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Low-dose γ-Interferon Therapy is Ineffective in Renal Cell Carcinoma Patients with Large Tumour Burden

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The efficacy and immunomodulatory effects of low-dose γ-interferon (γIFN) were investigated in an unselected population of patients with metastasising renal cell carcinoma. 36 patients suffering from metastasising renal cell carcinoma with a performance status exceeding Karnofsky index of 50 were entered into the open phase I/II trial. The majority of the patients recruited displayed a large tumour burden, and 28 patients (78%) had metastases involving two to six organ sites. Treatment was started with a 2-week cycle of either daily or weekly subcutaneous administration of either 100, 200 or 400 μg γIFN. After a therapy-free interval of 2 weeks treatment was switched to the alternate mode of administration. Subsequently, treatment was continued with the same dose applied once a week for a minimum of 3 months. Serum levels of neopterin and β-2-microglobulin, as well as flow cytometric analyses of peripheral blood mononuclear cells, were used for the assessment of biological response. Minimal antitumour activity was observed in this high-risk patient group and only 1 patient experienced a partial response (PR) lasting 36+ months. Comparison of the patients' characteristics to those of other low-dose γIFN trials revealed a highly significant difference in the tumour burden and clinical response. We conclude that patient selection is a decisive parameter for the outcome of treatment with low-dose γIFN, and that patients with poor prognostic features and a large tumour burden are not likely to respond to this almost atoxic treatment.
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INTRODUCTION

RENAL CELL carcinoma (RCC) is generally considered to be a promising target for treatment with biological response modifiers [1]. This assumption is based on two observations: spontaneous regressions are observed in a minority of patients and a variety of biological response modifying agents are effective in a minority of patients [2]. Comparable response rates with overlapping 95% confidence intervals have been reported for treatment with interleukin 2, α-interferon (αIFN), γIFN, adoptive immunotherapies with lymphokine activated killer cells, and various combinations of these substances [3–9]. However, the majority of the tumour responses are not durable. In addition, most of these therapies have applied doses close to the maximum tolerated dose. Therefore, for the entire group of patients treated, the clinical value of such therapies is limited by their significant toxicity, which severely compromises the quality of life in the majority of patients [10–12].

Recently, we and others have explored the antineoplastic activity and toxicity of biologically defined low doses of γIFN in patients suffering from metastasising RCC [13, 14]. In both clinical trials published, the patients were treated with 100 μg γIFN once a week. This dose was chosen as a 'biologically active dose', because a single injection of 100 μg γIFN caused nearly maximum biological response, defined by induction of β-2-microglobulin (β_2m), and was associated with minimal toxicity [15].

It was the objective of the present study to answer two questions: (i) to further define the optimal biological regimen

with low dose γIFN by comparing biological response to γIFN administered daily or once a week; and (ii) to investigate whether the favourable response rates observed in the previously published studies can also be achieved in an unselected patient population. Therefore all patients with a Karnofsky index of > 50% were included in the study. Using these selection criteria, more than 80% of RCC patients admitted to our centre were eligible for treatment according to the protocol.

MATERIAL AND METHODS

Patients

36 patients were treated according to the protocol. The study was performed in full conformity to the principles of the Declaration of Helsinki. The protocol was approved by the local ethics committee. All patients had given their informed consent prior to the study. 16 patients were treated with 100 μg, 9 with 200 μg and 11 with 400 μg γIFN. 29 of the patients completed both cycles of the phase I part of the study. The clinical characteristics of the patients are presented in Table 1. Criteria for patient selection included Karnofsky index of > 50%, progressive, metastasising renal cell carcinoma, age > 20 years, lack of CNS metastases and preserved function of major organ systems. During the entire study period only 4 advanced RCC patients admitted to our institution were not eligible due to the presence of CNS metastases or severe renal failure. Thus 89% of the patients with metastasising RCC who were admitted to our institution during the study were treated according to the protocol.

