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# Eosinophils and C4 predict clinical failure of combination immunotherapy with very low dose subcutaneous interleukin-2 and interferon in renal cell carcinoma patients

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ABSTRACT

***Background and Objectives.*** **The clinical and immunologic activities of interleukin-2 (IL-2) in cancer patients have been extensively studied and described; however, in most of these studies, IL-2 was administered by intravenous bolus or continuous infusion, while the immunologic effects of IL-2 given by the subcutaneous (s.c.) route have not yet been well studied.**

***Design and Methods.*** **The present study was aimed at evaluating the effects of IL-2, given at very low doses s.c. to patients with advanced renal cell carcinoma (RCC), on a number of immunologic parameters: number of total lymphocytes, number of CD4–, CD8–, CD25-positive cells, number of natural killer (NK) cells, titers of IL-2 soluble receptor (sIL-2R) and of C4, eosinophils, eosinophilic cationic protein (ECP) and eosinophilic protein X (EPX). Finally, a logistic regression model was performed to identify early immunologic parameters that correlate with a favorable or unfavorable treatment outcome.**

***Results.*** **Independently from the mere report of the changes induced by immunotherapy, the analysis showed that, within the pre-treatment model, a large eosinophil number predicts the failure of IL-2 treatment; in contrast, within the post-treatment model, high C4 serum titers and, again, a large number of circulating eosinophils predict immunotherapy failure.**

***Interpretations and Conclusions.*** **As far as concerns C4, its negative predictive value could be related to the fact that it is an indirect index of macrophage activation; thus, even though macrophages release substances with antitumor activity, they can also stimulate the release of sIL-2R, which may compete for exogenous IL-2. Some authors have postulated that macrophages may even stimulate tumor cell growth, or impair NK activity. Despite a great amount of uncertainty concerning the role of eosinophils, in our study, blood eosinophilia predicts a poor response to immunotherapy in patients with advanced RCC, thus supporting previous observations from our own group.**


Key words: immunotherapy, immunologic parameters, eosinophils, C4; predictors

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Interleukin-2 (IL-2), a cytokine secreted primarily by $CD4^+$ T-helper and $CD8^+$ cytotoxic T-cells in response to antigen and/or other cytokine stimulation,[1] exerts a variety of biological effects on hemopoietic cells, including activation of cytotoxic T-cells, helper T-cells, macrophages, natural killer (NK) cells and B-cells.[2] *In vitro* studies and *in vivo* adoptive immunotherapy studies have clearly shown that IL-2 can activate antigen-specific and non-specific T-cell responses against tumors,[3,4] exert a direct cytotoxic effect on human carcinoma cells, at least in certain tumor models,[5] and revert adenocarcinoma-derived mucin-related immunosuppression.[6] Furthermore, IL-2 can also induce eosinophilia, probably through the production of secondary cytokines, e.g., IL-5, IL-3 and GM-CSF, by stimulated lymphocytes.[7]

The clinical and immunologic activities of recombinant human IL-2 (rhuIL-2) in cancer patients have been extensively studied and described; however, in most of these studies, rhuIL-2 was administered using chemotherapy guidelines such that the maximum tolerable dose was given by intravenous bolus or continuous infusion over a few days.[8,9]

Atzpodien *et al.* demonstrated that low doses of subcutaneous rhuIL-2, are both clinically and immunologically effective;[10] more recently, Buzio *et al.* have demonstrated that very low doses of subcutaneous rhuIL-2 can induce persistent immunologic effects, with objective antitumor activity and low toxicity,[11] the latter probably due to reduced induction of nitric oxide synthesis.[12]

The present study was aimed at evaluating the effects of rhuIL-2, given at very low doses using the protocol described by Buzio *et al.*[11] to patients with advanced renal cell carcinoma, on each of the following immunologic parameters: number of total lymphocytes, number of CD4-, CD8-, CD25-positive cells, number of NK cells (identified as $CD3^-$ and $CD56^+$ cells), titers of IL-2 soluble receptor (sIL-2R) and of C4; particular attention was paid to eosinophils and their cytotoxic products, eosinophilic cationic protein (ECP) and eosinophilic protein X (EPX), since the role of these cells and proteins, relative to IL-2-induced antitumor activity, remains highly controversial.[13-17] Finally, a logistic regression model allowed us to identify early immunologic parameters that correlate with a favorable or unfavorable treatment outcome.

