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Original Paper

Phase I/II Trial of Autologous Activated Macrophages in Advanced Colorectal Cancer

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Autologous activated macrophage (AAM) therapy is an adoptive cellular therapy based on *ex vivo* differentiation and activation of autologous peripheral blood monocytes. This study was undertaken to evaluate the tolerance, efficiency and biological effects of AAMs in chemoresistant progressive colorectal cancers. From January 1993 to May 1995, 15 patients were treated. Mononuclear cells were collected six times by weekly apheresis, cultured for 7 days, and activated with interferon-γ. AAMs were then separated by elutriation and re-infused intravenously, with a mean total of 7.95×10^9 macrophages per patient. Clinical tolerance was good: toxicity consisted only of a World Health Organisation grade 2 fever after 28% of the infusions. Responses were not seen in the 14 evaluable patients, as expected with very bulky tumours: in 11, the tumours continued to progress, but disease was stabilised in 3 patients who experienced progression-free survival for 14, 12 and 12 weeks, respectively.

Key words: adoptive immunotherapy, autologous activated macrophages, metastatic colorectal carcinoma, phase I/II

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INTRODUCTION

SINCE THE introduction of 5-fluorouracil (5-FU) more than 30 years ago, little progress has been made in the treatment of advanced colorectal cancer (CRC). 5-FU remains the single most effective agent. Given as an i.v. push, 5-FU results in approximately 10% objective responses with short durations, few complete responses, and no improvement in overall survival [1]. The use of continuous i.v. infusion of 5-FU [2] or in combination with leucovorin [3] or methotrexate [4] can improve response rates in the 30–35% range, but the impact on survival has been minor with a median survival duration of only 11 months. Greater effectiveness was found with a 48-h schedule of 5-FU and leucovorin repeated on day 14 [5] or in a chronopharmacological trial with 5-FU, leucovorin and oxaliplatin [6], but further work is needed to fully establish the value of these particular combinations. Clinical trials for the examination of new agents or therapeutic approaches should therefore be continued.

Adoptive immunotherapy has received much attention during the last decade, after the initial report of Rosenberg [7]. However, the first clinical trials using lymphoid effector cells, lymphokine-activated killer cells and tumour-infiltrating lymphocytes, or cytokines alone were disappointing, with limited efficiency and a high, though reversible, toxicity [8, 9]. These observations have renewed interest in the therapeutic potential of macrophages. Cells of the macrophage lineage are considered to be of special importance in the host response to the neoplastic process, as indicated by reports that experimentally activated macrophages can display significant cytotoxicity towards neoplastic cells, while leaving non-transformed counterparts unharmed [10], most human tumours are infiltrated by macrophages [11], and agents that activate the tumoricidal properties of mononuclear phagocytes increase the host defences against tumours [12]. Regressions have been observed in tumour-bearing mice injected with syngeneic macrophages, activated *ex vivo* [13–15]. In terms of potential therapeutic use, it became important to obtain consistently a large number of

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macrophages. These differentiated immune cells are only present in tissues, and there is no convenient means of collecting them. However, blood monocytes cultured *ex vivo* undergo differentiation into macrophages and can be activated. In 1986, Stevenson and colleagues [16] reported on the first clinical phase I trial using human *ex vivo* activated monocytes: the monocytes were shown to move to the tumour and to function at least in a cytostatic manner. More recently, the potential antitumoral cytotoxicity of autologous activated macrophages (AAMs) has been confirmed *in vitro* and their production well established [17–21]. However, no threshold toxic cell dose has been attained in previous phase I clinical trials [16–18, 20], so we have carried out a phase I/II trial to evaluate the tolerance of larger numbers of AAMs and, eventually, their activity in a homogeneous group of patients with advanced chemoresistant and progressive CRC.