Treatment plan

At the start of treatment, the immunomodulatory and toxic effects of three different dose levels of γIFN, applied either daily or once a week, were determined. Treatment was started with either 100, 200 or 400 μg γIFN. This dose was applied subcutaneously during a 2-week cycle beginning with either daily (12 patients) or weekly administration (24 patients) of γIFN. The treatment was performed on an outpatient basis and compliance was controlled by assessment of the number of used vials. After a therapy-free interval of 2 weeks, the cycle was repeated by changing the frequency of administration. Subsequently, patients were treated with the same dose of γIFN once a week for a minimum of 3 months. For the patients achieving tumour response (complete or partial response) or stable disease, therapy was continued until progression of their disease.

Table 1. Clinical characteristics of the patients

Age	
Median	59.5 years
Range	33–77
Sex*	
Male	26
Female	10
Nephrectomy*	
Yes	28
No	8
Interval from diagnosis to study entry	
Median	10 months
Range	0–140
Karnofsky index*	
60	2 (6%)
70–80	21 (58%)
90–100	13 (36%)

*No. of patients.

Biological responses to the different schedules of administration were analysed by measurement of β_2m and neopterin serum levels in samples drawn immediately before and 2, 12, 24, 48 and 168 h after the first and last administration of each treatment cycle. In addition, the effect of γIFN on number and phenotype of peripheral blood lymphocytes was determined at the same time points.

Evaluation of tumour response was performed with serial CT scans after 3, 6, 9 and 12 months. Clinical efficacy was determined by assessment of the proportion of patients achieving complete or partial response. Complete response was defined as complete disappearance of all detectable lesions, partial response as a reduction of 50% or more in the size of the lesions measured by assessment of two diameters according to standard criteria [16].

Trial substance

γIFN produced by Biogen Inc was provided by Bioferon Inc (Laupheim, Germany) and displayed a specific activity of 2×10^7 U/mg protein.

Laboratory methods

Serum levels of β_2m and neopterin were determined by means of a commercially available radioimmunoassay (Pharmacia Inc, Uppsala, Sweden; Henning Inc, Berlin, F.R.G.). Lymphocyte subsets were determined by flow cytometric analysis of peripheral blood mononuclear cells using a FAC-SCAN (Becton Dickinson Inc, U.S.A.) according to standard methods. Briefly, after separation of peripheral blood mononuclear cells by density gradient centrifugation, the cells were stained by fluorochrome-labelled commercially available antibodies specific for CD4, CD8, CD54, Leu 11c, CD25 and CD3 (Dianova Inc, F.R.G.).

Statistical analysis

Data were analysed by means of descriptive statistical methods. To determine statistical significance of the differences in the values before and after treatment, the mean difference with 95% confidence intervals was calculated. Response rates were calculated with 95% confidence intervals. For the calculation of confidence intervals, the CIA statistical software package was used. Retrospective analysis of differences between populations of the two low-dose γIFN trials was performed by the chi square test.

RESULTS

Biological response to different schedules of low-dose γIFN

Significant dose-dependent increments of β_2m and neopterin serum levels were observed 48 h after the first injection of γIFN. Repeating the same dose three times at weekly intervals did not result in a downregulation of the β_2m response (Figure 1). After 48 h from the start of treatment with daily administration of γIFN, the induction of β_2m appeared to be within the range of