## Design and Methods

### *Patients' selection and treatment schedule*

Twenty-five patients (16 males and 9 females, average age: 59.6 yrs, range: 42-71) affected by advanced RCC were treated subcutaneously with very low doses of rhuIL-2 plus interferon, according to the treatment protocol originally proposed by Buzio *et al.*[11] All patients gave their informed consent to enrollment into both the clinical protocol and biological studies, according to institutional requirements.

Briefly, rhuIL-2 was given subcutaneously for 5 days per week, together with recombinant interferon-$\alpha$ (rIFN-$\alpha$) by intramuscular route twice weekly, for 4 consecutive weeks corresponding to one treatment cycle. The cycle was regularly repeated at 4 months' intervals in all patients, irrespective of clinical response.

rhuIL-2 was administered at the dose of 1 MU/$m^2$ every 12 hours, on days 1 and 2, followed by 0.5 MU/$m^2$ twice daily on days 3-5 of each week; concomitantly, rIFN-$\alpha$ was given as 1.8 MU/$m^2$ on days 3 and 5 of each week.

All blood samples were drawn from each patient prior to and about 60 hours after the end of the first treatment cycle.

### *Immunologic study*

Total white blood cell count was calculated using a cell counter (Contron, San Diego, CA, USA), while differential count was performed, by a single investigator, on peripheral blood smears stained with May-Grünwald-Giemsa; the absolute number of lymphocytes and eosinophils was thus extrapolated.

Whole blood was collected in preservative-free heparin and mononuclear cells were separated by centrifugation through Ficoll-Hypaque (Pharmacia-Upjohn, Nerviano, Italy) and immediately analyzed, while serum was isolated and stored at less than <20°C for subsequent titration.

### *Flow cytometry*

Immunophenotyping of mononuclear cells was performed using a staining panel which included monoclonal antibodies to the CD4, CD8, CD3, CD56 antigens and to the activation marker CD25 (Beckton-Dickinson, Mountain View, CA, USA). Since the CD25 antigen is expressed not only by lymphocytes, its percent and not its absolute number is considered. Both single and double staining, the latter for the NK subset, were used.

Stained cells were washed in phosphate-buffered saline (PBS) and resuspended in PBS containing paraformaldehyde. Flow cytometric analysis was performed using FACScan apparatus (Beckton-Dickinson, Mountain View, CA, USA).

### *Serum assay of sIL-2R and C4*

The sIL-2R level was evaluated using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cellfree IL-2R test kit, T-Cell Diagnostic Inc., Cambridge, UK).

C4, being an indirect index of macrophage activation,[17] was titered using an commercial immunochemistry kit (Beckman Diagnostic System Group, Brea, CA, USA).

### *Study of eosinophilic function*

Eosinophils were counted on a peripheral blood smear stained with May-Grünwald-Giemsa (Sigma Chemicals, St. Louis, MO, USA) at 25x magnification, while serum ECP and EPX were assayed using a sensitive radioimmune (RIA) method (Pharmacia-Upjohn, Nerviano, Italy). The technique was based on the double antibody: sample ECP (or EPX) competed with an unvarying amount of $^{125}$iodine-labeled ECP (or EPX). When an immunoadsorbent antibody was added, free myeloperoxidase could be separated from bound myeloperoxidase by centrifugation and then decantating. Pellet radioactivity was measured in a $\gamma$-counter and was inversely proportional to sample ECP (or EPX) amount.[18]

### *Treatment outcome*

Clinical response to treatment, i.e., objective responses [complete responses (CR) plus partial responses (PR), stable disease (SD) or progression (P)], was assessed using commonly accepted WHO criteria,[19] before the start of each treatment cycle.