PATIENTS AND METHODS

Patient population

Eligibility criteria for the trial comprised: measurable metastatic and progressive CRC not amenable to curative surgery, conventional chemotherapy failure, no treatment for at least 1 month prior to inclusion, World Health Organisation (WHO) performance status ≤ 2, age < 75 years, life expectancy > 3 months, a leucocyte count $> 4 \times 10^9$/l, a platelet count $> 120 \times 10^9$/l, haemoglobin levels > 6 mmol/l, serum bilirubin levels < 25μmol/l and creatinine levels < 150 μmol/l, negative serology for hepatitis B surface antigen and HIV, no history of cardiac failure and no serious disease or medical conditions requiring corticosteroids.

The following assessments and investigations were carried out before the protocol was started: complete history, physical examination, lung X-ray or chest computer tomography (CT) scan, abdomen and pelvis CT scan and liver doppler ultrasonography. Weight, physical examination, electrocardiogram, complete peripheral blood cell counts, peripheral blood mononuclear cell (MNC) subpopulations, coagulation parameters (prothrombin time, partial prothrombin time, fibrinogen, fibrin/fibrinogen degradation products), hepatic and renal function studies, C-reactive protein and carcinoembryonic antigen (CEA) were determined for initial assessment, poststudy evaluation and prior to each AAM infusion. Clinical examination and bacteriological controls were also performed for each course of therapy. Written, voluntary informed consent was obtained from each patient for the AAM protocol and for HIV testing before entry into the study. This protocol was approved by the 'Comité de Protection des Personnes dans la Recherche Biomédicale de Champagne-Ardenne'.

Preparation of activated macrophages

Leukapheresis. Collection of leucocytes was performed weekly by 3-h apheresis using the Cobe Spectra continuous flow cell separator (Cobe Lab., Lakewood, Colorado, U.S.A.) and acid/citrate/dextrose as anticoagulant. Seven to 9 litres of blood were processed at 50 ml/min. The apheresis was performed, aimed at collecting a maximum number of monocytes with low erythrocyte and polymorphonuclear contamination.

Cell culture and activation. MNCs were separated over a Ficoll–Hypaque gradient (d (density) = 1.077) using the Cobe 2991 blood cell processor, washed three times with a phosphate-buffered solution, resuspended at 5×10^6 cells/ml in Isocove's modified Dulbecco's medium (IMDM, Gibco, Cergy.Pontoise, France), supplemented with 3×10^{-5} M 2-mercaptoethanol, 1% non-essential amino acids, 2 mM L-glutamine, 2 mM sodium pyruvate, 5×10^{-6} M indomethacin, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2% heat-inactivated autologous human serum [20], and then seeded in 3000 ml Lifecell bags (Baxter, Maurepas, France) and incubated at 37°C in a 5% CO_2 humidified atmosphere for 7 days. Recombinant interferon-γ (IFN-γ) (Boehringer Ingelheim, Reims, France) was added at a final concentration of 250 IU/ml for the last 18 h of culture for macrophage activation. Total cell viability was assessed using the Trypan blue dye exclusion technique.

Purification of activated macrophages. On day 7, macrophages were separated by elutriation using a Beckman J6 ME centrifuge (Beckman, Gagny, France) equipped with a JE-5.0 rotor and a 40 ml elutriation chamber [21], resuspended in 4% human serum albumin (HSA) (Biotransfusion, Les Ulis, France) and re-infused within 4 h following completion of the preparation.

Flow cytometry analysis

Cell immunostaining was performed using mouse monoclonal antibodies followed by fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated affinity purified, isotype-specific goat antimouse antibodies (Immunotech, France). The following monoclonal antibodies were used: anti-CD3 (IgG1, Coulter, Margency, France), anti-CD45 (IgG1, Dako, Versailles, France), anti-CD14 (IgG2a, Dako), anti-HLA-DR (IgG2a, Becton Dickinson, Mountain View, California, U.S.A.). Samples were analysed on a flow cytofluorometer (XL Coulter) using propidium iodide to exclude dead cells.

Phagocytosis assay

Phagocytosis was assayed by the method of Dunn and Tyrer [22] using fluorescent microspheres. Cells (5×10^5/ml) were incubated and shaken at 37°C in water for 60 min in the presence of 5×10^6/ml fluoresbrite 1 μM diameter microspheres (OSI, Maurepas, France). The tubes were then placed on ice to stop phagocytosis, and the samples analysed by flow cytometry. The log of the fluorescent intensity was analysed as a function of the cell number, each peak corresponding to the number of cell-associated microspheres (Figure 1).