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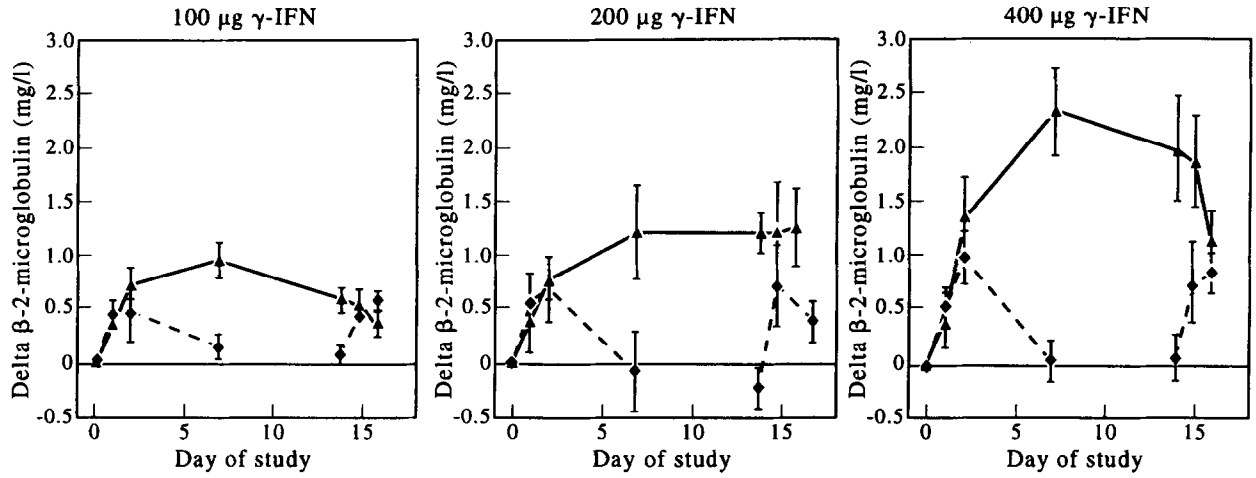

Figure 1. Biological response to γIFN: the differences of β-2-microglobulin serum levels to pretreatment levels measured during daily (solid line) and once weekly (broken line) treatment with of 100, 200 and 400 μg γIFN are shown. Lines represent the mean difference ± S.E.M. to pretreatment values. Statistical significance of these differences was calculated by estimating the 95% C.I. interval of the mean difference.

that observed after single injection. After 7 days of daily γIFN administration, however, HLA biosynthesis was further enhanced. At all dose levels tested, the serum levels of β_2m were higher after 7 days of daily γIFN administration than peak levels achieved upon weekly treatment. During the second week of the cycle no further increase was observed. The effect of γIFN administration on neopterin biosynthesis was comparable to that of the β_2m response. Significant increments of neopterin serum levels were observed after single injections of 100, 200 and 400 μg γIFN (Figure 2). Neopterin biosynthesis was also more effectively induced by daily treatment with γIFN. According to the small number of responding patients, no correlations have been investigated between biological and clinical response. However, the patient experiencing a partial remission showed the highest increments of β_2m serum levels among patients treated with 100 μg γIFN.

A decrease of leukocyte counts was observed both after daily and weekly administration of γIFN (Figure 3). Neither schedule, however, caused clinically significant leucopenia. Transient leucopenia was caused in part by a reduction in the number of CD8-positive lymphocytes. The decrease of the number of CD-positive cells was detectable within 12 h of subcutaneous γIFN administration and reached its nadir 12–24 h after injection of γIFN. No significant additional changes in the number of CD4-positive T-cells or natural killer cells were detected. In particular, the number of IL-2 receptor positive lymphocytes remained unaffected by γIFN therapy. We further analysed whether the decrease of CD8-positive lymphocytes was accompanied by increased expression of adhesion molecules. As shown in Figure 4, 4/5 individuals analysed showed a marked increase in the density of CD54 (ICAM 1) on peripheral blood lymphocytes. This increase was dose dependent and observed only upon treatment with 400 μg γIFN.

Clinical results

The patient group selected by the study criteria was predominantly composed of patients with large tumour burden and rapidly progressing widespread disease. The majority of patients (61%) suffered from disseminated disease affecting three or more organ sites (Table 2). In 55% of patients, the history of the disease was less than 2 years.

