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Granulocyte/macrophage-colony-stimulating-factor plus interleukin-2 plus interferon α in the treatment of metastatic renal cell carcinoma: a pilot study

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Abstract Granulocyte/macrophage-colony-stimulating factor (GM-CSF) plays a central role in the differentiation and function of dendritic cells, which are crucial for the elicitation of MHC-restricted T cell responses. Preclinical and the first clinical data provide a rationale for the application of GM-CSF in immunotherapy of cancer. Ten patients with renal cell carcinoma stage IV (Holland/Robson) were treated in this pilot study. Therapy was started with GM-CSF alone (2 weeks). Interleukin (IL-2) and interferon α (IFNα) were added sequentially (3 weeks GM-CSF plus IL-2 or IFNα, 3 weeks GM-CSF plus IL-2 plus IFNα). Therapy was performed on an outpatient basis. The cytokine regimen was evaluated for toxicity, clinical response and immunomodulatory effects [fluorescence-activated cell sorting analysis of peripheral blood mononuclear cells (PBMC), mixed-lymphocyte reaction and cytotoxicity of PBMC]. GM-CSF treatment caused a significant increase in the number of PBMC expressing costimulatory molecules. Addition of IL-2 and IFNα led to an increase in $CD3^+$, $CD4^+$, $CD8^+$ and $CD56^+$ PBMC in week 9. In an autologous mixed-lymphocyte reaction a 2.1-fold increase in T cell proliferation was observed after 2 weeks of GM-CSF treatment, and cytotoxicity assays showed changes in natural-killer-(NK)- and non-NK-mediated cytotoxicity in some patients. Two patients achieved partial remission, one patient had a mixed response. The toxicity of the regimen was mild to moderate with fever, flu-like symptoms and nausea being observed in most patients. Severe organ toxicity was not observed. We conclude that GM-CSF might be useful for immunotherapy of renal cell carcinoma, especially in combination with T-cell-active cytokines. Further studies are warranted.

Key words GM-CSF · Renal cell carcinoma · Cytokine therapy

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Introduction

Granulocyte/macrophage-colony-stimulating factor (GM-CSF) plays a crucial role in the proliferation and differentiation of dendritic cells [2, 24, 25]. Several animal models have provided evidence that such antigen-presenting cells (APC) can induce an MHC-restricted tumor-specific T cell response [19, 4, 14, 34]. Tumor vaccination using dendritic cells loaded with tumor antigen can induce both T cell immunity and clinical responses in patients with metastatic melanoma [18] or prostate cancer [30] and in advanced follicular lymphoma [9]. Gene-modified, GM-CSF-secreting tumor cells have conveyed protective immunity against a consecutive tumor challenge [6] and against preestablished small tumor burdens [8] in murine models. In a phase I clinical study using GM-CSF-transduced autologous tumor cells in patients with advanced melanoma, significant humoral and cellular antitumor immunity could be induced [28]. The vaccination efficacy of GM-CSF-transfected tumor cells is mainly attributed to recruitment of local APC at the vaccination site. Peptide vaccination together with systemic administration of GM-CSF could induce clinical remissions in melanoma patients [10]. In vitro, several immunomodulatory effects of GM-CSF on both humoral and cellular immune responses have been described: induction of lymphokine-activated killer cells [29], enhancement of T-cell-mediated cytotoxicity after bone marrow transplantation for hematological malignancies [22], augmentation of antibody-dependent cellular cytotoxicity [11], up-regulation of MHC molecule expression and increase in monocyte toxicity [32]. Therefore, both in vitro and in vivo evidence provides a rationale for the use of GM-CSF in the immunotherapy

of cancer. A combination with T-cell-active cytokines such as interleukin-2 (IL-2) and interferon α (IFNα) may result in synergistic effects.

In advanced renal cell carcinoma (RCC) cytokine therapy has activity in a yet poorly defined subgroup of patients. Rates of complete and partial responses do not substantially exceed 20–30% [16, 12]. Regimens with high-dose IL-2 and IFNα are associated with substantial toxicity, therefore low-dose cytokine regimens with IL-2 and IFNα have been evaluated. Although response rates seem to be somewhat lower with such regimens, toxicity could be considerably reduced [7, 21, 1, 31].

The primary objective of this phase I pilot study was to establish the feasibility, safety and toxicity of a combined treatment with GM-CSF, IL-2 and IFNα in RCC patients. The secondary objective was to evaluate the immunomodulatory effects of the treatment in patients with advanced RCC.

