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Follow up of soluble IL-2 receptor level in metastatic malignant melanoma patients treated by chemoimmunotherapy

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SUMMARY

Immunological parameters following chemoimmunotherapy combination were studied in 31 patients with metastatic malignant melanoma. They received Cisplatin (100 mg/m^2) on day 1 and 28, recombinant IL-2 (rIL-2; Eurocetus) in continuous infusion from day 3 to 6, 17 to 21, 31 to 34 and 45 to 49. Interferon-alpha (IFN-α; Roche) was given subcutaneously three times weekly. No significant change in CD4/CD8 ratio at onset or during treatment was observed between responder ($n=19$) and non-responder ($n=12$) patients. Regarding the IL-2 receptor (IL-2R) study, the percentage of cells expressing Tac (p55) receptor did not change either for healthy volunteers ($n=20$) and patients before any therapy, or between responder and non-responder patients. Concerning serum soluble IL-2R shedding before therapy, we observed a significant increase ($P=0{\cdot}001$) in patients (79 ± 40 pM) compared with healthy donors (30 ± 15 pM), but no significant variation was seen between responder and non-responder patients. In contrast, during the treatment, the soluble IL-2R level increased in both groups but, interestingly, a significant difference was found between responder and non-responder patients from day 7 ($P<0{\cdot}05$) to day 21 ($P\leqslant0{\cdot}01$), suggesting that the cells from non-responder may be slower in becoming stimulated. This finding is the most striking point of our study and suggests that sIL-2R might be an early predictive factor of the clinical response as obtained by logistic regression ($P=0{\cdot}0063$). Therefore patients with a serum soluble IL-2R level greater than 250 pM at day 21 have a 12-fold more chance of undergoing a clinical response.

Keywords metastatic malignant melanoma IL-2 chemoimmunotherapy soluble IL-2 receptor

INTRODUCTION

The ominous prognosis of metastatic malignant melanoma has led to a large number of clinical treatment trials. Many different chemotherapeutic combinations have been used [1–3], none of which has shown any real benefit.

Since Rosenberg [4] showed clinical improvement using IL-2 therapy in the treatment of malignant diseases, many attempts to treat with IL-2 have been undertaken with varying success. Immunotherapy has now become a recognized cancer therapy approach. High dose IL-2 alone or in combination with either other cytokines, lymphokine-activated killer cells (LAK) or chemotherapy have been widely used [5–7].

Some improvement in terms of response rate seems to have been achieved. From some studies [8–10] it has been established that IL-2 plays a pivotal role in the immune response, both in its own receptor expression and release of other cytokines. To exert its biological effect, IL-2 interacts with specific membrane receptors. Three types of IL-2 binding sites have been described: a 55-kD (α chain) protein low-affinity receptor unable to deliver a signal to the cell, a 70–75 kD (β-chain) intermediate-affinity receptor which can transduce a signal to the cell, and a high-affinity receptor made up of both the p55 and p75 molecules. More recently a 64-kD (γ chain) protein has been identified. This chain seems associated with the β chain and is required for signal transduction by either the high- or intermediate-affinity forms of the receptor [11]. Moreover, activated T cells release a truncated form of the p55 molecule as a soluble IL-2 receptor (sIL-2R), as well *in vitro* [12] into the culture supernatant as *in vivo* after the infusion of high dose IL-2 [13].

The present study evaluates immunological parameters following Cisplatin (CDDP), IL-2 and interferon-alpha (IFN-α) given in a sequential protocol to 31 patients [14]. We report here the dynamics of immunological events, both cellular and humoral, following this combination of chemoimmunotherapy.