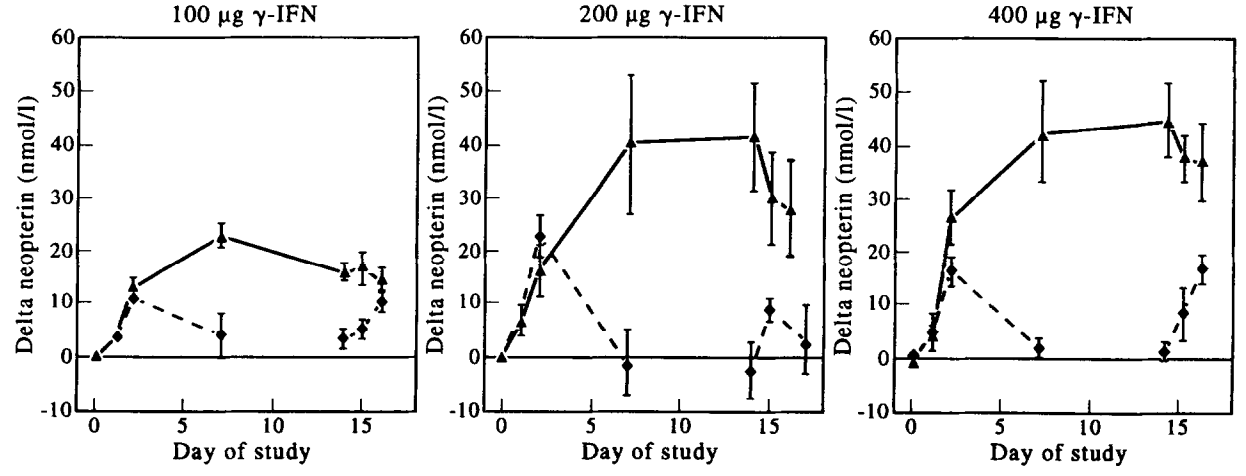

Figure 2. Biological response to γIFN: the differences of neopterin serum levels to pretreatment levels measured during daily (solid line) and once weekly (broken line) treatment with 100, 200 and 400 μg γIFN are shown. Lines represent the mean difference ± S.E.M. to pretreatment values. Statistical significance of these differences was calculated by estimating the 95% C.I. interval of the mean difference.

