Kircheis (Vienna) had used murine B16 melanoma cells engineered to express IL-2, IFNγ or both cytokines for prophylactic and therapeutic vaccination against the parental B16 tumour. A dose-dependent protection was found for vaccines expressing IL-2 or IFNγ or both cytokines in prophylactic vaccination models. In a therapeutic vaccination model, immunisation with cells expressing both cytokines, IL-2 and IFNγ, induced complete tumour regression in 50% of mice with measurable tumours. Vaccines consisting of IL-2 liposomes and irradiated melanoma cells protected mice against a lethal challenge with B16 melanoma cells as well as against IL-2-gene-modified B16 melanoma cells. It has also been shown that IL-2-gene-modified allogeneic melanoma cell vaccines can induce cross-protection against the syngeneic tumour. Efficacy of the modified allogeneic vaccines was comparable to that of the IL-2-gene-modified syngeneic vaccine.

Ron Apte (Beer-Sheva) compared the antitumour potential of IL-1α- and IL-1β-gene-transfected fibrosarcoma cells. Murine fibrosarcoma cells were transfected with cDNA of the precursor form of IL-1α, the mature form of IL-1β or a fusion cDNA of the IL-1β linked to the signal sequence of the IL-1Rα (ssIL-1β), to allow active secretion of the cytokine through the endoplasmic reticulum/Golgi pathway. Tumour cells over-expressing IL-1β in both forms did not manifest reduced tumorigenicity patterns; cells transfected with ssIL-1β were even more tumorigenic than the parental tumour. In contrast, cells over-expressing IL-1α manifested reduced tumorigenicity; they either did not grow in mice or grew and subsequently regressed. Regression of IL-1α-positive fibrosarcomas was mediated by antitumour immune responses, including the development of an immune memory, which protected mice from challenge with the wild-type tumour cells. Immuno-therapeutic effects were obtained when the wild-type-sarcoma-bearing mice were treated with cells from the same line engineered to express IL-1α. The results point to differential effects of the IL-1 molecules in inducing antitumour immunity; IL-1β may potentiate tumour development or angiogenesis, whereas IL-1α in its membrane-associated form may serve as an adhesion molecule, to promote cell-to-cell interactions between immune effector cells.

Vladimír Vonka (Prague) had developed a model for studying immune mechanisms involved in rejection of HPV-induced cancer. Kidney cells from Syrian hamsters and C57BL/6 mice were transformed by E6/E7 genes of HPV 16 and activated Ha-*ras* oncogene. From highly oncogenic hamster K3/II cells a TK^- subline was derived and the HSV TK gene was transduced to these cells. HSV TK^+ cells, denoted KL1/6, were susceptible to ganciclovir. Ganciclavir also suppressed the growth of KL1/6-induced tumours. This treatment resulted in development of immunity against the challenge with the original K3/II cells. Simultaneous administration of KL1/6 cells and K3/II cells followed by ganciclavir treatment suppressed not only the growth of KL1/6-induced tumours but also the growth of K3/II-induced tumours. An attempt was also made to induce antitumour immunity by DNA immunisation employing plasmids for the cell transformation. This procedure was more efficient in hamsters than in mice. In the latter system the immunisation resulted only in a delay of tumour development and slower growth of the tumours. The effect was more pronounced if plasmids containing genes for granulocyte/macrophage-colony-stimulating factor (GM-CSF) or IL-2 but not for B7.1 were administered simultaneously [30].