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PATIENTS AND METHODS

Patients

Thirty-one patients (15 male, 16 female) with metastatic malignant melanoma were included in this study. Median age

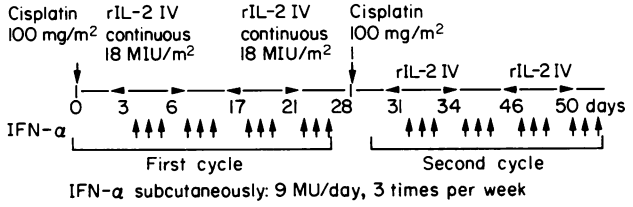

Fig. 1. Therapeutic protocol.

was 44 years (range 21–68 years), and median Eastern Cooperative Oncology Group (ECOG) performance status was 0 (range 0–2). Sites of metastatic disease included lymph nodes ($n=20$), skin/soft tissue ($n=17$), lung ($n=12$), liver ($n=2$), bone ($n=4$) and others ($n=4$). Exclusion criteria were evidence of serious active infection, corticosteroid treatment, significant past or current evidence of cardiovascular pathology, and concurrent other cancer. All the patients gave informed consent.

Protocol

Patients received 100 mg/m² of Cisplatin (CDDP) over 4 h on days 1 and 28, followed by 18 MIU/m² per day of IL-2 (Proleukin-Eurocetus, Amsterdam, The Netherlands) in continuous i.v. infusion from days 3 to 6, 17 to 21, 31 to 34 and 45 to 49. IFN-α (Roferon A; Roche, Neuilly, France) 9 MIU was given subcutaneously three times weekly (Fig. 1).

Cytofluorometric analysis

Flow cytometry analyses were performed on K3-EDTA anticoagulated peripheral whole blood from patients before treatment and at the end of each course.

Blood (100 μl) was incubated for 15 min at 4°C with conjugated isotype control or MoAbs against different cellular phenotypic markers conjugated either to fluorescein or to PE: T cell (anti-CD3), T helper/inducer cells (anti-CD4), T suppressor/cytotoxic cells (anti-CD8), activated T and B cells (HLA-DR), natural killer (NK) cells (anti-CD56, anti-CD16) and Tac IL-2 receptor (anti-CD25). All MoAbs were purchased from Coultronics (Margency, France) and Leu-11a (anti-CD16) from Becton Dickinson (Grenoble, France).

After two washings with cold PBS, the immunoprep Epics leucocyte was used for erythrocyte lysing and leucocyte fixing. Lymphocytes were analysed with an Epics Profile II flow cytometer (Coultronics) using a single 488-nm argon ion laser and logarithmic intensity scales. All samples were gated on forward and side scatter.

Median log fluorescence intensity (MFI) calculation was performed by subtracting the MFI of appropriate isotype control antibodies from the positively stained samples. The results are expressed as percentage of fluorescent cells.

Twenty healthy donors were also evaluated: they included 12 women and eight men with an age range of 25–55 years.

Cytokine assays

Sera were obtained from the whole blood laid on a serum separator tube (SST); they were aliquoted, stored at −20°C and examined for the presence of cytokines. Commercially available ELISA kits based on the antigenic detection of cytokines from Genzyme (Cambridge, UK) were used for detecting granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFN-γ; kits for serum soluble IL-2R and tumour necrosis factor-alpha (TNF-α) were purchased from Immunotech (Marseille, France) and from Endogen (Boston, MA) respectively.

All the results are expressed in pM. For studying the time course of sIL-2R levels, we expressed the results as a ratio R (or fold increase):

$$R=\frac{\text{level of sIL-2R (pM) at day } x}{\text{level of sIL-2R (pM) at day 0}}$$

Statistical analysis

Statistical significance of the differences between responder (R) and non-responder (NR) patients was determined by the non-parametric Mann–Whitney test and ANOVA test. Differences were considered as significant at $P\leqslant 0{\cdot}05$. Correlation coefficients were determined by the Spearman rank test. Data processing was carried out using the PCSM statistical package (Deltasoft, 1988).

Logistic regression has been used in order to determine predictive factors linked to clinical response (complete, partial response and stabilization *versus* progressive disease). Age, sex, metastatic site (cerebral, visceral, non-visceral) and serum sIL-2R levels have been taken into consideration. Patients with sIL-2R shedding from 15 to 250 pM were classified as group A, while patients with sIL-2R shedding from 251 to 550 pM were classified as group B. The cut-off of 250 pM is the mean value of sIL-2R shedding in non-responder patients at day 21. A stepwise selection (threshold 5%) has been performed in the logistic regression.