The aim of our study was to verify whether the biological modifications induced by the first treatment cycle only could be correlated with the best clinical outcome.

Furthermore, when the statistical analysis was performed according to treatment outcome, we considered objective response and stable disease together, because we reasoned that, especially in immunotherapy patients, a long-lasting control of tumor growth could be interpreted as a sign of immune response, as valuable as a tumor regression (and perhaps much more valuable than a short-lasting response).

### *Statistical analysis*

Data management and analysis were performed with the S-Plus statistical package.[20] Mean and standard deviation values were used as descriptive statistics. Paired and upaired *t*-tests were used to compare pre-treatment versus post-treatment values and responders versus non-responders, respectively. The correlation among variables was tested using the Pearson's product moment correlation coefficient. Finally, the independent effect of selected clinical variables with respect to clinical outcome (favorable - i.e., OR and SD, or unfavorable - i.e., P) was assessed by multiple logistic regression analysis.[20]

## Results

### *Clinical outcome*

Of the 25 treated patient, 6 (24%) had an objective response, equally distributed between CR and PR; 12 (48%) had stable disease, while 7 (28%) had progression despite treatment.

Patients' characteristics are summarized in Table 1.

Response to treatment was evidenced after the first cycle of immunotherapy in four patients; the other two responders had a delayed response, one after two cycles and one after three cycles.

The average duration of responses and stable disease were 14.6 (range: 8-24+) and 11 (range: 4-20) months, respectively.

Immunotherapy was well tolerated, WHO grade II fever being the most common side-effect observed, easily controlled by the administration of acetaminophene; other unwanted side-effects included flu-like syndrome, pain at injection site and peripheral edema requiring treatment with diuretics. Finally, no treatment discontinuations due to toxicity have been observed so far.

### *Immunologic study*

When all first cycles were considered together, independently of clinical response, rhuIL-2 + IFN treatment determined a statistically significant increase in the following immunologic parameters: number of lymphocytes, number of $CD4^+$, $CD8^+$ and NK cells, percent of $CD25^+$ cells, number of eosinophils, EPX, C4 and sIL-2R titers, while the variations in ECP titers did not reach statistical significance (Table 2).

When we compared first cycle data of patients developing a favorable clinical outcome, i.e., OR plus SD, against the data from patients with progressive disease, statistically significant post-treatment differences were shown for the number of lymphocytes, eosinophils, $CD4^+$ T cells and for the serum titers of C4 and sIL-2R, which were significantly higher in non-responding patients (Table 3).

Furthermore, when all patients were considered together, independently of the type of clinical response, C4 and sIL-2R post-treatment titers were strongly correlated ($p = 0.005$ by Spearman rank order correlation test). When response was taken into account, this correlation was significant in patients with progressive disease ($p = 0.00005$), but disappeared in the patients with OR or SD.

No significant differences were evidenced in terms of immunologic parameters between patients achieving an early or a delayed clinical response.

Multiple logistic regression analysis allowed us to identify some clinical variables as predictors of favorable or unfavorable outcome.

Indeed, eosinophils only predict progression despite treatment in the pre-treatment model (Table 4a), while, in the post-treatment model, C4 and eosinophils predict treatment failure (Table 4b).

The logistic regression model provides the probability of response as a function of the independent variables; considering the model to perform correctly when a responder is given a probability of response greater than 0.5, the performance of the pre-treatment model was 78%, while the performance of the post-treatment model was 85%.