Patients and methods

Patients

Ten patients with histologically confirmed advanced RCC (Holland/Robson stage IV) were entered into this immunotherapy protocol (Fig. 1). Inclusion criteria were age 18–80 years, measurable disease and a Karnofsky index of at least 70%. Exclusion criteria included CNS metastasis, other malignancies, inadequate renal, respiratory, bone marrow and hepatic function, heart failure above New York Heart Association grade II, ventricular arrhythmias above Lown grade III, autoimmune disorders, clinical evidence of coronary heart disease, cerebrovascular insufficiency and severe psychiatric disorders. Informed consent was obtained for participation in the study; the study protocol and patient information form were approved by the local ethics commitee. All patients had been previously nephrectomized, 7 had had additional surgery for metastasis and 3 patients had received (chemo)immunotherapy before being enrolled for the present protocol.

Treatment plan

Patients were treated in an outpatient setting. Immunotherapy (Fig. 1) consisted of a combined treatment with GM-CSF (Leucomax; Novartis Pharma, Nürnberg, Germany), IL-2 (Proleukin; Chiron Corp., Emeryville, Calif., USA), and recombinant IFNα2b (Intron A; Essex Pharma, München, Germany). GM-CSF was administered at 5 μg/kg s.c., twice per week in week 1 and three times per week in weeks 2–5 and 7–9. IL-2 was injected s.c. at 4×10^6 IU/m^2 five times per week in weeks 3–5 and 7–9. IFNα was applied at 5×10^6 U/m^2 three times per week in weeks 3–5 and 7–9. In order to monitor toxicity for the different cytokine combinations, cytokine treatment was performed sequentially. GM-CSF was given alone in weeks 1 and 2, IL-2 was added for 5 patients in weeks 3–5 while IFNα was added for another 5 patients. From week 7 to week 9, all patients received a combination of all three cytokines. The treatment plan is depicted in Fig. 1. During cytokine therapy, paracetamol, indomethacin and metoclopramide were used as concomitant symptomatic therapy as far as clinically needed.

Evaluation

Clinical and laboratory examinations (with assessment of toxicity) were performed weekly throughout the study. Monitoring consisted of clinical history and physical examination, laboratory tests (blood sedimentation rate, complete blood counts, thrombin time, partial thromboplastin time, electrolytes, bilirubin, transaminases, creatinine, amylase, lipase, lactate dehydrogenase), and immunological tests: fluorescence-activated cell sorting (FACS) analysis of peripheral blood mononuclear cells (PBMC), autologous mixed lymphocyte reaction and cytotoxicity assays (see below). Blood samples for immunological tests were taken before treatment and on day 1 of weeks 3, 6 and 10 (always before an additional cytokine was administered). Reevaluation of patients' tumor status was performed in week 10. A complete response (CR) was defined as the disappearance of all clinical and laboratory signs of disease. A partial response (PR) was a reduction of 50% or less in the sum of the products of the diameters of all measurable lesions without an increase in size of any lesion or the appearance of new lesions. No change (NC) was defined as a tumor reduction less than a PR in the absence of progressive disease. Progressive disease (PD) was defined as an increase of at least 25% in the sum of the products of the longest perpendicular diameters of measurable lesions, or the development of new lesions. A mixed response (MR) was defined as PR or CR in one organ together with SD or PD in another organ. Toxicity was evaluated according to the Common Toxicity Criteria (CTC).

FACS analysis of PBMC

Heparinized whole blood was treated with FACS lysing solution (Becton-Dickinson, Heidelberg, Germany) according to the manufacturer's instructions. Direct immunofluorescence staining was