Cytostasis assay

In this assay, 10^4 tumour (U937 or K562) cells were incubated with AAMs at a ratio of 1:1 and incubated at 37°C overnight. [^{3}H]thymidine was added 18 h later and its incorporation by tumour cells measured after a further 24 h incubation.

Cytolysis assay

Cytotoxicity was evaluated by an overnight ^{51}Cr-release assay. 10^6 exponentially growing target cells (T) were incubated at 37°C for 45 min with 200 μCi of ^{51}Cr. The cells were washed and seeded at 10^4 cells/well in 96-well flat microculture plates with macrophage effector cells (E) at E/T ratios ranging from 3/1 to 30/1. After 18 h incu-

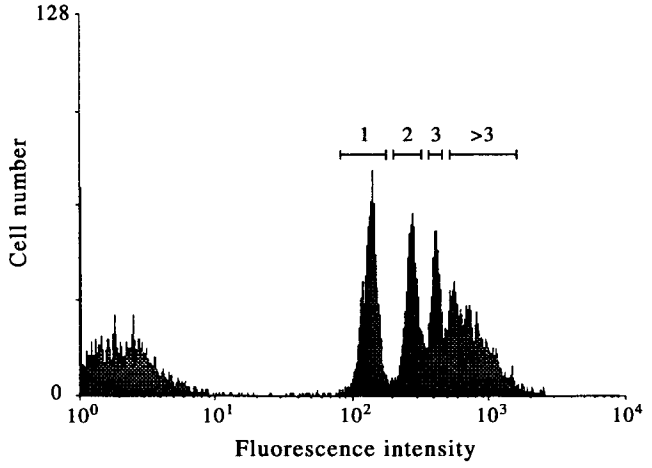

Figure 1. Phagocytosis assay. Analysis is expressed as a cytogram distribution of monocyte-derived macrophages that have ingested 1, 2, 3 and >3 fluorescent microspheres, each peak corresponding to the number of cell-associated microspheres.

bation at 37°C, the plates were centrifuged, 100 µl of each supernatant were collected and the ^{51}Cr-release from target cells was measured using an LKB 1277 gamma counter (Wallac Oy, Turku, Finland). All experiments were carried out in triplicate.

Data were expressed using the following formula:

$$\%\text{cytolysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100.$$

Treatment schedule

The therapeutic schedule included six leukaphereses, one per week for 6 consecutive weeks. Leukapheresis was performed in the morning; 3–4 h later, the patient was infused with AAMs derived from MNCs collected the week before. AAMs were administered into either large peripheral or central veins over a period of approximately 15 min using an infusion set for blood products with a filter (Baxter). After re-infusion, the remaining cells were recovered by rinsing the bag and the tubings with 100 ml of 4% HSA. The entire procedure was performed aseptically, under bacteriological control.

Evaluation of tolerance and efficiency

Standard WHO criteria were used for toxicity and response evaluation. Response was assessed 1 week after the sixth re-infusion and then at 4 week intervals for non-progressive patients. Progression-free survival was computed from the date of inclusion to the documentation of disease progression for stabilised patients.

Statistical analysis

According to the two-step Gehan method [23], the planned number of patients was 14 to detect a predicted response rate of 20% with 10% confidence limits and a β-risk ≤5%. Comparisons between results were performed using a two-tailed Student's *t*-test.

Table 1. Initial clinical characteristics of the patients

Characteristic	Study patients (n = 15)
Sex (number of patients)	
Men	11
Women	4
Age (years)	
Mean	61
Range	49–70
WHO performance status (number of patients)	
0	0
1	8
2	7
Primary site (number of patients)	
Rectum	2
Left colon	11
Right colon	2
Measurable disease (number of patients)	
Liver	13
Lung	4
Adrenal gland	1
Pelvis	1
Anastomotic recurrence	1

* World Health Organisation (WHO) performance status score.