**Table 1. Summary of patients' characteristics.**

| | |
|---|---|
| Sex | |
| Men | 16 (64%) |
| Women | 9 (36%) |
| Age (yrs) | |
| Median | 59.6 |
| Range | 42-71 |
| Previous nephrectomy | |
| Yes | 19 (76%) |
| No | 6 (24%) |
| Site of metastatic disease | |
| Lung | 11 (44%) |
| Liver | 4 (16%) |
| Bone | 9 (36%) |
| Nodes | 10 (40%) |
| Site of nephrectomy | 12 (48%) |
| Other | 3 (12%) |
| ECOG Performance status | |
| 0 | 10 (40%) |
| 1 | 8 (32%) |
| 2 | 7 (28%) |

**Table 2. When all patients were considered together, very-low dose s.c. rhuIL-2 plus IFN immunotherapy determined a statistically significant increase in the number of total lymphocytes, $CD4^+$, $CD8^+$, NK cells, eosinophils, percent of $CD25^+$ cells, and in the titers of C4 and sIL-2R.**

| *Variable* | *Pre-treatment* | | *Post-treatment* | | |
|---|---|---|---|---|---|
| | *mean* | *SD* | *mean* | *SD* | *p* |
| CD4 (Abs. #/µL) | 720.26 | 142.16 | 1100.15 | 341.11 | 0.005 |
| CD8 (Abs. #/µL) | 522.44 | 184.68 | 762.39 | 195.91 | 0.001 |
| CD25 (%) | 20.11 | 8.05 | 31.44 | 5.07 | 0.01 |
| NK (Abs. #/µL) | 285.39 | 94.60 | 815.19 | 188.50 | 0.001 |
| Eosinophils (Abs. #/µL) | 150.01 | 85.90 | 885.44 | 514.05 | 0.001 |
| Lymphocytes (Abs. #/µL) | 1,912.34 | 488.30 | 2,745.15 | 910.06 | 0.005 |
| ECP (µg/L) | 15.02 | 10.22 | 42.28 | 35.21 | n.s. |
| EPX (µg/L) | 28.44 | 22.66 | 102.44 | 44.26 | 0.005 |
| sIL-2R (U/mL) | 489.24 | 255.62 | 1,891.31 | 1022.35 | 0.001 |
| C4 (mg/dL) | 25.39 | 4.30 | 41.99 | 5.16 | 0.005 |

*Abs. #: absolute number.*

**Table 3. First cycle data of patients yielding a favorable clinical outcome (OR plus SD) compared to those of patients with progression showed statistically significant post-treatment differences for the number of lymphocytes, eosinophils, CD4+ T cells and for the serum titers of C4 and sIL-2R, which were significantly higher in non-responding patients.**

***Post-treatment values***

| *Variable* | *Favorable outcome* | | *Unfavorable outcome* | | |
|---|---|---|---|---|---|
| | *mean* | *SD* | *mean* | *SD* | *p* |
| CD4 (Abs. #/μL) | 828.45 | 358.21 | 1798.80 | 489.76 | 0.001 |
| sIL-2R (U/mL) | 1455.23 | 844.88 | 3012.65 | 1205.47 | 0.005 |
| C4 (mg/dL) | 40.45 | 3.51 | 45.92 | 4.50 | 0.001 |
| Lymphocytes (Abs. #/μL) | 2375.24 | 820.25 | 3696.34 | 958.18 | 0.005 |
| Eosinophils (Abs. #/μL) | 689.70 | 355.10 | 1388.77 | 891.56 | 0.01 |

*Abs. #: absolute number.*

**Table 4. Multiple logistic regression analysis: eosinophil titer predicts tumor progression in the pre-treatment model (a), while C4 and eosinophils predict tumor progression in the post-treatment model (b).**

| (a) | Pre-treatment model | | |
|---|---|---|---|
| | *Coefficient* | *t-value* | *p* |
| Intercept | 9.78 | 2.61 | < 0.01 |
| Eosinophils | - 0.012 | - 2.03 | < 0.05 |

| (b) | Post-treatment model | | |
|---|---|---|---|
| | *Coefficient* | *t-value* | *p* |
| Intercept | 8.05 | 1.95 | < 0.05 |
| Eosinophils | - 0.13 | - 2.41 | < 0.05 |
| C4 | - 0.24 | - 2.23 | < 0.05 |