Mario Colombo (Milan) reported that active immunisation with IL-12- but not IL-2-transduced C26 colon carcinoma cells can cure mice carrying lung metastases of the C51 colon carcinoma. C26 and C51 share the tumour-associated antigen encoded by the endogenous murine leukaemia virus *emv*-1 gene. The better performance of IL-12 vaccination was associated with antibody production of the IgG2a isotype. The C26 carcinoma cells were transduced with the gene coding for the human folate receptor (FR), an antigen over-expressed in several human tumours. The complete antigenic repertoire of C26/FR cells includes the above mentioned tumour-associated antigen coded by the *emv*-1 gene and another endogenous antigen, not yet cloned, recognised by a cytotoxic T lymphocyte (CTL) clone, plus the exogenous FR protein, which provides at least two different epitopes: one recognised in the context of $H\text{-}2K^d$, the other in the context of $H\text{-}2D^d$. BALB/c mice were injected i.v. with C26/FR to give lung metastases and vaccinated with C26/IL-12/FR on days 3, 6, 9 and 13. On day 16, mice were partially splenectomised and the splenic lymphocytes were frozen and used for retrospective study, in vitro, of the CD8 T cell response related to the treatment outcome. Vaccination cured 50% of mice and the effect was CD8-T-cell dependent. Mice either cured (responders) or not (non-responders) by vaccination developed tumour-specific CTL directed preferentially against endogenous C26-related tumour antigens or the FR antigen respectively. Tumours from vaccinated non-responding mice lost the expression of the FR transgene but maintained the expression of endogenous C26 antigens. Immuno-selection against FR antigen was not observed in tumours from non-vaccinated controls and from CD8-depleted vaccinated mice.

Richard Vile (Rochester) described, in a preclinical model system, viral transduction of tumour explants with cytokine genes for therapy of colorectal cancer. The efficacy of separate classes of therapeutic genes and delivery vectors, to induce antitumour responses, was compared. Two cytokines (IL-2 and GM-CSF) were compared with a costimulatory gene (B7.1) and a suicide gene (HSVtk). Efficacy against primary tumour growth was HSVtk[GCV], B7.1 > *puro*, IL-2 > GM-CSF, *neo* whereas efficacy in inducing antitumour immunity was GM-CSF, IL-2, > B7.1, HSVtk[GCV] > *puro*, *neo* in a prophylactic vaccination model. To exploit these data clinically, Vile demonstrated that colorectal tumours can be reproducibly explanted, established in short-term culture and rapidly transduced with adenoviral vectors,

so that as many as 90% of the cells can be engineered to express high levels of the most clinically relevant genes (GM-CSF or IL-2) within 1–2 weeks of surgery. Adenovirus-mediated gene delivery was reproducibly and significantly more efficient than retroviral transduction, suggesting that ex vivo, adenovirus-mediated vaccination of colorectal patients of the appropriate stage will be possible and effective.

Preclinical experience

J. Löhler (Hamburg) discussed the in vivo function of IL-2. IL-2-deficient mouse mutants were generated on a mixed (129/OlaxC57BL/6) genetic background and predominantly developed an ulcerative colitis, haemolytic anaemia and other auto-immune phenomena. To elucidate the complex disease syndrome of IL-$2^{-/-}$ mice, segregate individual traits, and to study the possible contribution of other genetic factors in its pathogenesis, the original IL-$2^{-/-}$ mice were bred to various genetic backgrounds. IL-$2^{-/-}$ mutants back-crossed to a homogeneous B6 background revealed a profound disturbance of the myeloid haematopoietic compartment, which, in the original knockouts, was only observed in a mild form. Introduction of the IL-2 deficiency into BALB/c mice by mating with the original mutant strain also led to a considerable modification of the phenotype. IL-$2^{-/-}$ BALB/c mice exhibited haemolytic anaemia, follicular hyperplasia of lymphoid organs, and inflammatory changes of the pancreas, liver, heart, lungs and thoracic blood vessels but not of the colon. In contrast to the initial IL-$2^{-/-}$ strain, which suffers from a relatively long lasting chronic disease, the BALB/c mutants all die within before they are 5 weeks old. The changes of the immune system are dominated by an uncontrolled, polyclonal activation and proliferation of T and B cells associated with increased production of auto-antibodies. Treatment of IL-$2^{-/-}$ BALB/c mice with anti-gp39 (CD40 ligand) antibody inhibited activation of B cells and $CD8^+$ T cells. However, it did not affect the activation of $CD4^+$ T cells. In such treated mice, amelioration of haemolytic anaemia but not of inflammatory lesions in most of the affected organs could be recognised. The primary immunological change of IL-$2^{-/-}$ mice was obviously an uncontrolled proliferation of activated $CD4^+$ T cells with an auto-reactive immune repertoire. According to the initially described in vitro functions of IL-2, it was expected that silencing of its gene should result in a profound immunodeficiency. By contrast, the findings in IL-2-deficient mutants suggest that, in vivo, this cytokine has a quite specific function in down-regulating immune responses and maintaining T cell homeostasis whereas other effects can be compensated by co-stimulatory signals or other cytokines.

