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# Phase I trial of intravenous infusion of ex-vivo-activated autologous blood-derived macrophages in patients with non-small-cell lung cancer: toxicity and immunomodulatory effects*

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**Summary.** The purpose of this phase I study was to evaluate the toxicity and biological activity of autologous blood-derived macrophages activated ex-vivo with recombinant human interferon γ (rhuIFNγ) [monokine-activated killer (MAK) cells] and administered intravenously to 11 lung cancer patients once a week for 6 consecutive weeks. Peripheral blood monocytes were collected by leukapheresis and then purified by counterflow elutriation. The MAK cells were generated by culturing the purified monocytes in Teflon bags for 7 days and adding rhuIFNγ to the cultured cells for the last 18 h. These MAK cells expressed differentiation-associated surface antigen MAX1, and were cytotoxic in vitro against tumour cell line U937. The MAK cells were infused at dose levels from $1 \times 10^7$ to $5 \times 10^8$ on an intrapatient dose-escalating schedule. No severe adverse side-effects occurred. Toxicity was mild to moderate [primarly fever (75%) and chills (32%)], non-dose-dependent, and non-cumulative. No consistent change in haemostatic function, or liver or renal function was observed. Dose-limiting toxicity was not reached at $5 \times 10^8$ cells (optimal dose reproduced for each patient). The maximum tolerated dose was not determined. The immunomodulatory activity of i.v. infused MAK cells was demonstrated both in vivo by significant increases in granulocyte count and neopterin level in the patients' peripheral blood post-infusion and in vitro by secretory products (IL-1. TNFα, neopterin, and thromboplastin-like substance) in the culture supernatants. The in vivo traffic patterns of autologous MAK cells labelled ex-vivo with $^{111}$In oxine were studied in 7 patients. Gamma imaging showed an immediate but transient lung uptake (<24 h), and a progressive uptake of radioactivity in the liver and spleen was seen from 6 h to 72 h post-infusion. Our results indicate that the preparation of high numbers of autologous, blood-derived MAK cells is a feasible procedure, and their transfusion is safe for patients. This immunotherapeutic approach seems to be encouraging from the point of view of establishing an adjuvant therapeutic modality in cancer patients with minimal residual disease.

**Key words:** Human macrophages – Interferon γ – Adoptive immunotherapy – Non-small-cell lung cancer

## Introduction

Early surgery is the only consistently effective method of treating lung carcinoma, which remains one of the most common causes of death in man [17]. The need for new effective adjuvant treatments is compelling because of the increasing incidence of local and systemic recurrence associated with surgical resection on its own. Currently available adjuvant chemotherapy, immunotherapy or radiotherapy has not proved to be effective thus far, and we have been encouraged to explore cellular adoptive immunotherapy as a new adjuvant modality in patients with resected lung cancer and high risk of metastases.

Initially it was reported that in vitro incubation of peripheral blood lymphocytes from cancer patients with interleukin-2 (IL-2) resulted in the generation of effector cells, named lymphokine-activated killer (LAK) cells, with lytic activity against fresh and cultured autologous tumour cells [13, 18, 20], and that adoptive immunotherapy with LAK effector cells in advanced cancer patients was effective for partial regression of some tumours [21]. However, these results of antitumour activity were obtained only at the expense of considerable toxicity such as IL-2-associated capillary-leak syndrome [19].

A large amount of data support the concept that the tumour-associated monocyte/macrophage system also

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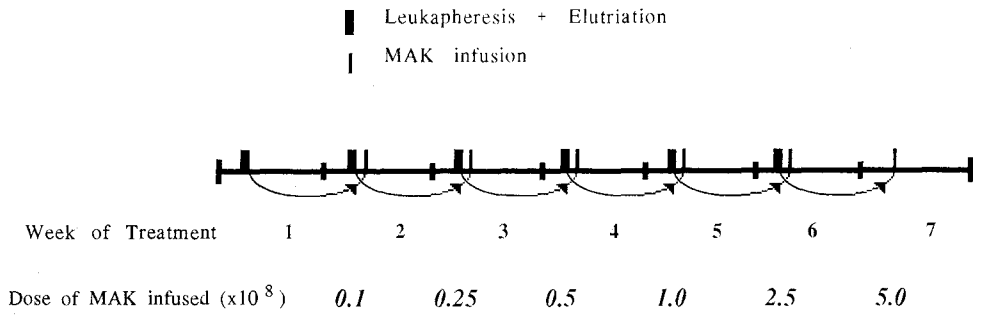


**Fig. 1.** Scheme of adoptive immunotherapy with recombinant-human-interferon-γ-treated monokine-activated killer (rhuIFNγ/MAK) cells

plays an important role in antitumour defence mechanisms [11]. Human monocytes/macrophages, when activated by recombinant human interferon γ (rhuIFNγ) develop cytotoxic properties against autologous tumour cell targets and allogeneic tumour cell lines [10, 16]. Chokri et al. [5] demonstrated that the adoptive transfer of human monocytes/macrophages plus interferon to mice resulted in the regression of lung metastases. However, reports of adoptive immunotherapy trials with monocytes or blood-derived macrophages and rhuIFNγ in patients are infrequent [2, 23] and our understanding of the in vivo effects of these adoptively transferred cells is limited.