RESULTS

Clinical response

All patients received the two induction cycles and were fully evaluable. Overall response rate was 61% (95% CI 38–70) with 4/31 patients achieving a complete response (CR) (13%) for 24+, 21+, 20+, 3 months and 15/31 (48%) a partial response (PR) with a median duration 5 months (2·5–20+). Complete and partial responses have been determined according to WHO criteria. Responding sites included lymph nodes ($n=16$), soft tissue ($n=12$), lung ($n=8$), bone ($n=1$) and others (two GI, one pleura, one head and neck); response rate was equivalent for visceral and non-visceral metastases. No difference in age and sex was observed between responder and non-responder patients.

Side effects

Fever, chills, nausea and vomiting, diarrhoea, fatigue, cutaneous toxicity were observed in most patients, and were moderate to severe (grade II–III) in intensity, but manageable and reversible on rIL-2 cessation. All patients experienced hypotension, including 17 grade III–IV cases, all well controlled with low-dose Dopamine. Renal toxicity was fully reversible. Haematologic toxicity consisted of anaemia (grade III–IV, $n=1$), leucopenia (grade II–IV, $n=1$) and thrombocytopenia (grade III–IV, $n=1$). No life-threatening toxicity was observed, but 24 h medical and nursing surveillance was provided during rIL-2 therapy.

Phenotypic lymphocyte subsets

CD4/CD8 ratio did not show any change between responder and non-responder patients, either at onset or during treatment.

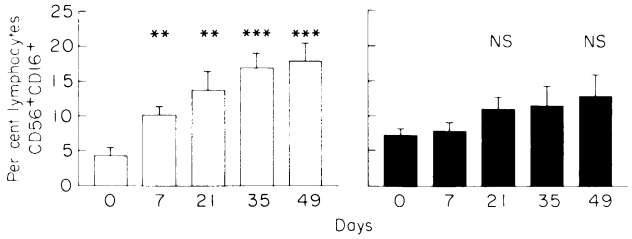

Fig. 2. Percentage of lymphocytes expressing $CD56^+$ $CD16^+$ in responder (□) and non-responder (■) patients. Mann Whitney test was performed for the statistical study. $^{**}P<0.001$; $^{***}P<0.0001$.

However, after the fourth course of IL-2, a generalized increase in CD8 cells was observed in all patients.

Concerning NK cells ($CD56^+$) and cells expressing the phenotype $CD56^+$ $CD16^+$, no significant difference was observed between responder and non-responder patients throughout the entire cycle. In contrast, cells expressing the $CD56^+$ $CD16^+$ phenotype (Fig. 2) seemed to increase linearly only in responder patients.

IL-2 receptors

The percentage of Tac-positive peripheral blood lymphocytes before treatment was $24.6 \pm 9.8\%$ in patients and $21.7 \pm 6\%$ in healthy volunteers. The serum level of soluble IL-2R was significantly higher ($P=0.001$) in patients (79 ± 40 pM) compared with healthy volunteers (30 ± 15 pM).

During treatment, the serum sIL-2R level and/or ratio increased in both groups, but, interestingly, a significant difference was found between responder and non-responder patients from day 7 ($P=0.045$) to day 21 ($P=0.016$) (Table 1 and Fig. 3).

The logistic regression shows that sIL-2R value at day 21 is significantly linked to the clinical response ($P=0.0063$), and odds ratio of response according to sIL-2R classification is equal to 11.9.

Cytokine release

TNF-α and GM-CSF were not detected at any time for either group of patients. IFN-γ was detected in all patients, but the release was variable among patients and not correlated with treatment response.

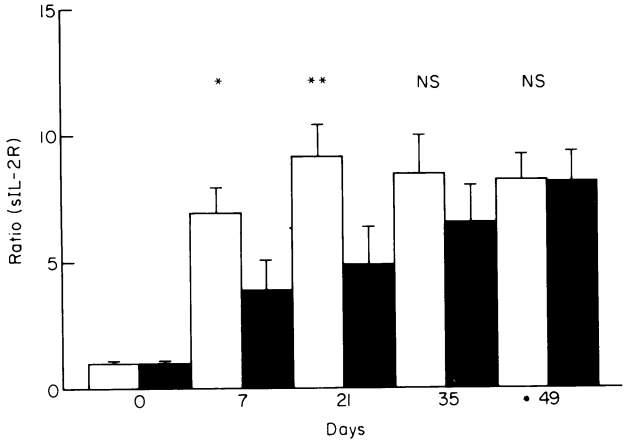

Fig. 3. Fold increase of soluble IL-2 receptor: comparison between responder (□) and non-responder (■) patients during chemoimmunotherapy. Mann Whitney and ANOVA tests were performed for the statistical study. * $P=0.045$; ** $P=0.016$.