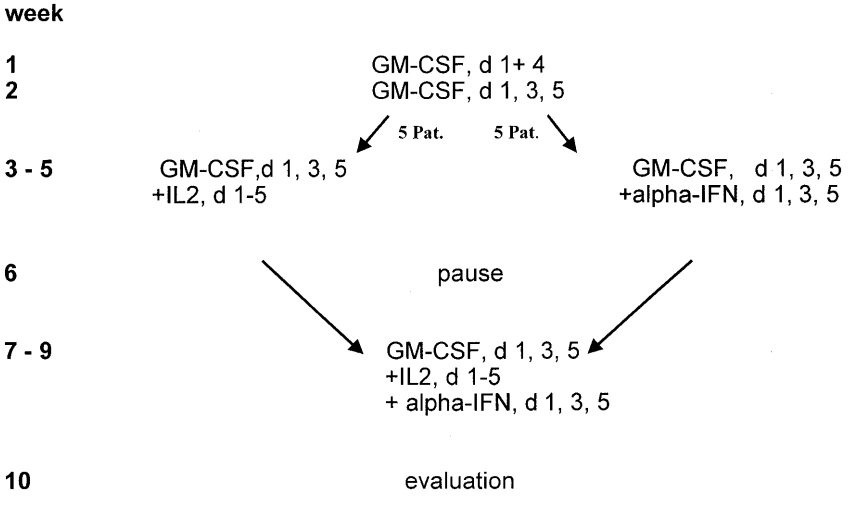

Fig. 1 Treatment schedule. *GM-CSF* granulocyte/macrophage-colony-stimulating factor, 5 μg/kg s.c.; *IL-2* interleukin-2, 4×10^6 IU/m^2 s.c.; *IFN* interferon α, 5×10^6 IU/m^2 s.c

performed in whole blood with the following phycoerythrin(PE)-conjugated monoclonal antibodies (mAb): CD3-PE, CD4-PE, CD8-PE, CD13-PE, CD14-PE, CD16-PE, CD19-PE, CD56-PE, CD80-PE, HLA-DR-PE, isotype-control-PE (Becton-Dickinson) and CD86-PE (Pharmingen, Hamburg, Germany); 10,000 cells were analyzed on a FACScan flow cytometer (Becton-Dickinson). Analysis was done on gated PBMC.

Absolute PBMC counts were calculated from the complete blood count by adding the mononuclear cell values (lymphocytes and monocytes).

Mixed lymphocyte reaction

PBMC from heparinized whole blood were prepared by density gradient centrifugation on Ficoll/Hypaque (Seromed, Berlin, Germany). Interphases were harvested and washed twice at low speed to remove platelets. T cells were isolated by a rosetting cycle of PBMC with sheep red blood cells treated with 2-aminoethyl-isothiouronium bromide (AET, Sigma, Deisenhofen, Germany) as described [3]. The stimulator cell fractions (non-T cells) were treated with 60 μg/ml mitomycin C (Sigma) for 60 min. After extensive washing, different numbers of stimulator cells were added to the culture wells containing 10^5 T lymphocytes well in RPMI-1640 medium supplemented with 4 mM L-glutamine (Gibco, Eggenstein, Germany), 50 μM 2-mercaptoethanol (Roth, Karlsruhe, Germany), 100 U/ml penicillin/streptomycin (Gibco) and 10% heat-inactivated fetal calf serum (Gibco), further referred to as complete medium. The final stimulator-to-responder ratio ranged from 1:10 to 1:312. The maximum proliferation rate was determined by cultivation of 10^5 T cells in the presence of IL-2 and phytohemagglutinin. During the last 16 h of 5 days of culture, 1 μCi/ml [^{3}H]-Thymidine (Amersham Life Science, Buckingham, UK) was added. Cells were harvested and radioactivity measured in a scintillation counter. To quantify the immunomodulatory effects of GM-CSF, cells for the autologous mixed-lymphocyte reaction were collected before treatment and after 2 weeks on GM-CSF, frozen and stored on liquid nitrogen. The test was performed at the end of the treatment protocol on both fractions simultaneously. In order to eliminate differences in proliferation values resulting from different qualities of patients' T cell preparations after freezing and thawing, the ratio T cell proliferation/maximum T cell proliferation was calculated for each stimulator cell concentration. The mean value of the different ratios (R_{mean}) was determined for the mixed-lymphocyte reaction curves before and after GM-CSF treatment. The change of proliferative capacity was expressed as a percentage, as R_{mean} after treatment/R_{mean} before treatment × 100.

Cytotoxicity assays

Cytotoxic activity of PBMC before treatment, after 2 weeks on GM-CSF and after combined therapy with GM-CSF + IL-2 + IFNα in week 10 was assayed by a JAM test [26]. A human renal carcinoma cell line (SLO), a natural-killer (NK)-cell-sensitive human myeloid leukemia cell line (K562) and a human B lymphoma cell line (Raji), which is NK-cell-resistant, were used as target cells. Two days before the assay, SLO cells were seeded in 96-well plates (Nunc) at 1×10^4 cells/well in complete medium. K562 and Raji cells were seeded at 3.5×10^5 cells/ml in 50-ml tissue-culture flasks (Nunc) in 5 ml complete medium 24 h prior to the assay. [^{3}H]-Thymidine labeling was performed at 5 μCi/ml for 7 h for all three target cell lines.

