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Abstract. *Purpose:* The purpose of this study was to investigate the biodistribution of mature dendritic cells (DCs) injected by various routes, during a cell therapy protocol.
Methods: In the context of a vaccine therapy protocol for melanoma, DCs matured with Ribomunyl and interferon-gamma were labelled with ^{111}In-oxine and injected into eight patients along various routes: afferent lymphatic vessel (IL) (4 times), lymph node (IN) (5 times) and intradermally (ID) (6 times).
Results: Scintigraphic investigations showed that the IL route allowed localisation of 80% of injected radioactivity in eight to ten nodes. In three cases of IN injection, the entire radioactivity stagnated in the injected nodes, while in two cases, migration to adjacent nodes was observed. This migration was detected rapidly after injection, as with IL injections, suggesting that passive transport occurred along the physiological lymphatic pathways. In two of the six ID injections, 1–2% of injected radioactivity reached a proximal lymph node. Migration was detectable in the first hour, but increased considerably after 24 h, suggesting an active migration mechanism. In both of the aforementioned cases, DCs were strongly CCR7-positive, but this feature was not a sufficient condition for effective migration. In comparison with DCs matured with TNF-α, IL-1β, IL-6 and PGE2, our DCs showed a weaker in vitro migratory response to CCL21, despite comparable CCR7 expression, and higher allostimulatory and TH1 polarisation capacities.
Conclusion: The IL route allowed reproducible administration of specified numbers of DCs. The IN route sometimes yielded fairly similar results, but not reproducibly. Lastly, we showed that DCs matured without PGE2 that have in vitro TH1 polarisation capacities can migrate to lymph nodes after ID injection.

Keywords: Dendritic cells – ^{111}In-oxine – Clinical trial – Migration – CC chemokine receptor 7

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Introduction

A recent review on anti-tumour vaccination trials using dendritic cells (DCs) referred to 98 published studies involving more than 1,000 patients [1]. This new therapeutic approach is encouraged, in particular, by the observation of induction of anti-tumour immune responses. However, many questions remain unanswered. There is no consensus on the optimal vaccine preparation or on its mode of injection [2]. At best, certain options may be considered preferable, such as the association of purified antigens with mature rather than immature DCs [3, 4], or the use of IL-12 p70 rather than of IL-10-secreting DCs [5]. The choice of procedures to allow DC migration towards lymph nodes likewise seems important. The main function of DCs is, indeed, the transport of peripheral antigens to lymph nodes where the primary immune response takes place, by activation of naive CD4+ and CD8+ T lymphocytes.

Expression of the CCR7 receptor plays an important role in regulating DC migration. CCR7 allows DCs to recognise the lymph node-directing chemokines CCL19 and CCL21 [6]. This receptor must be present on the DCs used for vaccination when they are injected intradermally (ID) or subcutaneously. Injecting the therapeutic product intranodally (IN) surmounts this constraint, since active cell migration is no longer necessary [7–9]. The same applies for intralymphatic vessel (IL) injections, as we have previously shown [10].

Whatever the injection route chosen, it is important to check for DC biodistribution and assess their effective presence in lymph nodes. Currently, radiolabelling is the only non-invasive technique to allow assessment of the fate of injected cells. We used this procedure in the framework of a cell therapy protocol for metastatic melanoma, in which mature DCs were loaded with tumour antigens. DC aliquots were labelled with ^{111}In-oxine, and cells were injected via three routes: IL, IN and ID. Study of the fate of DCs was an optional objective of the protocol and only eight patients were investigated. A total of four IL, five IN and six ID injections were carried out. For each injection, DCs were tested for CCR7 to establish a possible correlation between the level of receptor expression and the migratory capacity of the cells. The functional properties of DCs were also compared in vitro with those of DCs matured with TNF-α, IL1-β, IL-6 and PGE2—a cocktail that has been used in several clinical trials and is known to favour effective DC migration towards lymph nodes [11], but that contains PGE2, which is known to shift the T-helper cell response towards a TH2 type [12].

Materials and methods

Patients

Between December 2001 and May 2004, 14 patients affected by metastatic melanoma were enrolled on a phase II protocol of cell therapy. Among this group, eight patients received injections of DCs labelled with ^{111}In-oxine. For inclusion in the protocol, the patients had to be of the HLA-A*0201 subgroup, and their tumours had to express Melan-A and/or NA-17A antigens. A monthly injection was carried out by various routes: IL, IN guided by ultrasound or ID.

Dendritic cell preparation

DCs were prepared using the VacCell Processor system (IDM) [13]. In brief, leucocytes were collected by leukapheresis, before being cultured in plastic bags, in AIMV medium containing 500 U/ml GM-CSF and 50 ng/ml IL-13, with addition of IL-13 on day 4 of culture. On the seventh day, DCs were separated by elutriation. An aliquot was suspended in AIMV at a concentration of 2×10^6 DC/ml and incubated for 2 h with 50 μg/ml keyhole limpet haemocyanin (KLH, Immucothel Biosyn). After washing, DCs were matured overnight in AIMV, in the presence of 1,000 U/ml interferon-gamma (IFN-γ, Imukin Boehringer Ingelheim) and 1 μg/ml Ribomunyl (RBM, Pierre Fabre) [14]. The cells were then washed and exposed, for 2 h, to Melan-A and/or NA-17A (Clinalfa) tumour antigens, at a concentration of 50 μ*M*, before being injected by the IL route (injection week 0). Other DC aliquots were deep-frozen at the immature stage. These DCs were thawed in weeks 4 and 8, and then treated under the same conditions as on week 0, before being injected by the IN route. In weeks 0 and 12, intradermal reactions were performed with fresh or cryopreserved cells, incubated without KLH, matured and pulsed with the antigens. In all cases, cell viability was determined by the trypan blue dye exclusion test. Some ID injections included radiolabelled DCs, not previously exposed to KLH or tumour antigens. In all cases, the cells were suspended in HSA before being injected.