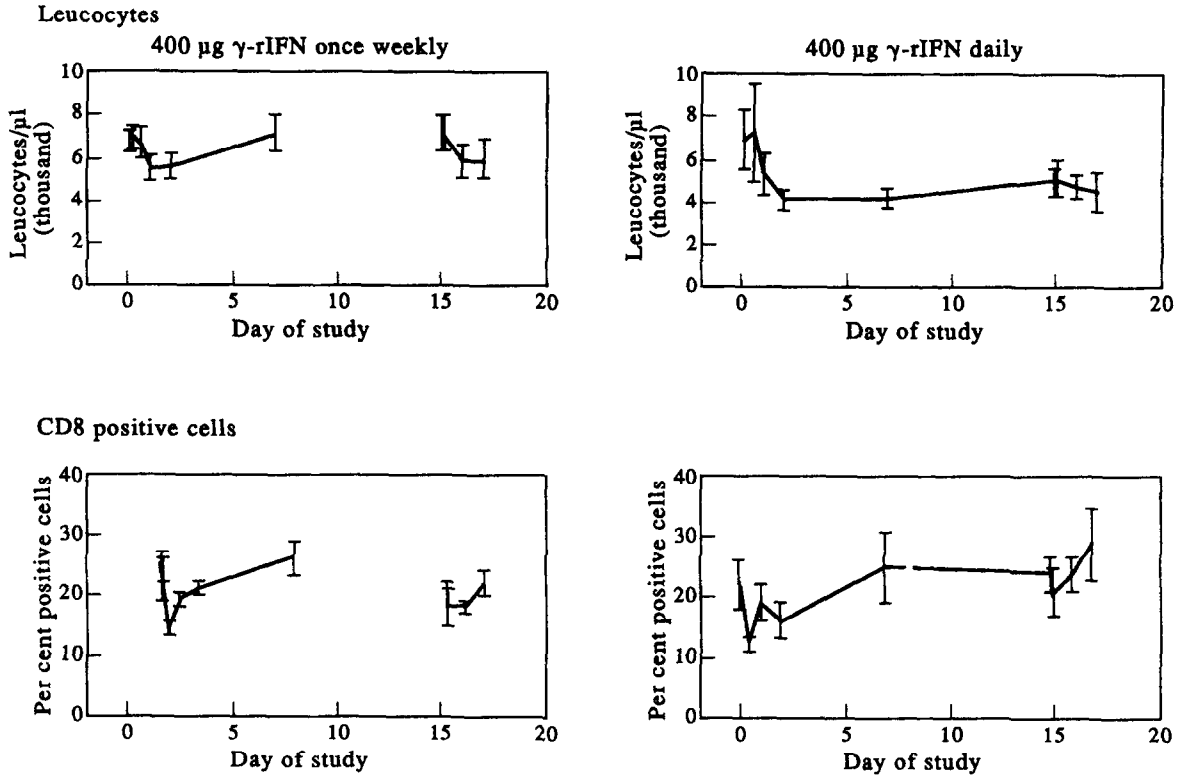

Figure 3. White blood cell counts and percentage of CD8-positive cells during treatment cycles with daily or once weekly administration of 400 μg γIFN. Lines represent mean values ± S.E.M.

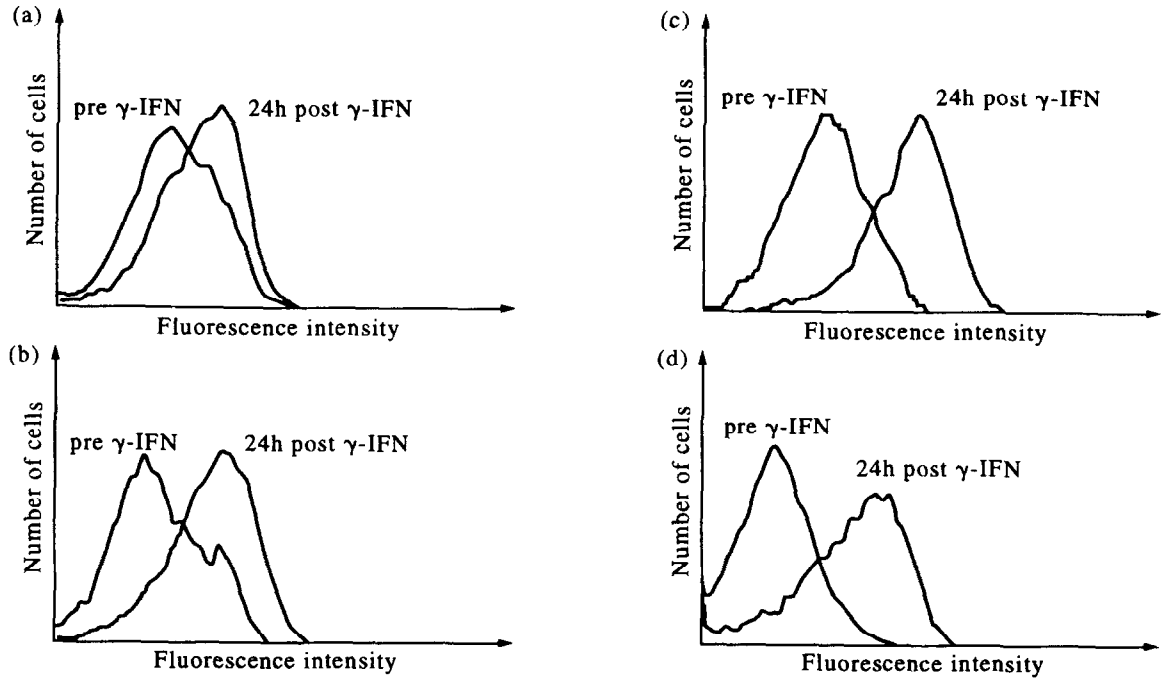

Figure 4. Induction of ICAM 1 by administration of γIFN. The figure shows ICAM 1 expression on peripheral blood mononuclear cells of 4 individuals (a–d) before and 24 h after γIFN.