The above considerations led us to conduct a phase I study of i. v. infused ex-vivo rhuIFNγ-activated autologous blood-derived macrophages, named monokine-activated killer (MAK) cells, in patients with non-small-cell lung carcinoma. The main objectives of this preliminary study were to determine the feasibility, toxicity and immunomodulating activity of this cellular adoptive immunotherapy modality.

## Patients and methods

### *Patient population*

Eleven patients with lung cancer were entered into this phase I trial. All 11 patients had histopathologically documented non-small-cell lung carcinoma for which no effective standard treatment was available after surgical resection. Eligibility requirements included a Karnofsky performance status greater than 70% and a life expectancy of at least 3 months, as well as a leukocyte count $>4 \times 10^9$/l, platelets $>100 \times 10^9$/l, serum total bilirubin <20 mmol/l, and serum creatinine <125 mmol/l. Exclusion criteria included severe current infection, significant cardiovascular abnormalities (e. g. congestive heart failure, unstable angina, prior myocardial infarction, arrythmias), severe obstructive pulmonary disease, or other prior immunotherapy within 12 weeks, and major surgery or chemotherapy or radiation therapy within 4 weeks before initiation of treatment. This phase I study was approved by the ethics committee of the University of Strasbourg and signed informed consent was obtained from all patients before entrance.

### *Study design*

Figure 1 illustrates the overall schedule of leukapheresis and macrophage infusions for all patients who entered into this trial. Each patient was treated once weekly with escalating dose levels (I, II, III, IV, V, VI) of autologous MAK cells administered by i. v. bolus infusion according to the following schedule: 0.1, 0.25, 0.50, 1.0, 2.5, and $5.0 \times 10^8$ cells/day. During the first week of treatment, patients were hospitalized to permit close monitoring of any adverse effects. Thereafter, patients were treated in the outpatient setting. The first patient entered in the study developed a grade III dyspnoea 5 min after the fourth i. v. bolus dose of $0.1 \times 10^8$ cells. Subsequently all cell preparations were filtered (40 μm-microaggregate Ultipor filter, Pall Biomed. Inc., Puerto Rico) before infusion. Patients received 5 mg dexchlorpheniramine i. v. for severe rigors and 500 mg paracetamol i. v. every 4 h as needed for fever >39° C. Peripheral blood was drawn at the baseline time and at 6, 24, 48, and 72 h post-infusion. Each sample was tested for the following parameters: blood cell counts, haemostatic parameters, cytokines, C-reactive protein and neopterin, as described below.

### *Preparation of activated macrophages*

*Blood monocyte isolation.* Leukapheresis was performed weekly to collect peripheral blood mononuclear cells (PMNC) as described previously [8, 9]. Briefly, Whole blood, 5–8 l, was processed using a Cobe 2997 cell separator (Cobe Lab., Lakewood, Colo.) and acid/citrate/dextrose as anticoagulant. Leukapheresed PMNC contained 50%–60% lymphocytes, 40%–50% monocytes and 5%–10% granulocytes (Table 3).

PMNC were then subjected to counterflow centrifugation-cell elutriation to isolate monocytes, as described previously [8], in the absence of a Ficoll/Hypaque density gradient centrifugation step. PMNC were loaded onto a JE-5.0 rotor with a large-capacity chamber using a J6M-E centrifuge (Beckman Instruments, Palo Alto, Calif.). $Ca^{2+}$- and $Mg^{2+}$-free Hank's balanced salt solution containing 2% human serum albumin was used as elutriation medium. Elutriated monocytes were usually more than 90% pure, as determined by morphology (May-Grunwald/Giemsa staining), cytochemistry (non-specific esterase staining) of the cytocentrifuged specimens. Elutriated monocytes were monitored for bacterial and fungal growth by microscopy, direct observation after Gram staining and culture assays in Castaneda vials at 37° C for 15 days. All reagents and media were free of endotoxin, as determined by a *Limulus* amoebocyte lysate kit using a chromogenic substrate assay (1 endotoxin unit = 12 ng/ml endotoxin; sensitivity = 0.125 EU/ml; Kabivitrum, Sweden). We have demonstrated elsewhere the functional integrity of these elutriated monocytes [8, 9].

*Monocyte culture and activation.* The freshly isolated monocyte suspensions were adjusted to $1 \times 10^6$ cells/ml in RPMI-1640 culture medium supplemented with penicillin (100 units/ml), streptomycin sulphate (100 mg/ml), glutamine (2 mM), pyruvic acid (2 mM), non-essential amino acids (1 ml), indomethacin (5 nM), mercaptoethanol (0.3 μM) (Vietech Laboratories, Lyon, France) and 2% (v/v) heat-inactivated autologous human serum. They were placed in hydrophobic Teflon culture bags (Stericell, Dupont de Nemours, France) allowing the cells to be cultivated in suspension. The bags, containing 100–300 ml suspension, were incubated for 7 days at 37° C in a 95% air and 5% $CO_2$ humidified incubator. Under these conditions monocytes differentiated into macrophages after 6 days of culture, as judged by morphology, cytochemistry, and the appearance of the macrophage specific membrane antigen MAX1 as discussed below. rHuIFNγ (specific activity = $20 \times 10^6$ units/mg protein) (Boehringer, Ingelheim, FRG) was added to the cultures at 500 U/ml 18 h before cell harvest. After 7 days, cells were harvested from the Teflon bags by gentle agitation at 4° C, counted for macrophages (as judged by morphology), washed twice in 4% human serum

albumin, and resuspended at a concentration of $1.0 \times 10^7$ cells/ml before in vivo infusion and in vitro studies.