Cell radiolabelling, cell injection and scintigraphic imaging

For radiolabelling, an aliquot of the cells was resuspended in 2 ml RPMI-1640 medium without serum or HSA, owing to the strong chelation of ^{111}In by these substances. The mixture was incubated for 15 min under gentle stirring, at room temperature, in the presence of ^{111}In-oxine (^{111}In, DRN 4908, Tyco-Mallinckrodt). To assess labelling stability, DCs obtained from healthy donors and prepared as for the clinical trial were radiolabelled, suspended in AIMV and incubated at 37°C in plastic bags. Aliquots were tested 2, 3, 5, 23 and 30 h after the beginning of the culture to assess activity linked to DCs. As shown in Fig. 1, after 30 h of culture, around 70% of the activity remained cell linked without increased mortality. The activities used for radiolabelling depended on the type of injection: 3.3–4.4 MBq for ID, 5.2–6.7 MBq for IN and 9.2–11.1 MBq for IL injections. The cells were then washed in RPMI-1640 and centrifuged at 400 *g*, before being suspended in HSA and injected. The labelling yield, i.e. the ratio of activity retained by the cells to total available activity, was calculated. For IL injections, radiolabelled cells mixed with non-radiolabelled cells were injected into a superficial lymphatic vessel of the foot, in a 10-ml volume, as previously described [10]. For IN injections, radiolabelled DCs were injected separately in a volume of 1 ml into one inguinal or crural lymph node, under ultrasound guidance. The observation of a node swelling ensured that the injection had been correctly performed. ID injections were carried out into the thigh, in a volume of less than 500 μl. Scintigraphic acquisitions were performed with a gamma camera equipped with a medium-energy collimator peaked for two photopeaks of ^{111}In (173 and 247 keV), 1 h and 24 h post injection. Five-minute acquisition images centred on the site of injection and related draining lymph nodes were first performed, followed by whole body images performed at a rate of 10 cm/min. Localisation of injection sites was marked with a ^{57}Co pen. The percentage of radioactivity that had spread from the injection site was calculated as follows: activity detected in the draining lymph nodes/activity detected in total body.

Immunolabelling, immunoassays

The following antibodies were used: mouse PE-labelled anti-CD83 and mouse anti-CCR7 (BD Biosciences Pharmingen, San Diego, USA), as well as the corresponding control isotypes. For the detection of CCR7, three-layer labelling was done to increase the sen-

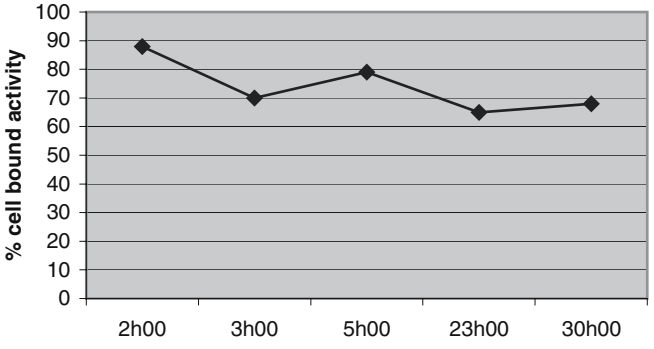


Fig. 1. Labelling stability of DCs. DCs were labelled with ^{111}In-oxine, washed, suspended in AIMV and cultured in a plastic bag for 30 h. At 1 h 45 min, 3 h, 5 h 45 min, 23 h 15 min and 30 h, cell aliquots were sampled and centrifuged to determine the activity in the supernatant and that linked to the cells

sitivity: anti-CCR7 IgM, biotinylated rat anti-mouse, PE-labelled streptavidin (BD Biosciences Pharmingen, San Diego, USA). The reading was carried out on a Facscan using Cellquest software (Becton Dickinson). For CCR7, the results were expressed in % positive cells as well as in mean fluorescence intensity (MFI) ratio of antibody-stained to isotype-stained population. The cytokines IL-10 and IL-12-p70 were assayed in the DC incubation medium after maturation of the cells by the ELISA technique using an Immunotech kit (Beckman Coulter).

In vitro assessment of functional properties of dendritic cells

Migratory, allostimulatory and TH1 polarisation capacities were tested in vitro with DCs produced from peripheral blood mononuclear cells of healthy donors, as described above. Maturation with RBM/IFN-γ was compared to maturation with 10 ng/ml TNF-α, 2 ng/ml IL-1β, 1,000 U/ml IL-6 (all purchased from CellGenix) and 1 μg/ml PGE2 (Prostine Pharmacia). Cells were tested after 18 and 48 h of maturation. For the 48-h point, DCs matured for 18 h in the presence of RBM/IFN-γ were centrifuged, washed and resuspended in fresh medium, in contrast to DCs matured with TNF-α, IL-1β, IL-6 and PGE2, which were left in the original medium.

Characterisation of the migratory properties of DCs To assess the migratory capacities of DCs we used a 12-well Transwell microplate with a 5-μm pore size (Corning Costar) that precluded passive diffusion but allowed active migration of DCs. DCs were suspended in AIMV medium plus 10% AB serum at 1×10^6 cells/ml. Lower chambers of the Transwell were filled with AIMV plus nothing or plus 250 ng/ml CCL21 (AbCys). One hundred microlitres of the matured or non-matured DCs was added into the upper chamber and allowed to migrate for 3 h at 37°C. Cells that had migrated to the lower chamber were counted by flow cytometry for a constant period of 90 s. Each experiment was performed in duplicate. The results are presented as follows: mean number of DCs that migrated in response to CCL21 minus mean number of spontaneously migrated DCs.

Characterisation of allostimulatory capacity Lymphocytes enriched by elutriation were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and plated at 1×10^5 per well in 96-well microplates with various numbers of DCs. After 3–4 days of culture in X-VIVO plus 5% AB serum, cells were collected and stained with CD3-PC5 (Beckman Coulter) before analysis by flow cytometry. The decrease in CFSE staining was used to assess T cell division in response to DCs.

Determination of TH1 polarisation In order to determine TH1 polarisation, 1×10^5 allogenic $CD4^+$ T cells, purified using $CD4^+$ magnetic beads (Miltenyi, Paris), were plated onto 96-well microplates with various numbers of DCs. After 6 days of culture in X-VIVO plus 5% AB serum, stimulated T cells were expanded with 50 U/ml IL-2 (Proleukin, Roche, Basel) and analysed for intracellular IFN-γ production between days 10 and 11. For this determination, cells were first stimulated with 50 ng/ml PMA and 500 ng/ml ionomycin (both from Sigma-Aldrich, St Quentin, Fallavier) for 4 h. Golgi stop (Becton-Dickinson) was added for the last 2 h. The cells were then intracellularly stained for IFN-γ using the intracellular cytokine staining kit (Becton-Dickinson), according to the manufacturer's instructions. In some experiments, cells that had migrated in response to CCL21 were compared with unmigrated cells. A single ratio of 1 DC for 40 lymphocytes was tested because of the low number of DCs harvested after migration.

