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Acknowledgement—We thank Prof. J.G.P. Tijssen of the Department of Clinical Epidemiology and Biostatistics for critically reading this manuscript.

European Journal of Cancer Vol. 32A, No. 4, pp. 735–736, 1996.

Printed in Great Britain
0959-8049/96 $15.00 + 0.00

0959-8049(95)00651-6

Home Therapy with Autologous Tumour-infiltrating Lymphocytes and Subcutaneous Interleukin-2 in Metastatic Melanoma

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THE FIRST results for melanoma immunotherapy with TIL (tumour infiltrating lymphocytes), reported by Rosenberg and associates in 1988, showed an overall response rate of 55% [1]. Subsequent clinical studies gave results ranging from 17 to 35% [2–6]. However, the high intravenous IL-2 (interleukin-2) doses used in previous TIL immunotherapy protocols required hospitalisation, most often in intensive care units, because of severe cardiovascular toxicity.

The purpose of the present work was to assess the feasibility and tolerance of home adoptive immunotherapy in patients with stage IV melanoma who received TIL and IL-2 (the latter administered subcutaneously in low doses) in association with interferon-α (IFN-α). Moreover, for TIL production, a simpler, easier reproducible culture method was developed than that described in earlier studies.

6 patients were included in this study, all with stage IV malignant melanoma. Therapeutic targets were subcutaneous in 3 patients, pulmonary in 2, involved intra-abdominal nodes in 2, bone in 1 and mucous in 1. The characteristics of patients are summarised in Table 1. TIL were extracted from a nodal metastasis in 3 cases and from a subcutaneous metastasis in the other 3 cases. Our laboratory methodology used to produce TIL has previously been reported [7, 8]. Approximately 4 weeks before the first re-injection of TIL, a single cisplatin injection (100 mg/m^2) was performed for immunosuppressive purposes.

Administration of recombinant IL-2 (Proleukin*, Eurocetus*), begun the evening after TIL reinjection, consisted of a subcutaneous injection of 3.6×10^6 IU/m^2 per day, 5 days per week, for 2 weeks. Recombinant interferon-α2a (Roferon-A*, Roche*), started at the same time, consisted of a subcutaneous injection of 3×10^6 IU per day, 3 days per week, in continuous treatment. If cell expansion permitted, a second reinjection of TIL in association with IL-2 was performed 1 month later. Thus, each patient was hospitalised for only 24 h to receive TIL and initiate cytokine therapy. The following subcutaneous injections of cytokines were carried out at home by a nurse. The maintenance cycles consisted of continuous subcutaneous IL-2 doses (3.6×10^6 IU/m^2 per day), 5 days per week, 2 weeks per month. Treatment was stopped in case of progression or stabilisation at the end of the first maintenance cycle.

Among the 6 patients treated, there was one complete remission (CR) (subcutaneous metastases) lasting 4 months and 3 cases of >50% partial remission (PR) (subcutaneous, mucous metastases and pulmonary metastases) lasting 5, 5 and 7 months. No grade III or IV toxicity was noted during treatment. All patients were able to receive therapy at home with a good quality of life. The most common side-effects were erythematous subcutaneous nodules at the IL-2 injection site, which disappeared spontaneously within a few weeks, and a pseudo-flu syndrome.

Our culture method, involving short exposure to phytohaemagglutinin and the use of feeder cells (an EBV-transformed B line), enabled us to achieve sufficient TIL expansion *in vitro* (5 of the 6 patients received two reinjections, with a mean number of 17×10^9 cells) with moderate IL-2 doses (150 IU/ml), hopefully providing for more specific cytotoxic activity [7–9]. Analysis confirmed that only T lymphocytes were present in all cases since 100% of the cells were labelled by CD3. TIL were predominantly CD8 lymphocytes for patients 1 (PR) and 5 (progression), and CD4 for patients 2 (PR) and 4 (progression). Patient 3 (CR) had approximately equal proportions of CD4 and CD8, and for patient 6 (PR), the CD4/CD8 ratio differed between the first and second

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Received 19 Jul. 1995; revised 22 Nov. 1995; accepted 24 Nov. 1995.
*No specific mean.

Table 1. Patient characteristics

Patient no./ Age/Sex	Prior therapy	Tumour resection for TIL culture	Site(s) of relapse	Cells infused ($\times 10^9$)	Phenotype CD4/CD8 (%)	% Cytotoxicity* K562/Daudi/ Autologous	Results
1/24/M	Cervical LN dissection IFN-α Chemotherapy Radiotherapy	Skin met.	Lung (multiple)	A: 10 B: 6	40/60 39/61	4/0/42 0/0/44	PR for 7 months DOD 11 months
2/45/M	WLE facial primary Cervical LN dissection IFN-α Chemotherapy	Skin met.	Skin mucous	A: 6 B: 7.2	70/32 74/32	9/10/ND 1/3/ND	PR for 5 months DOD 16 months
3/35/F	WLE facial primary Cervical LN dissection IFN-α Chemotherapy	Skin met.	Skin	A: 4.9	47/46	24/34/ND	CR for 4 months AWD 31 months
4/35/M	WLE arm primary Axillary LN dissection IFN-α adjuvant Chemotherapy adjuvant	LN met.	Skin Bone Intra-abdominal LN	A: 15 B: 3.4	59/26 91/9	11/49/ND 8/24/ND	PD DOD 6 months
5/38/F	WLE thigh primary Inguinal LN excision without dissection	LN met.	Lumbo-aortic LN	A: 13.5 B: 8	15/84 29/74	5/12/56 21/52/56	PD DOD 9 months
6/23/F	WLE thigh primary Inguinal LN dissection	LN met.	Lung (multiple)	A: 9 B: 7	30/65 54/42	3/4/ND 14/18/ND	PR for 5 months AWD 23 months

WLE, wide local excision; LN, lymph node; met., metastasis; PR, partial remission; CR, complete remission; PD, progression disease; DOD, dead of disease; AWD, alive with disease, survival duration was determined from the beginning of adoptive immunotherapy; ND, not done; A = first re-injection of TIL; B = second re-injection of TIL. *Cytotoxicity results are expressed as a percentage of lysis and concern an E/T (effector cell/target cell ratio) of 25/1.

injections. Cytotoxicity assays showed that, at the time of re-injection, patients 1 and 5 had TIL levels cytotoxic for autologous tumour cells, and TIL levels in patient 5 were also cytotoxic for the Daudi cell line. In two studies (including ours), the point of TIL origin appeared to be associated with response. We observed remission in all patients who received TIL extracted from subcutaneous nodules, and Rosenberg and associates [2] found that 49% of patients re-injected with TIL extracted from subcutaneous metastases responded to treatment (compared to 17% of those whose TIL were extracted from metastatic lymph nodes).

Our study demonstrated a clinical effect of TIL combined with low doses of IL-2 in an ambulatory treatment. However, an important point for the efficacy of TIL might be the tumoral burden. We are currently considering this point within a clinical research protocol comparing the effect of IL-2 alone or associated with TIL in patients with stage III melanoma after total nodal removal. The tolerance of our immunotherapy protocol with TIL and the reproducibility of our culture method enable us to carry out this therapeutic trial while ensuring patients a reasonable quality of life.

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Acknowledgements—We thank Dr Errera (Chiron) for providing us with interleukin 2 and Miss Arlette Bénardin for her excellent technical assistance.