Table 1. Follow up of serum sIL-2R level in responder (R) and non-responder (NR) patients

Day	sIL-2R (pM) R ($n=19$)	sIL-2R (pM) NR ($n=12$)	P
0	60 ± 29	77 ± 49	NS
7	252 ± 120	150 ± 100	0.045
21	330 ± 140	215 ± 102	0.016
35	310 ± 106	278 ± 110	NS
49	320 ± 112	337 ± 125	NS

Results are expressed in pM as mean ± s.d.
P = Mann–Whitney test.

DISCUSSION

In this study we tried to answer one of the questions asked by clinicians: is there any early predictive factor for the clinical response to chemoimmunotherapy? We report here the results of measuring different immunological parameters following CDDP, IL-2, IFN-α combination in 31 patients with metastatic malignant melanoma. Taken together, these data suggest that soluble IL-2 receptor level is significantly correlated to the clinical response during the first two courses of therapy, and might be an early predictive factor.

Clinical trials of IL-2-based therapy have confirmed the original findings of Rosenberg *et al.* [4] to the extent that objective responses do occur in malignant metastatic diseases, and especially in melanoma and advanced renal carcinoma. With the aim of selectively enhancing the biological and anti-tumour effects of IL-2, one widely used approach has been addition of other therapeutic agents. The ability of IFN to modulate cellular responses to IL-2 based on experimental studies [15,16] has provided the rationale for combining IL-2 and IFN.

Among cytotoxic drugs added to IL-2 treatment, cyclophosphamide [17] and dacarbazine [18] have been effectively used with acceptable tolerance, but in all cases the response rates were less than 30%. Our present study evaluates the potential synergism of CDDP, IL-2 and IFN-α. We obtained 61% overall responses with 13% complete responses [14].

It is well known that IL-2-induced tumour regressions are mediated by the host's biological response, but which immunological parameter is predictive of the clinical response remains to be established. According to the results reported by Lissoni *et al.* [19] and Favrot *et al.* [20] immunophenotypic lymphocyte modifications do not seem to correlate to the clinical response, which is totally in agreement with our own results concerning $CD3^+$, $CD4^+$, $CD8^+$, $CD25^+$ lymphocyte expression, both at the beginning or during treatment. Indeed, in responder patients we obtained a negative correlation ($r_{ho}=-0.37$; $P=0.05$) between the absolute number of cells expressing $CD25^+$ and the shedding of soluble IL-2R in the serum which is not found in non-responder patients; this last point might be explained by a dysfunction of the soluble IL-2 receptor shedding.

In contrast with most of the published data, we found a

significant difference in the serum sIL-2R levels between responder and non-responder patients during the first two induction courses, suggesting that the non-responder lymphocytes may be slower in becoming stimulated as assessed by CD25 shedding. It might be the most striking point of our study since, to our knowledge, no one to date has mentioned this type of variation. Indeed, Lissoni *et al.* [19] reported opposite results regarding serum soluble IL-2R release between responder and non-responder patients. However, their study used low dose s.c. IL-2 alone in advanced renal cancer. Moreover, the small number of patients ($n = 13$; R = 4, NR = 9) did not allow them to draw definite conclusions about the relationship between immune changes and clinical response. In the same manner, in a retrospective study on patients with hairy cell leukaemia, Richards *et al.* [21] reported that improved clinical status was associated with decreasing serum sIL-2R levels, whereas disease relapse was associated with increasing levels. But it is important to note that, in this particular case, the involvement of an immune system defect might explain the results, since it has been shown *in vitro* that hairy leukaemia cells release the soluble form of IL-2R [22].