Results

Characteristics of dendritic cells used for injection

Radiolabelling and number of cells injected

As indicated in Table 1, excellent labelling yields (average of 86%) were obtained in 14 of 15 cases. The worst yield, for the ID route in patient 5, may be explained by poor cell viability before radiolabelling, since this preparation was the only one with a viability lower than 70%. An average of 44 (33–50)$\times10^6$, 14 (7–25)$\times10^6$ and 19 (8–34)$\times10^6$ DCs with a mean ^{111}In activity of 7.5, 2.9 and 1.4 MBq was injected for the IL (n=4), IN (n=5) and ID (n=6) routes, respectively.

Phenotype

Dendritic cells prepared according to the IDM technique displayed a "dendritophage" phenotype pattern [13]: in particular, they did not completely lose CD-14 expression upon differentiation (not shown). In addition, as shown in Fig. 2, DCs incubated overnight in the presence of Ribomunyl and IFN-γ expressed CD83. Of the 15 injections performed, all but 2 (IL route for patient 3 and ID route for patient 8) yielded a CD83 expression higher than 50%. In contrast, expression of CCR7 was more variable, ranging from 3% to 75% of positive cells, with MFI ratios varying from 1 to 5.1. It should be noted that differences in expression of CCR7 and CD83 were sometimes found in the same patient when two different preparations were admin-

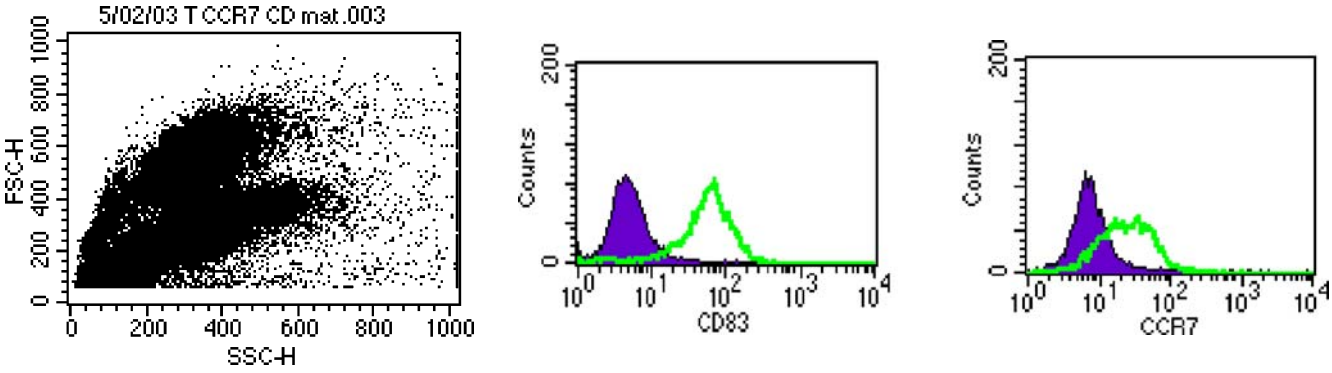
Table 1. Radioactivity and number of cells injected

Patient no.	Type of injection	No. of DCs injected ($\times10^6$)	Viability (%)[a]	Labelling yield (%)	Activity injected (MBq)	Migration to lymph nodes
1	IL	45	92	92	6.51	Yes
2	IL	50	77	85	7.14	Yes
3	IL	50	76	91	7.7	Yes
6	IL	33	83	94	8.47	Yes
1	IN	25	80	94	3	No
4	IN	8	75	91	3.73	No
5	IN	10	75	89	2.62	No
6	IN	7	79	82	0.93	Yes
8	IN	20	70	91	4.46	Yes
2	ID	25	73	77	0.92	Yes
3	ID	20	83	83	2.7	Yes
5	ID	10	47	31	0.44	No
6	ID	8	90	90	1.89	No
7	ID	16	77	84	0.67	No
8	ID	34	90	69	1.67	No

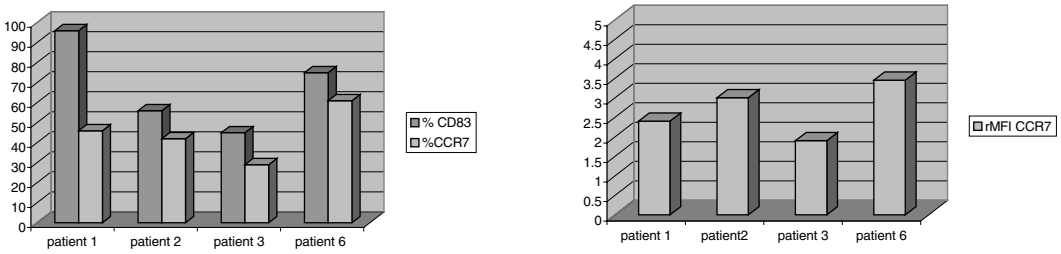
IL intralymphatic, *IN* intranodal, *ID* intradermal