After labelling, cells were washed three times and seeded at 1×10^4 cells/well in 96-well plates in 100 μl Iscoves modified dulbecco's medium (IMDM, Gibco) containing 10% human AB serum and 1:500 thymidine (Sigma). Effector cells (PBMC of patients) were added at an effector: target ratio of 10:1 in 100 μl IMDM. Cells were coincubated for 20 h, then harvested on fiberglass filters type 6–7 (Inotech). The percentage specific killing was calculated from DNA retained (cpm) in the filters [13].

Statistical analysis

Changes in absolute numbers of distinct-surface-marker-positive PBMC during cytokine therapy and increases in proliferation in the autologous MLR were statistically evaluated according to the Wilcoxon matched-pair analysis rank test (MPAR test).

Results

Treatment toxicity

The treatment could be performed on an outpatient basis. The overall systemic toxicity of the treatment was mild to moderate with CTC-NCI grade 1 or 2 fever, flu-like symptoms and nausea being observed in most of the patients. One patient developed grade 3 fever while on both GM-CSF + IL-2 and GM-CSF + IL-2 + IFNα; no other grade 3 or 4 toxicities were observed. Fatigue and flu-like symptoms were generally more pronounced when the three cytokines were given concurrently. No severe organ toxicity occurred. There was a slight increase of creatinine level (up to 1.5 times the pretreatment value) in 6 patients and grade 1–2 hepatic toxicity (elevated transaminases) in 2 patients during combined treatment with GM-CSF + IL-2 + IFNα. Table 1 lists the systemic toxicity in the different phases of the study.

Subcutaneous injection of IL-2 caused transient erythema and induration at the injection site in most patients.

Of the 10 patients, 8 were considered evaluable for response at the end of the protocol in week 10: in 1 patient, treatment was discontinued in week 7 because of newly developing, clinically symptomatic cerebral metastasis that had been undetectable in a computed tomography scan 6 weeks before. In another patient, treatment was stopped in week 5 because of leukocytosis with massive eosinophilia (white blood cells 49,000/μl, eosinophils 30,380/μl). There was no change of the pulmonary and bone metastases in this patient, and the eosinophil counts returned to normal after discontinuation of the treatment.

Six of the 10 patients received 100% of the scheduled cytokine dose. Because of leukocytosis higher than 2×10^4/μl, GM-CSF was reduced from a weekly dose of 3×5 μg/kg to 2×5 μg/kg in 1 patient during week 2 and in 2 patients during week 7 of the treatment. In 1 patient IFNα was reduced from three doses of 9×10^6 U to three doses of 4.5×10^6 U in week 8 because of increasing transaminases.

Treatment response

Two PR and one MR could be documented. Both patients undergoing PR had had no prior treatment apart from tumor nephrectomy. One patient only had lung metastasis, the other lung and liver metastasis as well as a massive local relapse. Remarkably, in the second patient there was complete response of the local relapse

Table 1 Toxicity (according to CTC-NCI). The table shows toxicity for each individual patient during the different phases of the study: granulocyte/macrophage-colony-stimulating factor (GM-CSF) alone, GM-CSF plus interleukin (*IL-2*) or interferon α (*IFNα*) and GM-CSF plus IL-2 plus IFNα. Toxicity is indicated as overall (*O*), fever (*F*), gastrointestinal (*G*), cutaneous (*C*), followed by CTC grade 1–4

Patient no.	GM-CSF alone	GM-CSF + IL-2 or GM-CSF + IFNα	GM-CSF + IL-2 + IFNα	Remarks
1		O (1) F (1) C (1/2)	O (1) F (1) C (1/2)	
2		G (1) F (2) C (1/2)	O (1) G (1) F (2) C (1/2)	
3	O (1) G (1) F (2)	O (2) G (1) F (2) C (1/2)	O (2) G (1) F (2) C (1/2)	GM-CSF twice per week because of leukocytosis (week 7)
4		O (1) G (1) F (2) C (1/2)	O (1) G (1) F (2) C (1/2)	GM-CSF twice per week because of leukocytosis (week 7)
5	O (1) G (1) F (1)	O (2) G (1) F (3) C (1/2)	O (2) G (1) F (3) C (1/2) L (1)	
6		O (1) G (1) F (2) C (1/2)	O (1) G (1) F (2) C (1/2)	GM-CSF twice per week because of leukocytosis (week 2)
7			F (2)	Off study in week 7 because of CNS metastasis
8		O (2) G (1) F (2) C (1)	O (2) G (1) F (2) C (1)	
9		O (1) G (1) F (1)	O (1) G (1) F (1) L (2)	IFN dose reduction (50%) week 8 because of liver toxicity
10		O (2) G (1) F (2) C (1/2)		Off study in week 5 because of massive leukocytosis with eosinophilia