RESULTS

Patient characteristics

From January 1993 to May 1995, 15 patients (aged 49–70 years; 11 men, 4 women), whose characteristics are listed in Tables 1 and 2, were included in the study. All had undergone surgical resection of their primary lesions (13 colon carcinomas and 2 rectal carcinomas) and had received prior systemic 5-FU chemotherapy. One patient withdrew from the study after two re-infusions for bowel obstruction secondary to an anastomotic recurrence, leaving 14 evaluable patients.

Characteristics of treatment

Monocyte–macrophage collection. Leukapheresis products contained a mean number of 8.01×10^9 leucocytes with 87% MNCs (Table 3a). Cultures were initiated with

Table 2. Initial biological characteristics of the patients

Characteristic	Mean levels (range)*
Haemoglobin (mmol/l)	7.8 (6.1–9.2)
White blood cells (10^9/l)	8.3 (3.1–12.7)
Monocyte cells (10^9/l)	0.8 (0.2–1.5)
Thrombocytes (10^9/l)	301 (120–716)
Serum creatinine (µmol/l)	77 (48–99)
Serum bilirubin (µmol/l)	13 (5–24)
Serum ASAT (IU/l)	46 (18–124)
Serum ALAT (IU/l)	34 (12–83)
Serum alkaline phosphatase (IU/l)	234 (63–608)
Serum LDH (IU/l)	871 (230–1902)
Serum CRP (mg/l)	69 (1–212)
CEA (ng/ml)	1649 (9–16 000)

* Before first cycle of treatment for the 15 patients.
ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; LDH, lactate dehydrogenase; CRP, C reactive protein; CEA, carcinoembryonic antigen.

Table 3. Yield of monocyte-derived macrophage preparations

Cell preparation steps ($n = 86$)	Cell number or percentage (mean ± S.D.‖)
(a) Cell collection (day 0)*	
White blood cells ($\times 10^9$)	8.01 ± 3.65
Mononuclear cells ($\times 10^9$)	6.75 ± 2.63
(b) Cell culture (day 0)†	
Total cells seeded ($\times 10^9$)	4.90 ± 2.66
% of monocytes	41.3 ± 13.7
Number of monocytes ($\times 10^9$)	2.23 ± 1.78
(c) Cell culture (day 7)‡	
Total cells recovered ($\times 10^9$)	4.31 ± 1.91
% of macrophages	36.8 ± 11.4
Number of macrophages ($\times 10^9$)	1.63 ± 0.94
(d) Cell elutriation§	
Total cells recovered ($\times 10^9$)	1.63 ± 0.94
% of macrophages	83.5 ± 12.3
Number of macrophages ($\times 10^9$)	1.37 ± 0.82

* Number of collected cells at each leukapheresis; † mononuclear cells were purified from the cytopheresis product by Ficoll–Hypaque centrifugation in a Cobe 2991 blood cell processor; ‡mononuclear cells were cultured for 6 days followed by 18-h activation with 250 IU/ml IFN-γ. On day 7, cells were harvested and counted separately for lymphocytes and monocyte-derived macrophages (AAMs); §cells recovered were subjected to countercurrent centrifugal elutriation for purification of AAMs; ‖S.D., standard deviation.

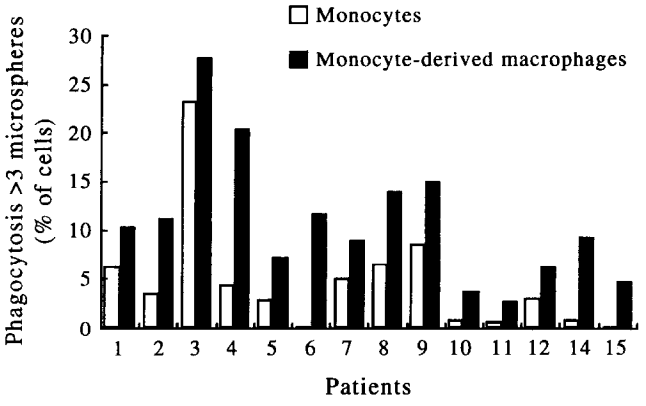

Figure 2. Relative phagocytic capacities of the monocytes and monocyte-derived macrophages. Monocytes and monocyte-derived macrophages were incubated with fluorescent microspheres and further analysed using flow cytometry (see Figure 1). Results are expressed as the mean of cells that ingested more than three microspheres. Autologous activated macrophages exhibited a highly increased phagocytic capacity ($P < 0.001$).