### *Toxicity assessment*

Baseline studies included a physical examination, blood cell count with leucocyte differentials, clotting studies, a complete automated blood chemical profile and urinalysis (creatinine clearance and 24 h urinary protein), chest X-ray, and electrocardiogram.

After each infusion, patients were assessed for evidence of subjective or objective toxicity on a daily basis during the 6 weeks of treatment according to the World Health Organization (WHO) toxicity grading scale. Dose escalation was permitted in the absence of grade III toxicity. The dose-limiting toxicity was defined as toxicity of grade III or higher by WHO criteria. The maximum tolerated dose was defined as the dose level that produced grade III toxicity in 50% of patients.

### *Characterization of cells*

*Cell counts.* Cell counts were obtained using a Coulter counter model ZM (110-µm-diameter orifice) (Coulter Coultronics, France). Leucocyte differentials were determined from May-Grunwald/Giemsa (MGG)- and non-specific-esterase-stained cytocentrifuge smears. Cell membrane integrity was checked by the trypan blue dye exclusion method. Elutriated monocytes were analysed for cell-surface CD14 antigen expression by immunofluorescence using anti-LeuM3 fluorescein-isothiocyanate-conjugated (Becton Dickinson, France) monoclonal antibody. Differentiated macrophages recovered from the Teflon bags were characterized for the expression of a specific membrane antigen MAX1 using the immunoperoxidase slide technique [1] (anti-MAX1 monoclonal antibody, a mouse IgG1*K*, was a generous gift from R. Andreesen, Medizinische Universitäts Klinik, Albert Ludwig Universitäts, Freiburg i. Breisgau, FRG).

*Phagocytosis.* Monocyte and macrophage ability for phagocytosis was determined by using yeast particles as previously published [6]. Cell suspensions (1 ml) with a density of $5 \times 10^5$ cells/ml were incubated for 2 h in petri culture dishes. Adherent cell monolayers were then incubated for 2 h at 37° C with heat-inactivated yeast particles (*Saccharomyces cerevisae*, 30 particles/cell), washed with phosphate-buffered saline, and stained with MGG. Each preparation was judged with regard to the phagocytosed particles divided by the number of counted cells, which gives the phagocytic index.

*Procoagulant activity.* The capacity of MAK cells to shorten the clotting time of human plasma was evaluated by a one-stage recalcification time as previously described [8, 9]. Samples of 100 µl macrophage suspension ($5 \times 10^6$ cells/ml) were added to 100 µl citrated control human plasma and the mixture was incubated for 5 min at 37° C. Clotting was triggered by the addition of 100 µl 20 mM $CaCl_2$. A calibration curve of clotting time was constructed using dilutions of a rabbit brain thromboplastin suspension (Dade, Düdinger, Switzerland).

*Monokine release.* Human interleukin-1β (IL-1β), tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6) levels were monitored in the culture supernatants and in the patient's sera before and 6, 24, 48, 72 h after MAK infusions. Levels of IL-1β and TNFα were measured by a radioimmunoassay (IL-1βIRMA, TNF-α IRMA, Medgenix Diagnostics, Brussels, Belgium; detection limits = 1 pg/ml and 0.3 pg/ml respectively). Levels of IL-6 were measured by an enzyme sandwich immunoassay (InterTest 6, Genzyme Corporation, Boston, Mass.; detection limit = 0.16 ng/ml).

*Antitumour activity in vitro.* The assay was a standard 24-h [$^3$H]thymidine incorporation cytostasis assay using the tumour cell line U937 as target. Cytostasis effects were expressed as the percentage inhibition of [$^3$H]thymidine incorporation by the treated tumour cells compared to the control tumour cells as previously reported [6]. The cytostasis effects were correlate with the cytotoxic effects, measured by the exclusion of typan blue dye, instead of thymidine incorporation by the tumour cells. Thymidine incorporation was measured by liquid scintillation in a Beckman scintillation counter. The effector cells ($10^4$ cells) were activated by exposure to 500 units/ml rhuIFNγ for 18 – 24 h before adding the tumour cells ($10^4$ cells in log-phase growth).

### *In vivo kinetics of $^{111}$In-labelled autologous macrophages*

For the in vivo traffic studies, blood monocytes from 7 study patients were collected 1 week after completing the phase I trial. Autologous macrophages were generated by methods similar to those used for the phase I trial. Among the 7 patients, 4 received autologous MAK cells and 3 autologous non-activated macrophages. Samples of 10 ml $^{111}$In-labelled washed macrophage suspension ($2 \times 10^8$ cells) in Tyrode/albumin solution (about 200 – 300 µCi) were infused over 1 min through a 19-gauge butterfly needle into a peripheral vein, and followed by a normal saline flush of 20 ml. Gamma camera imaging was carried out during infusion, every 10 s for 10 min after infusion, and at 1, 3 and 7 days after infusion a gamma camera APEX 415 (Elscint, Israel) linked with a medium-energy collimator. Following the gamma camera examination, gamma counting was carried out on serial whole-blood samples, drawn on EDTA from a separate peripheral venipuncture site on days 1 and 3 after infusion, for analysing radioactivity using a 1282 Compugamma counter LKB (LKB Wallac, Finland).