and of the liver metastasis, but owing to residual lesions in the lungs, the response was formally classified as PR. In 1 patient, a "mixed response" was seen: there was PR of the pulmonary and mediastinal metastases; the bone metastasis was progressive. This patient had had nephrectomy, metastasis surgery and radiation as the previous treatment. In 3 patients there was no change in the number and size of metastases after therapy; 1 of these patients was excluded from the protocol because of leukocytosis with massive eosinophilia in week 6. Four patients had progressive disease, including the patient who was excluded in week 7 because of a newly developing cerebral metastasis. Another patient had had progressive disease during treatment with IFNα before enrollment in this protocol. The clinical responses are listed in Table 2.

Analysis of leukocyte subsets

After 2 weeks of treatment with GM-CSF alone, the white blood cell count mean rose from 6278 ± 1597/μl to 14,930 ± 6443/μl. The eosinophil count increased slightly from 142 ± 56/μl before treatment to 253 ± 173/μl under GM-CSF alone. After addition of IL-2 or IFNα significant eosinophilia occurred (mean eosinophil count 1493 ± 955/μl); after administration of all three cytokines 1824 ± 738 eosinophils/μl were counted. The lymphocyte count rose from 1204 ± 314/μl before treatment to 2661 ± 1536/μl in week 10.

FACS analysis of patients PBMC was carried out before treatment, after 2 weeks of GM-CSF, after 3 weeks of GM-CSF + IL-2 or GM-CSF + IFNα and finally after three weeks of GM-CSF + IL-2 + IFNα. The expression of surface molecules involved in antigen presentation and T cell activation, such as CD80 (B7.1), CD86 (B7.2) and HLA-DR was analyzed and subpopulations, including T cell ($CD3^+$) CD4- and CD8-subsets, B cells ($CD19^+$), NK cells ($CD56^+$) and monocytes ($CD14^+$) were quantified. The absolute number of these cells was calculated from complete blood counts and the percentage of marker-positive PBMC on FACS analysis.

There was a marked increase in $CD80^+$ ($P = 0.02$) and $CD86^+$ ($P = 0.03$) PBMC after 2 weeks of treatment with GM-CSF alone. Whereas $CD80^+$ cells showed a further increase after GM-CSF + IL-2 or GM-CSF + IFNα and reached their highest values in week 10 after administration of all three cytokines, addition of IL-2 and/or IFNα caused no further increase in the number of $CD86^+$ cells (Fig. 2).

There was no significant change in overall HLA-DR^+ PBMC after 2 weeks on GM-CSF; only the determination of HLA-DR on gated monocytes showed a 75% increase of HLA-DR^+ cells (data not shown).

As far as the effector cell populations are concerned (Fig. 2), there was a transient decrease in the absolute number of T cells under GM-CSF alone. Addition of IL-2 and/or IFNα was followed by a significant increase of $CD3^+$ ($P = 0.008$; not shown), $CD4^+$ ($P = 0.001$) and $CD8^+$ ($P = 0.02$) cells. No significant influence on the number of $CD19^+$ B cells could be documented. The time course of the number of $CD56^+$ cells was similar to that of T cells: a slight decrease when GM-CSF was administered alone was followed by up to a 2.4-fold increase in $CD56^+$ cell count ($P = 0.05$) after addition of IL-2/IFNα.