Experimental animals

Willem Den Otter (Utrecht) had showed, in experimental animals, that local IL-2 therapy is far more effective than systemic IL-2 therapy and leads to systemic immunity. Cures were obtained in mice with breast cancer, lung cancer, rats with bladder cancer, guinea pigs with liver cancer and mice with mastocytoma, fibrosarcoma and lymphoma. Mice with very large infiltrated and disseminated tumour loads can be cured. Surprisingly, about 70% of the mice can also be cured with a single IL-2 injection. Acute and late toxic effects of *local* IL-2 therapy are negligible (review: [12]).

Jeroen van Moorselaar (Utrecht) had studied the therapeutic effect of IL-2 in rats with transplanted bladder cancer. IL-2 was given three times weekly intratumorally in bladder tumours in a total of ten injections. Treatment started when tumours reached a size of 0.2 cm^3. All tumours gradually decreased in size, and 60 % of the rats were free of tumour at day 80.

Robert Van Es (Utrecht, The Netherlands) had studied the effect of perilesional IL-2 injections in the VX2 head and neck cancer of the rabbit. VX2 squamous cell carcinoma was transplanted into both auricles of rabbits. Peritumoral injections were started when one of the tumours exceeded 2 cm^3 in size. Complete remissions of the primary tumours with simultaneous complete regression of the non-treated contralateral tumour occurred in 4 out of 12 rabbits.

Kees De Groot (Utrecht) presented data on the therapeutic application of biodegradable hydrogels for slow release of IL-2 in mice. IL-2-containing dextran-based hydrogels were prepared. IL-2 is slowly released from such gels by diffusion and by degradation of the hydrogel in the case of a biodegradable polymer. Macroscopic IL-2-loaded gels and IL-2-loaded injectable microspheres were developed. This allows a single implantation or single injection of IL-2-containing gels instead of IL-2 injections on 5 consecutive days. Results showed that these IL-2-containing hydrogels exert therapeutic effects similar to those of injections of free IL-2 during 5 consecutive days.

Conclusion

These papers show that, in experimental animals, local IL-2 therapy is effective against a variety of transplanted tumour types, that systemic effects are obtained, and that vast tumour loads can regress completely. Finally, a single injection of IL-2 or IL-2-containing hydrogel can cure a mouse.

Clinical experience with veterinary cancer

Rachel Stewart (Harare) had performed trials of IL-2 therapy of ocular squamous cell carcinoma [10] and vulval papilloma carcinoma complex [16] in cattle in Zimbabwe. Cattle with bovine ocular squamous cell carcinoma were treated with peritumoral IL-2 injections on 10 days in 2 weeks. Depending on the doses of IL-2, 50%–90% complete tumour regression was obtained. In addition, preliminary results suggest that single-dose

therapy is as effective as multiple-dose therapy with fewer side-effects. Cattle with bovine vulval papilloma carcinoma complex were treated with a single dose of IL-2. They have been followed for 9 months so far and 50% have shown partial regression, but none complete regression.

Wim Klein (Utrecht) had treated sarcoids in horses with (IL-2). Sarcoids are fibroblastic skin tumours with a variable epithelial component. It is the most frequent neoplasm in horses. Sarcoids were treated with IL-2 injections at the base of the tumour for 5 or 10 days. After injections on 5 consecutive days there were 42% complete remissions, 25% partial remissions and 33% without regression. The results with ten injections were not better than those with five injections.