### *Statistics*

Results are expressed as means ± standard deviations. Statistical analysis was performed using Student's *t*-test for paired samples.

## Results

### *Patient characteristics*

The characteristics of all 11 patients entered in this study are summarized in Table 1. All of them had received surgical therapy for their malignancies. Of the 11 patients treated in this phase I study, 10 completed the 7-week treatment protocol. One patient was removed from the protocol after three cycles because of perisurgical blood-transfusion-related EBV hepatitis but was still included in our evaluation of toxicity and immunomodulatory effects. These 11 patients received a total of 63 separate infusion courses. The starting cell dose for initial patient entries was $0.1 \times 10^8$, with subsequent entries at $0.25 \times 10^8$, $0.5 \times 10^8$, $1 \times 10^8$, $2.5 \times 10^8$ and $5 \times 10^8$ cells (Table 2).

### *Preparation of ex-vivo-activated cytotoxic macrophages*

Leukapheresis was performed once weekly during the 6-week study period. MGG differential data pooled from the 64 different leukaphereses of the patients participating in this phase I study are presented in Table 3. The leukapheresis product obtained by the Cobe 2997 cell separator contains predominantly PMNC cells. Granulocytes accounted for less than 10% of the final product. The red/white blood cell ratio and the platelet/white blood cell ratio were in the ranges of 3 – 5/1 and 5 – 10/1 respectively. On the basis of these data, the blood leukapheresed buffy

**Table 1.** Patient characteristics

| | |
|---|---|
| No. of patients entered | 11 |
| Sex (M/F) | 10/1 |
| Median age (years; range) | 59 (50–67) |
| Tumour types | |
| Epidermoid carcinoma | 8 |
| Adenocarcinoma | 3 |
| Tumour staging (TNM system)[a] | |
| T1N1 | 5 |
| T2N1 | 6 |
| Prior therapy | |
| Pneumonectomy | 5 |
| Lobectomy | 6 |

[a] American Thoracic Society: clinical staging of primary lung cancer [22]

**Table 2.** Number of patients treated at various dose levels

| Dose level | $10^{-7} \times$ MAK[a] cells infused | Entered | Completed one full cycle |
|---|---|---|---|
| I | 1.0 | 11 | 11 |
| II | 2.5 | 11 | 11 |
| III | 5.0 | 11 | 11 |
| IV | 10.0 | 11 | 10 |
| V | 25.0 | 11 | 10 |
| VI | 50.0 | 11 | 10 |

[a] MAK, monokine-activated killer

**Table 3.** Obtention of blood-derived autologous MAK cells

| Parameter | Cytapheresis concentrate[a] ($n = 63$) | Elutriation product[a] ($n = 63$) |
|---|---|---|
| Blood processed (l) | 5.6 ±1.2 | |
| Leucocytes | 82.21±29.67($\times 10^9$/l) | 1.64±0.69($\times 10^9$) |
| PMNC[b] (%) | 9.1 ± 7.8 | 5.5 ±3.1 |
| Lymphocytes (%) | 44.6 ±15.3 | 5.6 ±1.4 |
| Monocytes (%) | 46.2 ±13.2 | 89.7 ±5.8 |
| RBC: WBC[c] | 3.9 : 1 | 0.0003 : 1 |
| PLT: WBC[d] | 37.4 : 1 | 0.7 : 1 |

| | Cells at various times of culture[a] | | |
|---|---|---|---|
| | 0 days | 6 days | 7 days |
| $10^{-9} \times$ Leucocytes ($l^{-1}$) | 1.646±0.572 | 1.051±0.428 (64.8 ±20.5)[e] | 0.871±0.365 (42.33±25.9)[e] |
| PMNC (%) | 5.5 ±3.1 | 0.3 ± 0.1 | 0.1 ±0.1 |
| Lymphocytes (%) | 5.6 ±1.4 | 6.7 ±1.5 | 5.8 ± 2.3 |
| Mo/Mφ(%)[f] | 89.7 ±5.8 | 93 ± 2.2 (60.4 ± 7.5)[e] | 94.2 ± 1.7 (42.5 ±10.5)[e] |

[a] All values are expressed as mean ± standard deviation
[b] Peripheral blood mononuclear cells
[c] Red blood cell to white blood cell (leucocyte) ratio
[d] Platelet to white blood cell (leucocyte) ratio
[e] In parentheses are expressed the cell recoveries with time in culture
[f] Monocyte/macrophage ratio

coats were not preseparated on a Ficoll/Hypaque density gradient prior to elutriation.