[a]Determined by the trypan blue dye exclusion test

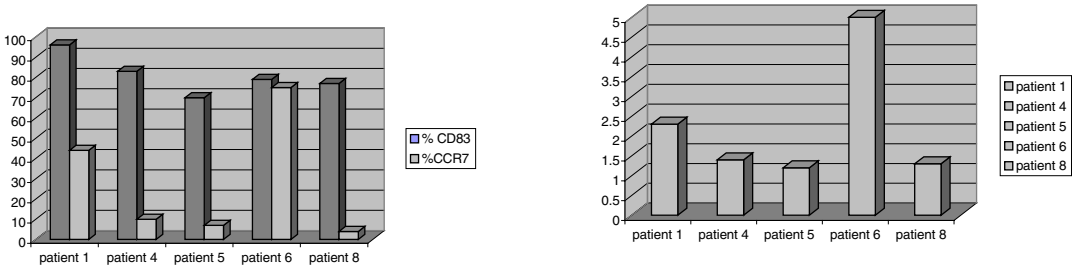
a



b intra-lymphatic injections



c intranodal injections



d intradermal injections

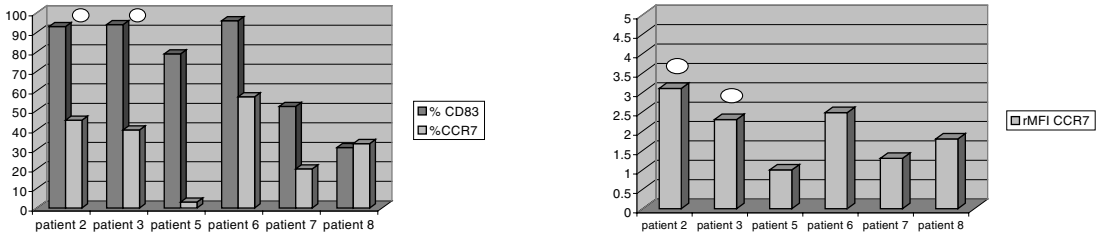


Fig. 2. Phenotype characteristics of DCs. **a** Example of graphs obtained for one patient. **b**–**d** CD83 and CCR7 results for cells injected via the IL (**b**), IN (**c**) and ID (**d**) routes. Results are expressed in percentages of positive cells (*left row*) or as CCR7:isotype MFI ratio (*right row*). *White circles* indicate patients exhibiting DC migration after ID injection

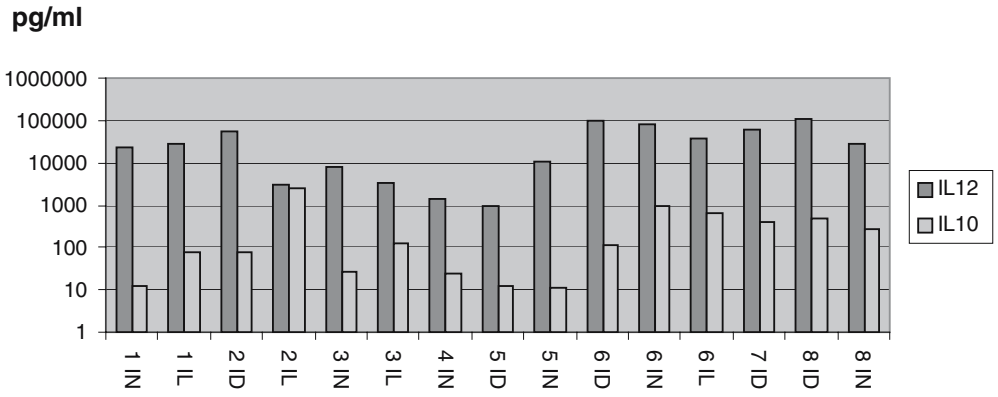


Fig. 3. IL-12 p70 and IL-10 secretion by DCs (*numbers* indicate patients). IL-12 p70 and IL-10 were assayed in supernatants after DC maturation with IFN-γ and Ribomunyl

istered. This was unrelated to the duration of maturation, which was 16±2 h.

Secretion of IL-12 p70 and IL-10

Cells allowed to mature overnight in the presence of Ribomunyl and IFNγ secreted IL-12 p70 and IL-10 cytokines in culture medium. As shown in Fig. 3, IL-12/IL-10 ratios remained positive for all the preparations. The capacity to secrete IL-12 is particularly important for induction of a T cell response of the TH1 type [5].

Scintigraphic imaging after DC injection

Injections by the IL route

IL injections allowed visualisation of retention of DCs in eight to ten inguinal, crural and iliac nodes. Images from the first hour after injection already showed the lymph nodes, as well as the pathway of the lymphatic vessel. Images obtained at 24 h were not very different, except that the lymphatic pathway was no longer revealed. Radioactivity recovered in the nodes corresponded to 80% of that injected (Fig. 4). These results were very similar to those

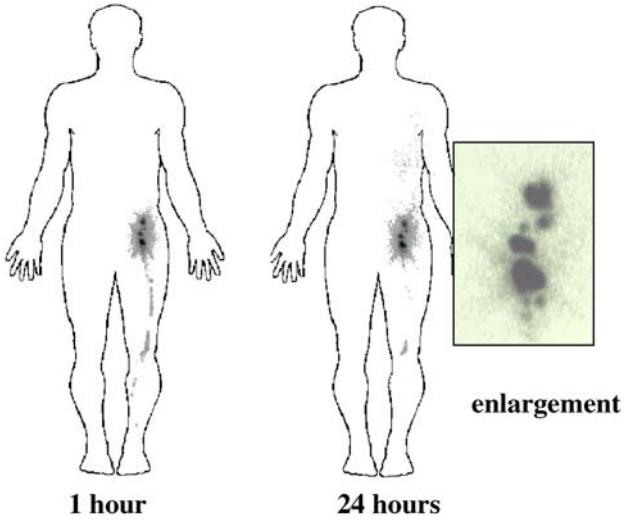


Fig. 4. Representative example of DC biodistribution after IL injection. As soon as the first hour post injection, approximately 80% of the activity injected was found in draining nodes. Note that the lymphatic pathway was visible on the 1-h image and disappeared thereafter

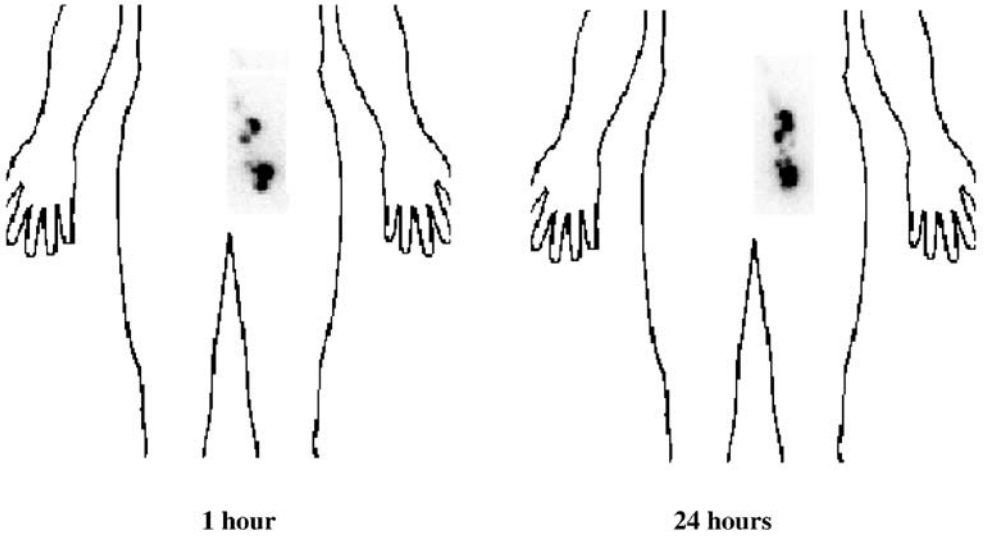


Fig. 5. Biodistribution of DCs for patient 8 after IN injection. From the first hour onwards, radioactivity diffused towards several nodes