4.90×10^9 MNCs after purification by Ficoll separation, and included 2.23×10^9 monocytes on average (Table 3b). After a 7-day culture, 4.31×10^9 cells could be harvested, containing 1.63×10^9 macrophages (Table 3c), which were purified by elutriation to approximately 84%, giving a mean of 1.37×10^9 AAMs (Table 3d). The maximum number of AAMs generated from one single preparation cycle was 4.2×10^9 and the mean total for six infusions was 7.95×10^9. Table 4 shows infusion data for each individual patient.

Comparative biological characterisation of fresh monocytes and AAMs. Phagocytic activity, *in vitro* tumoricidal capacity and cell surface phenotypes were systematically determined ($n = 84$). *Ex vivo* activation of the cells was shown by increased phagocytosis of unopsonised fluorescein-labelled latex beads (Figure 1). As seen in Figure 2, the phagocytic capacities of the monocytes and AAMs were quite different for the 14 evaluable patients. However, for all 14 cases, AAMs had a greater ability to ingest more than three fluorescent microspheres than freshly isolated monocytes ($P < 0.001$). The tumoricidal capacity of AAMs was investigated using cytostasis and cytolysis *in vitro* assays. Whereas fresh monocytes did not spontaneously lyse cultured tumour cells or inhibit their proliferation, AAMs from all patients displayed a high capacity for inhibiting the proliferation of U937 cells (Figure 3). As expected, AAMs from patients developed only weak tumour cell killing activity against the K562 cell line, which is known to be particularly sensitive to natural killer (NK) cell activity.

Similar densities of CD45 and CD14 were expressed on the monocytes and cultured AAMs (data not shown). By

Table 4. Characteristics of the re-infused macrophages

Patient	Number of therapies	AAM number/infusion ($\times 10^9$)	Total AAMs ($\times 10^9$)	Purity (%)*
1	6	1.86 (1.03–2.4)†	11.14	82
2	6	0.79 (0.49–1.17)	4.74	86
3	6	0.9 (0.5–1.2)	5.4	91
4	6	1.73 (1.42–2.16)	10.4	88
5	6	1.51 (1.02–2)	9.06	92
6	6	1.27 (0.85–1.73)	7.62	87
7	6	0.73 (0.05–1.7)	4.36	76
8	6	1.12 (0.69–1.43)	6.72	83
9	6	1.08 (0.58–1.55)	6.48	84
10	6	2.82 (2.13–3.84)	16.9	83
11	6	0.89 (0.67–1.14)	5.33	74
12	6	1.45 (0.62–3.04)	8.71	84
13‡	2	3.33 (2.46–4.2)	6.66	91
14	6	0.51 (0.2–0.74)	3.03	84
15	6	1.89 (0.52–3.35)	11.35	74

* Mean percentage of autologous activated macrophages (AAMs) in the re-infused suspension as assessed by flow cytometry; † mean AAM number of all therapies performed per patient (range); ‡ withdrawn from the study after two infusions (bowel obstruction/anastomotic recurrence).