Rafael Nickel (Norderstedt and Utrecht) had treated lower urinary tract carcinomas in dogs with local IL-2 therapy. Transitional cell carcinomas are the commonest primary tumours in the lower urinary tract. Tumours stage T1–T2, N0–1, M0 were treated by endoscopy-assisted injections of IL-2-containing liposomes into the tumour tissue. Two injections were given in 1 week. Of eight animals that entered the trial, five were still alive (mean 9 months) and two had to be humanety killed (9 and 14 months after treatment); in one of these dogs the problems leading to euthanasia were not tumour-related. Although tumour remission was not observed, the clinical condition of the dogs was improved or did not deteriorate and there was no radiological sign of lung metastasis. These preliminary results suggest a beneficial effect of IL-2 in dogs with transitional cell carcinoma of the lower urinary tract.

Paul Ziekman (Berkel-Enschot and Utrecht) had treated tumours in companion animals with local low-dose IL-2 therapy. Ten dogs and six cats with a variety of tumours were treated with IL-2. Complete remissions were obtained in four out of ten dogs; interestingly these four dogs were the only ones that had been treated with debulking surgery before local treatment of the remnant of the tumour with IL-2. In addition, there were two partial remissions. There were two complete remissions in cats.

Conclusion

The data obtained in veterinary cancer show that local treatment of a variety of cancer types with IL-2 leads to high percentages of cures and complete and partial remissions.

Clinical experience with human cancer

Guido Forni (Torino) discussed the possible use of cytokines to help the immune recognition of tumour antigens [25] and emphasised that it is the *local* cytokine that dictates the Th1 or Th2 immune memory mechanisms elicited by prompting tumour antigen presentation by different sets of antigen-presenting cells and inducing the release of distinct repertoires of secondary factors. In a series of parallel clinical studies, the Forni group has shown that low doses of IL-2 injected around the lymph nodes draining inoperable relapses of squamous cell carcinoma of the head and neck (SCCHN) often elicit complete and partial responses [9]. However, after a disease-free interval of 3–5 months, all these tumours relapsed, suggesting that immunosuppression bars the establishment of a long-lasting systemic immunity. It was felt that minimal residual disease after conventional management of primary tumours is a more appropriate setting for exploration of the ability of IL-2 to delay local relapses. Of the 220 patients recruited with a resectable SCCHN and oropharynx, 101 were randomly assigned to surgery and radiotherapy (if required) and 100 to IL-2 plus surgery and radiotherapy (if required). The patients of the IL-2 group received 5000 U IL-2 injected around the ipsilateral cervical lymph node chain daily for 10 days before surgery. After surgery (and radiotherapy), 5-day IL-2 courses were administered monthly for 1 year. Statistical analysis showed that patients receiving IL-2 displayed a significantly extended disease-free interval that led to longer overall survival. These data indicated that perilymphatic administration of low, non-toxic doses of IL-2 is a simple and manageable way to delay SCCHN relapses.

Reinder Bolhuis (Rotterdam) described the capacity of bi-specific monoclonal antibodies (bs-mAb) to induce lymphocyte activation and to trigger the lytic and lymphokine-producing machineries of T cells for in vivo preclinical and clinical studies. Bolhuis reported on the first international multicentre study of locoregional – i.e. intraperitoneal – treatment of ovarian cancer in patients with advanced disease. Immunotherapy consisted of two 5-day cycles of treatment with daily intraperitoneal injections of in vitro activated and expanded autologous T cells targeted with bs-mAb specific for CD3 on T cells and the foliate receptor, which is overexpressed on ovarian carcinoma cells. An impressive intraperitoneal response of 27% was observed, with 3 patients even showing complete remissions in the intraperitoneal cavity.

Dainius Characiejus (Vilnius) reported the negative results of i.p. IL-2 treatment of 4 patients with malignant ascites accompanying an advanced ovarian carcinoma. His review of the literature (seven papers) showed that 23% objective responses were obtained.