During the elutriation procedure, a highly enriched monocyte population was obtained in the rotor-off fraction with a low contaminating cell fraction, mostly comprising granulocytes and lymphocytes (Table 3). The red/white blood cell ratio and the platelet/white blood cell ratio were in the ranges of 0.0005–0.0001/1 and 0.5 to 1/1 respectively. The yield (mean ± SD) of purified blood monocytes per elutriation procedure was $1.64 \pm 0.69 \times 10^9$. Viability in all elutriated cell fractions was 98% or greater. All cell fractions were negative for bacterial or fungal contamination.

Following a 6-day culture period and a 18-h exposure to rhuIFNγ, cell recoveries in the Teflon culture bags are shown in Table 3. Macrophage recovery was 42.5±2.5% (mean ± SD) and their purity was 94.2±1.7% (mean ± SD). These monocyte-derived cells showed a morphologically characteristic picture of differentiated macrophages and expressed maturation-associated MAX1 antigen on their cellular membranes. They were fully adherent, presented a high ability of yeast phagocytosis and had a potent secretory capacity (neopterin, procoagulant activity).

Analysis of secretory products in the culture supernatants shows that no additional release of IL-1, IL-6 and TNFα occurred after pretreatment with rhuIFNγ (Table 4).

The in vitro cytotoxicity of blood-derived macrophages cultured for 7 days in Teflon bags and used for adoptive immunotherapy was tested against U937 tumour cells at a low (1/1) effector/target ratio in the presence or absence of rhuIFNγ (Table 4). Macrophages inhibited by 20% the uptake of [$^3$H]thymidine by the U937 tumour cells while rhuIFNγ at concentrations of up to 500 U/ml, when added to the macrophages, increased their cytotoxic effect to 80% inhibition of [$^3$H]thymidine uptake by tumour cells. This inhibition of thymidine incorporation was well correlated with loss of tumour cell viability, as assessed by the trypan blue exclusion test, in accordance with previous studies [6]. MAK cell cytotoxicity was variable from patient to patient, and from dose to dose in the same patient but not as a function of the length of treatment (Fig. 2).

### *Toxicity*

The side-effects observed in the patients are summarized in Table 5. The 11 patients received 63 evaluable courses of therapy. Infusions of autologous IFNγ-MAK cells at the dose levels tested were generally well tolerated. None of the 11 evaluable patients exhibited signs or symptoms of severe or life-threatening toxicity. Most patients experienced at all dose levels mild to moderate chills followed by transient fever, with a severity not clearly dose-related and generally responding to paracetamol. Typically patients developed rigors or shaking chills within 0.5–2 h followed by transient fever. All patients with documented fever had specimens taken for microbiological examination and none of them was shown to have developed a gram-positive or -negative infection. One patient (the first patient treated) experienced grade 3 dyspnoea, which responded promptly

**Table 4.** Functional characterization of blood-derived autologous MAK cells cultured in Teflon bags

| Parameter | Time in culture | | |
|---|---|---|---|
| | 0 days | 6 days Before activation[a] | 7 days After activation[a] |
| Mo/Mφ content(%)[b] | 89.7 ±5.7 | 95.8 ±3.2 | 96.4 ± 3.5 |
| Phagocytosis[c] | 0.8 ±0.1 | 0.9 ±0.2 | 2.8 ± 0.2 |
| Tumour cytostasis[d] | ND | 19.4 ±5.1 | 82.5 ±13.6 |
| Secretory products in supernatants[e] | | | |
| Neopterin (ng/ml) | ND | 4.6 ±2.45 | 6.76± 2.8 |
| IL-1 (ng/ml) | ND | 9.11±1.64 | 12.68± 2.65 |
| TNFα (ng/ml) | ND | 5.79±1.26 | 8.19± 2.31 |
| IL-6 (ng/ml) | ND | 8.9 ±6.36 | 7.8 ± 5.73 |
| PCA(U/5 × $10^6$ cells) | 0.06±0.01 | 45.51±5.11 | 50.12± 5.31 |

[a] Before and after activation with rhuIFNγ
[b] Monocyte/macrophage content; means ± SD of 63 experiments
[c] Mean of yeast phagocytosed per monocyte or macrophage. Results are the means ± SD of 18 experiments
[d] Cytostasis was evaluated by inhibition of thymidine incorporation at a 1/1 effector: target ratio. Results are the means ± SD of 18 experiments
[e] Means ± SEM of 11 experiments. IL-2, interleukin-2; TNFα, tumour necrosis factor α; PCA, procoagulant activity

**Table 5.** Clinical side-effects observed during the i.v. rhuIFNγ/MAK immunotherapy phase I trial

| Side-effect | No. patients suffering side effects in cycles I–VI | | | | | |
|---|---|---|---|---|---|---|
| | I 0.1[a] (11) | II 0.25[a] (11) | III 0.5[a] (11) | IV 1.0[a] (10) | V 2.5[a] (10) | VI 5.0[a] (10) |
| Fever[b] | | | | | | |
| WHO grade I | 2 | 3 | 5 | 4 | 4 | 3 |
| WHO grade II | 1 | 5 | 4 | 5 | 5 | 6 |
| Chills | 1 | 3 | 2 | 3 | 6 | 5 |
| Dyspnoea | | | | | | |
| WHO grade II | 0 | 0 | 0 | 0 | 1 | 0 |
| WHO grade III | 0 | 0 | 0 | 1 | 0 | 0 |
| Headache | 0 | 1 | 0 | 0 | 1 | 1 |
| Nausea | 0 | 1 | 0 | 1 | 0 | 0 |