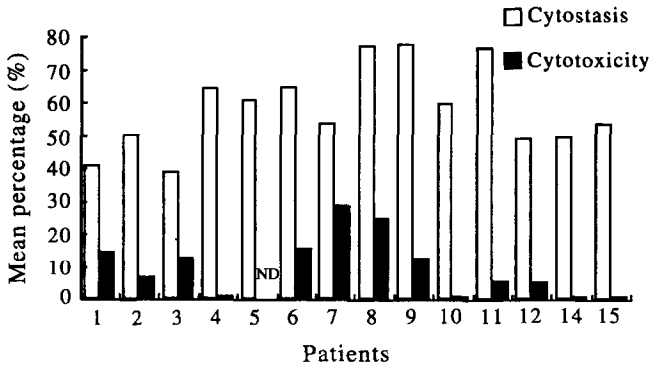

Figure 3. Cytostatic capacity and cytolytic activity of the monocyte-derived macrophages. Values for the cytostasis capacity are expressed as mean inhibition percentages of U937 tumour cell proliferation. Results for the cytolytic activity are expressed as mean percentages of specific releases by U937 cells with ^{51}Cr-release assay.

contrast, the expression of HLA-DR markedly increased on AAMs to a mean of 89.77 ± 9.73 (S.D.) versus 55.08 ± 30.25 for the monocytes ($P < 0.001$). A detailed phenotype analysis of the AAMs is shown in Table 5.

Clinical results

Tolerance. The weekly infusions of macrophages were well tolerated and the patients were discharged from hospital 2 h after completion of infusion. Grade 2 fever was observed in the night following 24/86 (28%) infusions in 9/15 patients. This resolved spontaneously or with paracetamol. No other toxicity was recorded.

Tumour response. 3 patients had stable disease (SD), which led to progression-free survival for 14, 12 and 12 weeks, respectively. Of these patients, 1 had some lesions that decreased, but by less than 25% (patient 14). The other 11 evaluable patients progressed without a change in rate (Table 6).

Usual biological and immunological parameters. No consistent change in liver or renal functions was observed. Data analysis indicated no significant variations in the haematological and haemostatic parameters investigated after six infusions, as compared with the pretherapeutic levels.

DISCUSSION

The present article reports on the results of a phase I/II trial of adoptive immunotherapy using autologous IFNγ-activated macrophages. This treatment was delivered in a routine hospital setting, and intensive care monitoring was not needed. Up to 4.2×10^9 AAMs were infused, i.e. 1.4 times more cells than previously, yet the clinical toxicity was mild: 28% of the 86 re-infusions were followed by a transient grade 2 fever reversible within some hours. The fever was inconsistent and easily controlled with paracetamol. Hence, these data demonstrate the excellent clinical tolerance of patients to repeated intravenous infusions of very large quantities of AAMs. The procedure we used for *ex vivo* generation of the AAMs is based on the procedures of previous phase I trials [17, 18, 20] and work from our group to optimise the technique of producing large amounts of activated macrophages [21].

*Table 5. Phenotype analysis of monocyte-derived macrophages from treated patients**

Antibody	Positive AAM (%)†
Anti-CD3	<1
Anti-CD45	97.7 ± 1.1‡
Anti-CD14	94.1 ± 3.7
Anti-HLA-DR	90.2 ± 9.5

* Data were derived from tested autologous activated macrophages (AAMs) in the 14 treated patients (total of 84 infusions analysed); † as determined by flow cytometry; ‡ mean ± standard deviation.

Table 6. Characteristics of the response to treatment

Patient	Measurable metastases: Liver	Other sites	Response
1	yes	Lung	PD
2	yes	–	SD
3	yes	–	PD
4	yes	–	PD
5	yes	Lung	PD
6	–	lung, adrenal	SD
7	yes	–	PD
8	–	Lung, pelvis*	PD
9	yes	–	PD
10	yes	–	PD
11	yes	–	PD
12	yes	–	PD
13	yes	Anastomotic recurrence	NE
14	yes	–	SD
15	yes	–	PD

* Irradiated lesion; SD, stable disease; PD, progressive disease; NE, not evaluable.