It is known that serum sIL-2R concentrations rapidly increase in response to IL-2 infusion, and remain high until the end of the IL-2 cycle. Furthermore, we obtained a significant dissociation in serum sIL-2R expression between R and NR patients, and this only up to day 21, suggesting that serum sIL-2R level might be an early predictive factor of the clinical response. Indeed, the statistical analysis based on logistic regression using different factors (sex, age, metastatic site and serum sIL-2R level) on clinical response concerns this assumption. Patients with level greater than 250 pM at day 21 have 12-fold more chance of undergoing a clinical response.

It has been established that IL-2 directly activates NK cells, resulting in proliferation and induction of cytotoxic activity [23]. In fact, we observed during the first induction cycle that NK cells expressing both $CD56^+$ and $CD16^+$ from responder patients significantly increased (Fig. 2), whereas the NK cells from non-responder patients slightly increased only at the end of the treatment. Weil-Hillman *et al.* [24] and Ellis & Fisher [25] have previously shown that, following IL-2 administration, the predominant cell population mediating LAK activity is NK cell since they express a high density of $CD56^+$ antigen [26]. Among these $CD56^+$ cells, 90% express low-affinity Fcγ receptor (i.e. $CD16^+$), whereas a minority ($< 10\%$) are $CD16^-$ [27]. The functional role of these distinct NK cell subsets is still unclear, although the same authors suggested that these subsets might define a portion of the NK cell differentiation pathway.

IL-2 has been shown to stimulate TNF-α production *in vivo*, which also plays a role in the necrosis of some tumours. In contrast with previous data reported by Blay [28] on circulating TNF both before any therapy and during IL-2 infusion to patients with metastatic renal cell carcinoma, we did not detect TNF-α release at any time in either group of patients. Furthermore, our results are in agreement with McIntyre *et al.* [10], who found detectable serum TNF-α only in one patient out of 12 treated with rIL-2 for malignant melanoma or renal cell carcinoma.

Although the above results must be interpreted with caution, we speculate from this study that serum soluble IL-2R level may be of potential value in the follow up of patients with metastatic malignant melanoma receiving chemoimmunotherapy.

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REFERENCES

1 Legha SS. Current therapy for melanoma. Semin Oncol 1989; **16**: 34–44.
2 Gundersen S. Dacarbazine, vindesine and cisplatinum combination chemotherapy in advanced malignant melanoma: a phase II study. Cancer Treat Rep 1987; **71**:997–9.
3 Carey RW, Anderson JR, Green H *et al.* Treatment of metastatic malignant melanoma with Vinblastine, Dacarbazine and Cisplatine: a report from the cancer and leukemia Group B. Cancer Treat Rep 1986; **70**:329–31.
4 Rosenberg SA, Mulé JJ, Spiess PJ *et al.* Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin-2. J Exp Med 1985; **161**:1169–88.
5 Rosenberg SA, Lotze MT, Muul LM *et al.* A progress report on the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukin-2 or high dose interleukin-2 alone. N Engl J Med 1987; **316**:889–97.
6 Weiner LM, Padavic-Shaller K, Kitson J *et al.* Phase I evaluation of combination therapy with interleukin-2 and γ-interferon. Cancer Res 1991; **51**:3910–8.
7 Rosenberg SA, Lotze MT, Yang JC *et al.* Combination therapy with interleukin-2 and α-interferon for the treatment of patients with advanced cancer. J Clin Oncol 1989; **12**:1863–74.
8 Urba WJ, Steis RG, Longo DL *et al.* Immunomodulatory properties and toxicity of interleukin-2 in patients with cancer. Cancer Res 1990; **50**:185–92.
9 Gottlieb DJ, Prentice HG, Heslop HE *et al.* IL-2 infusion abrogates humoral immune responses in humans. Clin Exp Immunol 1992; **87**:493–8.
10 McIntyre CA, Chapman K, Reeder S *et al.* Treatment of malignant melanoma and renal cell carcinoma with recombinant human interleukin-2: analysis of cytokine levels in sera and culture supernatants. Eur J Cancer 1992; **28**:58–63.
11 Voss S, Sondel P, Robb R. Characterization of the interleukin 2 receptors (IL2R) expressed on human natural killer cells activated *in vivo* by IL2: association of the p64 IL2R γ chain with the IL2R β chain in functional intermediate affinity IL2R. J Exp Med 1992; **176**:531–41.
12 Rubin L, Jay G, Nelson D. The released interleukin-2 receptor binds interleukin-2 efficiently. J Immunol 1986; **137**:3841–4.
13 Voss SD, Hank JA, Nobis C *et al.* Serum levels of the low-affinity interleukin-2 receptor molecule (TAC) during IL2 therapy reflect systemic lymphoid mass activation. Cancer Immunol Immunother 1989; **29**:261–9.
14 Khayat D, Borel Ch, Tourani JM *et al.* Sequential chemoimmunotherapy with cisplatin interleukin2 and interferon alpha-2-a for metastatic melanoma. J Clin Oncol 1993; **11**:2173–80.
15 Itoh K, Shiiba K, Shimizu Y *et al.* Generation of activated killer (AK) cells by recombinant interleukin-2 (rIL2) in collaboration with interferon-γ (IFNγ). J Immunol 1985; **134**:3124–9.
16 Weiner LM, Steplewski Z, Koprowski H *et al.* Divergent dose-related effects of γ-interferon therapy on *in vitro* antibody-dependant cellular and nonspecific cytotoxicity by human peripheral blood monocytes. Cancer Res 1988; **48**:1042–6.