[a] $10^{-8}$ × no. MAK cells administered; total no. patients in parentheses
[b] Fever: WHO grade 0, none; grade I, <38° C; grade II, 38–40° C; grade III, >40° C

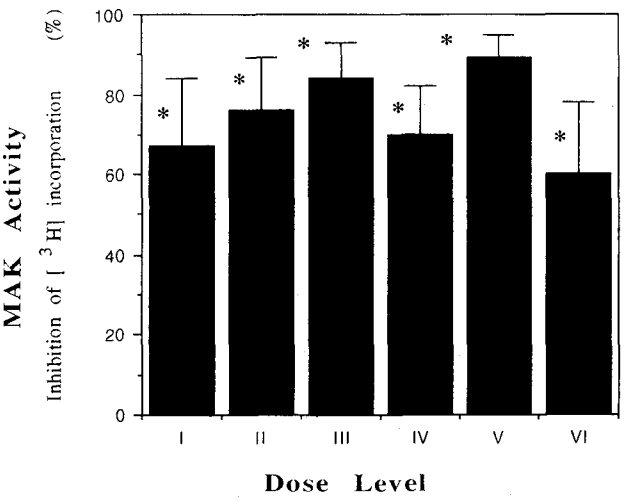


**Fig. 2.** In vitro antitumoral activity of rhuIFNγ-MAK cells during the 6-week period of adoptive immunotherapy. Macrophages were activated with rhuIFNγ (500 U/ml) for 18 h and cytotoxic activity of rhuIFNγ-MAK cells against the U937 tumour cell line was measured by [$^3$H]thymidine incorporation at a 1/1 effector/target ratio. The results represent means ± standard errors (SEM) of six independent experiments. Statistical analysis by Student's *t*-test: * $P$ <0.001, compared to the corresponding untreated tumour cells

to antihistamines and corticosteroids. This was seen after the fourth dose in the patient given 1 × $10^8$ MAK cells. The similarity of these symptoms to those of a granulocyte transfusion reaction suggested that microaggregates were probably responsible for these effects. Afterwards the other courses were done with microfiltered autologous MAK cells. Despite the fact that patient variability was an important feature of these toxicities, the macrophage immunotherapy regimen used in this study has not been associated with excessive morbidity and repeated outpatient treatment was possible without treatment-related loss of performance status. None of these side-effects was dose-limiting at the cell doses studied and there was no treatment-related infection or significant blood pressure change. The maximum-tolerated dose, defined as the dose that produced grade III toxicity in 75% of patients, was not reached during this study. There was no hepatic or renal toxicity and there were no significant effects on haemoglobin, or platelet counts. Almost all the patients studied showed no significant changes in prothrombin or partial thromboplastin time, euglobulin lysis time, or fibrinogen in response to therapy. Similarly, there were no significant changes in protein C, plasminogen, antithrombin III, or fibrin/fibrinogen degradation product (FDP) levels throughout the study period. Nevertheless, one patient treated with dose levels IV, V and VI showed slight modifications in favour of biological consumption (highest FDP level = 1024 μg/ml; highest reduction of prothrombin time, fibrinogen, and plasminogen to 40% of pretreatment values). These changes were transitory, with onset at 6 h post-infusion, their peak value at 24 h post-infusion, and a return to the normal values at 48 h post-infusion. They were not dose-related or correlated to any particular dramatic change in the procoagulant activity of the macrophages infused in this patient. In any event, no patient experienced clinical thrombosis or haemorrhagic incidents or evident signs of intravascular coagulation during the course of therapy.

### *Immunomodulatory effects*

*Blood cell counts.* An increase in mean absolute white blood cell and granulocyte counts was observed during infusion therapy with significant transient increases occurring at doses greater than 1.0 × $10^8$ cells (Fig. 3). The optimal values were observed 6 h after each dose escalation with a return to pre-infusion baseline values 24 h post-infusion.

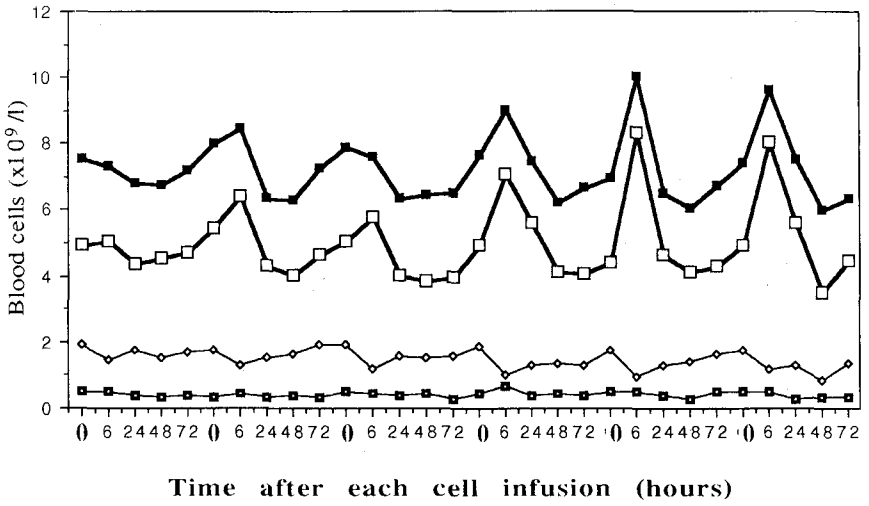


**Fig. 3.** Haematological changes during the 6-week period of adoptive immunotherapy with rhuIFNγ-MAK cells. ■, leucocytes; □, granulocytes; ◇, lymphocytes; ◘, monocytes