One patient showed partial remission following treatment with 100 μg γIFN (3%, 95% C.I. 0–15%). This patient suffered from a large single local recurrence of his tumour at the site of nephrectomy. After 3 years of continuous treatment, this patient has been free from progression and is clinically well. All the other patients showed progressive disease after a median of 3 months. 2 patients experienced prolonged stabilisation of the course of their disease, but the tumour progressed after 6 and 12 months, respectively.

The acute toxicity of both daily or weekly treatment was

Table 2.

	No of patients (%)
Site of metastases	
Lung	27 (75%)
Lymph node	
Thoracic	12 (33%)
Other	14 (39%)
Liver	11 (31%)
Bone	11 (31%)
Local recurrence	11 (31%)
Primary tumour	8 (22%)
Soft tissue	6 (17%)
Other	8 (22%)
Number of sites	
1	8 (22%)
2	6 (17%)
3–6	22 (61%)

moderate. Tolerance of the treatment during weekly administration was excellent. Moderate constitutional symptoms and minor febrile reactions were dose dependent and were observed in the majority of patients. After weekly treatment with γIFN, 13% of the patients receiving 100 μg, and 50% of the patients receiving 200 and 400 μg γIFN experienced WHO grade II febrile reactions. Constitutional symptoms were more marked during treatment cycles using daily injections of γIFN: WHO grade 2 was noted in 7% of the patients receiving 100 μg and in 87% of the patients receiving 200 and 400 μg γIFN. No severe adverse drug reactions were observed.

DISCUSSION

One major objective of this study was to further define the optimum biological treatment schedule with low dose γIFN. Serum levels of β_2m were used for assessment of biological response as a measure of *in vivo* HLA biosynthesis. Daily subcutaneous injections of γIFN for 1 week induced higher β_2m serum levels than weekly administration. Similarly, a more marked biological response to daily administration was also observed with neopterin serum levels. This difference was detectable at all dose levels tested. Continuation of daily γIFN administration for more than 1 week did not further increase β_2m serum levels. Therefore, it seems that cyclic daily treatment with γIFN is optimal for induction of HLA synthesis in patients with renal cell carcinoma. After the highest dose level applied, a minor trend towards downregulation of the β_2m synthesis was observed. Whether this represents a significant problem for long-term treatment with γIFN remains to be answered. Induction of anticytokines by γIFN might be one explanation for such a downregulation of biological responses. Recently, enhancement of the synthesis of such an inhibitory protein, the interleukin 1 receptor antagonist protein, by γIFN treatment has been described [17].

A transient decrease of CD8-positive cells was the only significant effect on peripheral blood cells detectable, subsequent to both daily and weekly administration of γIFN. Such a temporary reduction of certain subtypes of peripheral blood lymphocytes was observed by several investigators including the authors, following single injection of γIFN, interleukin 2, αIFN and αTNF (tumour necrosis factor) [18–20]. This reduction was accompanied by an increased expression of the adhesion molecule ICAM 1 on the surface of peripheral blood cells by cytokine treatment. This would imply that cytokine-responsive cells, in particular, change their homing patterns. These cells might then shift by adherence to the vessel wall or migration into the tissues. These results indicate that, after injection of cytokines, peripheral blood cells might be preferentially depleted of cytokine-responsive cells. Thus, it might be misleading to study the biological effects of treatment with γIFN on the function or phenotype cells when using samples derived from peripheral blood.