17 Mitchell MS, Kempf RA, Harel W *et al.* Effectiveness and tolerability of low-dose cyclophosphamide and low-dose intervenous interleukin-2 in disseminated melanoma. J Clin Oncol 1988; **6**:409–24.

18 Hayat K, Rodgers S, Bruce L *et al.* Malignant melanoma and renal cell carcinoma: immunological and haematological effects of recombinant human interleukin-2. Eur J Cancer 1991; **27**:1009–14.

19 Lissoni P, Barni S, Ardizzoia A *et al.* Second line therapy with low-dose subcutaneous interleukin-2 alone in advanced renal cancer patients resistant to interferon-alpha. Eur J Cancer 1992; **28**:92–96.

20 Favrot MC, Combaret V, Negrier S *et al.* Functional and immunophenotypic modifications induced by interleukin-2 did not predict response to therapy in patients with renal cell carcinoma. J Biol Response Mod 1990; **9**:167–77.

21 Richards JM, Mick R, Latta JM *et al.* Serum soluble interleukin-2 receptor is associated with clinical and pathologic disease status in hairy cell leukemia. Blood 1990; **76**:1941–5.

22 Chilosi M, Semenzato G, Cetto G *et al.* Soluble interleukin-2 receptor in the sera of patients with hairy cell leukemia: relationship with the effects of recombinant alpha interferon therapy on clinical parameters and on natural killer *in vitro* activity. Blood 1987; **70**:1530–5.

23 Lanier LL, Benike CJ, Phillips JH *et al.* Recombinant interleukin-2 enhanced natural killer cell-mediated cytotoxicity in human lymphocyte subpopulations expressing the Leu 7 and Leu 11 antigens. J Immunol 1985; **134**:794–801.

24 Weil-Hillman G, Fish P, Prieve AF *et al.* Lymphokine-activated killer activity induced by *in vivo* interleukin-2 therapy: predominant role for lymphocytes with increased expression of CD2 and Leu19 antigens but negative expression of CD16 antigens. Cancer Res 1989; **49**:3680–8.

25 Ellis TM, Fisher RI. Functional heterogeneity of Leu19 (bright$^+$) and Leu19 (dim$^+$) lymphokine-activated killer cells. J Immunol 1989; **142**:2949–54.

26 Lanier LL, My Le, Civin CI *et al.* The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. J Immunol 1986; **136**:4480–6.

27 Nagler A, Lanier LL, Cwirla S *et al.* Comparative studies of human FcRIII-positive and negative natural killer cells. J Immunol 1989; **143**:3183–91.

28 Blay JY, Favrot MC, Negrier S *et al.* Correlation between clinical response to interleukin-2 therapy and sustained production of tumor necrosis factor. Cancer Res 1990; **50**:2371–4.