Mixed lymphocyte reaction

To determine whether 2 weeks treatment with GM-CSF had an influence on the proliferative capacity of T cells and/or the stimulatory ability of non T stimulator cells, an autologous mixed-lymphocyte reaction was performed before and after treatment with GM-CSF. This reaction contains fetal calf serum as a source of foreign

Table 2 Patient characteristics and clinical response. *PR* partial response, *MR* mixed response, *NC* no change, *PD* progressive disease

Patient no.	Gender	Age (years)	Date of primary diagnosis–diagnosis of metastasis	Site of metastasis	Prior treatment	Response
1	Male	49	3/95–3/95	Lung, liver, spleen local relapse	Pulmonary surgery	PD
2	Male	61	2/93–11/94	Lung	None	PR
3	Male	63	11/92–11/92	Lung, liver	Metastasis surgery α-interferon	PD
4	Female	59	3/91–3/94	Lung, thyroid gland	Thyroidectomy, radiation	NC
5	Male	48	8/95–8/95	Lung, bone, soft tissue, mediastinum	Bone surgery, radiation	MR
6	Male	52	1989–7/90	Lung	Metastasis surgery, α-interferon	NC
7	Male	51	1/95–12/95	Lung, pleura, bone	None	Off study
8	Female	60	12/95–12/95	Lung, liver	Hemicolectomy	PD
9	Female	69	1993–2/96	Lung, liver, local relapse	None	PR
10	Female	54	6/93–6/93	Lung, bone	Metastasis surgery α-interferon + vinblastine	Off study

antigen and, in 7 patients, data were evaluable. After GM-CSF therapy, a 2.1-fold increase in autologous T cell proliferation could be observed. Figure 3 shows the data of all evaluable patients.

Cytotoxicity assays

The cytotoxic activity of PBMC against the different cell line targets K562, SLO and Raji was determined before therapy, after 2 weeks of GM-CSF treatment and at the end of the protocol after GM-CSF/IL-2 and IFNα. Data from 6 patients were evaluable. GM-CSF alone was able to modulate the cytotoxic activity of PBMC; an increase of cytotoxicity could be measured in 2 patients against K562, in 4 patients against SLO and in 3 patients against Raji. Of the 4 patients who showed increased cytotoxicity against SLO, 2 responded to therapy. After addition of IL-2 and IFNα, there was a further increase in cytotoxicity in some patients. Compared to pretherapeutic values, an augmented cytotoxic activity could be measured in most patients (Table 3).

Discussion

Considering the disappointing 5 year survival rates [16], advanced RCC remains a tumor with a bad prognosis. Systemic cytokine treatment using IL-2 and IFNα can achieve objective response rates of only 20%–30%. Therefore, a continued search for novel treatment strategies and for novel agents with better antitumor activity is needed.

In recent years, GM-CSF has become a promising cytokine in immunotherapeutic approaches, since there are preclinical [6, 8, 10, 29, 22, 11, 32] and the first clinical data [28] suggesting induction of immune-mediated antitumor activity by GM-CSF. Since GM-CSF alone has only little antitumor activity in RCC patients [33, 23], we decided to evaluate a combined treatment using GM-CSF along with cytokines able to trigger T cells such as IL-2 and IFNα. This strategy aimed to augment antigen presentation and T cell activation in order to elicit a tumor-specific T cell response.

We could demonstrate that a combined cytokine treatment with GM-CSF, IL-2 and IFNα is feasible and safe; no severe toxicity was observed. Objective clinical responses (PR) were seen in 2 out of 10 evaluable patients, and 1 patient was classified as having a MR, which, in our opinion, warrants further investigation.

Immunological data show that GM-CSF alone leads to a significant increase in $CD80^+$ and $CD86^+$ PBMC. These costimulatory molecules are expressed on APC and are crucial for T cell activation. It can be speculated that systemic administration of GM-CSF may recruit APC in peripheral blood as well as in tissues, thereby increasing the presentation of tumor-specific antigens. In animal models it could be demonstrated that intraperitoneal administration of GM-CSF leads to an expansion of dendritic cells [5]. In our patients, the increase of $CD80/CD86^+$ PBMC is probably caused by a phenotypically heterogenous cell population: a high number of $CD14^+$ monocytes and a low number of peripheral blood dendritic cells, which can hardly be detected because of their low frequency and the lack of truly lineage-specific markers for all maturation stages of dendritic cells. Since data suggest that monocytes differentiate into dendritic cells in vivo by transendothelial trafficking [20] and since GM-CSF is a maturation factor that, in association with IL-4, drives the dendritic differentiation of peripheral monocytes, the increase of $CD\text{-}14^+$ cells may lead to an increase of fully competent APC in tissues.