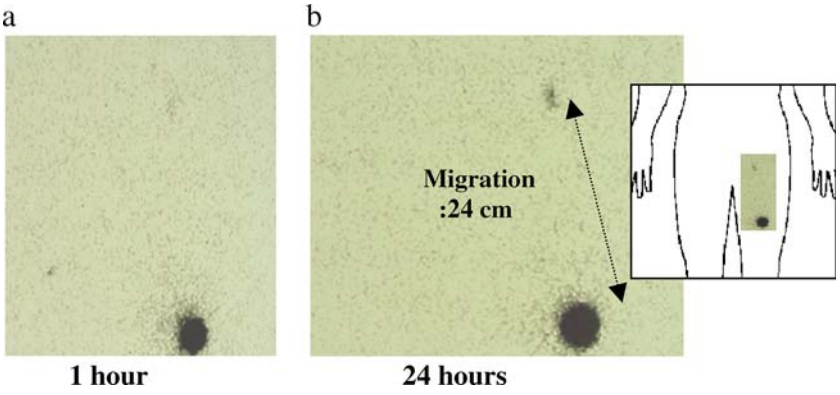


Fig. 6. Biodistribution of radiolabelled DCs after intradermal injection in patient 2

obtained previously when injecting tumour antigen-loaded macrophages [10].

Injections by the IN route

In three of the five IN injections, no image of radioactivity was detected apart from at the site of injection. In two cases, migration starting from the target node was observed. For patient 6, 3% of injected activity reached an adjacent lymph node at 1 h (4% at 24 h), and for patient 8, 23% of activity spread in several adjacent nodes at 1 h (26% at 24 h) (Fig. 5). No clear correlation was observed between the phenotype of the cells injected and whether they migrated or not. For example, patient 6 presented strong expression of CCR7, whereas patient 8 did not. Strong expression of CCR7 was observed for patient 1, although no migration could be detected (Fig. 2).

Injections by the ID route

Six patients received ID injections of DCs labelled with ^{111}In-oxine. In only two cases (patients 2 and 3), could migration towards a proximal lymph node be visualised. The migration was detectable in the first hour, but appeared much more clearly at 24 h, suggesting an active and progressive mechanism. DC migration to the lymph node

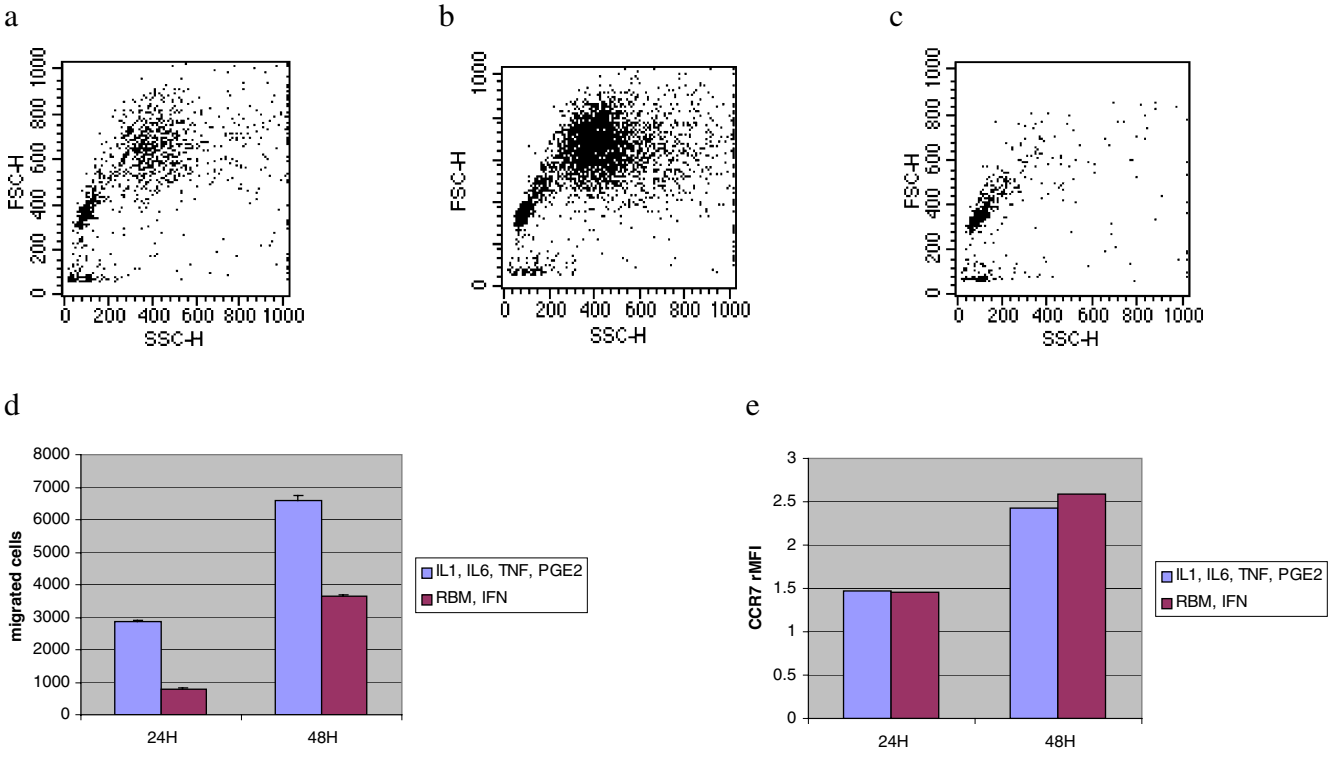


Fig. 7. a, b Migration in response to CCL21 of DCs submitted to two different maturation stimuli. Cytograms of migrating DCs matured with RBM and INF-γ (**a**) or with IL1-β, IL-6, TNF-α and PGE2 (**b**). **c** Control, no CCL21. **d** Number of migrating DCs matured for 24 and 48 h. Specific migration is depicted, i.e. number of DCs that migrated in response to CCL21 minus number of DCs that migrated spontaneously. **e** CCR7 expression in unsorted DCs matured for 24 and 48 h. One representative experiment out of three is shown