The rationale for this therapeutic approach was derived from the assumption that the macrophage system plays an essential role in the prevention of the occurrence and spread of malignant tumours. This is supported by evidence that activated macrophages are able to kill tumour cells *in vitro* [10] by at least two mechanisms. The first requires an intimate cellular contact at an E/T ratio of 1/1 [15]; the second is induced by cytokines involving mainly tumour necrosis factor-α (TNF-α) [24]. Adoptive transfer of activated macrophages to nude mice bearing subcutaneous tumours has been shown to elicit tumoral regressions when injected either locally or intravenously [15]. However, cells of the mononuclear phagocyte lineage can infiltrate tumours in relatively high proportions and are not necessarily able to eradicate them. Tumour-associated macrophages (TAMs) have a complex relationship with the malignant cells inside the tumour and, at least for some established solid tumours, seem to co-exist in a symbiotic manner [25]. Many TAM products may even support tumour development by stimulating growth of the malignant cells [26]. An ambivalent role could be suspected, but in fact, depends on the number of monocytes gathered *in situ*, their state of differentiation, and activation dictated by tissue- and tumour-specific stimuli in the local microenvironment. The *in vitro* generation of AAMs provides a means to by-pass the local tumoral factors and to obtain immune cells in a particular state of differentiation and activation, circumventing therapeutic use of heterogeneous TAMs with possible defects relevant to the local neoplastic tissue. AAMs obtained by this technique were shown to be highly cytostatic but weakly cytolytic *in vitro*. The degree of difference between these two functions high-

lights the difficulty of defining the antitumoral activity of AAMs. Conflicting results exist in the literature, but studies suggest that in addition to TNF-α [24], other unidentified molecules could play a role in the tumoricidal activity of activated macrophages [27].

Although several authors have reported that macrophages generated by culturing blood monocytes from cancer patients could be activated *in vitro* and rendered cytotoxic to a variety of cultured tumour cells [28–30] or non-cultured autologous tumour cells [31], only a small number of clinical trials [16–18, 20] have been previously undertaken using AAMs: they were phase I studies using local or systemic infusions in patients with either one [16, 18] or a variety of tumour types [17, 20]. Our study represents the first homogeneous reported phase I/II trial of AAMs in the treatment of human CRC.

In a previous phase I study [18], ^{111}In-labelled autologous macrophages infused i.v. were detected in the lungs in the first 24-h postinfusion period; thereafter, macrophages migrated to the liver and spleen, remaining in these organs for several days. This led us to treat patients with advanced CRC whose preferential metastatic sites included the liver and lungs. No response, according to WHO criteria, was observed in these organs for our patients. Yet, they were bearing very bulky tumours and it is currently believed that immunotherapy has the best impact on residual disease [32]. Nevertheless, 3 patients had SD as judged on measurable lesions and CEA. In no case was an accelerated growth rate observed, eliminating a possible humoral stimulation, i.e. the facilitation sometimes described in experimental conditions, as indicated by the increase or stabilisation of the CEA doubling time (data not shown).

The administration schedule and factors affecting the traffic of transferred cells to tumour cells may also alter the response rate. Stevenson and colleagues [16] re-infused AAMs intraperitoneally weekly in patients with peritoneal colorectal carcinomatosis and this schedule seemed to improve the prognosis: 4/7 patients were free from local relapse 17 months after therapy, a benefit also described by Andreesen and associates [17]. Research [17, 20] using i.v. infusions showed a weaker action, but design or total duration of treatment were different and the patient population more heterogeneous. Even if first observations emphasise the value of local administration over several weeks, the optimal route has still to be established for each tumour type.

The major limitation, however, remains the low numbers of AAMs available for re-infusion in patients bearing an important tumoral mass, the E/T ratio used being insufficient. At present, the inability to expand these cells *in vitro* shows a need for an improvement in culture conditions to increase the cellular yields. Moreover, the multiple *in vitro* assays used in our trial are strongly needed to define the re-infused products and could be the basis of safety controls in cellular therapies.

In conclusion, the good tolerance and the relative activity noted in the 3 patients with SD indicate that further studies are required to optimise generation of AAMs and the schedule used. Patients with advanced disease are less likely to benefit from immunotherapy, but there is certainly a need to use this approach when minimal residual disease exists or even as postoperative adjuvant therapy for high-risk CRC. In addition, as well as their therapeutic value on their own, macrophages could also represent vectors for effective gene therapies, and modification of their *in vivo* antitumoral potentialities by gene transfer is already under investigation [33].

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