GM-CSF alone did not significantly affect the number of other effector cells. After addition of IL-2 or IFNα, increases of $CD3^+$, $CD4^+$, $CD8^+$ and $CD56^+$ PBMC occurred and reached significant levels in weeks 7–10 under combined cytokine treatment. An autologous mixed-lymphocyte reaction was used as a surrogate parameter for T cell function, whereby fetal calf serum, a component of the cell culture medium, likely serves as a source of foreign antigen [27]. After 2 weeks of GM-CSF

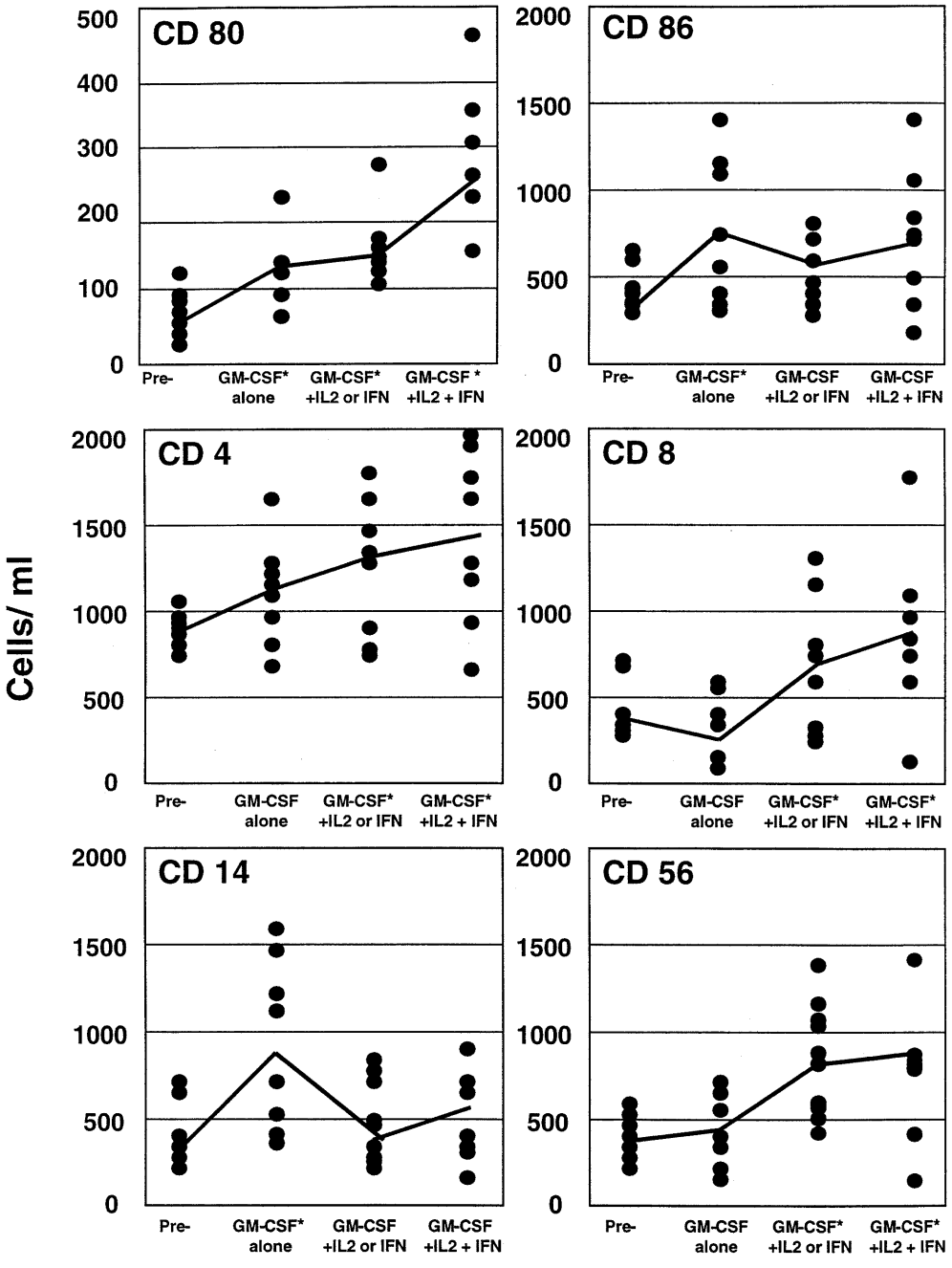

Fig. 2 Surface markers on peripheral blood mononuclear cells (marker-positive cells/μl) during the different phases of the study (*pre* pretreatment, GM-CSF alone, GM-CSF plus IL-2 or IFNα and GM-CSF plus IL-2 plus IFNα). Data for all patients are given for each assay time. Mean values for each assay time are connected by a line. *Significant increase compared with pretreatment value ($P < 0.05$)

treatment, patients' T cells showed a 2.1-fold higher level of proliferation than before treatment. This could be due to an augmentation of both antigen presentation and T cell proliferative capacity.