The response rate observed in this study was clearly inferior to the response rates reported for low-dose γIFN treatment of renal cell carcinoma. In order to analyse potential causes for this discrepancy, we compared the results of two low-dose γIFN trials performed by our group (Table 3). No significant differences in age, sex distribution and the interval from diagnosis to start of treatment were observed. However, there was a striking difference in the tumour burden for the two patient populations: only 22% of the patients presenting the disease restricted to one organ were entered into this trial. The proportion of comparable patients with localised disease was more than 70% in our previous γIFN study. Accordingly, the 95% confidence intervals of the response rates in this subgroup of patients in both studies are overlapping. Thus, prospective application of a different level of the patients' performance status as a selection criterion resulted in recruitment of two patient groups, which greatly differed with respect to their tumour burden. This most likely explains the discrepant response rates observed in these two studies. To further test the hypothesis of whether low-dose γIFN is clinically active in patients with a low tumour burden, we have initiated a third clinical trial. Only patients with renal cell carcinomas restricted to one site are recruited in this study. This trial is still in progress but preliminary results are promising

Table 3. Comparison of the clinical characteristics of the study population of two consecutive low-dose γIFN studies

	Ref. 13	Present study
IFN dose (μg/dose)		
Phase I	10–500	100–400
Phase II	100	100–400
IFN source	Gentech	Biogen
n	22	36
Number of sites		
1	19 (70%)	8 (22%)
2	7 (26%)	6 (17%)
3–6	1 (4%)	22 (61%)
χ^2	22.91	$P<0.0001$
Interval diagnosis treatment		
<24 months	16 (58%)	20 (55%)
24–48 months	3 (11%)	3 (8%)
>48 months	8 (30%)	13 (36%)
χ^2		n.s.
Clinical response (PR+CR)	6	1
% (95% C.I.)	17 (11–50)	3 (0–15)
		$P<0.05$
Patients with disease restricted to one site		
n	14	10
PR+CR	5	1
% (95% C.I.)	35% (13–65)	10% (3–44)
		n.s.

*Lung and thoracic lymph node metastases were considered as a single site. n.s., non-significant.

and indicate response rates of approximately 30% in this highly selected group of RCC patients (W. Avlitzky, personal communication). We conclude that patient selection is crucial for clinical results in the treatment with low-dose γIFN in renal cell carcinoma, and low dose γIFN might represent an alternative treatment exclusively for patients with a low tumour burden.

The question arises whether patient selection is of similar importance for clinical responses observed with other biological response modifiers. Variable response rates ranging from 0 to 40% and more, with overlapping confidence intervals, were observed in clinical studies with interleukin 2, αIFN and γIFN. Multivariate analyses in large patient populations indicate that, preferentially, patients without clinical symptoms, localised disease and slow progression might benefit from biotherapies [21, 22]. According to these data, it appears that the features predicting sensitivity to γIFN are also observed in patients responding to therapy with IL-2 or αIFN. This suggests that the capacity to induce clinical remission in RCC with biological response modifiers is not solely dependent on type, dose and schedule of treatment, but rather upon the selection of patients with low-risk features. In the past, numerous studies have attempted to correlate the biological response to various cytokines with clinical outcome. In some of them, correlations between clinical response and induction of several immune parameters by biological response modifiers have been described [13, 23, 24]. Unfortunately, very few studies have analysed the impact of histological subclassification, cytogenetics or molecular characteristics on the response to immunotherapy. The question of whether biological response modifier sensitive renal cell carcinomas represent a specific disease entity with distinct molecular pathogenesis and a unique response to various biological therapies remains unclear. Interpretation of different phase II trials is not possible before this question is answered. We conclude that efforts should be intensified to further characterise the clinical, molecular, cytogenetic and immunological features of biological response modifier responsive RCC. Such studies are currently in progress in our institution.

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