Willem Den Otter (Utrecht), Marek Zembala and Zygmund Dobrowolski (Craców) had treated patients with recurrent bladder carcinoma with locally applied low-dose IL-2 therapy. Ten patients with T1, G1/2, N0, M0 papillary bladder carcinoma were treated by five instillations of recombinant IL-2. Treatment started 2 days after incomplete transurethral resection and was delivered over 5 consecutive days. A marker lesion (<10 mm) was left to assess the effect of IL-2 at 2 months. The marker lesion had regressed completely after 2 months in 8 out of 10 patients. Marker lesions that had not regressed were surgically removed. One of these 2 patients has been tumour-free for 6 years. Four

of the 8 patients whose marker tumour regressed have been tumour-free for 4–6 years [11].

Three groups reported treatment of non-resectable and histologically confirmed liver metastases of different origin with local repeated intra-arterial injection of IL-2 with or without interferon γ, and combined with lipiodol. Catheters were inserted either percutaneously or by operation into the liver or spleen. Nick Lygidakis (Athens) had used his own regional treatment on 499 patients with different primary tumours metastatic to the liver [23]. Out of this group 70% of patients with liver metastases from colorectal cancer were objective responders or at least stable (complete response, CR 12%; partial response, PR 38%; stable disease, SD 20%). Their median survival was 25 months, and they included 8 patients with histologically confirmed complete remission who had received a long-term follow-up of 3–6 years. Sigmund Pomer (Heidelberg) treated 12 patients with metastatic renal cell carcinoma (RCC) and achieved 2 partial remissions and 8 stable conditions for a median of 32.5 months (range, 24–72 months). Hartmut Kirchner (Hanover) reported on a single case, a patient with progressive liver metastases from rectal carcinoma, who had been heavily pretreated and reached partial remission after treatment on the schedule suggested by Lygidakis.

Wolfgang Scheef (Bonn) demonstrated treatment of bone metastases with an instrument designed specifically for the injection of cytokines into the metastases. He showed impressive responses by comparing X-ray pictures taken before and after local cytokine injection (IL-2 or interferon β). In his experience with several hundred patients who had bone metastases of different origin, treatment resulted in pain reduction and recalcification in most of patients and complete responses in some.

Christian Hofer (Munich) presented an account of transdermal application of IL-2 with new ultra-deformable carriers called transfersomes. The system has not been in clinical use, but in mice it led to measurable uptake of IL-2 through the skin into the vascular system.

Paolo Lissoni (Milan) reported that the application of IL-2 and IL-12 separately in patients who had RCC led to significantly increased and decreased mean lymphocyte numbers respectively. Lymphocyte mean number achieved after IL-2 plus IL-12 was significantly higher than with IL-2. However, the mean lymphocyte numbers were higher than they were after IL-2 alone. This underlined a rationale for a possible association of these two major antitumour cytokines.

Probably the largest clinical experience in regional IL-2 application is related to *inhalation in treatment for pulmonary tumours* [1, 13, 15, 18, 20–23, 26–28, 32]. Twelve groups reported on inhalation of IL-2 for treatment of pulmonary or mediastinal metastases or primary tumours. Pulmonary metastases of renal-cell carcinoma make up by far the largest group.

Detailed information is available on local modulatory effects of exclusive inhalation of IL-2.