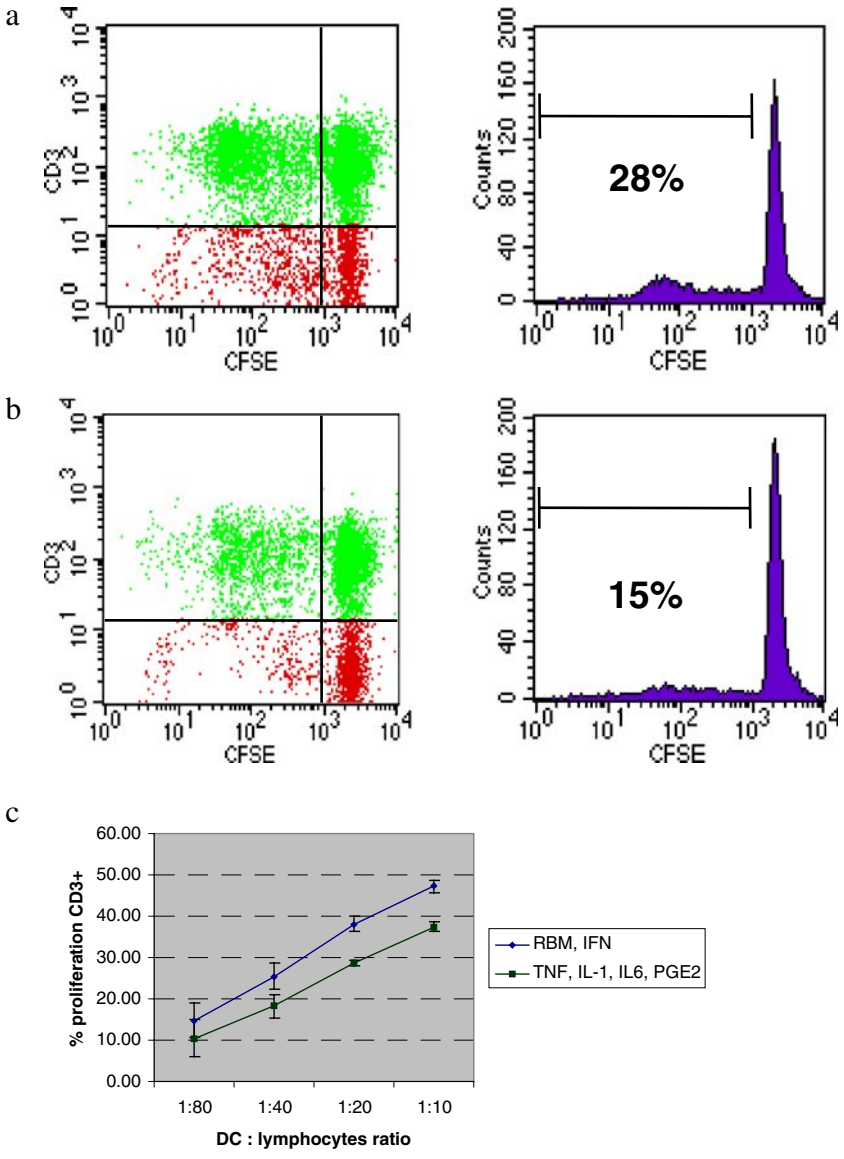


Fig. 8. T cell stimulatory capacity of DCs. Matured DCs were mixed with allogenic T cells intracellularly stained with CFSE. On day 3 or 4, T cells were analysed using flow cytometry. The percentages indicated correspond to the weakly stained proliferating cell fraction. **a, b** Representative plots for DCs matured with RBM and INF-γ (**a**) or with IL1-β, IL-6, TNF-α and PGE2 (**b**) for 18 h and cultured with lymphocytes in a 1:40 DC to lymphocyte ratio. **c** Results for various DC to lymphocyte ratios (means of triplicate measurements ± SEM). One representative experiment out of three is shown

increased from 0.75% at 1 h to 1.4% at 24 h for one patient and from 1% to 1.8% for the other patient, representing an increase of 80% (Fig. 6). For these two injections, the cells showed strong CCR7 expression. Nevertheless, no migration could be observed for patient 6, who showed similar phenotype characteristics.

No other spots of radioactivity were seen on whole body images, except in patient 5 following ID injection. A hepatic image was recorded in this patient, whose DCs showed poor viability The hepatic image might reflect the biodistribution of free ^{111}In.

In vitro comparison of DCs matured with IL-1β, IL-6, TNF-α and PGE2

It seemed interesting to compare the functional properties of the DCs used in this study with those of DCs matured with IL-1β, IL-6, TNF-α and PGE2, a cocktail used in several clinical trials and known to favour effective DC migration towards lymph nodes.

In vitro migration in Transwell chambers

As expected, DCs matured in the presence of IL-1β, IL-6, TNF-α and PGE2 showed a higher migratory response to CCL21 than DCs matured with RBM and IFN-γ, despite comparable levels of surface membrane CCR7. Migratory capacities were higher with DCs matured for 48 h than with DCs matured overnight (Fig. 7).

Allostimulatory response

The allostimulatory ability of DCs matured with RBM and IFN-γ was higher than that of DCs matured for 24 h with