Cytotoxicity assays with different target cells demonstrate that GM-CSF alone is able to modulate cytotoxicity of NK and non-NK effector cells. Out of 8 patients, 6 could be evaluated; PBMC of 2 patients could not be evaluated for technical reasons. After 2 weeks on GM-CSF, 2 patients with a response and 1 patient with no change showed an increase in cytotoxic activity against an allogeneic RCC cell line, whereas there was no increase in cytotoxicity against the NK cell target K562. However, in 3 out of 6 patients, an increased NK cell cytotoxicity could be demonstrated under combined GM-CSF/IL-2/IFNα therapy. Owing to the small number of patients, a correlation with clinical outcome could not be demonstrated.

Our data confirm immunomodulatory activity and low toxicity of GM-CSF when given three times per week at a dose of 5 μg/kg. Wos et al. [33] used GM-CSF alone at a dose of 3 μg/kg daily for the treatment of advanced RCC in a phase II trial and observed PR in 2/26 previously treated patients. Toxicity was mild in this study. Rini et al. [23] also administered GM-CSF alone at a dose of 10 μg/kg per day for the treatment of

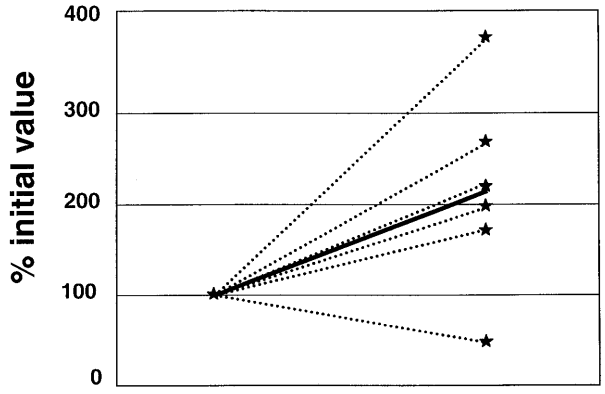

Fig. 3 Autologous mixed lymphocyte reaction. T cell proliferation (percentage of the initial value). Data are given for each evaluable patient (···) before treatment (=100%) and after 2 weeks of treatment with GM-CSF. —— The mean increase in T cell proliferation ($P < 0.05$)

Table 3 Cytotoxicity of peripheral blood mononuclear cells against an allogenic renal carcinoma cell line (*SLO*), a natural killer (*NK*) cell target (*K562*) and a NK-resistant cell line (*Raji*). Specific cytotoxicity is indicated separately for each evaluable patient, each target cell line and the different phases of the study: pretreatment/GM-CSF alone/GM-CSF plus IL-2 plus IFNα

Patient	Specific cytotoxicity (%)		
	SLO	K562	Raji
1	10/6/13	20/25/60	16/14/22
2	15/38/17	15/2/14	12/38/13
4	5/15/25	25/26/25	6/20/10
5	2/14/14	3/2/2	2/2/2
6	8/6/4	8/23/43	6/12/10
8	2/35/60	15/15/25	12/5/20

advanced RCC in a phase II trial and observed some antitumor activity but increased toxicity at this dose. Schiller et al. [26] used combined treatment with GM-CSF (2.5, 5 and 10 μg/kg daily) and IL-2 (4.5×10^6 or 9×10^6 IU/m^2 daily) in 34 patients with different refractory cancers. This study included 15 patients with advanced RCC, 11 of whom were not nephrectomized. Although none of them qualified as PR because of a lack of response in their primary tumor or at other metastatic sites, 5/15 patients had more than 50% reduction in the size of their lung metastasis, showing antitumor activity for this regimen. The rationale for combining GM-CSF with T cell cytokines is emphasized by this study. The combination with both IL-2 and IFNα might yield better results; an overall survival benefit has recently been demonstrated for IFNα treatment in a randomized trial with 335 evaluable patients [15], furthermore combined IL-2/IFNα therapy gave better results than when each cytokine was used alone in a prospective randomized study in 425 patients [17].

The question whether GM-CSF is able to augment the antitumor effects of IL-2 and IFNα is being addressed in a current clinical study using sequential application of the cytokines.

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