Joachim Müller-Quernheim (Borstel) reported local immunomodulatory effects of inhalation of IL-2. He had analysed the accessory function, measured as the accessory index (AI), of alveolar macrophages [32] and of peripheral blood monocytes (PBM) in patients treated exclusively with inhalation of IL-2 before and after 2 weeks of treatment, comparing pre-treatment indices of alveolar macrophages and PBM with those after administration of low, medium or high doses given five times daily. Inhaled IL-2 enhanced significantly and in a dose-dependent manner the accessory function of alveolar macrophages and, to a smaller extent, that of PBM. Spontaneous cytokine release (tumour necrosis factor α, IL-6, IL-8, MIP 1α) of alveolar macrophages remained unaltered. Müller-Quernheim's method of evaluation might be valuable in optimising clinical studies to improve therapeutic efficacy. It has proved sensitive enough to detect differences in response to different doses [23], and possibly to allow a titration of optimal doses in individual patients or for individual tumours. Rex Yung (Los Angeles) presented a dose escalation study using a single inhalation of IL–2 per day (doses of 2.5×10^6–20×10^6 IU/m^2). Extensive pulmonary-function tests were performed under therapy. The analysis is of special interest because it involves the only high-dose single daily inhalations; other groups have used multiple daily inhalations (three to five per day). So far, Rex Yung reported, there had been no dose-limiting toxicities or consistent lymphocytosis or eosinophilia. Serial inhalations of technetium 99-m diethylenetriaminepentaacetate after 3 weeks showed a decrease in tracer half-time in 75% of the patients; this suggests tracer uptake possibly due to an increase in alveolar permeability. There was no pulmonary oedema or impaired pulmonary function except that 2 of 12 patients had grade I reversible bronchospasm (shortness of breath), which responded to the use of a β-2 bronchodilator.

Use of the same dose (for example 36×10^6 IU Proleukin) as a single daily dose or split into five applications per day seemed to result in different levels of immunomodulation, and they probably cannot be seen as identical doses. Once completely saturated, IL-2 receptors do not respond to a further dose increase, which induces no additional immunomodulation. This could explain the lack of dose-limiting toxicity. Repeated stimulation with lower doses was considered possibly more effective.

Edith Huland (Hamburg) reported treatment of pulmonary metastases of renal cell carcinoma in 188 patients who had proven progression. High-dose inhalation of IL-2 (18–36 MIU/day, 6 days/week) had an explicit influence on pulmonary metastatic growth. IL-2 inhalation was given five times a day according to a standard operation protocol of the University of Hamburg [18, 21]. In 68% of patients, progressive disease was stopped (CR 3%, PR 10%, SD > 3 months occurred in 55% of patients for a median duration of 16.8, 10.3 and 7 months respectively). The median survival of 5.3 months, expected on the basis of risk analysis, was increased to 17.2 months (range, 0.9–67.3 months). A survival benefit was also detectable in patients in high-risk groups. Treatment was given until metastases either

disappeared or progressed, and patients were able to perform social roles (within their job, family, and so on) during a median treatment time of 7.2 months. The costs of such long-term cytokine therapy have to be discussed in relation to its low complication rate, good social performance status, minimal requirement of co-medication or co-treatment, and good quality of life.

Hans Heinzer (Hamburg) reported the first long-term prospective analysis of subjective and objective quality of life in 15 patients treated mainly with inhalation (18–36 MIU/day) and 10 patients treated intravenously (9×10^6 IU IL-2 m^{-2} day^{-1}). Questionnaires indicated an excellent acceptance except in 3 patients who felt too impaired by intravenous therapy to answer. Inhalation of IL-2 led to a moderate but significant decrease in quality of life to minus 15% after 1 month of treatment. Quality of life at months 3, 6, 9, and 12, however, was comparable with that before treatment. Subdomain analysis revealed a tendency to improved psychological-distress scores at months 6 and 9, probably because progressive tumour growth was controlled. Quality of life was stable for a mean of 13.4 months [15].

Axel Heidenreich (Marburg) reported that treatment for pulmonary metastatic RCC according to the Hamburg schedule prevented progression in 84.7% of patients (15.4% PR and 69.3% SD >3 months) for a mean response duration of 9 months (3–22 months). Toxicity did not exceed WHO grade II except in 1 patient.

Zoltan Varga (Marburg) described 14 patients with metastatic RCC and pulmonary metastases treated only with inhalation of IL-2 (3×10^6 IU/day, 5 days/week) and systemic IFNα (5 x 10^6 IU/m^2, three times/week). In addition, patients received vinblastine at 0.1 mg/kg every third week. Two CR, 6 PR, and 2 SD were observed for 3–36 months. Side-effects of inhalation were minimal compared with those of systemic therapy.