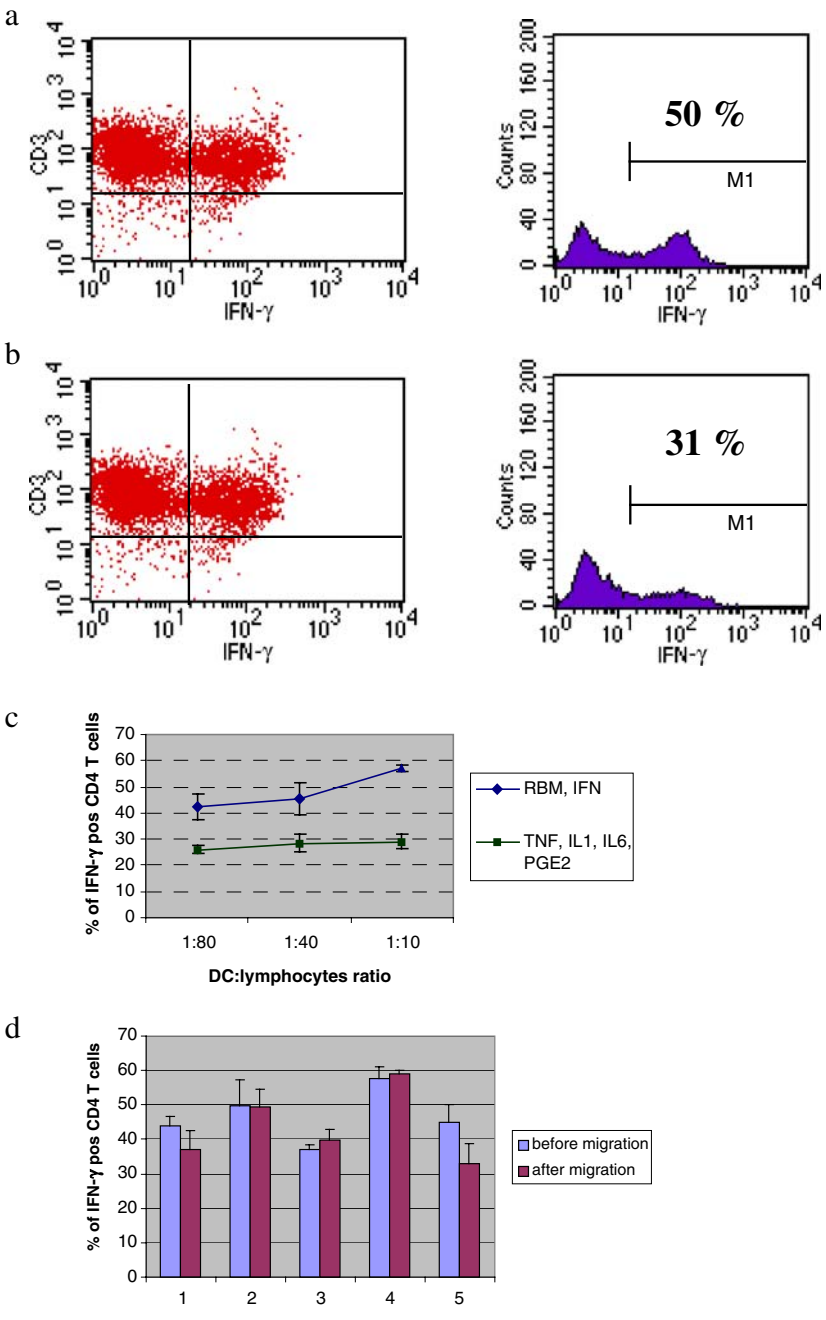


Fig. 9. T cell polarisation of DCs. Matured DCs were co-cultured with CD4-positive T cells for 10–11 days. The cells were harvested, stained for CD3 and intracellularly for INF-γ, and analysed by flow cytometry. **a, b** Representative plots for DCs matured with RBM and INF-γ (**a**) or with IL1-β, IL-6, TNF-α and PGE2 (**b**) for 18 h and cultured with lymphocytes in a DC to lymphocyte ratio of 1:40. The percentages represent IFN-γ-secreting lymphocytes. c Percentage of IFN-γ-secreting CD4-positive T cells according to the DC to lymphocyte ratio. **d** In some experiments DCs matured with RBM and IFN-γ were tested before and after migration in response to CCL21. Due to the limited numbers of migrated DCs, the experiments were only carried out at a ratio of 1:40. Experiments were performed after 24 h (*1–3*) or 48 h (*4 and 5*) of maturation. Data are expressed as the mean of duplicate measurements ± SEM

IL-1β, IL-6, TNF-α and PGE2 (Fig. 8). The same results were obtained after maturation for 48 h.

Induction of T helper cell polarisation by DCs

The ability to induce the TH1 phenotype was evaluated by measuring IL-12 p70 in the maturation culture medium and by evaluating the capacity to trigger IFN-γ production by CD4+ T cells. As expected, DCs matured with IL-1β, IL-6, TNF-α and PGE2 produced no IL-12 p70, in contrast to DCs matured for 24 h with RBM and IFN-γ (results not shown), and the percentage of IFN-γ-producing allogenic lymphocytes was more markedly elevated upon stimulation by RBM and IFN-γ-matured DCs (Fig 9). The same applied to DCs matured for 48 h (results not shown). Interestingly, DCs matured with RBM/IFN-γ taken from the Transwell lower chamber, and thus selected for their ability to migrate in response to CCL21, presented similar antigen-presenting and TH1 polarisation capacities to unselected cells.

Discussion

We investigated the biodistribution of mature DCs injected by various routes, during a cell therapy protocol. Two radioisotopes are mainly used for cell labelling, ^{99m}Tc-

hexamethylpropylene amine oxime (HMPAO) and ^{111}In-oxine. ^{99m}Tc-HMPAO can be used with higher activities and, therefore, has the theoretical advantage of a greater sensitivity. However, ^{99m}Tc labelling is less stable than ^{111}In labelling. As shown in the study of Blocklet et al. [15], more than half of the radioactivity is released from the cells 16–21 h after DC labelling with ^{99m}Tc-HMPAO, as against less than 30% after labelling with ^{111}In-oxine, a result in agreement with our data. The technetiated derivative released from the site of injection is cleared by the bladder. This derivative could also be responsible, at least partly, for the lymph node images obtained after ID injection of immature ^{99m}Tc-HMPAO-labelled DCs [16], the molecule being drained partially by the lymphatic vessels. These considerations led us to favour the labelling of DCs with ^{111}In-oxine.

Injections into an afferent lymphatic vessel allowed visualisation of about ten lymph nodes, 1 h after the end of the injection. The cells deposited in the lymphatic system probably moved due to the pressure generated by the injection and arrived at the nodes, which seemed to act as filters. The transport was passive, so the cells did not require particular migratory properties. This is why equivalent images were obtained during a previous trial, in which CCR7-negative macrophages were administered by the same IL route [10]. Such a route appears of interest for transporting a definite number of DCs to the nodes. The disadvantage of the technique is the cost of its implementation, since it requires the services of a radiologist for several hours. Moreover, IL injection does not involve any selection of the DCs transported to lymph nodes, in contrast to the ID route. The rare (1–3%) DCs recruited for migration after ID injection might have functional properties particularly adapted to the induction of an immune response. We tried to investigate this problem by in vitro migration experiments. In these experiments, migrated cells showed TH1 polarisation capacities similar to those of the whole cell population. This favours the hypothesis that migration and ability to induce TH1 polarisation are unrelated properties. Moreover, using blood circulating DCs exposed to PAP protein, Fong et al. compared IL and ID routes in a clinical trial on prostate cancers and showed that IL as well as ID injections induced an immune reaction to PAP protein [17].