## Discussion

rhuIL-2 has usually been administered in the clinical setting by the intravenous route or subcutaneously at high doses;[4,8-10] in both cases, antitumor activity has been well documented and the immunologic effects of the cytokine have been widely studied and described.[21-25] In contrast, the use of very low doses of subcutaneous rhuIL-2 has only recently been proposed[11] and, while its antitumor activity and low toxicity profile have been clearly documented,[11,12] the effects that such a treatment can induce on the immune system of the host receiving this cytokine, have not been specifically investigated yet. The rationale of the long-term stimulation of the immune system of cancer patients by rhuIL-2, as proposed by Buzio *et al.*, relies on the assumption that a continuously activated immune system could counteract active tumor proliferation, leading to, at least, reduced tumor growth.[11] Indeed, independently of clinical outcome, rhuIL-2 administration was continued in all patients, even in those with progressive disease, since we reasoned[11] that they may benefit from slower tumor growth, resulting from a sustained immunologic attack, no other active treatment option being currently available.[26]

Furthermore, the activation of cellular immunity has been proposed to play a major role in inducing tumor dormancy;[27] thus, Wheelock *et al.* first stressed the importance of cellular immunity in inducing dormancy in a murine lymphoma model.[28] In their study, growth restraint was dependent on both cytolytic T-lymphocytes and macrophages; the secretion of IL-2 and IFN-γ by T-cells induced the activation of cytotoxic T-cells in the early stage of induction of dormancy followed by activated macrophages at a later stage. Studies in mice with leukemia showed that allogeneic bone marrow transplantation provided an anti-leukemic effect[29,30] and that such mice carried cell cycle-arrested dormant tumor cells for long periods of time.[31] Khazaie *et al.*[32] showed that injecting live B-lymphoma cells at a site refractory to tumor growth led to a long lived T-cell response that correlated with the persistence of dormant tumor cells.

Indeed, besides OR, patients had fairly long-lasting stable disease (on average: 11 months), with 8 patients showing SD for at least one year, a figure which is hardly explainable simply by slow neoplastic growth.

We investigated the changes induced by a single rhuIL-2 plus IFN treatment on some immunologic parameters in a population of patients with metastatic renal carcinoma; besides this conventional immunologic study, we also evaluated the effects of immunotherapy on eosinophil production and activity, a subject of great current interest and of questionable interpretation. The mere report of the changes induced by very low dose rhuIL-2 treatment, independently of response, showed a statistically significant increase in the number of NK, CD4+, CD8+ cells, total lymphocytes and eosinophils, as well as in EPX, C4 and sIL-2R serum titers and in the percent of CD25+ cells. The percent of cells expressing the CD25 antigen was preferred to the absolute number of CD25 lymphocytes, because of the heterogeneity of cells capable of expressing this activation marker, which is also present on the surface of non-lymphocytic cells.

Pre- and post-treatment immunologic data were analyzed separately by multiple logistic regression analyses, and two different statistical models were made to assess which parameter(s) could predict clinical response, or vice versa, treatment failure.

The analysis showed that, within the pre-treatment model, a large eosinophil number predicts the failure of rhuIL-2 treatment.

In contrast, within the post-treatment model, high C4 serum titers and, again, a large number of circulating eosinophils predict neoplastic disease progression, that is immunotherapy failure.