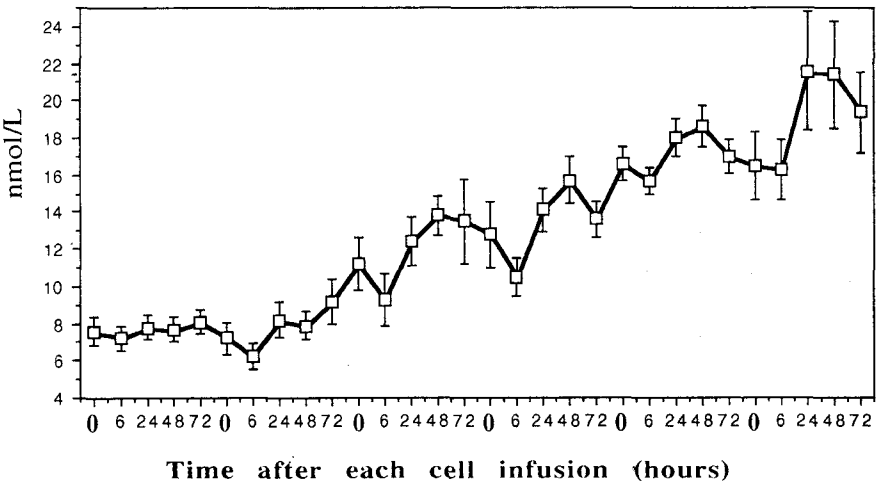


**Fig. 4.** Changes in neopterin during the 6-week period of adoptive immunotherapy with rhuIFNγ-MAK cells

*Neopterin.* Neopterin levels were increased 24–48 h after each dose of IFNγ-MAK cells with the exception of the first dose (Fig. 4). The maximal increase (32%) in neopterin was seen after the sixth dose and this was significantly greater than the increases seen after the four previous doses. There appeared to be cumulative elevation of neopterin levels since the serum neopterin level generally did not returned to baseline values by the next dose.

*C-reactive protein.* Serum levels of C-reactive protein were examined at baseline, 24, 48 and 72 h after each dose of IFNγ-MAK cells. Slight and insignificant increases in C-reactive protein were observed 24 h after each cell dose with a return to baseline within 48 h of the cell dose. The maximal increase (32%) in C-reactive protein was seen after the sixth dose.

*Cytokines.* IL-1, TNF and IL-6 levels were measured in serum to monitor the bioreactivity of autologous MAK cell infusions. Only slight insignificant intra-individual variations in IL-6, IL-1β and TNFα levels occurred in sera 6 h or 24 h post-infusion. Cytokine levels were variable from patient to patient, and from dose to dose in the same patient but without an apparent dose/response relationship.

*In vivo traffic of* $^{111}$ *In-labelled autologous macrophages*

Representative gamma camera images are presented in Fig. 5. In all patients studied, gamma camera images showed early distribution to lungs, liver, and spleen with slight body background activity by 1 h post-infusion. Lungs showed the greatest amount of uptake at that time and a substantial clearance during the first 24 h post-infusion. Liver and spleen uptake showed a steady rise over the first 24 h after the initial uptake measured at 5 min, which then declined steadily from 72 h post-infusion. The counting of peripheral blood samples drawn revealed no significant plasma-associated radioactivity as measured by a gamma counter following infusion with $^{111}$ In-labelled autologous MAK cells or $^{111}$ In-labelled autologous non-activated macrophages.

## Discussion

In a previous report we demonstrated that activated macrophages generated by culturing cancer patients' blood monocytes with rhuIFNγ produced marked selective lysis of experimental and human cancer cells in vitro [6]. The