Another way of conveying DCs to their site of action is to inject them intranodally. We used such an approach for the second and third therapeutic injections in our clinical trial. Migration of radioactivity from the injected node to neighbouring ones was sometimes observed. No correlation with CCR7 expression on DCs was found, and migration was clearly detected as soon as the first hour onwards, with kinetics close to that observed after IL injection, suggesting that the movement from one node to another was passive and probably followed the efferent vessel route. A different interpretation was provided by de Vries et al. [18], who reported observations comparable to ours. Following IN injections, the authors did not show any significant difference in migration between immature and mature DCs. They proposed that migration could originate from destruction of the architecture of the node, potentially induced by the injection, explaining why DCs were no longer retained in situ. We believe, on the contrary, that radioactivity diffusion from one node to another is rather indicative of compliance with the node architecture. Whatever lymph node architecture modifications there might be, DCs injected by the IN route seem able to perform their function of presentation to lymphocytes. The first clinical trial using this route to inject semi-mature DCs, indeed, pointed out some immune and clinical responses [8, 19]. A second trial compared IL, ID and IN routes for the injection of DCs matured in the presence of calcium ionophore, as well as of IL-2 and IL-12. All three routes allowed the induction of an immune response with the emergence of specifically antigen-reacting lymphocytes. Nevertheless, the IN route provided the best results for T cell sensitisation [9].

After injection by the ID route, two cases of migration of about 1–2% of the injected activity towards a single proximal node were observed. The weak radioactivity recorded raises the question of its cell association, a question difficult to answer in the absence of histological investigations. Such a histological approach has been conducted by others and has demonstrated cell migration both in the mouse by autoradiography [20] and in the chimpanzee using injections of fluorescently labelled DCs [21]. Furthermore, in man, de Vries et al [18] injected ^{111}In-labelled DCs, via either the ID or the IN route. They showed by autoradiographic techniques the presence of labelled cells in lymph nodes distant from the injection site. These arguments strongly suggest that cell-bound radioactivity was taken up by the nodes in our investigation. The uptake of free ^{111}In seems unlikely because, owing to its binding to transferrin, free ^{111}In would have yielded bone marrow and hepatic images. Such localisations were never observed on the whole body scintigraphic images, except in the case of one patient (no. 5) whose cell viability was very poor. However, the possibility that some radioactivity, attached to cell-derived colloidal particles, reached the lymph nodes cannot be excluded.

The biodistribution of ID-injected ^{111}In-labelled DCs has been investigated by several groups. Morse et al. [22], using immature DCs, observed variable migration to lymph nodes (two cases out of four) of 0.1% and 0.4% of the injected radioactivity, with a maximum being reached at 24 h. Blocklet et al. [15] compared immature DCs (n=3 patients) with DCs at the beginning of maturation (n=3 patients). They did not record any migration with immature DCs, while in one patient injected with DCs at the beginning of maturation, a weak migration (0.25% of the injected activity) was observed. Two other studies [18, 23] used DCs matured in a mixture containing PGE2. This substance has been shown, in in vitro experiments, to favour the migration of DCs [24, 25], but it does not favour the induction of a TH1-type lymphocyte response [26]. We observed similar results when our DCs generated in plastic bags, in the presence of GM-CSF and IL-13, were matured with IL1-β, IL-6, TNF-α and PGE2. In six patients, de Vries et al. [18] noted a migration ranging from less than 0.8% to 4% of the cells injected (average 1.8%). By

comparison, the average migration for immature DCs was 0.3%. Interestingly, mature DCs more commonly migrated towards several nodes, and their migration tended to increase between 24 and 48 h after injection. Similar results were reported by Nair et al. [23], in six patients: there was migration of 0.5–2.0% of the quantity injected and observation of activity in a second node after 21 h. In our study, we never observed diffusion towards more than one node. It is remarkable that our maturation "cocktail" favoured induction of DCs that initiated a strong TH1-type immune reaction. The present study reports migration to lymph nodes of ID-injected DCs matured in the presence of agents that favour a pro-inflammatory rather than a migratory profile. In the two cases of migration observed, more than 40% of cells expressed the CCR7 receptor, but only a few cells migrated to the lymph node. DCs of another patient with a high level of CCR7 expression did not migrate. These results suggest that there is no strict correlation between CCR7 expression and migration. This was also the case in our in vitro model, corresponding to previous observations by others [25, 27]. Expression of this receptor is a necessary but insufficient condition for migration to occur. We cannot exclude the possibility that only cells with high expression of CCR7 migrate after ID injection.

It seems unrealistic to expect that large quantities of DCs will be conveyed to lymph nodes after a single inoculation. Indeed, various studies in animals show that increasing the number of DCs injected subcutaneously leads to a saturation phenomenon, whereby only 3% of the injected DCs are found in the nodes [28, 29]. It appears possible to increase this percentage appreciably by conditioning the site of injection with pre-injection of DCs, which increases the local secretion of inflammatory cytokines, and that of CCL21 by endothelial lymphatic cells [28]. In another animal model, however, using a different adjuvant pretreatment of the skin, this approach only worked for immature DCs [23]. Passive injection of cells via the IN or IL route would have the advantage of exceeding the ceiling of 3% mentioned above. It remains to be ascertained, however, whether this type of injection would be effective in improving immunisation and clinical outcome. It should be recognised that this was not the case in our clinical trial, the results of which will be published: among 13 patients who completed the protocol, no objective response was reported, three were stabilised, and the others showed disease progression. Therefore, present knowledge suggests that provision of high numbers of DCs to lymph nodes will not play a key role in improving the effectiveness of cancer vaccines.

In conclusion, the labelling of DCs by ^{111}In is a tool that allows visualisation of the biodistribution of injected DCs in humans. In this study, we showed that an IL injection can convey a large and precise quantity of DCs in a reproducible way to around ten nodes. In some cases, injection by the IN route gives a similar result, but this technique is not reproducible. Finally, we showed, for the first time in humans, that DCs with TH1 cell polarisation capacities can migrate to lymph nodes after ID injection.

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