As far as C4 is concerned, its negative predictive value could be related to the fact that it is considered as an indirect index of macrophage activation;[17] thus, even though macrophages certainly release substances with antitumor activity such as TNF-α, IL-1,

nitric oxide, free radicals and proteases, and can function as antigen-presenting cells,[33] they can also stimulate the release of sIL-2R, which may compete for rhuIL-2 with IL-2 surface receptor, thus diminishing the clinical efficacy of rhuIL-2.[34,35] Indeed, in our study, C4 and sIL-2R titers were strongly correlated when all cycles were considered together, independently of the type of clinical response; when response was taken into account, this correlation was significant only in patients with progressive disease. Furthermore, some authors have postulated that macrophages may even stimulate tumor cell growth,[36,37] or impair NK activity, the latter effect probably by interfering with post-binding events through the production of PGE2 and TGF-β 1.[38] Finally, current literature data stress an urgent need for a thorough revision of previous paradigms regarding the role of the TNF cytokine family in IL-2-induced antitumor activity.[39,40]

The value of eosinophils as effector cells in antitumor immune response remains highly controversial. Thus, eosinophils are considered beneficial when present within tumors, while they are often interpreted as indicators of poor prognosis when present in high concentrations in the peripheral blood of patients with neoplasia.[41-44]

Direct[13] or antibody-dependent[14] cancer lysis by eosinophils has been demonstrated in *in vitro* models and eosinophilic cationic proteins have been proposed as possible mediators of this antitumor activity of eosinophils,[45] even though their behavior in cancer patients is far from being well defined or understood.[46] However, we have recently demonstrated that eosinophils are unlikely to play a direct cytotoxic role on tumor cells through the release of their cationic proteins ECP and EPX, at least *in vitro*.[15]

Moreover, in our study, blood eosinophilia predicts a poor response to low dose rhuIL-2 + IFN immunotherapy in patients with advanced RCC.

The conflicting results reported in the literature, taken together with our findings, clearly indicate that the precise role of eosinophils in IL-2-treated cancer patients is far from understood and deserves further thorough investigations. In particular, since it is not absolutely obvious that the blood parameters observed after the first cycle of treatment remain substantially unmodified in subsequent cycles, a longitudinal study is warranted to clarify the relationship existing between duration of response and modifications of immunologic parameters.

### Contributions and Acknowledgments

*CP and MM had the original idea of the study and wrote the paper; MDA and MAC performed all laboratory investigations; SQ and MDA performed the statistical analyses; CP and CB enrolled patients into the study. Finally, CB reviewed the manuscript and, as Senior Author, is cited last.*

### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

### Manuscript processing

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### Potential implications for clinical practice

- On the basis of the results of the above study, biological predictors of failure to respond to very low dose s.c. IL-2 and interferon-α therapy are now available, at least in patients with renal cell carcinoma; indeed, hypereosinophilia and the activation of macrophages are negative prognostic factors.
- Thus, new immunologic approaches to the treatment of these neoplasms should also consider the modulation of both eosinophil and macrophage activity. The role of these cells and their relationship with the tumor on the one hand, and with the effector cells of the immune system on the other, deserves further studies.