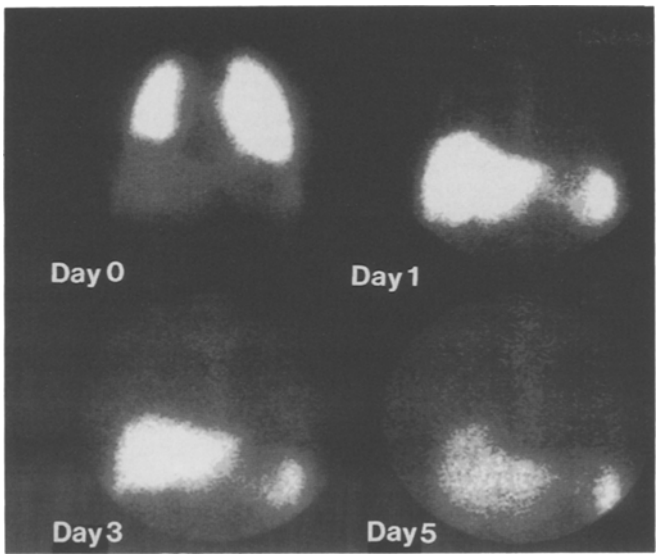


**Fig. 5.** In vivo traffic of $^{111}$ In-labelled autologous MAK cells administered intravenously. Gamma camera images of the chest and the abdomen in one patient with lung cancer (after right lobectomy) following i.v. infusion of $^{111}$ In-labelled autologous MAK cells. Uptake of activity is detected in the lungs (greatest uptake), liver, and spleen during the first 24 h post-infusion. By 24 h the lung activity had cleared while the activity in the liver and spleen declined steadily afterwards from 72 h post-infusion

present phase I study summarizes our preliminary clinical experience with the adoptive immunotherapy of cultured blood-derived differentiated cytotoxic macrophages in lung cancer patients. This represents the first reported phase I trial of IFNγ-activated autologous macrophages in the treatment of human lung cancer. Our experimental setting of culturing elutriated blood monocytes free of other contaminating mononuclear cells or platelets is basically different from the method recently reported by Andreesens et al. [2]. Weekly i.v. infusion of IFNγ-activated autologous macrophages with intrapatient dose escalation at doses from $0.1 \times 10^8$ to $5.0 \times 10^8$ cells was feasible and well-tolerated. Our dose escalation was stopped at a dose of $1.0 \times 10^8$ cells for the first patient entered in the study because of grade III toxicity. Subsequent macrophage preparations were microfiltered before infusion. Afterwards, side-effects (primarly fever and chills) were inconsistent, not dose-related, and not cumulative. Therapy was delivered in a routine hospital setting, without the need fo intensive-care monitoring. Clinical toxicities and laboratory abnormalities were transient and showed evidence of reversal within hours of the end of each treatment phase. In a previous report, we described fewer transient and more significant clinical and biological toxicities resulting from loco-regional adoptive immunotherapy using autologous blood monocytes activated by liposomal MTP-PE (muramyl tripeptide-phosphatidyl ethanolamine. In any case, these associated toxicities were significantly less severe than those reported previously in clinical trials with LAK+IL-2 [19]. In our previous study [9], the side-effects were attributed to the release of soluble mediators (IL-1, TNF, IL-6) by the activated monocytes. The systemic administration of blood monocyte-derived activated macrophages was not accompanied by a similar significant increase of these mediators in the patients' sera and C-reactive protein, known to be an IL-6-associated acute-phase reactant [12, 14, 24], remained almost stable throughout the study. On the other hand, the incubation of macrophages with rhuIFNγ did not induce a significant increase of IL-1 and TNFα in the culture supernatants. This suggests, as reported previously [6], that tumoricidal activated macrophages interact primarly by cell-to-cell contact with the target tumoral cells in contrast to monocytes, which act by releasing soluble cytokines.

The rhuIFNγ-activated macrophages have been shown to release neopterin in vitro [3, 15]. In our study, a secretion of neopterin was shown in the MAK cell supernatants in vitro, and a pronounced increase in neopterin levels occurred in vivo from 24 h to 48 h after MAK cell infusions. Furthermore, a cumulative effect seemed to occur during the 6-week period of therapy, which suggests that the in vivo secretion of neopterin is substantially promoted by a triggering effect of the infused MAK cells.

Bartholeyns and al. [4] have demonstrated that local or systemic administration of human macrophages produced inhibition of the growth or regression of pulmonary metastases in experimental mouse models. In our study, macrophages derived from the blood of cancer patients' were able to induce significant killing of tumour cells in vitro. Although we have found a significant macrophage-mediated U937 cell line cytotoxicity after IFNγ in the majority of our patients, we observed significant variability in cytotoxicity within the same patient during treatment.

The kinetics of $^{111}$ In-labelled autologous macrophages infused intravenously demonstrated that these cells localize very rapidly in the lungs with a marked uptake of radioactivity during the first 24 h post-infusion. Afterwards this radioactivity is detectable in the liver and spleen for 5 days. These findings should be taken into account in any future phase II/III studies of adoptive immunotherapy in patients with primary or secondary lung or liver tumours.

In conclusion, this phase I study has clearly demonstrated that i.v. infusion of autologous activated macrophages is safe and feasible on a once-a-week schedule for 6 consecutive weeks. However, one of the remaining problems is the improvement of culture conditions in order to generate greater quantities of non-adherent mature and cytotoxic human macrophages for adoptive immunotherapy. To this end, other studies, since completion of this phase I study, have shown that serum-free medium [25] and growth factors, such as granulocyte/macrophage-colony-stimulating factor [7] could improve the yields of in vitro cultured blood-derived macrophages in suspension. Neither the dose-limiting toxicity nor the optimal immunomodulatory dose has been defined in our phase I study. Further studies are required (a) to define the optimal infusion schedule and route of administration and (b) to evaluate whether such an immunotherapy modality can be used in an immunoadjuvant setting for the treatment of non-small-cell lung cancer in humans.

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