Petro Petrides (Berlin) had investigated the efficacy of an alternative regimen [27], which consisted of daily subcutaneous application of 18×10^6 IU IL-2 on days 1–14 and daily inhalation of 18×10^6 IU on days 16–26.

Two groups reported the use of IL-2 inhalation in renal cell carcinoma as second-line therapy in patients whose tumours had already progressed under systemic immunotherapy.

Jens Atzpodien (Hanover) had used inhaled IL-2 in heavily pretreated patients in addition to chemoimmunotherapy as developed by the Hanover group. The safety and tolerability of therapy were good. He reported long-term stabilisation in 30% of patients and a dramatic reduction of pulmonary tumour load in individual patients. Therefore, in 1998 he had initiated a prospective randomised clinical trial that combined inhalation of IL-2 with the Hanover schedule in a first-line setting.

Jan Roigas (Berlin) reported the use of inhalation therapy in a setting similar to the Hamburg schedule (36×10^6 IU/day split into six parts with five parts inhaled and one part applied subcutaneously) in 7 patients with metastatic RCC who had been pretreated unsuccessfully with chemoimmunotherapy (IL-2, IFNα, 5-fluorouracil). Toxicity did not exceed grades I and II except for coughing, and 2 patients were able to continue working. Four PR and 2 stable conditions with a median response time of 8 months were observed in the 7 patients. Tolerability and effectivity of inhaled IL-2 in patients with renal cell carcinoma has also been reported by others [22, 26].

Three groups discussed their experience with tumours other than renal-cell carcinoma.

Johannes Kullmer (Marburg) had treated patients for bronchio-alveolar carcinoma, a rare malignancy, with cycles of inhalation consisting of three 2-week courses of inhalation therapy ($3 \times 6 \times 10^6$ IU/day, 5 days/week) with a 1-week rest between the 2-week courses. All patients, except 1 who was stable for 170 days, had tumour progression. Side-effects were mild. Kullmer stated that the study design did not allow an answer to the question of whether higher dosages, a different schedule [20], or combination with other cytokines might have improved results, or whether the tumour itself was resistant to immunotherapy.

Alexander H. Enk (Mainz) presented the results of the first study [13] to use inhalation of IL-2 in patients with stage IV melanoma metastatic to the lung; many of them had been pretreated unsuccessfully with dacarbazine chemotherapy. A group of 27 patients were treated with 36×10^6 IU/day for 6 months in addition to four bolus infusions of dacarbazine (850 mg/m^2) every 4 weeks. Side-effects were minimal. Five patients experienced complete remission, 8 partial remission and 5 stable disease. He concluded that inhalation therapy for lung metastases is a promising addition to the therapeutic arsenal against malignant melanoma.

Uwe Wagner (Tübingen) was the first to combine inhalation of IL-2 with chemotherapy for treating patients who had gynaecological tumours metastatic to the lung [28]. Eight patients with breast carcinoma and 2 patients with ovarian carcinoma received 9×10^6 IU IL-2 in addition to different chemotherapy protocols four times per day 5 days/week during cytotoxic treatment. All patients had been pretreated, in general, with chemotherapy; some had systemic immunomodulation without success. Toxicity was limited to WHO grade I and grade II, mainly coughing. Progressive pulmonary metastases showed a partial response in 7 of 10 patients with a mean duration of 5.4 months. Two patients showed no change (7.5 months) and 1 patient had tumour progression. Wagner concluded that inhalation is feasible and constitutes an additive treatment for pulmonary metastases of gynaecological cancers in patients under chemotherapy for further tumour control [19].

Conclusion

Inhalation of IL-2 by cancer patients is reported to be well tolerated with good quality of life during treatment and might improve survival. Patients not eligible for systemic IL-2 can benefit from regional treatment [1].

Cytokines are physiologically local hormones, and their regional use could lead to good patient outcome (as measured, for example, by survival and quality of life)

according to ASCO guidelines 1996 [3]. Regional immunotherapy extends the possibility of IL-2 treatment beyond highly selected patients and seems to be a promising tool that will provide many patients with effective and tolerable therapy.

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