## References

1. Theze J, Alzari PM, Bertoglio J. Interleukin 2 and its receptors: recent advances and new immunological functions. Immunol Today 1996; 17:481-6.
2. Hoon DSB, Morisaki T, Essner R. Inhibition of non-hemopoietic cancer cell growth by interleukin-4 and related cytokines. In: Dalgleish AG, Browning M, eds. Tumor immunology. Immunotherapy and cancer vaccines. Cambridge: University Press; 1996. p. 219-40.
3. Schwartzentruber DJ, Hom SS, Dadmarz R, et al. In vitro predictors of therapeutic response in melanoma patients receiving tumor-infiltrating lymphocytes and interleukin-2. J Clin Oncol 1994; 12:1475-83.
4. Oppenheimer MH, Lotze MT. Interleukin-2: solid-tumor therapy. Oncology 1994; 51:154-69.
5. Weidmann E, Sacchi M, Plaisance S, et al. Receptors for interleukin 2 on human squamous cell carcinoma cell lines and tumor in situ. Cancer Res 1992; 52: 5963-70.
6. Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nat Med 1998; 4:43-9.
7. Weller PF. Cytokine regulation of eosinophil function. Clin Immunol Immunop 1992; 62:S55-9.
8. Rosenberg SA, Lotze MT, Muul LM, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 1987; 316:889-97.
9. West WH, Tauer KW, Yannelli JR, et al. Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 1987; 316: 898-905.
10. Atzpodien J, Körfer A, Franks CR, Poliwoda H, Kirchner H. Home therapy with recombinant interleukin-2 and interferon-α 2b in advanced human malignancies. Lancet 1990; 335:1509-12.
11. Buzio C, De Palma G, Passalacqua R, et al. Effectiveness of very low doses of immunotherapy in advanced renal cell cancer. Br J Cancer 1997; 76:541- 4.
12. Porta C, Moroni M, Bobbio-Pallavicini E, Tinelli C, Regazzi-Bonora M. Nitrate plasma level as a marker of nitric oxide production after subcutaneous interleukin-2 immunotherapy. J Natl Cancer Inst 1997; 89:1545.
13. Huland E, Huland H. Tumor-associated eosinophilia in interleukin-2-treated patients: evidence of toxic eosinophil degranulation on bladder cancer cells. J Cancer Res Clin 1992; 118:463-7.
14. Rivoltini L, Viggiano V, Spinazzè, et al. In vitro anti-tumor activity of eosinophils from cancer patients

treated with subcutaneous administration of interleukin 2. Role of interleukin 5. Int J Cancer 1993; 54:8-15.
15. Moroni M, Porta C, Gritti D, et al. Cationic protein-rich supernatants of cultured eosinophils from IL-2-treated patients have no cytotoxic activity on human renal cell carcinoma and melanoma cells: a preliminary report. Ann NY Acad Sci 1997; 832:295-303.
16. Arinaga S, Karimine N, Takamuku K, et al. Correlation of eosinophilia with clinical response in patients with advanced carcinoma treated with low-dose recombinant interleukin-2 and mitomycin C. Cancer Immunol Immun 1992; 35:246-50.
17. Hartung HP, Zanker B, Bitter-Suermann D. Influence of macrophage activation on the synthesis of complement components C2, C3, C4. Adv Exp Med Biol 1982; 155:525-9.
18. Confalonieri M, Tacconi F, De Amici M, et al. Benign idiopathic hypereosinophilia: a feeble masquerader or a smouldering form of the hypereosinophilic syndrome? Haematologica 1995; 80:50-3.
19. World Health Organization. WHO Handbook for reporting results of cancer treatment. Offset publication 48. Geneva, World Health Organization, 1979.
20. Becker RA, Chambers JM, Wilks AR, eds. The new S language: a programming environment for data analysis and graphics. Pacific Grove: Wadsworth & Brooks; 1988.
21. Fiorentino B, Di Stefano P, Giuliani C, et al. Immunological effects of alternative weekly interferon-alpha-2b and low dose interleukin-2 in patients with cancer. Br J Cancer 1992; 66:981-3.
22. Hermann GG, Geertsen PF, von der Maase H, et al. Recombinant interleukin-2 and lymphokine-activated killer cell treatment of advanced bladder cancer: clinical results and immunological effects. Cancer Res 1992; 52:726-33.
23. Schneekloth H, Korfer A, Hadam M, et al. Low-dose interleukin-2 in combination with interferon-α effectively modulates biological response in vivo. Acta Haematol 1993; 89:13-21.
24. Schiller JH, Hank J, Storer B, et al. A direct comparison of immunological and clinical effects of interleukin 2 with and without interferon-alpha in humans. Cancer Res 1993; 53:1286-92.
25. Mertens WC, Banerjee D, al-Mutter N, Stitt L, Bramwell VH, Lala PK. High-dose continuous venous infusion of interleukin-2: influence of dose and infusion rate on tumoricidal function and lymphocyte subsets. Cancer Immunol Immun 1995; 41:271-9.
26. Sokoloff MH, deKernion JB, Figlin RA, Belldegrun A. Current management of renal cell carcinoma. CA Cancer J Clin 1996; 46:284-302.
27. Uhr JW, Scheuermann RH, Street NE, Vitetta ES. Cancer dormancy: opportunities for new therapeutic approaches. Nat Med 1997; 3:505-9.
28. Wheelock EF, Weinhold KJ, Levich J. The tumor dormant state. Adv Cancer Res 1981; 34:107-40.
29. Siu H, Vitetta ES, May RD, Uhr JW. Tumor dormancy. I. Regression of BCL1 tumor and induction of a dormant tumor state in mice chimeric at the major histocompatibility complex. J Immunol 1986; 137:1376-82.
30. Weiss L, Morecki S, Vitetta ES, Slavin S. Suppression and elimination of BCL1 leukemia by allogeneic bone marrow transplantation. J Immunol 1983; 130:2452-5.
31. Uhr JW, Tucker T, May RD, Siu H, Vitetta ES. Cancer dormancy: studies of the murine BCL-1 lymphoma. Cancer Res 1991; 51:5045s-5053s.
32. Khazaie K, Prifti S, Beckhove P, et al. Persistence of dormant tumor cells in the bone marrow of tumor cell-vaccinated mice correlates with long-term immunological protection. Proc Natl Acad Sci USA 1994; 91:7430-4.
33. Vile RG, Chong H, Dorudi S. The immunosurveillance of cancer: specific and nonspecific mechanisms. In: Dalgleish AG, Browning M, eds. Tumor immunology. Immunotherapy and cancer vaccines. Cambridge: University Press; 1996. p. 7-38.
34. Lissoni P, Tisi E, Brivio F, et al. Increase in soluble interleukin-2 receptor and neopterin serum levels during immunotherapy of cancer with interleukin-2. Eur J Cancer 1991; 27:1014-6.
35. Lissoni P, Barni S, Rovelli F, et al. Neuroimmunotherapy of advanced solid neoplasms with single evening subcutaneous injection of low-dose interleukin-2 and melatonin: preliminary results. Eur J Cancer 1993; 29A:185-9.
36. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. Immunol Today 1992; 13:265-70.
37. Klein E, Mantovani A. Action of natural killer cells and macrophages in cancer. Curr Opin Immunol 1993; 5: 714-8.
38. Lauzon W, Lemaire I. Alveolar macrophage inhibition of lung-associated NK activity: involvement of prostaglandins and transforming growth factor-beta 1. Exp Lung Res 1994; 20:331-49.
39. Mier J, Lawrence D, Unrau M, Atkins M. The role of tumor necrosis factor (TNF) family members in IL-2 toxicity and tumor regression. Int J Immunopathol 1996; 2:39-42.
40. Nagata S. Fas ligand and immune evasion. Nat Med 1996; 2:1306-7.
41. Flamm J. Tumor-associated tissue inflammatory reaction and eosinophilia in primary superficial bladder cancer. Urology 1992; 40:180-5.
42. Thompson AC, Bradley PJ, Griffin NR. Tumor-associated tissue eosinophilia and long-term prognosis for carcinoma of the larynx. Am J Surg 1994; 168:469-71.
43. Leighton SE, Teo JG, Leung SF, Cheung AY, Lee JC, van-Hasselt CA. Prevalence and prognostic significance of tumor-associated tissue eosinophilia in nasopharyngeal carcinoma. Cancer 1996; 77:436-40.
44. Samoszuk M. Eosinophils and human cancer. Histol Histopathol 1997; 12:807-12.
45. Trulson A, Nilsson S, Venge P. The eosinophil granule proteins in serum, but not the oxidative metabolism of the blood eosinophils, are increased in cancer. Br J Haematol 1997; 98:312-4.
46. Porta C, Moroni M, De Amici M. Eosinophils and serum eosinophilic cationic proteins in interleukin-2-based immunotherapy for cancer. Br J Haematol 1998; 100:607-9.